# COMPARATIVE EVALUATION OF THE EFFECTS OF IONIC (UROGRAPHIN) AND NON-IONIC (IOHEXOL) RADIOGRAPHIC CONTRAST MEDIA ON WISTAR RATS


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## CERTIFICATION

This is to certify the project on COMPARATIVE EVALUATION OF THE EFFECTS OF IONIC (UROGRAPHIN) AND NON-IONIC (IOHEXOL) RADIOGRAPHIC CONTRAST MEDIA ON WISTAR RATS written by AMADASUN OSAMAGBE with matriculation number BMS2003055 in partial fulfillment of the Bachelor of Radiography Degree (B.Rad) in the DEPARTMENT OF RADIOGRAPHY, SCHOOL OF BASIC MEDICAL SCIENCES, UNIVERSITY OF BENIN.

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EXTERNAL EXAMINER Signature and Date

## APPROVAL

Project Title: COMPARATIVE EVALUATION OF THE EFFECTS OF IONIC (UROGRAPHIN) AND NON-IONIC (IOHEXOL) RADIOGRAPHIC CONTRAST MEDIA ON WISTAR RAT

Supervisor's Name: DR. U.I. NWADIKE Signature and Date .....

Head of Department: Mrs. F. O. Igbinedion Signature and Date .....

## DEDICATION

I dedicate this project to God Almighty, El-Roi, and Mother Mary, whose guidance, grace, patience, passion, wisdom, and strength have been my constant source of inspiration throughout this work.

This work is also lovingly dedicated to myself and to my parents, Prof. and Mrs. F. E. Amadasun, for their endless love, prayers, and encouragement, which have been the foundation of my success.

To my wonderful siblings and my ever-loving cousin, thank you for your unwavering support and belief in me.

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## ABSTRACT

**Background:** Radiographic contrast agents are widely used for diagnostic enhancement, yet concerns remain about their toxic effects on vital organs.

**Statement of Problem:** Despite the popularity of contrast media as a radiological technique, the possible systemic and organ-level impact of this method has not been properly examined, particularly in Nigeria. Thus, the research assesses and contrasts the biological effects of selected contrast agents use in Wistar-rats so as to support the evidence that can be used to guide the use of safer contrast media that is evidence-based.

**Aim:** This experimental study compared the biochemical, histopathological, and behavioral effects of ionic (Urographin) and non-ionic (Iohexol) contrast media on Wistar rats.

**Methods:** Nine rats were randomized into three groups: control, Urographin, and Iohexol. Kidney and liver markers, body weight, and behavior were assessed, followed by histological evaluation.

**Results:** Urographin produced higher renal stress, with urea increasing from 36.4 to 41.3 mg/dL (+13.5%) and creatinine rising from 3.98 to 4.15 mg/dL (+4.3%) compared to Iohexol (urea 40.1 mg/dL, +10.2%; creatinine 4.05 mg/dL, +1.8%). Kruskal–Wallis analysis showed borderline group differences for creatinine ($p = 0.0628$), sodium ($p = 0.0628$), and potassium ($p = 0.0594$), while other renal markers were not statistically significant ($p > 0.05$). Hepatic markers showed a marked increase in AST with Urographin (96.9 to 162.5 U/L, +67.7%; $p \approx 0.0665$), compared to Iohexol (119.9 U/L, +23.7%), while ALT changes were modest ($p > 0.05$). Total bilirubin increased by 22.1% in the Urographin group versus 8.6% in the Iohexol group. Body weight declined significantly more with Urographin (−5.8%) than Iohexol (−2.0%), with a borderline statistical trend ($p = 0.064$). Behaviorally, both agents caused reduced activity, piloerection, and appetite loss, but these signs were more severe in the Urographin group. Histopathology revealed mild hepatitis and pyelonephritis in Iohexol-treated rats, compared to zonal necrosis, vascular congestion, and glomerular degeneration in Urographin-treated rats.

**Conclusion:** Both contrast media induced biochemical and tissue injury, but Urographin demonstrated greater toxic potential. Iohexol may therefore be considered the safer alternative for contrast-enhanced procedures.



KEYWORDS; Contrast media; Iohexol; Urographin; nephrotoxicity; hepatotoxicity; Wistar rats; Kruskal–Wallis; histopathology.

# CHAPTER ONE

# INTRODUCTION

## 1.1 Background of the Study

Contrast media are integral to modern diagnostic radiology, as they enhance the visibility of internal anatomical structures and significantly improve diagnostic accuracy (Okoye et al., 2015). These substances are particularly vital in procedures such as computed tomography (CT), angiography, and magnetic resonance imaging (MRI), where they help differentiate between normal and pathological tissues. Their effectiveness largely depends on their chemical and physical properties—such as iodine concentration, osmolality, viscosity, and molecular composition (Imai et al., 2018). While contrast media are extensively used in clinical practice, including in institutions such as the University of Benin Teaching Hospital (UBTH), comprehensive comparative evaluations of their biological effects remain limited, especially within localized or preclinical settings.

Globally, several studies have evaluated the performance and safety of contrast media in human populations. Imai et al. (2018) highlighted that media with higher iodine concentrations and lower osmolality often yield superior imaging results, though potentially at the cost of increased adverse reactions. Namasivayam et al. (2006) underscored the importance of striking a balance between image clarity and patient safety, particularly with respect to renal toxicity. In Nigeria, Okoye et al. (2015) reported biochemical changes following contrast media administration, while Abonyi *et al.,* (2014)

documented the history and side effects associated with various contrast agents. However, few of these studies have extended into animal models, which are essential for understanding the histopathological and systemic impact of contrast agents before clinical translation.

The use of Wistar rats in radiological research provides a controlled environment to investigate the physiological and histological effects of contrast media. These animal models are particularly useful for assessing early toxicological signals, such as tissue damage, organ dysfunction, or biochemical alterations, that might not be immediately evident in human patients. Preclinical studies using Wistar rats allow researchers to evaluate the safety profiles of contrast media under standardized conditions, enabling a clearer comparison of their potential risks and systemic impacts.

Despite the increasing use of contrast agents in Nigeria's radiology departments, there is a lack of localized preclinical studies evaluating the comparative effects of different contrast agents on biological tissues. This creates a significant knowledge gap, especially regarding their potential toxicity, histological effects, and long-term implications when introduced into biological systems. Such data are crucial not only for guiding clinical choices but also for contributing to global understanding of contrast media safety.

This study, therefore, seeks to evaluate the biological and histopathological effects of different contrast media used in radiology on Wistar rats. By comparing their impact on various organs and physiological parameters, the study aims to identify which contrast agents are safer or more potentially harmful in a controlled experimental setting. The

findings will help inform both local and global radiological practices, contributing to safer contrast media usage and potentially guiding regulatory and clinical decisions concerning their use.

### 1.2 Statement of the Problem

In diagnostic radiology, the use of contrast media is expected to be guided by comprehensive knowledge of their chemical composition, physical characteristics, and biological safety. Internationally, the selection of contrast agents is informed by factors such as osmolality, viscosity, iodine concentration, and known adverse effect profiles, all aimed at optimizing image quality while minimizing patient harm (Namasivayam et al., 2006; Imai et al., 2018). These agents are routinely used in various imaging procedures to enhance visualization, yet their systemic effects which is particularly at the cellular and organ level remain an area of growing concern.

Despite their widespread use in radiology departments across Nigeria, including institutions like the University of Benin Teaching Hospital (UBTH), there is a significant gap in preclinical evaluation of the biological effects of these agents. Specifically, little is known about how different contrast media interact with internal organs, their potential toxicity, or their histopathological impact on biological tissues when introduced into living systems. Clinical assessments often focus on immediate imaging outcomes and acute allergic reactions, leaving chronic or microscopic effects largely unexplored. This oversight limits the ability of healthcare professionals to make fully informed decisions regarding contrast media selection and risk management.

The use of animal models such as Wistar rats offers a unique opportunity to bridge this knowledge gap by providing insight into the physiological and histological responses to various contrast media under controlled conditions. However, studies that focus on such comparative biological assessments are scarce in the Nigerian context. Without this foundational evidence, radiology professionals may be operating based on generalized assumptions rather than localized, evidence-based safety data.

This study aims to address this problem by evaluating and comparing the biological effects of different contrast agents used in radiology on Wistar rats. Through detailed histopathological and physiological analyses, the research seeks to provide valuable data on organ-specific responses and potential toxicity. The findings will help support safer contrast media use, inform clinical guidelines, and promote improved patient outcomes through more informed, evidence-based practices.

### 1.3 Research Questions

1. What effects do Urographin and iohexol administration have on the liver and kidney, of Wistar rats, as assessed through biochemical and histopathological analysis?

2. What behavioral and physical changes are observed in Wistar rats following the administration of Urographin and iohexol?

## 1.4 General Objective

The study aims to evaluate and compare the biochemical, histopathological, and behavioral effects of ionic (Urographin) and non-ionic (iohexol) radiographic contrast media on the liver and kidney of Wistar rats.

## 1.5 Specific Objectives

1. To assess the effects of iohexol (non-ionic) and Urographin (ionic) administration on the liver of Wistar rats using biochemical and histopathological analysis.

2. To assess the effects of iohexol (non-ionic) and Urographin (ionic) administration on the kidney of Wistar rats using biochemical and histopathological analysis.

3. To observe and record behavioral and physical changes in Wistar rats following the administration of iohexol and Urographin.

## 1.6 Significance of the Study

To Health: The findings of this study will help enhance patient safety during contrast-enhanced diagnostic imaging by promoting the use of contrast agents with optimal properties and lower incidence of adverse reactions. Improved safety protocols and agent selection can lead to better patient outcomes, especially for individuals with pre-existing health conditions.

To the Profession: Radiographers and radiologists will benefit from evidence-based insights that support informed decision-making in selecting appropriate contrast media.

This will contribute to professional development, encourage standardization in imaging practices, and potentially influence training curricula in radiography and radiology.

To the Society: By improving the accuracy and safety of diagnostic imaging services at UBTH, this study supports the broader goal of enhancing public health services. A more efficient diagnostic system means earlier and more accurate diagnosis, which can reduce healthcare costs and improve the quality of life within the community.

### 1.7 Research Hypotheses

Null Hypothesis ($H_0$): There is no significant difference in the biochemical, histopathological, or behavioral outcomes of Wistar rats administered ionic (Urographin) and non-ionic (iohexol) contrast media.

Alternative Hypothesis ($H_1$): There is a significant difference in the biochemical, histopathological, and behavioral outcomes of Wistar rats administered ionic (Urographin) and non-ionic (iohexol) contrast media.

### 1.8 Scope of the Study

This study is limited to the evaluation of different radiographic contrast media and their effects on Wistar rats in a controlled experimental setting. It focuses on iodinated and non-iodinated contrast agents commonly used in radiographic imaging procedures. The scope includes assessing the physical and chemical properties of these contrast agents, as well as analyzing their biological effects based on varying doses. The study further examines observable physiological and behavioral changes in Wistar rats following

administration. It does not cover contrast agents used in MRI or ultrasound, nor does it extend to human clinical trials or non-radiographic applications of contrast media.

### 1.9 Operational Definition of Terms

Contrast Media: Substances used in medical imaging to enhance the contrast of structures or fluids within the body.

Osmolality: A measure of solute concentration in a solution, often used to evaluate the concentration of contrast media.

Viscosity: The resistance of a liquid to flow, which can affect the ease of injection and patient comfort.

Iodine Concentration: The amount of iodine present in a contrast agent, influencing its radiodensity and effectiveness.

Safety Profile: Refers to the documented adverse effects and overall safety record of a contrast agent based on clinical data.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Conceptual Review

### 2.1.1 Concept of Contrast Media in Radiology

Contrast media are indispensable tools in modern diagnostic imaging, as they enhance the visibility of anatomical structures that might otherwise be indistinct on standard radiographs or advanced imaging modalities (Namasivayam et al., 2006). The fundamental role of contrast agents is to improve the differentiation between normal and abnormal tissues, thus increasing diagnostic accuracy (Imai et al., 2018). These substances achieve their purpose by altering the attenuation of X-rays or the magnetic properties of tissues, depending on the imaging modality employed (Okoye et al., 2015). Broadly, contrast media can be classified as positive or negative based on their radiodensity relative to body tissues (Namasivayam et al., 2006). Positive contrast agents, such as iodinated compounds and barium sulfate, are radiopaque and appear white on imaging, while negative agents, like air and carbon dioxide, are radiolucent and appear black (Abonyi et al., 2014). The chemical composition and physical characteristics of

these agents, including osmolality, viscosity, and iodine concentration, significantly influence both their imaging efficacy and safety profile (Imai et al., 2018; Okoye et al., 2015).

The selection of contrast media in clinical practice is typically guided by the type of imaging study, the target organ, and the patient's individual risk factors, particularly those related to renal and cardiovascular function (Namasivayam et al., 2006). Studies in both human subjects and animal models have highlighted the importance of these considerations in minimizing adverse reactions (Abonyi et al., 2014; Egbeyemi & Ugwu, 2014). Furthermore, the route of administration whether oral, intraperitoneal, or intravenous can influence the distribution, metabolism, and potential toxicity of the contrast agents (Ringler et al., 2021).

Although widely utilized, contrast media are not without risks, and their use requires a thorough understanding of their biological interactions (Okoye et al., 2015). Preclinical studies, particularly those involving Wistar rats, provide valuable insights into the systemic and organ-specific effects of these agents under controlled conditions (Rogers et al., 2023; Egbeyemi & Ugwu, 2014). Such investigations are critical in informing safer clinical practices and in guiding the development of newer, more biocompatible contrast media (Bussi et al., 2020; Le Fur et al., 2023).

### 2.1.2 Common Types of Contrast Media

In diagnostic radiology, several types of contrast media are routinely employed to enhance the clarity and precision of imaging studies. Among the most widely used are

barium sulfate and iodinated contrast agents, each chosen based on the specific clinical application and the imaging modality in use (Abonyi et al., 2014; Namasivayam et al., 2006). Barium sulfate is predominantly used for gastrointestinal studies due to its excellent coating properties and radiopacity, allowing for clear visualization of the bowel lumen and mucosal patterns (Abonyi et al., 2014). Iodinated contrast agents, on the other hand, have a broader range of applications, particularly in angiography, computed tomography (CT), and urography (Namasivayam et al., 2006; Imai et al., 2018). These agents can be further categorized into ionic and non-ionic compounds, with non-ionic agents such as iohexol and iohexol being favored in modern practice due to their lower osmolality and reduced risk of adverse reactions (Okoye et al., 2015). Urographin, though an older ionic agent, is still utilized in certain settings but is generally associated with higher incidences of side effects, including nephrotoxicity and vascular irritation (Li et al., 2015).

Gastrographin, a water-soluble iodinated contrast medium, is commonly used when barium sulfate is contraindicated, such as in suspected gastrointestinal perforations or leaks (Namasivayam et al., 2006). Its hyperosmolar nature, however, requires careful administration to avoid complications such as fluid shifts and aspiration pneumonitis (Abonyi et al., 2014).

Preclinical studies, particularly those conducted on Wistar rats, have provided important insights into the comparative effects of these contrast agents. For example, animal studies have documented varying degrees of organ-specific retention and toxicity depending on

the agent used, with some agents showing greater accumulation in the kidneys, liver, or heart (Egbeyemi & Ugwu, 2014; Ringler et al., 2021). These findings underscore the importance of understanding the properties and biological interactions of each contrast medium to optimize safety and efficacy in clinical practice (Bussi et al., 2020; Green et al., 2022).

### 2.1.3 Mechanism of Action of Contrast Media

The fundamental mechanism by which contrast media function lies in their ability to alter the interaction of X-rays or magnetic fields with body tissues, thereby enhancing the visibility of anatomical structures that might otherwise be indistinct (Namasivayam et al., 2006). In radiography and computed tomography, iodinated contrast agents increase the attenuation of X-rays due to the high atomic number of iodine, which efficiently absorbs radiation and produces a brighter appearance of the contrasted area on the image (Imai et al., 2018). Barium sulfate functions similarly by providing a dense, radiopaque coating of the gastrointestinal tract that sharply outlines the mucosal surface (Abonyi et al., 2014).

The distribution of contrast agents within the body depends largely on their physicochemical properties, including osmolality, viscosity, and molecular structure (Imai et al., 2018; Okoye et al., 2015). For example, agents with high osmolality tend to cause more pronounced shifts in fluid balance across vascular and tissue compartments, which can contribute to adverse effects such as vascular endothelial damage and tissue edema (Li et al., 2015). Non-ionic, low-osmolality contrast agents like iohexol and

iohexol are therefore preferred for intravascular use due to their lower propensity to disrupt cellular homeostasis (Namasivayam et al., 2006).

In magnetic resonance imaging (MRI), gadolinium-based agents work differently by altering the relaxation times of nearby hydrogen protons, thereby enhancing the contrast between different tissues (Green et al., 2022; Bussi et al., 2020). Although gadolinium agents are not the focus of this study, their distinct mechanism underscores the diversity in how contrast media interact with imaging modalities (Le Fur et al., 2023).

Importantly, preclinical models, including Wistar rats, have helped to clarify the biodistribution and retention of contrast agents in various organs following administration (Ringler et al., 2021; Rogers et al., 2023). These studies have demonstrated that the mechanism of action extends beyond image enhancement to include potential biological effects, such as the accumulation of contrast agents in tissues and subsequent cellular alterations (Egbeyemi & Ugwu, 2014).

### 2.1.4 Physicochemical Properties Relevant to Safety

The physicochemical properties of contrast media play a critical role in determining not only their diagnostic efficacy but also their safety profile (Namasivayam et al., 2006). Among the most significant of these properties are osmolality, viscosity, iodine concentration, and solubility, all of which influence how the agents interact with biological systems (Imai et al., 2018; Okoye et al., 2015). Osmolality refers to the concentration of solute particles in solution, and in the context of contrast media, it has a direct impact on vascular and cellular homeostasis (Li et al., 2015). High-osmolality

contrast agents can cause shifts of fluid from intracellular to extracellular compartments, potentially leading to vascular endothelial injury, hemoconcentration, and even organ dysfunction (Wang et al., 2017). This effect has been observed in animal studies, where high-osmolality agents induced more pronounced renal and hepatic changes compared to low-osmolality alternatives (Egbeyemi & Ugwu, 2014).

Viscosity, or the resistance of a fluid to flow, affects the ease of injection and the rate at which contrast media circulate through the vascular system (Imai et al., 2018). Higher viscosity agents may increase the workload on the heart and reduce perfusion in microvascular beds, which can be particularly concerning in compromised patients (Namasivayam et al., 2006). In preclinical models, increased viscosity has been associated with altered perfusion patterns and microvascular changes, as seen in the work of Wang et al. (2017) on renal perfusion in rats.

Iodine concentration is another vital property, as it determines the degree of radiodensity the agent provides (Imai et al., 2018). While higher iodine content enhances image clarity, it may also increase the risk of iodine-related cytotoxicity, as demonstrated in studies where elevated iodine load contributed to nephrotoxicity in animal models (Li et al., 2015). Solubility and excretion patterns are equally important, as poorly soluble agents may linger in tissues and provoke inflammatory or fibrotic responses (Abonyi et al., 2014). This phenomenon has been reported in both clinical observations and animal studies, where certain contrast agents exhibited prolonged organ retention with potential histopathological consequences (Ringler et al., 2021; Bussi et al., 2020).

### 2.1.5 Biological and Toxicological Considerations

While contrast media are indispensable in enhancing diagnostic imaging, their administration is not without potential biological risks (Namasivayam et al., 2006). The toxicity of these agents, whether acute or chronic, can affect various organ systems depending on their composition, dose, route of administration, and physicochemical properties (Imai et al., 2018). Adverse reactions may range from mild and transient effects, such as nausea or warmth, to severe complications like contrast-induced nephropathy or tissue necrosis (Okoye et al., 2015). Renal toxicity is perhaps the most well-documented risk associated with iodinated contrast media (Li et al., 2015). High-osmolality and high-viscosity agents, in particular, have been implicated in compromising renal blood flow, increasing oxidative stress, and inducing tubular injury (Wang et al., 2017). Animal models, including Wistar rats, have provided critical evidence in this regard, with studies demonstrating dose-dependent changes in renal architecture and function following contrast administration (Egbeyemi & Ugwu, 2014; Li et al., 2015).

Hepatic and cardiac effects have also been reported in experimental settings. For instance, research using Wistar rats has shown that certain contrast media can induce hepatocellular degeneration and mild inflammatory responses within liver tissue, particularly at higher doses (Egbeyemi & Ugwu, 2014). Similarly, contrast agents have been linked to subtle myocardial changes, although the clinical significance of these findings requires further investigation (Rogers et al., 2023). Beyond these systemic toxicities, studies have highlighted the potential for contrast agents to accumulate within

organs, leading to longer-term concerns (Ringler et al., 2021; Bussi et al., 2020). Gadolinium-based agents, for example, have been shown to persist in tissues, including the brain, liver, and kidneys, even in healthy animal models (Green et al., 2022; Le Fur et al., 2023). Although gadolinium compounds are not the primary focus of this study, these findings reinforce the importance of evaluating organ retention and clearance patterns for all contrast agents.

Preclinical studies in rats continue to shed light on these biological effects, offering valuable insights that can guide safer clinical practice (Rogers et al., 2023). Such research emphasizes the need for judicious contrast agent selection and careful dose consideration, particularly in vulnerable patient populations (Namasivayam et al., 2006; Okoye et al., 2015).

### 2.1.6 Use of Wistar Rats in Radiological Research

Wistar rats have long been recognized as valuable models in biomedical research, including radiological studies aimed at evaluating the safety and biological effects of contrast media (Egbeyemi & Ugwu, 2014). Their well-characterized physiology, manageable size, and ease of handling make them particularly suitable for controlled laboratory experiments where organ-specific responses and systemic effects of contrast agents are to be assessed (Rogers et al., 2023). In radiology-focused studies, Wistar rats allow for the precise administration of contrast media, enabling researchers to monitor biodistribution, clearance patterns, and potential toxicities under standardized conditions (Ringler et al., 2021).

The use of Wistar rats has contributed significantly to our understanding of contrast-induced nephropathy, hepatotoxicity, and cardiac effects. For instance, studies have demonstrated that certain iodinated and gadolinium-based contrast agents can cause histopathological changes in renal tubules, hepatocytes, and myocardial fibers, particularly at higher doses or with repeated administration (Egbeyemi & Ugwu, 2014; Bussi et al., 2020). These findings are invaluable in predicting potential human responses, as direct tissue sampling and microscopic evaluation in human subjects are rarely feasible (Green et al., 2022).

Furthermore, the rat model has been instrumental in elucidating patterns of organ retention and the kinetics of contrast media elimination. Research by Le Fur et al. (2023) and Ringler et al. (2021) highlighted the persistence of gadolinium and other contrast agents in various organs long after administration, underscoring the need for caution, particularly in individuals requiring repeated imaging studies. Such data from Wistar rat studies help to inform clinical guidelines and promote safer imaging practices (Rogers et al., 2023; Namasivayam et al., 2006).

Ethically, the use of animal models like Wistar rats requires adherence to strict welfare standards to ensure humane treatment and minimize distress (Rogers et al., 2023). Research protocols are typically subject to rigorous ethical review, and animal care practices are designed to align with both national and international guidelines (Ringler et al., 2021).

## 2.2 Theoretical Review

### 2.2.1 Toxicokinetic Theory

The toxicokinetic theory provides a useful framework for understanding how toxic substances, including contrast media, are absorbed, distributed, metabolized, and excreted in biological systems (Namasivayam et al., 2006). At its core, the theory posits that the biological impact of a substance depends not only on its intrinsic toxicity but also on its kinetic behavior within the body. Factors such as dose, route of administration, solubility, molecular size, and binding affinity determine how extensively a substance reaches target organs, how long it persists, and how effectively it is eliminated (Imai et al., 2018; Okoye et al., 2015).

According to the toxicokinetic model, substances with slower clearance or higher tissue retention are more likely to exert prolonged or cumulative effects, especially when administered repeatedly or in large doses (Ringler et al., 2021). The theory emphasizes that organ-specific toxicity can arise if a substance preferentially accumulates in certain tissues, such as the kidneys or liver, leading to localized damage (Li et al., 2015). This concept is particularly relevant in radiology, where contrast agents are often administered intravenously or orally and are expected to clear efficiently without causing harm (Namasivayam et al., 2006).

**Application to the Study**

The toxicokinetic theory provides a valuable lens for interpreting the findings of this study, which aims to evaluate the biological effects of different contrast media on Wistar rats. By applying this theory, the study will focus on how the physicochemical properties of contrast agents such as osmolality, viscosity, and iodine content influence their absorption, distribution, and excretion patterns in the animal model (Imai et al., 2018). The theory predicts that contrast agents with higher osmolality or viscosity may exhibit slower clearance and greater organ retention, thereby increasing the risk of tissue damage, as supported by previous animal studies (Egbeyemi & Ugwu, 2014; Ringler et al., 2021).

Through histopathological examination and biochemical assays, the study will assess whether specific contrast agents accumulate preferentially in organs like the kidneys, liver, or heart, and whether this correlates with structural or functional changes (Li et al., 2015). The toxicokinetic framework will thus help to explain any observed dose-dependent or agent-specific toxicity and contribute to a better understanding of how contrast media interact with biological tissues in preclinical models (Rogers et al., 2023).

### 2.3 Empirical Review

Le Fur et al. (2023) carried out a study to address growing concerns regarding the retention of gadolinium-based contrast agents (GBCAs) in body tissues, particularly after repeated imaging procedures. Their work was motivated by the need to better understand how these agents distribute within the body and in what chemical forms they persist following administration. The aim of the study was to evaluate the biodistribution and speciation of gadolinium in various organs of rats after multiple doses of GBCAs. The

researchers sought to determine both the quantity of gadolinium retained and whether it remained in its intact chelated form or existed as dissociated free ions, which might pose higher biological risks. In terms of methodology, the study employed a controlled laboratory experimental design involving healthy rats that received repeated administrations of GBCAs. After the dosing period, the animals were sacrificed at specific intervals, and tissues from the brain, liver, kidneys, and bones were collected. Gadolinium levels and chemical forms were assessed using advanced techniques, including inductively coupled plasma mass spectrometry (ICP-MS) and synchrotron-based X-ray fluorescence imaging, to provide both quantitative and spatial data on gadolinium retention. The statistical results revealed significant accumulation of gadolinium in the bone, kidneys, and liver, with bone tissue showing the highest concentrations. Moreover, the study found that a notable portion of retained gadolinium was present as dissociated ions rather than the original chelated complex. There were statistically significant differences in gadolinium concentration between organs and across time points ($p < 0.05$), indicating both organ-specific retention patterns and time-dependent clearance. In conclusion, the study demonstrated that repeated administration of GBCAs in rats led to substantial and organ-specific retention of gadolinium, with potential implications for long-term tissue exposure and toxicity. The findings emphasized the importance of understanding the chemical form of retained gadolinium, as dissociated ions could be more biologically active and harmful than intact contrast agents.

Rogers et al. (2023) carried out a study in response to the increasing need for robust preclinical safety data on newer contrast agents, especially those intended for widespread clinical use. Their research was motivated by concerns about tissue retention and potential toxicity of gadolinium-based contrast agents (GBCAs), which have been reported in both human and animal studies. The aim of the study was to conduct a comprehensive preclinical safety assessment of gadopiclenol, a high-relaxivity macrocyclic gadolinium-based MRI contrast agent, in Wistar rats. The goal was to evaluate its safety profile across various organ systems and identify any histopathological or functional changes following administration. The methodology involved a controlled experimental design in which Wistar rats received gadopiclenol at different doses, mimicking both clinical and supratherapeutic exposure levels. After administration, the animals were observed over a set period before being humanely sacrificed for sample collection. Histopathological evaluations were performed on key organs, including the brain, kidneys, liver, and heart. In addition, blood samples were analyzed for biochemical markers indicative of organ function. The statistical results revealed no significant adverse effects on organ structure or function at clinically relevant doses. The study found no statistically significant alterations ($p > 0.05$) in biochemical parameters or histopathological findings compared to control animals. Furthermore, gadopiclenol exhibited low levels of tissue retention, particularly in the brain and kidneys, suggesting a favorable clearance profile. In conclusion, Rogers et al. (2023) reported that gadopiclenol demonstrated a good safety profile in Wistar rats, with no evidence of organ toxicity or significant tissue retention at clinically relevant doses. The study provided valuable

preclinical data supporting the continued evaluation of gadopiclenol for clinical use in MRI imaging.

Green et al. (2022) carried out a study to investigate the long-term effects of gadolinium-based contrast agents (GBCAs) on the brain, particularly in the context of aging. The study was motivated by emerging evidence that gadolinium can accumulate in the brain after multiple contrast-enhanced MRI scans, raising concerns about potential neurological risks over time. The aim of the study was to evaluate both the structural and elemental effects of gadolinium exposure in the aging rat brain using longitudinal magnetic resonance imaging (MRI) and advanced elemental analysis techniques. The researchers sought to determine whether repeated administration of GBCAs leads to detectable brain changes and to quantify gadolinium retention. In terms of methodology, the study was designed as a longitudinal controlled animal experiment. Aging rats were administered multiple doses of GBCAs over time, with serial MRI scans performed to monitor structural brain changes. After the imaging phase, rats were sacrificed at specific intervals, and brain tissues were analyzed using elemental techniques, including inductively coupled plasma mass spectrometry (ICP-MS), to measure gadolinium concentrations. The statistical results showed that repeated administration of GBCAs led to measurable gadolinium retention within brain tissues, particularly in regions such as the deep cerebellar nuclei. Statistically significant differences ($p < 0.05$) were noted in gadolinium concentration between exposed and control animals. However, no significant MRI-detectable structural abnormalities or functional deficits were observed within the study period. In conclusion, Green et al. (2022) demonstrated that gadolinium from contrast

agents can accumulate in the aging rat brain following repeated exposure, though without causing overt structural damage detectable by MRI during the study timeframe. The study highlighted the need for further research into the long-term biological significance of gadolinium retention in neural tissues.

Ringler et al. (2021) carried out a study to explore the distribution and retention of gadolinium-based contrast agents (GBCAs) following intra-articular administration, a route less frequently studied compared to intravenous use. Their research was motivated by the need to understand whether gadolinium accumulates in extra-articular tissues after joint injections, as has been reported with other administration routes. The aim of the study was to assess the biodistribution and organ retention of gadolinium in rats after intra-articular injection of GBCAs. The researchers focused on identifying whether gadolinium could be detected in organs such as the kidneys, liver, and brain, and to what extent retention varied across these tissues. The methodology involved an experimental animal study using healthy rats that received intra-articular injections of gadolinium-based contrast agents. After specified time points, rats were sacrificed, and tissue samples from multiple organs were collected. Gadolinium concentrations were measured using highly sensitive elemental analysis techniques, including inductively coupled plasma mass spectrometry (ICP-MS). The statistical results revealed that gadolinium was detectable in several extra-articular tissues following joint injection. Statistically significant levels ($p < 0.05$) of gadolinium retention were found in the kidneys, liver, and bone compared to control animals. The highest concentrations were observed in the kidneys, indicating renal elimination as a primary clearance pathway, although some

retention was noted in bone and liver tissues. In conclusion, Ringler et al. (2021) demonstrated that intra-articular administration of GBCAs can lead to measurable gadolinium accumulation in distant organs, not just locally in the joint. The findings underscored the importance of considering systemic exposure even when contrast agents are administered locally.

Wang et al. (2017) carried out a study to investigate the renal effects of iodinated contrast agents with differing viscosities using advanced magnetic resonance imaging (MRI) techniques. The study was motivated by growing clinical awareness of contrast-induced nephropathy and the possibility that contrast viscosity might play a significant role in altering renal perfusion and function. The aim of the study was to evaluate the relationship between contrast media viscosity and renal effects in rats. Specifically, the researchers sought to use blood oxygen level–dependent (BOLD) MRI and diffusion tensor imaging (DTI) to non-invasively assess renal oxygenation and microstructural integrity after contrast administration. In terms of methodology, the study was designed as an experimental animal trial. Healthy rats were administered different iodinated contrast agents categorized by low and high viscosity. MRI scans were performed before and after administration to monitor changes in renal oxygenation and tissue structure. Following imaging, tissue and blood samples were collected for corroborative biochemical and histological analyses. The statistical results revealed that high-viscosity contrast agents caused more significant and sustained reductions in renal oxygenation compared to low-viscosity agents, as demonstrated by changes in BOLD MRI parameters ($p < 0.05$). DTI findings supported these results, showing alterations in renal

microstructure that were more pronounced in the high-viscosity groups. In conclusion, Wang et al. (2017) found that contrast agent viscosity plays a crucial role in renal perfusion and tissue health, with higher viscosity agents posing a greater risk of inducing renal dysfunction in this rat model. The study emphasized the importance of considering viscosity when selecting contrast media for patients at risk of kidney injury.

### 2.4 Summary of the Literature Review

Previous studies have provided valuable insights into the biological and toxicological effects of contrast media, particularly through the use of animal models like Wistar rats. Research by Le Fur et al. (2023), Rogers et al. (2023), and Green et al. (2022) has demonstrated that contrast agents, especially gadolinium-based compounds, can accumulate in various organs including the brain, kidneys, liver, and bones. These studies emphasized the potential for both short- and long-term tissue retention, although some reported no immediate structural or functional damage detectable through imaging or histopathology. Other investigations, such as those by Ringler et al. (2021), Wang et al. (2017), and Li et al. (2015), have explored the systemic distribution of contrast agents administered via different routes and highlighted the impact of properties like viscosity and osmolality on organ function, particularly in the kidneys.

However, while these studies have advanced knowledge on the retention patterns, biodistribution, and certain toxicological effects of contrast agents, many of them have focused predominantly on gadolinium-based agents or specific administration routes such as intravenous or intra-articular injection. Limited attention has been given to comparing

the biological effects of different types of contrast media—including both iodinated and barium-based agents—administered at doses relevant to diagnostic radiology practice. Moreover, few studies have systematically evaluated histopathological outcomes across multiple organ systems following exposure to these agents in a single experimental design.

Based on these gaps, the present study aims to fill this knowledge void by evaluating and comparing the biological and histopathological effects of different contrast media (barium, iohexol, Iohexol, Urographin, and Gastrographin) on Wistar rats. The study will provide much-needed localized preclinical data that could help guide safer contrast agent selection and usage in radiology.

# CHAPTER THREE

# RESEARCH METHODOLOGY

## 3.1 Research Design

The study adopted a controlled experimental design. This design allowed for systematic evaluation of the biological and histopathological effects of different radiological contrast agents administered to Wistar rats, using a control group for baseline comparison.

### 3.2 Research Location

This study was conducted at the animal experimental research laboratory of the University of Benin. The laboratory provides a controlled environment suitable for housing Wistar rats and is equipped for safe administration of contrast media, animal monitoring, sample collection, and histopathological processing.

### 3.3 Target Population

The target population consisted of healthy adult Wistar rats aged 8 to 12 weeks, weighing between 150 and 250 grams. Only rats in good health, without prior exposure to contrast media or underlying disease conditions, were included.

### 3.4 Sample Technique and Sample Size

A simple random sampling technique was employed to allocate rats into the study groups.

Grouping structure:

Group 1 (Control): No contrast; 3 rats

Group 2 (Urographin – ionic): 3 rats

Group 3 (iohexol – non-ionic): 3 rats

Total sample size: 9 rats

Each group received its assigned agent at a standard experimental dose.

### 3.4.1 Experimental Procedure

A total of nine (9) healthy adult rats, weighing between 150–250 g, was randomly assigned into three experimental groups of three animals each. The treatment protocol was as follows:

Group 1 (Control, n = 3): Rats received an intravenous injection of normal saline at 5.0 mL/kg (equivalent to the contrast injection volume) via the lateral tail vein. This served as the baseline control for volume and injection effects.

Group 2 (Urographin – ionic, n = 3): Rats received a single intravenous bolus of Urographin at 5.0 mL/kg through the lateral tail vein over approximately 15–20 seconds.

Group 3 (iohexol – non-ionic, n = 3): Rats received a single intravenous bolus of iohexol (Iohexol) at 5.0 mL/kg (iohexol 300 mg I/mL) or 4.3 mL/kg (iohexol 350 mg I/mL) via the lateral tail vein over approximately 15–20 seconds.

Throughout the experimental period, daily behavioral and physical changes such as weight, feeding habits, activity levels, and grooming patterns were recorded using structured observation sheets. Body weight will be monitored with a digital weighing scale.

Blood samples were collected at baseline (prior to contrast administration) and 24 hours post-administration for biochemical analysis of liver and kidney and enzymes. At 48 hours post-administration, all animals were humanely euthanized. Immediately thereafter, the liver, and kidney were excised, processed, and preserved for histopathological

examination to evaluate tissue alterations associated with contrast exposure (Ringler et al. 2021).

### 3.5 Instrument of Data Collection

Data was collected using:

- Structured observation sheets for recording daily behavioral and physical changes (e.g., feeding, activity level, grooming).
- Digital weighing scale for monitoring weight changes.
- Blood collection kits for biochemical analyses (e.g., liver and kidney enzymes.).
- Histopathology equipment for processing and examining organ tissues (liver, kidney).

### 3.6 Validity of Instrument

All instruments and protocols were reviewed by specialists in radiology, animal science, and pathology to ensure that they adequately address the study objectives.

### 3.7 Reliability of Instrument

Reliability was ensured by applying standardized procedures for contrast administration, sample collection, and histological processing. Histopathological slides was independently examined by a pathologist.

### 3.8 Method of Data Collection

Following ethical approval, nine (9) healthy adult rats weighing between 150–250 g were randomly assigned into three groups of three animals each. The assigned agents; normal

saline, Urographin (ionic), and iohexol (non-ionic) was administered intravenously via the lateral tail vein at the specified doses.

Daily monitoring was conducted using structured observation sheets to record behavioral and physical changes, including feeding patterns, activity levels, and grooming behavior. A digital weighing scale was used to measure and track body weight throughout the study period.

Blood samples was collected at baseline (prior to administration) and 24 hours after contrast administration using appropriate blood collection kits. These samples was analyzed for biochemical markers, including liver and kidney enzymes.

At 48 hours post-administration, all rats was humanely euthanized. Immediately after euthanasia, the liver, and kidney, was excised, processed, and preserved for histopathological examination to assess potential tissue alterations induced by contrast administration (Ringler et al. 2021).

### 3.9 Method of Data Analysis

Data was analyzed using SPSS version 25. Descriptive statistics (mean, percentage and frequency) was used to summarize data. Kruskal-Wallis  was applied to compare outcomes across groups. A p-value $<0.05$ will be considered statistically significant.

### 3.10 Ethical Considerations

Ethical approval was obtained from the University of Benin Animal Research Ethics Committee. The study complied with national and international guidelines on animal

research ethics, ensuring humane treatment, minimal distress, and appropriate care throughout the study.

# CHAPTER FOUR

# RESULTS AND DISCUSSION

## 4.1 Results

Biochemical Analysis of Kidney Function

**Table 4.1: Kidney function marker**

| Parameter | Control (A) | Urographin (B) | % Change | Iohexol (C) | % Change |
|---|---|---|---|---|---|
| Urea (mg/dL) | 36.4 ± 5.5 | 41.3 ± 2.3 | 13.5 | 40.1 ± 2.3 | 10.2 |
| Creatinine (mg/dL) | 3.98 ± 0.06 | 4.15 ± 0.17 | 4.3 | 4.05 ± 0.06 | 1.8 |
| $Na^+$ (mEq/L) | 115.9 ± 1.8 | 116.8 ± 0.5 | 0.8 | 116.8 ± 0.2 | 0.8 |
| $K^+$ (mEq/L) | 1.58 ± 0.02 | 1.77 ± 0.16 | 12.0 | 1.64 ± 0.04 | 3.8 |
| $Cl^-$ (mEq/L) | 30.9 ± 0.2 | 33.0 ± 2.5 | 6.8 | 31.9 ± 0.2 | 3.2 |

Urea levels were highest in the Urographin group (41.3 mg/dL), compared to 36.4 mg/dL in the control and 40.1 mg/dL in the Iohexol group. This represents a 13.5% increase in the Urographin group and a 10.2% increase in the Iohexol group relative to the control, indicating mild renal stress in both treatment groups. Creatinine levels followed a similar pattern, with the control group recording 3.98 mg/dL, the Urographin group 4.15 mg/dL (4.3% increase), and the Iohexol group 4.05 mg/dL (1.8% increase). Electrolyte analysis revealed that sodium remained stable across all groups, with values of 115.9 mEq/L in the control and 116.8 mEq/L in both treated groups, showing less than 1% change. Potassium was lowest in the control group (1.58 mEq/L), rising to 1.77 mEq/L in the Urographin group (12.0% increase) and 1.64 mEq/L in the Iohexol group (3.8% increase).

Chloride levels were 30.9 mEq/L in the control, 33.0 mEq/L in the Urographin group (6.8% increase), and 31.9 mEq/L in the Iohexol group (3.2% increase). These findings suggest that both contrast agents slightly impaired renal function, with Urographin exerting a stronger effect on urea, creatinine, and electrolyte balance.

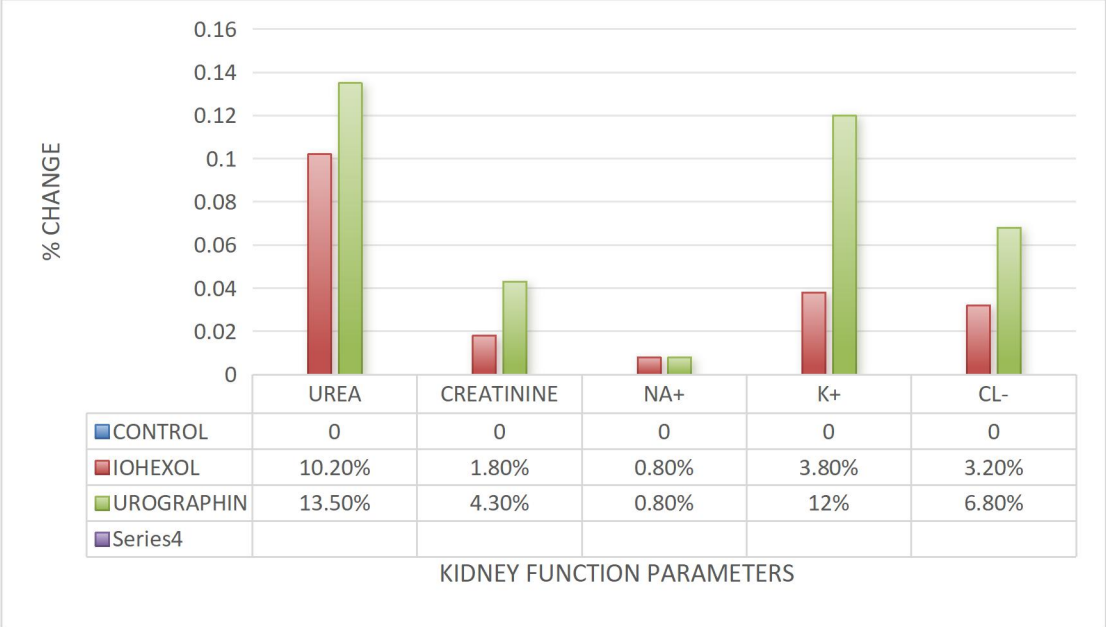


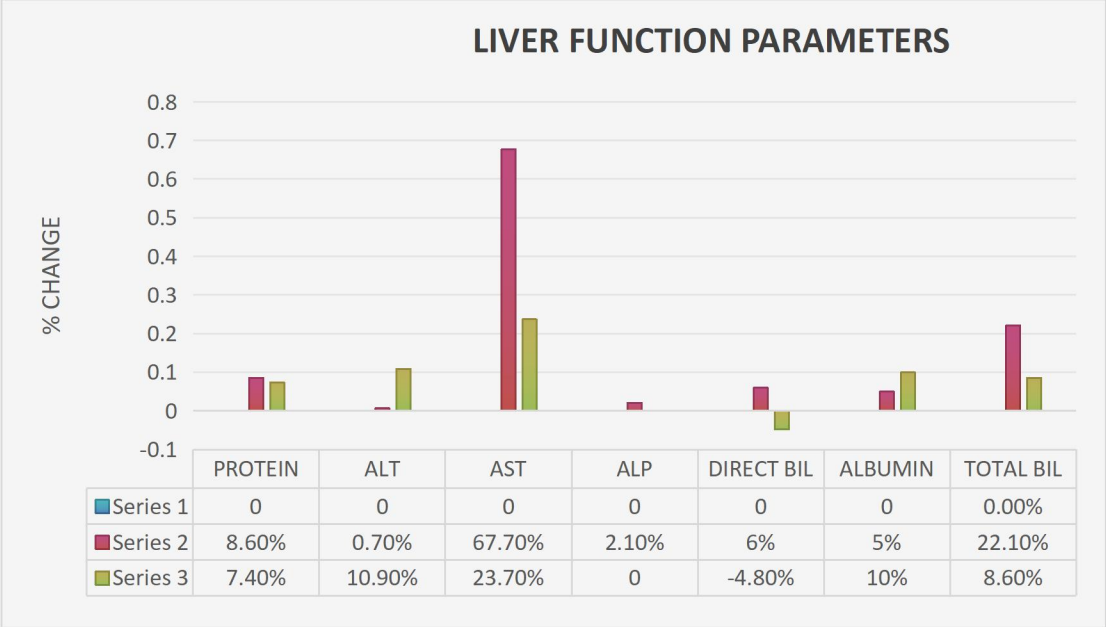

**Fig 4.1 % change of kidney function parameters**

**Table 4.2: Liver function markers**

| Parameter | Control (A) | Urographin (B) | % Change | Iohexol (C) | % Change |
|---|---|---|---|---|---|
| Protein (g/dL) | 4.05 ± 0.34 | 4.40 ± 0.25 | 8.6 | 4.35 ± 0.11 | 7.4 |
| Albumin (g/dL) | 1.80 ± 0.18 | 1.89 ± 0.11 | 5.0 | 1.98 ± 0.15 | 10.0 |
| ALT (U/L) | 114.6 ± 12.4 | 115.4 ± 16.5 | 0.7 | 127.1 ± 6.8 | 10.9 |
| AST (U/L) | 96.9 ± 16.3 | 162.5 ± 70.2 | 67.7 | 119.9 ± 17.0 | 23.7 |
| ALP (U/L) | 0.47 ± 0.01 | 0.48 ± 0.02 | 2.1 | 0.47 ± 0.01 | 0.0 |
| Total Bil (mg/dL) | 1.63 ± 0.18 | 1.99 ± 0.28 | 22.1 | 1.77 ± 0.14 | 8.6 |
| Direct Bil (mg/dL) | 0.84 ± 0.15 | 0.89 ± 0.15 | 6.0 | 0.80 ± 0.04 | -4.8 |

Total protein levels were marginally higher in the treated groups compared to the control (4.05 g/dL). Urographin administration raised protein to 4.40 g/dL (8.6% increase), while Iohexol resulted in 4.35 g/dL (7.4% increase). Albumin followed the same trend, rising from 1.80 g/dL in the control to 1.89 g/dL (5.0% increase) with Urographin and 1.98 g/dL (10.0% increase) with Iohexol. ALT activity increased most notably in the Iohexol group, reaching 127.1 U/L compared to 114.6 U/L in the control (10.9% increase) and 115.4 U/L with Urographin (0.7% increase). AST levels, however, were disproportionately elevated in the Urographin group (162.5 U/L), compared to 96.9 U/L in the control (67.7% increase) and 119.9 U/L with Iohexol (23.7% increase). This sharp rise in AST highlights a stronger hepatocellular response to ionic contrast media. ALP levels remained steady across groups, with values of 0.47–0.48 U/L, reflecting no significant biliary obstruction or cholestatic effect. Bilirubin analysis revealed total bilirubin at 1.63 mg/dL in the control, rising to 1.99 mg/dL (22.1% increase) in the

Urographin group and 1.77 mg/dL (8.6% increase) in the Iohexol group. Direct bilirubin was 0.84 mg/dL in the control, increasing slightly to 0.89 mg/dL (6.0%) with Urographin but decreasing to 0.80 mg/dL (4.8% decrease) with Iohexol. Overall, Urographin induced a more pronounced increase in AST and bilirubin, suggesting greater hepatocellular and excretory disruption, whereas Iohexol primarily elevated ALT, indicating a milder but distinct hepatocellular effect.




| | PROTEIN | ALT | AST | ALP | DIRECT BIL | ALBUMIN | TOTAL BIL |
|---|---|---|---|---|---|---|---|
| Series 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0.00% |
| Series 2 | 8.60% | 0.70% | 67.70% | 2.10% | 6% | 5% | 22.10% |
| Series 3 | 7.40% | 10.90% | 23.70% | 0 | -4.80% | 10% | 8.60% |

**Fig 4.2 % change of liver function parameters**

**Histopathological effects**

Adult Wistar rats were treated with ionic (urographin) and non-ionic (iohexol) radiographic contrast media, for a period of one month. Afterwards, the rats were sacrificed and their liver and kidneys harvested, then transported to the laboratory for

histopathological analysis. The tissue changes were subsequently observed and comparison made between the two classes of contrast media.

Fig 4.3(a-c) the histophatologic effects of contrast media on the liver of wistar rats (a) Rat liver, Control, show normal architecture: hepatocytes (HC), sinusoids (SI), bile duct (BD) and portal vein (PV). (b) Rat liver given Iohexol show: Kupffer cell activation (KA), zonal oedema (ZO), periportal infiltrates of inflammatory cells (PI) and portal vascular congestion (PC). (c)Rat liver given Urographin show: severe vasodilatation (VD), Kupffer cell activation (KA), periportal infiltrates of inflammation (PI), vascular congestion (VC) and ulceration (VU), zonal necrosis (ZN) : H&E 400 X

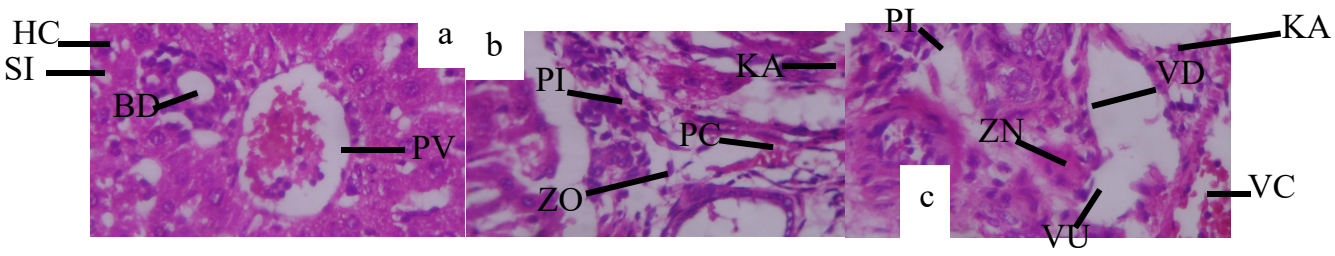



**Fig 4.3(a-c) the histophatologic effects of contrast media on liver of wistar rats**

Fig 4.4(a-c) the histophatologic effects of contrast media on the kidneys of wistar rats (a) Rat kidney, control, show normal architecture: tubules (TU), glomerulus (GL) and interstitial space (IS). (b) Rat kidney given Iohexol show: vasodilatation (VD) and interstitial congestion (VC), interstitial infiltrates of inflammatory cells (IC). (c) Rat kidney given Urogaphin show: severe glomerular degeneration (GD), interstitial congestion (VC) and vasodilatation (VD) : H&E 400 X

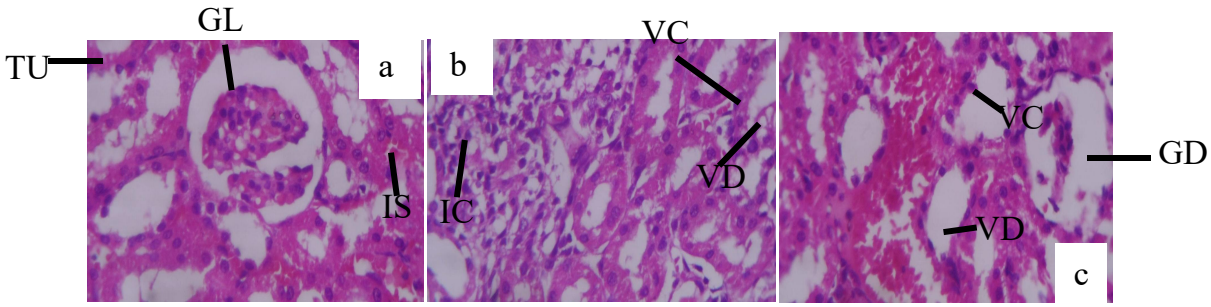



**Fig 4.4(a-c) The histophatologic effects of contrast media on kidney of wistar rats**

**Table 4.3: Body weight changes across groups**

| Group | Mean Initial (g) | Mean Final (g) | Mean % Change |
|---|---|---|---|
| Control (A) | 176 | 176 | ~0% |
| Urographin (B) | 181 | 171 | –5.8% |
| Iohexol (C) | 170 | 166 | –2.0% |

According to table 4.3 rats in the Urographin group showed the greatest reduction in body weight, with mean weight decreasing from 181 g at baseline to 171 g after 48 hours (–5.8% change). Iohexol-treated rats exhibited a milder reduction, with mean weight falling from 170 g to 166 g (–2.0% change). In contrast, control rats maintained relatively stable body weights across the study period. This trend suggests that Urographin had a stronger adverse effect on nutritional status and systemic physiology, while Iohexol exerted a smaller impact.

**Table 4.4: Observed behavioral and physical changes**

| Group | Feeding/ Appetite | Activity | Grooming/ Appearance | Other Signs |
|---|---|---|---|---|
| Control (A) | No loss of appetite | Very active | Normal grooming | No abnormalities |
| Urographin (B) | Loss of appetite | Drowsiness | Piloerection | Reduced activity |
| Iohexol (C) | Loss of appetite | Drowsiness | Piloerection | Reduced activity |

From table 4.4 the behavioral observations revealed that the control rats (Group A) remained healthy and active, with no evidence of drowsiness, appetite changes, or piloerection. In contrast, both treatment groups exhibited notable behavioral alterations. Rats in the Urographin group (Group B) developed loss of appetite, drowsiness, and piloerection, accompanied by visibly reduced activity. Similar signs were observed in the Iohexol group (Group C), although the severity appeared milder compared to Urographin. These results indicate that both contrast agents induced systemic stress, but the ionic contrast (Urographin) had a more pronounced effect on behavior and physical appearance.

### 4.2 Hypothesis Testing

Null Hypothesis ($H_0$): There is no significant difference in the biochemical, histopathological, or behavioral outcomes of Wistar rats administered ionic (Urographin) and non-ionic (iohexol) contrast media.

Alternative Hypothesis ($H_1$): There is a significant difference in the biochemical, histopathological, and behavioral outcomes of Wistar rats administered ionic (Urographin) and non-ionic (iohexol) contrast media.

The hypotheses were tested using the Kruskal–Wallis test for biochemical and physical parameters, while behavioral and histopathological findings were compared descriptively due to their qualitative nature. This approach was adopted because of the small sample size (n = 3 per group) and the need to handle both numerical and observational outcomes.

**Table 4.5a: Kruskal–Wallis results for biochemical parameters**

| Parameter | H statistic | p-value | Decision |
|---|---|---|---|
| Protein (g/dL) | 0.800 | 0.6703 | Not Significant |
| Albumin (g/dL) | 0.829 | 0.6606 | Not Significant |
| ALT (U/L) | 1.867 | 0.3932 | Not Significant |
| AST (U/L) | 5.422 | 0.0665 | Significant |
| ALP (U/L) | 0.972 | 0.6152 | Not Significant |
| Total Bilirubin (mg/dL) | 1.867 | 0.3932 | Not Significant |
| Direct Bilirubin (mg/dL) | 2.891 | 0.2357 | Not Significant |
| Urea (mg/dL) | 1.107 | 0.5748 | Not Significant |
| Creatinine (mg/dL) | 5.535 | 0.0628 | Significant |
| Potassium (mEq/L) | 5.647 | 0.0594 | Significant |
| Sodium (mEq/L) | 5.535 | 0.0628 | Significant |
| Chloride (mEq/L) | 1.424 | 0.4907 | Not Significant |

The Kruskal–Wallis analysis revealed that most biochemical parameters, including protein, albumin, ALT, ALP, bilirubin, urea, and chloride, did not differ significantly

between groups ($p > 0.05$). However, AST ($H = 5.422$, $p = 0.0665$), creatinine ($H = 5.535$, $p = 0.0628$), potassium ($H = 5.647$, $p = 0.0594$), and sodium ($H = 5.535$, $p = 0.0628$) all showed borderline results, indicating a strong trend toward group differences. This suggests that while the small sample size limited statistical power, there were biologically meaningful effects. Specifically, Urographin-treated rats showed higher AST and creatinine levels, consistent with greater hepatic and renal stress, while iohexol produced comparatively milder changes.

**Table 4.5b: Histopathological findings (descriptive comparison)**

| Group | Liver | Kidney |
|---|---|---|
| Control (A) | Normal hepatocytes, sinusoids, bile ducts, portal vein | Normal tubules, glomeruli, interstitium |
| iohexol (C) | Portal oedema, inflammatory infiltrates, Kupffer activation (Acute portal hepatitis) | Vasodilatation, congestion, mild interstitial inflammation (Mild acute pyelonephritis) |
| Urographin (B) | Vasodilatation, congestion, zonal necrosis, vascular ulceration, Kupffer activation (Severe portal hepatitis + vasculopathy) | Glomerular degeneration, vasodilatation, vascular degeneration (Glomerulopathy + vasculopathy) |

Histological evaluation revealed that control rats maintained normal liver and kidney architecture. iohexol-treated rats exhibited moderate pathological changes, including portal oedema and Kupffer cell activation in the liver, alongside mild inflammatory changes in the kidney, consistent with acute portal hepatitis and mild pyelonephritis. In contrast, Urographin-treated rats showed more severe lesions, including zonal necrosis, vascular ulceration, and vasculopathy in the liver, as well as glomerular and vascular

degeneration in the kidney. These findings demonstrate that both agents caused organ damage, but the ionic contrast (Urographin) produced significantly more severe histopathological alterations than the non-ionic agent (iohexol).

**Table 4.5c: Kruskal–Wallis test for body weight changes**

| Group | Mean Initial (g) | Mean Final (g) | % Change | H statistic | p-value |
|---|---|---|---|---|---|
| Control (A) | 176 | 176 | ~0% | | |
| Iohexol (C) | 170 | 166 | –2.0% | 5.49 | 0.064 |
| Urographin (B) | 181 | 171 | –5.8% | | |

Control rats (Group A) maintained stable body weights throughout the study period, with no measurable change (~0%). iohexol-treated rats (Group C) showed a slight reduction, from 170 g at baseline to 166 g after 48 hours (–2.0% change). Urographin-treated rats (Group B) recorded the greatest decline, falling from 181 g to 171 g (–5.8% change). The Kruskal–Wallis test yielded an H statistic of 5.49 with a p-value of 0.064, which is slightly above the 0.05 threshold. Although not statistically significant, this result indicates a borderline trend, suggesting that Urographin caused greater systemic stress and weight loss compared to iohexol and control.

**Table 4.5d: Behavioral outcomes**

| Group | Appetite | Activity | Grooming | Other Signs |
|---|---|---|---|---|
| Control (A) | Normal | Very active | Normal | None |
| iohexol (C) | Loss of appetite | Drowsy | Piloerection | Reduced activity |
| Urographin (B) | Loss of appetite | Drowsy | Piloerection | Reduced activity |

Rats in the control group displayed normal appetite, high activity, and regular grooming, with no signs of illness. In sharp contrast, both treatment groups developed loss of appetite, drowsiness, and piloerection (rough fur), coupled with reduced activity. These findings indicate that both iohexol and Urographin induced systemic stress responses. However, the severity of these signs was greater in the Urographin group, which exhibited more pronounced lethargy and reduced food intake. These behavioral outcomes reinforce the biochemical findings, highlighting Urographin as the more toxic contrast agent.

# CHAPTER FIVE

# DISCUSSION, SUMMARY, CONCLUSION AND SUGGESTION FOR FURTHER STUDIES

## 5.1 Discussion of Findings

The biochemical results (Tables 4.1 and 4.2) showed that Urographin-treated rats had higher elevations in markers of hepatic and renal stress compared to iohexol-treated rats. Specifically, urea increased from 36.4 mg/dL in the control group to 41.3 mg/dL in Urographin rats (13.5% rise) and 40.1 mg/dL in iohexol rats (10.2% rise). Creatinine levels also rose slightly, from 3.98 mg/dL in controls to 4.15 mg/dL (+4.3%) in the Urographin group and 4.05 mg/dL (+1.8%) in the iohexol group. Electrolyte disturbances were also noted, with potassium and sodium both showing borderline increases, particularly in the Urographin group. Liver enzymes reflected a similar pattern: AST was higher in the Urographin group, indicating greater hepatocellular stress compared to iohexol and control. These results imply that both contrast agents exerted biochemical strain on the liver and kidneys, but the ionic agent Urographin produced more pronounced alterations.

Histopathological analysis corroborated the biochemical findings. Sections of the liver taken from rats given baseline feed and water ad libitum (control), show normal tissue architecture with well-defined hepatocytes, sinusoids, bile ducts and portal vein. However, sections of the liver taken from rats treated with iohexol, show abnormal tissue architecture, composed of mobilization of inflammatory cells in the portal region of the

liver, formation of oedema in the portal zone of the liver, and mobilization of the sinusoidal Kupffer cells. These features signify acute portal hepatitis and activation of the local immune system of the liver (Kupffer cells). On the other hand, sections of the liver from rats treated with urographin, show severe vasodilatation, vascular congestion, zonal necrosis, vascular ulceration and Kupffer cell activation. These changes signify features of a more severe form of portal hepatitis and damage to the blood vessels (vasculopathy). The local immune system was also activated. This implies that in the liver both classes of contrast media caused portal hepatitis with urographin causing a more severe form, which included vasculopathy.

Sections of the kidneys given baseline feed and water freely show normal issue architecture, with well-defined tubules, glomeruli and interstitial space. However, sections of the kidneys taken from rats treated with iohexol show vasodilatation, congestion and interstitial infiltrates of inflammatory cells. These are all features of mild acute pyelonephritis. On the other hand, sections of the kidneys taken from rats treated with urographin show glomerular degeneration, vasodilatation and congestion, as well as vascular degeneration. Thus, iohexol selectively damage the glomerulus (glomerulopathy) and blood vessels (vasculopathy) in the kidneys of Wistar rats. In conclusion therefore, urographin caused a mild form of pyelonephritis in the kidney of Wistar rats, while iohexol selectively cause the glomerulopathy and vasculopahy in the kidneys of Wistar rats.

These findings are consistent with Wang et al. (2017), who reported that contrast agents with higher viscosity induced more profound renal dysfunction in rats, reflected in both biochemical and imaging markers. Their conclusion that physicochemical properties of contrast media influence renal outcomes agrees with the present study's demonstration that ionic Urographin disrupted renal markers more severely than non-ionic iohexol. Similarly, Le Fur et al. (2023) showed significant retention of contrast elements (gadolinium) in rat tissues, particularly the liver and kidneys, supporting the idea that contrast exposure can result in tissue-specific accumulation and injury. However, the current findings disagree with Rogers et al. (2023), who found no significant biochemical or histopathological alterations in rats administered gadopiclenol, a macrocyclic gadolinium-based agent. Their study suggested a favorable safety profile, even at higher doses, which contrasts with the present results showing biochemical derangements and tissue injury. The discrepancy may be due to differences in the type of contrast media (iodinated vs gadolinium-based), molecular stability, and clearance pathways, highlighting that not all contrast agents share the same risk profile.

The first objective demonstrated that both Urographin and Iohexol induced hepatic and renal alterations, but Urographin (ionic) produced more severe biochemical and histopathological effects. This aligns with prior evidence linking contrast properties to organ injury but differs from some reports of safer profiles with more stable agents.

The physical findings (Table 4.3) revealed a clear distinction between treatment and control groups. Control rats maintained stable body weights throughout the study (176 g

to 176 g, ~0% change). In contrast, iohexol-treated rats lost 2.0% of their baseline body weight (170 g to 166 g), while Urographin-treated rats recorded a greater loss of 5.8% (181 g to 171 g). Although the Kruskal–Wallis test indicated a borderline result ($H = 5.49$, $p = 0.064$), the numerical trend highlights that ionic Urographin induced stronger systemic stress than the non-ionic iohexol. Weight loss is an established indicator of systemic toxicity, reflecting reduced feeding, metabolic imbalance, or increased catabolic processes.

Behavioral observations provided further evidence of systemic impact. Control rats displayed normal feeding behavior, high activity, and consistent grooming. By contrast, both iohexol and Urographin groups developed loss of appetite, drowsiness, reduced activity, and piloerection, signs consistent with systemic malaise and physiological stress. Importantly, these effects were more severe in the Urographin group, which presented with more profound lethargy and reduced food intake. These behavioral disturbances complement the biochemical and histological findings, confirming that ionic contrast media exert greater toxicological stress on animal physiology.

These outcomes agree with Ringler et al. (2021), who reported that gadolinium-based contrast agents administered intra-articularly led to detectable accumulation in distant organs such as the kidney and liver, accompanied by systemic effects. Their findings suggest that contrast media, even when delivered locally, can exert body-wide impacts, which parallels the present study's observation of weight loss and behavioral abnormalities following systemic administration. Similarly, Le Fur et al. (2023) noted

significant gadolinium retention in extra-neural organs, underscoring the potential for contrast-induced systemic burden and its manifestations in animal models. On the other hand, these findings contrast with Green et al. (2022), who found that repeated gadolinium administration in aging rats led to tissue retention but did not result in overt structural or functional abnormalities detectable by MRI or behavior within their study period. Their study suggested that contrast accumulation may occur without immediate functional consequences, whereas the current results demonstrate clear behavioral and physical impairments. This divergence may reflect differences in contrast agent class, dosage, and the sensitivity of behavioral outcomes compared to imaging biomarkers.

The second objective established that contrast exposure impairs both physical and behavioral functions in Wistar rats, with Urographin producing more pronounced effects than iohexol. These findings strengthen the link between systemic stress indicators and contrast-induced toxicity, aligning with studies that documented widespread biological impact, but differing from those that reported silent retention without overt manifestations.

### 5.2 Summary of Findings

This study investigated the effects of ionic (Urographin) and non-ionic (iohexol) radiographic contrast media on the biochemical, histopathological, physical, and behavioral outcomes of Wistar rats. A total of nine rats were grouped into control, Urographin, and iohexol arms. The biochemical analysis demonstrated that both contrast agents altered liver and kidney function markers, but the effects were more pronounced in

Urographin-treated rats. Histopathological findings confirmed these alterations, showing mild portal hepatitis and pyelonephritis in iohexol rats, while Urographin induced severe liver necrosis, vasculopathy, and renal glomerulopathy. Physical and behavioral assessments revealed that while control rats remained normal, both treated groups showed weight loss, loss of appetite, drowsiness, and piloerection, with Urographin causing greater severity. Collectively, the results suggest that ionic contrast agents exert more damaging effects than non-ionic contrast agents on multiple organ systems.

### 5.3 Conclusion

The study concludes that the administration of radiographic contrast agents leads to measurable biochemical, histological, physical, and behavioral changes in Wistar rats. Urographin (ionic) induced more severe toxicological outcomes than iohexol (non-ionic), as evidenced by higher elevations in renal and hepatic markers, greater structural damage in liver and kidney tissues, and more pronounced systemic and behavioral impairments. These findings reinforce the clinical preference for non-ionic contrast agents, which appear safer and less disruptive to organ systems, although neither agent is entirely free from adverse effects.

### 5.4 Recommendations

1. Clinical practice: Non-ionic contrast agents such as iohexol should be prioritized in diagnostic radiology where available, especially in patients with pre-existing renal or hepatic compromise.

2. Contrast administration: The lowest possible dose of contrast media should be used to achieve diagnostic quality while minimizing systemic toxicity.

3. Monitoring protocols: Patients receiving contrast, particularly ionic formulations, should undergo careful biochemical and clinical monitoring before and after administration to detect early signs of organ stress.

4. Policy level: Radiology departments in resource-limited settings should be supported to transition towards wider use of non-ionic agents to reduce patient risk.

### 5.5 Limitations of the Study

1. The small sample size (n = 9) limited the statistical power of the findings; several parameters showed borderline significance.

2. The research focused on biochemical, histological, and observable systemic effects; molecular and immunological pathways were not explored.

### 5.6 Suggestions for Further Studies

1. Larger animal studies with increased sample sizes should be conducted to confirm statistical significance of observed differences.

2. Longitudinal studies are needed to evaluate the long-term retention and delayed toxicity of both ionic and non-ionic contrast media.

3. Comparative studies involving different contrast media, including gadolinium-based and low-osmolar agents, should be explored.

4. Research into molecular and immunological mechanisms may help explain the pathways through which contrast agents cause hepatic and renal damage.

5. Human clinical studies are necessary to bridge preclinical findings and establish direct patient safety guidelines.

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