EFFECT OF THE AQUEOUS EXTRACT OF *Myristica fragrans* (NUTMEG SEEDS) ON THE KIDNEY OF ADULT WISTAR RATS

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BENIN CITY, EDO STATE

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A PROJECT SUBMITTED TO THE DEPARTMENT OF ANATOMY, SCHOOL OF BASIC MEDICAL SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA.

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NOVEMBER 2025

CERTIFICATION

This is to certify that this project work titled **EFFECT OF THE AQUEOUS EXTRACT OF *Myristica fragrans* (NUTMEG SEEDS) ON THE KIDNEY OF ADULT WISTAR RATS.** was carried out by **OSAH JOHNSON OMOAREBU** with Matriculation Number **BMS2102142**, of the Department of Anatomy, School of Basic Medical Science, University of Benin City, Edo State, Nigeria.

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EXTERNAL EXAMINER **DATE**

DEDICATION

This work is dedicated to God Almighty, the Giver of life for keeping me alive throughout my academic journey and especially throughout the duration of this research.

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I wish to express my profound gratitude to **Almighty God** for the gift of life, wisdom, and strength granted to me throughout the course of this research work. His divine guidance made the successful completion of this project possible.

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ABSTRACT

Myristica fragrans (nutmeg) is a tropical evergreen tree commonly used as a culinary spice and traditional remedy for its stimulant, carminative, and antioxidant properties. Its seeds contain phytochemicals such as myristicin, elemicin, safrole and eugenol which possess both beneficial and potentially toxic effects. Although nutmeg is widely consumed, high or prolonged intake has been linked to renal toxicity mediated by oxidative imbalance. This study investigated the effects of aqueous *Myristica fragrans* seed extract on renal function, oxidative stress biomarkers, and kidney histoarchitecture in adult Wistar rats. Twenty adult Wistar rats weighing were divided into four groups [A,B,C,D] where group A [serves as Control] receives animal feed [grower mash] with distilled water for 28 days while groups B, C and D were administered with 200mg/kg, 750mg/kg and 1000mg/kg respectively of the aqueous extract of Myristica fragrans for 28days. An Orogastric tube was used for daily administration of the extract to the rats. Rats were sacrificed on the 29th day. Upon sacrifice, renal function was assessed using serum urea and creatinine levels, oxidative stress was evaluated via markers like superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), and malondialdehyde (MDA), and structural integrity was examined through histopathological analysis of kidney tissues. The results shows that the aqueous *Myristica fragrans* extract exerts dose-dependent renal alterations encompassing biochemical, oxidative, and histological dimensions. While low-dose exposure initiates mild oxidative and vascular stress, higher doses lead to significant renal dysfunction characterized by elevated urea, antioxidant depletion and lipid peroxidation. Histopathological examination revealed a dose-dependent progression of injury from normal architecture in the control group to mild degenerative changes (swelling and congestion) at 200mg/kg, advancing to perivascular fibrosis (scarring) at 750mg/kg, and culminating in severe, active peritubular inflammatory infiltrates at the highest dose of 1000mg/kg. The findings underscore the importance of dose regulation and controlled use of *Myristica fragrans* in traditional and dietary applications, as chronic or excessive intake may compromise renal health despite its known therapeutic potential.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

For centuries, nature has remained a fundamental source of therapeutic agents, with medicinal plants serving as the foundation of both traditional and modern healthcare systems (Nair *et al*., 2005 and Ghorbani, 2005). These plants are highly valued because of their rich phytochemical composition, which provides numerous compounds useful in the prevention and treatment of diseases as well as in the development of pharmaceutical drugs (Chukwuma *et al*., 2015 and Lifongo *et al*., 2014). Described as a "treasure trove" of bioactive substances, plants have been employed by ancient civilizations for treating illnesses and improving well-being (Akintonwa *et al*., 2009). Historically, they constituted the primary source of medicinal preparations before the emergence of synthetic drugs (Ayyanar and Ignacimuthu, 2011).

Herbal medicines, also referred to as phytomedicines or botanical drugs, continue to play a significant role in healthcare worldwide. They are generally perceived to be safer, more affordable, and more accessible than synthetic alternatives, while also offering therapeutic benefits with fewer side effects (Yadav and Agarwala, 2011; Simmler *et al*., 2018 and Ichim, 2019). In addition, plant-based foods and spices are widely recognized as rich sources of essential nutrients and bioactive compounds that contribute to overall health and well-being (Hirschi, 2009). However, it is important to note that while these natural products offer health benefits, their misuse or excessive consumption may result in adverse health outcomes, since

some plants possess toxic properties when ingested in large quantities (Mounanga *et al.,* 2015).

Among these medicinal plants, nutmeg (*Myristica fragrans*), an evergreen tree of the family Myristicaceae, is one of the most widely known and valued spices. It originated in the Moluccas (Spice Islands) of Indonesia but is now cultivated extensively in tropical regions including India, Sri Lanka, Malaysia, Grenada, and parts of the Caribbean (Parthasarathy *et al*., 2008). The nutmeg seed, which is the dried endosperm of the fruit, is enclosed in a hard, wrinkled, brown shell and contains aromatic volatile oils, while the surrounding red aril (mace) is also used as a spice of economic importance (Jaiswal *et al.,* 2009 ; Agbogidi and Okonta, 2010). Traditionally, nutmeg has been used in Ayurvedic, Unani, and Siddha medicine to relieve digestive disorders, insomnia, rheumatism, and pain, as well as serving as a sedative and aphrodisiac (Bordoloi *et al.,* 2021 and Tajuddin *et al.,* 2003).

Phytochemical studies show that nutmeg contains a wide variety of bioactive compounds, including alkaloids, saponins, anthraquinones, cardiac glycosides, and flavonoids, although tannins are absent in aqueous preparations (Bordoloi *et al.,* 2021). It also contains significant amounts of fixed oils (25–40%) and volatile oils (5–15%), which are rich in compounds such as myristicin, safrole, and elemicin (Gupta *et al.,* 2016). These constituents are largely responsible for its aromatic and psychoactive properties. While nutmeg exhibits antioxidant potential and free radical scavenging activity that suggest possible protective effects against oxidative stress, it has also been associated with toxic effects at high doses, including hallucinations, cytotoxicity, and organ damage in animal studies (Gupta *et al.,* 2016; Adjene and Nwose, 2010).

In traditional medicine, nutmeg is often consumed in aqueous forms such as teas or decoctions, prepared for their ease of use and perceived safety. These extracts retain important phytochemicals like flavonoids and alkaloids, making them highly relevant for pharmacological and toxicological research (Bordoloi *et al.,* 2021). However, the potential nephrotoxic effects of nutmeg remain a concern, as some of its volatile constituents may induce oxidative stress, inflammation, or direct cytotoxic action on renal cells (Gupta *et al.,* 2016). Conversely, the antioxidant activity of nutmeg could also provide renal protection, as observed in related studies where plant antioxidants, such as those from *Nigella sativa*, reduced gentamicin-induced kidney injury (Yaman and Balikci, 2010).

This dual nature of nutmeg therapeutic at low or moderate doses, but potentially toxic at higher concentrations warrants systematic investigation. Considering its widespread use as both a food spice and a traditional remedy, assessing the renal effects of aqueous extracts of nutmeg seed in experimental models such as adult Wistar rats is crucial. Such studies will provide important insights into its safety profile and therapeutic potential, especially regarding kidney function and toxicity.

1.2 STATEMENT OF RESEARCH PROBLEM

The increasing use of herbal remedies, including nutmeg (*Myristica fragrans)*, has raised concerns about their safety, particularly for vital organs like the kidneys. Nutmeg seeds are widely used in traditional medicine and culinary practices, yet scientific evidence on the safety of their aqueous extract, especially regarding kidney function, remains limited Experimental studies have demonstrated that high doses of aqueous nutmeg extract can cause significant renal alterations, including glomerular degeneration, tubular necrosis, and

increased renal biomarkers such as urea and creatinine (Alalwani, 2013). With chronic kidney disease affecting millions globally (WHO, 2019) and the kidneys' critical role in filtering toxins, evaluating the safety of widely used herbal extracts is essential. This study aims to investigate the effects of aqueous nutmeg seed extract on kidney function and histology in adult Wistar rats, assessing serum creatinine and urea levels and histopathological changes to determine potential nephrotoxic or nephroprotective effects

1.3 AIM OF THE STUDY

To investigate the effect of aqueous extract of *Myristica fragrans* (Nutmeg seed) on the kidney of adult Wistar Rat.

1.4 SPECIFIC OBJECTIVES

To verify the aim, the following objectives were employed:

1. To investigate the effects of aqueous extract of *Myristica fragrans* on the body weight of adult Wistar rats.
2. To investigate the effects of aqueous extract of *Myristica fragrans* on the kidney weight of adult Wistar rats.
3. To investigate the effects of aqueous extract of *Myristica fragrans* on the histological features on the kidney of adult Wistar rats
4. To determine the biochemical effect of the extract on oxidative stress markers such as Superoxide Dismutase and Catalase.

1.5 SIGNIFICANCE OF THE STUDY

This study holds significant value for public health and pharmacological research by elucidating the safety of nutmeg (*Myristica fragrans*) aqueous extract, a commonly used spice and remedy. With chronic kidney disease affecting over 850 million people globally (WHO, 2019), ensuring the renal safety of herbal products is critical. Findings from this study, using Wistar rats as a reliable toxicological model, could guide safe nutmeg consumption practices, inform healthcare providers about potential renal risks, and support regulatory standards for herbal medicines. By advancing knowledge of nutmeg's nephrotoxic or nephroprotective effects, the study may influence safer use of herbal remedies and contribute to reducing kidney-related health risks.

CHAPTER TWO

REVIEW RELATED LITERATURE

This chapter comprises of detailed description of the aqueous extract, including reviews of the previous work involving the plant extract, and the organ

2.1 *Myristica Frangrans* [nutmeg seeds]:

Nutmeg (*Myristica fragrans*) is an evergreen tree from the **Myristicaceae** family. Originally native to the Banda Islands of Indonesia, known historically as the "Spice Islands," it is now widely cultivated in Southeast Asia, India, Sri Lanka, Grenada, Malaysia, and across the Pacific (Barceloux, 2009 and ITIS, 2023). The tree thrives best in hot, humid climates with fertile, well-drained volcanic soils, making it a major spice crop in several producing regions (Olaleye *et al.*, 2006 and Pham *et al.*, 2000).

Typically, nutmeg trees grow between **9 and 12 meters tall**, although under favorable conditions they may grow even higher. The species is **dioecious**, meaning that male and female flowers are borne on separate trees, both of which are necessary for fruit production (ITIS, 2023). Its leaves are glossy, dark green, and alternately arranged, measuring **5–15 cm long** and **2–7 cm wide**, while its flowers are small, fleshy, pale yellow, and bell-shaped (Barceloux, 2009). Trees usually begin producing fruit after about **six to eight years**, and under good management they can remain productive for more than **50 years** (ITC, 2003).

The **fruit** itself resembles a peach or apricot, with a smooth greenish-yellow outer covering. When ripe, the fruit naturally splits to reveal the **nutmeg seed** enclosed by a scarlet-red covering known as **mace** (ITIS, 2023). The nutmeg seed is oval, hard, and brown with a

wrinkled outer surface and a highly aromatic kernel inside (Barceloux, 2009). After harvesting, the seeds are dried for several weeks until the kernels rattle inside their shells; they are then separated and sold as nutmeg (FAO, 1994).Both **mace and nutmeg** are treated as separate spices. Mace offers a delicate flavor, while nutmeg is valued for its warm, sweet fragrance, making it a staple in many global cuisines (ITC, 2003). Beyond culinary use, nutmeg seeds have long been important in **traditional medicine, trade, and cultural rituals**, which enhances the economic and cultural value of the tree (Freedman, 2015; Ogawa and Ito, 2019).

Fig 2.1 Nutmeg tree

Fig 2.2 Nutmeg fruit and Mace

2.2 History and Significance of nutmeg seed

Nutmeg is a *Myristica fragrans* fruit seed that has a yellow peach-like appearance and grows from *Myristica fragrans* tree. The crop is an evergreen, fragrant dioecious tree commonly cultivated in tropical regions, particularly Southeast Asian countries, tropical America, and the Pacific Islands. It is indigenous to the Indonesian Banda Island which is called the Maluku or Spice Islands. Historically, the Arab introduced the nutmeg to Europe until the finding of the nutmeg trees by the Portuguese on Banda Island in the 15th century (Payne, 1963). Later, in the 17th century, when the Dutch occupied the Spice Islands, and they had the monopoly in trading spices, and until the end of the 18th century, when the British acquired nutmeg seedlings cultivated in the Banda Islands (Barceloux, 2009). In the recent past, there has been

a need for nutmeg seeds in developed countries. Germany, Japan, the United States, and Europe are among the top nutmeg seed destination importers. Different countries, like Indonesia, India, Sri Lanka, and Grenada, are renowned for the exportation of nutmeg (Purba *et al.*,2021; Gordon, 2020 and Private Sector Trade Note, 2009). Today, quality nutmeg is in growing demand because of its vast worth in cosmetics, pharmaceutical, and bakery industries. The specific composition of fixed oil, essential oil, and oleoresin found in nutmeg seed extract makes it a sought-after industrial product in foods and pharmaceuticals. Nutmeg seed oil and its by-products are utilized vastly as a flavoring agent in food products, while the nutmeg oleoresin is a substitute for the dry seeds. The fixed oil makes up around 20–40 % and possesses a strong aroma and contributes 8–15 %of the mixture. The nutmeg oil production can, therefore, replace the dry seeds as this commodity is greatly aromatic and non-aflatoxin (FAO,1994).

2.3 Description of Nutmeg seeds

Nutmeg is the dried seed of *Myristica fragrans*, known for its sweet taste and distinctive fragrance. The seed is oval in shape, light brown in color, and has a wrinkled outer surface. When fully ripe, the seed appears whitish, firm, and fleshy, marked with reddish-brown streaks. It is encased in a bright red, fleshy covering called the aril, which is commonly known as mace. After harvesting, both the nutmeg seed and mace are dried separately and processed for use as spices.

Fig 3 Nutmeg seeds

Table 1. Taxonomic description	
Taxonomic rank	Taxon
Kingdom	Plantae
Sub kingdom	Viridiplantae
Division	Tracheophyta
Class	Magnoliopsida
Order	Magnoliales
Family	Myristicaceae
Genus	Myristica
Species	Fragrans
Common name	Nutmeg
Hindi/Urdu name	Jaiphal

2.4 Taxonomical classification and background of nutmeg seeds

The scientific name of nutmeg is *Myristica fragrans*, which belongs to the Myristicaceae family. It is also recognized by different local names around the world. In the United Kingdom it is called nutmeg or mace, in Indonesia bunga pala, in Germany nuez moscada,

in France muscadier, in India jaiphal, in Uruguay and Spain Muskatbaum, and in Arabic-speaking countries jawzat altayib.The M. fragrans tree is widely cultivated across Southeast Asia and other regions such as Indonesia, Malaysia, Grenada, Sri Lanka, India, and Vietnam (Olaleye *et al.*, 2006; Pham *et al.*, 2000; Gils and Cox, 1994 and Al-Rawi *et al.*, 2013). It is originally native to the Banda Islands in Indonesia, also known as the Spice Islands.This tropical, evergreen tree is aromatic and typically grows between 9–12 meters tall, with scattered branches. It is dioecious, meaning male and female flowers occur on separate trees. The leaves are dark green, alternately arranged along the branches, and measure about 5–15 cm long and 2–7 cm wide. The flowers are fleshy, waxy, bell-shaped, and light yellow in color. The tree usually begins to bear fruit after six years and continues to produce throughout the year for 20–75 years (ITC, 2003 and Barceloux, 2009). The fruit is fleshy, green or yellow, and resembles an apricot or peach. When ripe, it splits open into two halves, revealing a shiny purplish-brown seed that is surrounded by a bright scarlet aril, which is known as mace.

2.5 Composition of Nutmeg Seed (*Myristica fragrans*)

Studying the composition of oils provides valuable insight into their potential uses in various fields, as well as their role in promoting health and treating certain diseases. Raw nutmeg seeds generally contain about 30–55% oil and 45–60% solid matter, mainly cellulose. The oil is made up of two main components: a crude fixed oil called nutmeg butter, which accounts for 20–40%, and an essential oil, which contributes 8–15%. Together with other oleoresins, these compounds give nutmeg its strong aroma due to the presence of aromatic groups (Parthasarathy *et al.*, 2008).In addition, nutmeg seeds contain protein, starch, and

lipids (Rahardiyan *et al.*, 2020). Nutmeg oleoresin is well known for its intense fragrance and flavor, which makes it widely used as an alternative to dried nutmeg seeds in industries such as pharmaceuticals and food production (ITC, 2003). Therefore, nutmeg butter, essential oils, and oleoresins are considered effective substitutes for whole nutmeg in commercial markets. Nutmeg oil can be extracted in different ways, with the fixed oil usually obtained through hydraulic pressing with heat or Soxhlet extraction. These methods produce a semi-solid, aromatic, orange-colored product known as nutmeg butter. The extraction process strongly affects the chemical composition and quality of the yield (Ibrahim and Al-Rawi, 2018; Matulyte *et al.*, 2019). For example, Soxhlet extraction yields about 34% oil, while supercritical extraction yields about 38.8% (Al-Rawi *et al.*, 2013).In contrast, essential oil is extracted by steam distillation, resulting in either a colorless or pale-yellow liquid with the characteristic flavor and aroma of nutmeg (Umayah and Marhaendro, 2021). This oil is soluble in alcohol but not in water, and because it is sensitive to light and air, it must be stored in a tightly sealed container.

2.6 Chemical Properties of Nutmeg Seed

Identifying the chemical constituents of nutmeg extracts is important for their confirmation and standardization (Radzali *et al*., 2022). Several analytical techniques, including GC, GC–MS, HPLC, and GC–TOFMS, have been used to study its composition. Ibrahim and Al-Rawi (1918) analyzed the chemical makeup of supercritical nutmeg extract using GC–TOFMS.In recent years, nutmeg essential oil has attracted more scientific interest compared to nutmeg butter or fixed oil. Although its concentration is lower than that of the fixed oil in the seeds, essential oil is more popular because it contains bioactive compounds with pharmacological

and industrial importance. The major components of nutmeg essential oil have been reported in multiple studies (Kapoor *et al.*, 2013; Yuan *et al.*, 2006; Ginting *et al.*, 2017; García-Díez *et al.*, 2017 and Maya *et al.*, 2004). Muchtaridi *et al.* also studied the composition of nutmeg seed essential oil, while Subaddarage *et al.* (1985) examined the chemical and physical properties of Sri Lankan nutmeg oil. Nutmeg extracts are known to contain phenolic compounds, terpenoids, flavonoids, and fatty acids (Spricigo *et al.*, 1999). However, their concentration and presence vary depending on factors such as geographical origin, freshness of the seeds, extraction methods, and extraction conditions (Jaiswal and Williams, 2017). Machmudaha *et al.* (2006) demonstrated that nutmeg supercritical extracts share many components with essential oil, including terpenes, polyphenols, and limonene. Across different studies, the number of chemical compounds identified in nutmeg seeds ranges from 10 to more than 50. For example, Morsy (2016) detected 53 compounds in nutmeg oil, including myristicin, sabinene, elemicin, limonene, terpinen-4-ol, myristic acid, α-pinene, and β-pinene. The concentration of these compounds varies greatly depending on the extraction method and cultivation site. For instance, myristicin levels were found to range between 2% and 42%, but were absent in some oils. Additionally, minor compounds such as anisole, camphor, cumene, copaene, cyclamen aldehyde, α-sarone, menthyl isovalerate, and menthone were detected by GC–TOFMS but not by other techniques (Ibrahim and Al-Rawi, 1918).

2.7 Pharmacological and Biological Properties of Nutmeg Seed

Medicinal plants are widely recognized as valuable sources of natural products with significant pharmacological effects, including anti-inflammatory, antioxidant, antimicrobial,

antidiabetic, and anticancer activities. Nutmeg seeds play an important role in disease prevention and treatment due to the presence of bioactive compounds such as secondary metabolites, flavonoids, terpenes, alkaloids, phenols, and fatty acids. These natural antioxidants and phytochemicals have generated increasing interest in combining conventional pharmaceuticals with plant-based therapies. Among its many applications, nutmeg seed extract is particularly important, as it contains the major bioactive constituents responsible for its pharmacological effects (Grover *et al.*, 2002). For this reason, nutmeg continues to attract attention as a promising natural source for therapeutic and pharmaceutical development, highlighting the need for more detailed research to explore its full potential.

2.7.1 Antimicrobial Properties of Nutmeg Seed

Infections arise when harmful microorganisms such as bacteria, viruses, fungi, or parasites invade the body and cause disease. Over time, many bacteria have developed resistance to antibiotics, which has led to increased interest in natural alternatives (Jalal *et al.*, 2023; Ahmed and Ganjo, 2019). Essential oils and extracts from aromatic plants, including nutmeg, have demonstrated strong antibacterial and antifungal activities (Hanif *et al.*, 2010).Nutmeg seeds contain bioactive compounds such as myristicin, carvacrol, cymene, pinene, and caryophyllene, which contribute to their antimicrobial potential. Methanolic extracts of *Myristica fragrans* were found to inhibit the growth of Helicobacter pylori strains at concentrations as low as 12.5 µg/mL, suggesting its effectiveness in treating gastrointestinal conditions such as gastritis, peptic ulcer disease, and gastric carcinoma (Mahady *et al.*, 2005). Additionally, the essential oil of nutmeg has been shown to exert broad-spectrum inhibitory effects against foodborne pathogens, plant and animal disease-causing bacteria, and spoilage

organisms (Dorman and Deans, 2000). A separate study revealed that volatile oils from nutmeg inhibited pathogenic strains of Escherichia coli O157, while non-pathogenic strains remained unaffected. Interestingly, O157 strains were more sensitive to beta-pinene compared with non-pathogenic E. coli strains, indicating selective antibacterial activity (Takikawa *et al.*, 2002).

2.7.2 Anti-inflammatory Properties of Nutmeg Seed

Inflammation is the body's natural defense response to infections, tissue damage, or injuries (Chen *et al.*, 2018). However, persistent or uncontrolled inflammation is a major factor in the progression of several diseases, including cancer, autoimmune disorders, rheumatoid arthritis, cardiovascular conditions, hypertension, liver damage, and obesity (Tsai *et al.*, 2019; Wu *et al.*, 2021; Ostro *et al.*, 2014; Hage *et al.*, 2017; Laird *et al.*, 2013). The inflammatory response is generally characterized by swelling, heat, pain, redness, and impaired tissue function. It is primarily mediated by cytokines such as interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-1β (IL-1β), interleukin-1 (IL-1), and nitric oxide (NO) (Di *et al.*, 2018; DiNatale *et al.*, 2010; Manohar *et al.*, 2018).Inflammatory processes are activated through various signaling pathways, including MAPK, NF-κB, and JAK-STAT (Torres *et al.*, 2011; Li *et al.*, 2022; Lee *et al.*, 2020). Many medicinal plants are widely utilized for their anti-inflammatory effects, and nutmeg is one of them (Francis and Sankari Malaiappan, 2022; Dkhil *et al.*, 2019). Studies have shown that nutmeg seeds possess significant anti-inflammatory activity. For instance, Lee and Park (2011) demonstrated that myristicin, one of nutmeg's bioactive compounds, reduces the release of cytokines,

chemokines, nitric oxide, and growth factors in double-stranded RNA (dsRNA) stimulated macrophages through calcium pathway regulation.

Nutmeg essential oil was also shown to relieve joint swelling, nerve pain, and hypersensitivity in rats by inhibiting cyclooxygenase-2 (COX-2), a key enzyme in inflammation (Zhang *et al.*, 2016). Similarly, in-silico studies revealed that myristicin could bind and block VEGFA, COX-1, EGF, and HIF enzymes, suggesting its potential therapeutic targets (Al-Rawi *et al.*, 2023b). Ethanolic extracts of nutmeg were also found to suppress the release of inflammatory markers, including TNF-α, nitric oxide, IL-6, and IL-1β, in a dose-dependent manner (Cao *et al.*, 2013; Dewi *et al.*, 2015).Traditionally, nutmeg has been applied as a topical remedy for muscle, joint, and nerve pain due to its anti-inflammatory effects (Al-Rawi *et al.*, 2013). In addition, eugenol, another compound present in nutmeg, has demonstrated strong anti-inflammatory activity and has been widely used in dentistry, analgesic ointments, and treatments for sprains and rheumatic pain (Buckle, 2014; Al-Rawi *et al.*, 2011). Compounds such as myrisfrageal A and B, and dehydrodiisoeugenol, were shown to inhibit nitric oxide overproduction by downregulating iNOS mRNA expression, with IC50 values of 18.5 and 21.2 μM respectively (Cao *et al.*, 2015).Furthermore, flavonoids found in nutmeg are effective in blocking enzymes responsible for inflammation, including nitric oxide synthase (NOS), cyclooxygenase (COX), phospholipase A2, and lipoxygenase, thereby reducing the formation of inflammatory mediators such as prostaglandins, leukotrienes, arachidonic acid, and nitric oxide (Akinwunmi andand Oyedapo, 2014). Macelignan, another nutmeg compound, has been reported to provide anti-inflammatory and antioxidant benefits, further enhancing nutmeg's role as a therapeutic agent (Lee *et al.*, 2012).

2.7.3 Antidiabetic Aspects of Nutmeg Seed

Diabetes is a long-term metabolic disorder that affects millions worldwide and results from either insufficient insulin production or the body's reduced sensitivity to insulin, leading to poor control of blood sugar levels (CDC, 2022). While synthetic drugs are widely used in diabetes treatment, they often cause unwanted side effects, which has encouraged the exploration of natural products such as medicinal plants and spices as alternative therapies. Nutmeg (*Myristica fragrans*) seeds have shown promising antidiabetic effects. Extracts of nutmeg have been reported to stimulate insulin signaling, enhance glucose uptake in body cells, and lower blood glucose levels (Broadhurst *et al.*, 2000). The extracts also provided β-cell protection by improving lipid metabolism and correcting hyperglycemia (Arulmozhi *et al.*, 2007).Nutmeg was also shown to activate AMP-activated protein kinase (AMPK) in skeletal muscle cells (C2C12), a process vital for managing obesity, diabetes, and other metabolic disorders (Nguyen *et al.*, 2010). Another important mechanism is the inhibition of α-amylase, an enzyme responsible for breaking down starch into glucose. By slowing this process, nutmeg helps reduce glucose spikes after meals. For instance, water extracts of nutmeg inhibited α-amylase activity by 28.96% at 1 mg/ml (Bhutkar *et al.*, 2018). Similarly, benzene extracts showed stronger inhibition (57.80% with IC50 of 2.25 ± 0.28 mg/ml), while methanol extracts showed weaker activity (16.20% at 2.5 mg/ml) (Hemlata *et al.*, 2019).Additionally, nutmeg seeds lowered serum insulin levels, making them particularly effective in managing hyperinsulinemia (Pereira *et al.*, 2019). Overall, nutmeg seeds may improve insulin sensitivity, regulate glucose metabolism, and reduce the risk of diabetes-related complications. These findings highlight nutmeg's potential role in developing natural

antidiabetic drugs. However, further studies are necessary to establish its safety and therapeutic efficacy.

2.7.4 Antioxidant activity of nutmeg seed

Natural antioxidants are gaining popularity all over the world as health-benefiting bioactive molecules. They are usually extracted from numerous parts of the plants, i.e., vegetables, fruits, herbs, and spices (Anwar *et al.*, 2018). Antioxidants represent a large category of chemicals that include vitamins, minerals, phenols, and carotenoids. It shows a wide variety of therapeutic activities, i.e., antiaging, antimicrobial, antidegenerative disorders, antiinflammation, and anticancer effects (Adwas *et al.*, 2019). Seed oils have been used for thousands of years as a treatment in traditional medicine (Liu, *et al.*, 2022; Ahmad, 2009). Seed oils rich free carrier capacity and natural antioxidants composition. It has various mechanisms such as neutralization free radicals, lipoperoxidation reduction, and enhancing our natural enzymatic defenses (Moussa *et al.*, 2019). In addition, free radicals, and reactive oxygen species (ROS) are pivotal in ion transport, gene expression, cancer, and apoptosis (Azad and Iyer, 2014). They interact with a number of cellular molecules and metabolites, leading to cellular damage and disease (Asif, 2015). Antioxidants are associated with health promotion by restricting the formation of free radicals and ROS (Noguchi and Niki, 2019; L 'Lopez-Pedrouso *et al.*, 2022). The N-hexane extract from nutmeg seeds contains high levels of various antioxidants (Parle *et al.*, 2004). Alkaloids and flavonoids have been identified to be primary antioxidants in nutmeg (Spricigo *et al.*, (1999). Flavonoids are antioxidants derived from polyphenolic composites-based. It is commonly well documented to cure cancer due to its potential in cancer growth inhibition (Davis, 2001). Murcia *et al.*, measured

the antioxidant activity of nutmeg in 2004. Comparative study and assessment were made on nutmeg, propyl gallate (E-310), BHA butylated hydroxyanisole (E-320) and BHT butylated hydroxytoluene (E-321). The nutmeg antioxidant activity using the Trolox equivalent antioxidant capacity (TEAC) assay was more than that of BHT. They identified that nutmeg seeds possessed the greatest protective feature via deoxyribose assay. They also confirmed that nutmeg enhanced the stability and oxidation of oils at 110 C such as olive oil, corn oil, sunflower oil, margarine, and butter. Likewise, the antioxidant and radical-scavenging action of nutmeg via DPPH radical assay has been confirmed in other oils by Tomaino *et al.*, (2005). They showed good antioxidant and free radical-scavenging activity at room temperature with DPPH radical assay. Effect varied between the oils while nutmeg was more active than basil, oregano, and thyme. In general, the nutmeg antioxidants activity makes it a potential broadcast candidate and agent for prevention and treatment of many health diseases.

2.7.5 Apoptotic and Anti-Cancer Properties of Nutmeg

Cancer is one of the leading causes of death worldwide (Lim, 2002). Among them, breast cancer is the primary cause of cancer-related mortality in women, accounting for about 25% of all reported cases (Lei *et al.*, 2021). Interestingly, epidemiological studies show variations in cancer prevalence among different populations. Countries with high spice consumption often report lower cancer incidence, suggesting dietary factors may play a protective role. Apart from genetic factors (5–10%), lifestyle and diet contribute to 90–95% of cancer cases (Anand, 2008; Monahan *et al.*, 2020). Moreover, migration studies demonstrate that individuals moving to new regions tend to acquire the cancer prevalence of the host country, emphasizing the strong influence of environment and diet (Al-Rawi *et al.*, 2011).For this

reason, plants, seeds, and herbal extracts have been widely studied for their anticancer potential due to their pharmacological safety and effectiveness (Hanif *et al.*, 2023; Al-Rawi *et al.*, 2011; Ibrahim *et al.*, 2011). The method of extraction also plays a role in enhancing activity yields (Ab Rahman *et al.*, 2011).

Nutmeg essential oils and seed extracts have shown therapeutic and protective effects against carcinogenesis. Nutmeg extract inhibited B16-F10 melanoma cells with IC_{50} values of 21.83 µg/mL (ethanol), 21.66 µg/mL (ethyl acetate), and 47.53 µg/mL (n-hexane), inducing apoptosis via caspase-3 (Susianti *et al.*, 2021). Similarly, compounds isolated from nutmeg seeds displayed strong cytotoxicity against oral cavity KB cancer cell lines and lung NCI-H187 cancer cell lines with IC_{50} values of 5.9 µM and 6.3 µM, respectively (Chumkaew and Srisawat, 2019). Methanolic extracts inhibited Jurkat cell growth and induced apoptosis in human leukemia cells at concentrations of 50 and 100 µg/mL (Chirathaworn *et al.*, 2007). Mechanistic studies suggest that nutmeg's polyphenols may exert anticancer effects through Sirtuin 1 (SIRT1) mRNA downregulation, a pathway linked to apoptosis (Ibrahim *et al.*, 2017). Supercritical extraction methods also influence the bioactivity of nutmeg extracts, although their effects against MCF7 breast cancer and HCT116 colorectal cancer cells were less significant (Al-Rawi *et al.*, 2011; Al-Rawi *et al.*, 2023c). Nutmeg extract additionally showed strong anti-angiogenic activity, inhibiting blood vessel formation in a 3D rat model at 100 µg/mL, thereby reducing breast cancer cell growth (Al-Rawi *et al.*, 2023a). Anti-angiogenic therapy is a promising approach not only in cancer treatment but also for diseases such as rheumatoid arthritis, diabetic retinopathy, cardiovascular disease, obesity, and lymphopenia (Al-Rawi, Ibrahim and Ahmed, 2023). This effect is attributed to nutmeg's

bioactive compounds, including aromatic ethers, terpenes, flavonoids, and phenolics (Al-Rawi *et al.*, 2011; Crozier *et al.*, 2006). Myristicin, a major benzodioxole compound in nutmeg, has been shown to suppress lung tumor growth in mice (Ahmad *et al.*, 1997), induce apoptosis in K562 leukemia cells via DNA damage and mitochondrial pathways (Martins *et al.*, 2014), and exhibit hepatoprotective effects by inhibiting TNF-α–induced apoptosis (Morita *et al.*, 2003). However, it also demonstrated toxicity against SK-N-SH neuroblastoma cells (Lee *et al.*, 2005). Other compounds such as sabinene, sesquiterpenes, and phytosterols contribute to nutmeg's anticancer activity by inhibiting cancer cell proliferation, angiogenesis, and by inducing apoptosis (Modzelewska *et al.*, 2005; Dahham *et al.*, 2018). Overall, nutmeg contains a diverse range of phytochemicals with anticancer and apoptotic properties, supporting its potential role in complementary cancer prevention and therapy.

2.7.6 Cardioprotective Effects of Nutmeg Seed

Chronic diseases such as heart disease, hypertension, chronic kidney disease, arthritis, cancer, and diabetes are among the leading causes of disability and mortality worldwide. They account for nearly seven out of every ten deaths globally (Raghupathi and Raghupathi, 2018). Although chronic conditions are generally manageable, they are often not curable (Allegrante *et al.*, 2019). Natural products and functional foods contain bioactive compounds that have long been used in disease management (Zhu *et al.*, 2022; Kim *et al.*, 2018). These compounds regulate essential pathophysiological processes including oxidative stress, inflammation, fibrosis, and hypoxia (Islam, 2022). Nutmeg seed possesses a rich chemical composition with the potential to prevent cardiovascular disorders, diabetes, hyperlipidemia,

and lipid oxidation (Pratiwi *et al.*, 2018; Parvin *et al.*, 2023). Several studies highlight its traditional and modern use as a natural therapeutic agent for cardiac diseases. Extracts of nutmeg seed are particularly rich in bioactive molecules with cardioprotective effects, suggesting strong potential as a therapeutic candidate for cardiovascular disorders (Yang *et al.*, 2022; Pashapoor *et al.*, 2020; Sharma *et al.*, 1995). The protective properties of nutmeg are attributed to compounds such as myristicin, safrole, and eugenol. Myristicin, the principal constituent, demonstrates antioxidant and anti-inflammatory activity, enabling it to mitigate hyperlipidemia, neurotoxicity, hyperglycemia, myocardial damage, and hepatotoxicity (Liu *et al.*, 2022). It has also been linked to improved cardiac metabolism, which is beneficial in cardiovascular disease management (Liu *et al.*, 2022). Additionally, nutmeg contains flavonoids, alkaloids, and other phytochemicals that contribute to antioxidant and biochemical effects relevant to chronic conditions such as cardiovascular disease and Alzheimer's disease (Zhang *et al.*, 2015; Deng *et al.*, 2022). Quercetin, a flavonoid found in nutmeg, plays a vital role in enhancing cardiac metabolism. It exhibits multiple biological activities, including antiplatelet, anti-inflammatory, estrogenic, antimicrobial, antiviral, antioxidant, and antimutagenic properties (Roy *et al*., 2022; Salehi *et al*., 2020). In summary, nutmeg seed extract demonstrates significant cardioprotective potential and may serve as a natural alternative to conventional therapies for heart disease. However, further studies are essential to confirm its efficacy, establish safe dosage levels, and evaluate its long-term safety as a therapeutic agent.

2.7.7 Cognitive and Neurological Effects

Nutmeg has been reported to enhance memory, learning ability, and overall mental alertness. Experimental studies show that administration of nutmeg extracts improves spatial memory and learning performance in animal models by modulating neurotransmitters like acetylcholine and dopamine (Hosseinzadeh and Parvardeh, 2004). The cholinesterase inhibitory activity of nutmeg is especially important in the prevention of neurodegenerative diseases such as Alzheimer's and Parkinson's disease. Active compounds such as α-pinene, sabinene, elemicin, and myristicin help prevent the breakdown of acetylcholine, thus improving cognitive function (Torić *et al.*, 2023). Nutmeg also shows neuroprotective and anxiolytic effects, reducing anxiety and depression-like behaviors in experimental models. These benefits are attributed to its ability to regulate the GABAergic system, enhance brain antioxidant enzymes, and reduce oxidative stress in neural tissues (Dhingra and Sharma, 2006). Furthermore, nutmeg's myristicin and macelignan demonstrate protective activity against β-amyloid-induced neurotoxicity, suggesting a potential role in slowing Alzheimer's progression (Zhang *et al.*, 2015)

2.7.8 Anti-convulsant and Hepatoprotective Effects

Nutmeg extract has long been used in traditional medicine as an anti-epileptic agent. Modern studies confirm that it exhibits anticonvulsant properties, especially against chemically induced seizures in animal models. These effects are thought to occur via the enhancement of GABAergic transmission and suppression of excessive neuronal firing (Dhingra and Sharma, 2006). In terms of liver protection, nutmeg helps reduce hepatic oxidative stress by lowering levels of lipid peroxidation and enhancing antioxidant enzyme activity such as

superoxide dismutase (SOD) and catalase (CAT) (Yuliana *et al.*, 2019). The liver-protective activity is attributed to compounds like myristicin, elemicin, and lignans, which reduce pro-inflammatory mediators such as TNF-α, IL-6, and nitric oxide. These actions help prevent hepatotoxicity caused by alcohol, drugs, or environmental toxins (Nagaraju *et al.*, 2012). Additionally, nutmeg seed extracts show promise in managing non-alcoholic fatty liver disease (NAFLD) by regulating lipid metabolism and lowering cholesterol levels, making it a strong candidate for liver health management (Liu *et al.*, 2022).

2.7.9 Psychotropic effect of nutmeg seed

Consumption of nutmeg has been associated to cause the similar kind of symptoms of anticholinergic poisoning, e.g., giddiness, tingling, euphoria, and hallucinations such as time–space distortion, detachment from reality, separation of limbs, and death fear (Pawar, 2023; Desai, 2016). Nutmeg has the reputation of being sedative, hallucinogenic, and anticholinergic poisoning in nature (Hausner, and Poppenga, 2012). Though, for inducing these psychogenic effects, about 1–3 seeds or 5 g to 30 g of powdered nutmeg seeds is needed, as 7 g of powdered nutmeg = 1 tablespoon (Holstege, 2005). There has been a recent finding where n-hexane extract of nutmeg has been found to be highly safe with LD50 > 2000 mg/kg, while the nutmeg extract showed antidepressant effects in various nervous components of serotonin and norepinephrine. (Iwata *et al*., 2022). Nutmeg is also said to possess antidepressant and anxiogenic properties. In a recent study, nutmeg extract had a marvelous effect on the nervous system, and was capable of curing anxiety, behavioral agitation, and insomnia (El-Alfy, *et al.*, 2019). This is due to the properties of nutmeg components, which have the ability to stimulate the serotonin secre action, which induces

sedation or relaxation. This effect is induced by the action of nutmeg on the brain's endogenous cannabinoid system. The endocannabinoid system (ECS) is understood to control a number of physiological processes such as immune response, appetite, mood, and sleep. Nutmeg extract has been documented to inhibit monoacylglycerol lipase (MAGL) and the endogenous cannabinoid enzyme fatty acid amidase hydrolase (FAAH) (El-Alfy, *et al.*, 2016). MAGL inhibition has been shown to produce anxiolytic and anti-nociceptive anti-emetic effects (Mulvihill and Nomura, 2013). Moreover, inhibition of MAGL has a significant influence in the brain levels of precursor for inflammation, causing lowered neuroinflammation (Kasatkina *et al.*, 2021). This is due to the elevation of 2-AG (2-arachidonoylglycerol) and a significant drop of AA, which is a key prostaglandin building block, a pro-inflammatory mediator (Deng, and Li, 2020). This will result in a significant decrease in neuro inflammation. Additionally, the nutmeg n-hexane extract significantly enhanced the memory and learning activity of mice at 5 mg/kg body weight (Parle *et al.*, 2004). The improvement effect of nutmeg extract was because it had procholinergic activities and contained various antioxidant compounds individually or synergistically. Nutmeg seeds were found to provide relief from chronic and chronic pain in different parts of the human body (Zhang *et al.*, 2016). Though caution needs to be exercised when applying nutmeg in the long term because it can lead to negative undesirable effects on the auditory responsiveness of human beings (Adjene, and Nwose, 2010). In summary, the use of nutmeg in drug development is highly recommended. However, it is advisable to state that the psychoactive nutmeg ingredient may require modification or mixing with other materials to achieve effective therapy while limiting potential side effects and abuse. Therefore,

additional research is necessary to determine the best conditions for formulating medication from nutmeg.

2.8 Toxicological properties of Nutmeg seeds

Nutmeg seed has historically been associated with toxicity and narcotic effects (Weil, 1971). Although rare, several cases of nutmeg intoxication have been reported. For example, a 23-year-old college student developed symptoms after ingesting powdered nutmeg (Abernethy and Becker, 1992), and a 16-year-old student experienced poisoning following consumption of ground raw seed (McKenna *et al.*, 2004). Another report described a 17-year-old boy who became disoriented, exhibited repetitive movements, and engaged in self-directed speech after an overdose of nutmeg (Beckerman and Persaud, 2019). Despite these reports, nutmeg consumption alone is rarely fatal, and symptoms usually resolve within 24 hours (Smith, 2014). Toxic effects are typically observed with ingestion of 5–30 g of powdered nutmeg, although even higher doses of 20–80 g have generally not resulted in life-threatening outcomes (Brenner *et al.*, 1993; Stein, Greyer, and Hentschel, 2001). These toxic effects are mainly linked to raw nutmeg seed rather than to its extracts or oils. Modern extraction techniques, particularly supercritical extraction, allow the preparation of safe and toxin-reduced nutmeg extracts (Zhang *et al.*, 2019; Ibrahim *et al.*, 2017). Adjusting extraction parameters produces fractions with different chemical profiles and lower toxicity (Ibrahim and Al-Rawi, 2018; Al-Rawi *et al.*, 2011). Moreover, supercritical nutmeg extract and the bioactive compound myrislignan have demonstrated strong protective effects against thioacetamide-induced liver damage in vivo (Yang *et al.*, 2018). Overall, nutmeg extracts hold potential as safer, biologically active products for use in the pharmaceutical and food

industries. However, further studies are needed to confirm their efficacy, determine optimal dosage, and ensure long-term safety.

2.9 Organ of Study – Kidney

The kidneys are a pair of excretory organs located on the posterior abdominal wall, one on each side of the vertebral column, positioned behind the peritoneum. Their primary function is to remove metabolic waste products along with excess water and salts from the blood, while also maintaining the body's acid–base balance (Chaurasia, 2006). Each kidney forms the main organ of the urinary system and lies in close contact with the suprarenal gland at its superomedial pole. The right kidney is slightly lower than the left (about 1–1.2 cm) due to displacement by the liver. In an adult, each kidney measures approximately **11 cm in length, 6 cm in width, and 4 cm in thickness**, with an average weight of **130–150 g** (Ellis, 2006). Anatomically, the kidneys are positioned in the paravertebral gutter. At the medial border lies the hilum, a vertical slit through which renal vessels, nerves, and the renal pelvis (origin of the ureter) pass. This hilum is directed slightly forwards and medially, giving the kidney a rotated appearance. As a result, an anteroposterior radiograph shows the kidney in a foreshortened view of its width (Chummy, 2011). In relation to surface anatomy, the hilum of the right kidney lies just below the transpyloric plane, whereas that of the left lies slightly above it, both about 5 cm from the midline. The right kidney's inferior position is largely attributed to the bulk of the liver. The upper pole of the left kidney corresponds to the level of the 11th rib, while the upper pole of the right kidney aligns with the 12th rib. During respiration, both kidneys exhibit a vertical movement of about 2 cm, following the excursion of the diaphragm (Chummy, 2011).

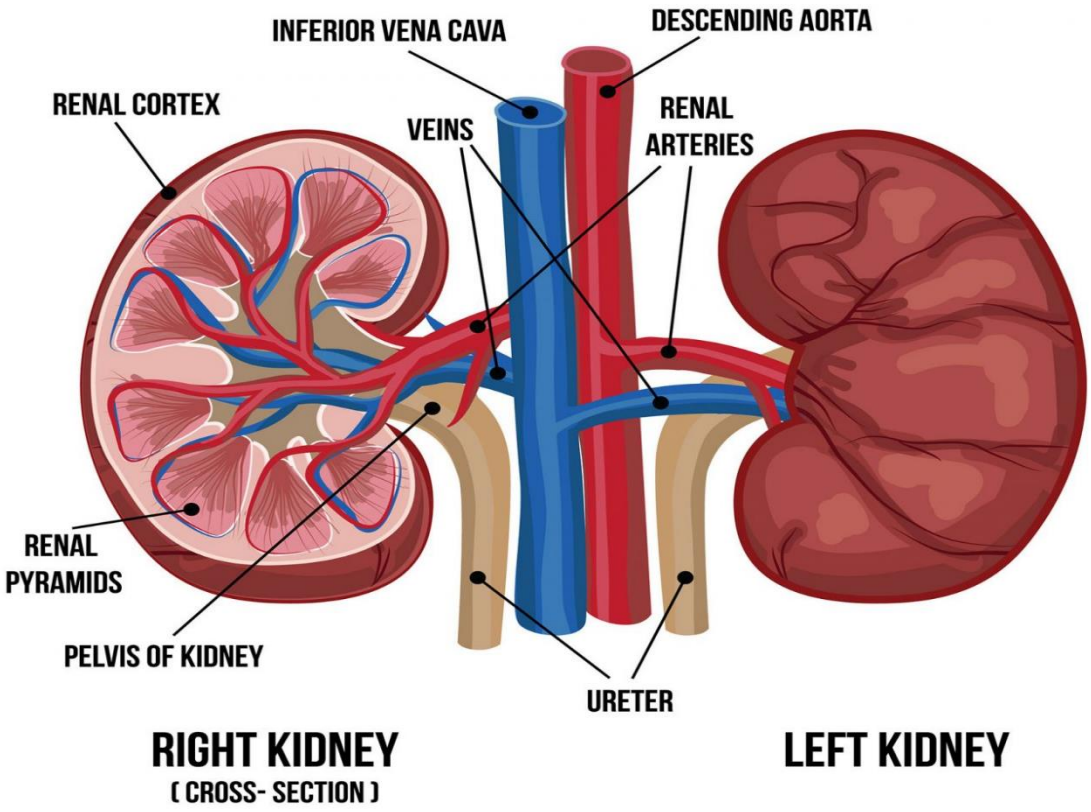

Fig 2.4 THE KIDNEY

2.9.1 Renal Fat and Fascia

The kidneys are embedded in a thick cushion of fat known as **perinephric fat**, which is enclosed within the **renal fascia**. Superiorly, this fascia merges with the diaphragm's fascia but forms a distinct compartment for the suprarenal gland, allowing the gland to be separated during nephrectomy. Medially, it connects with the sheaths of the aorta and inferior vena cava, while laterally it is continuous with the transversalis fascia. Inferiorly, however, it remains relatively open, extending around the ureter into the pelvis [Ellis, 2006].

The kidney is surrounded by **three layers (capsules):**

1. The **renal fascia** (outermost covering)
2. The **perinephric fat** (fatty layer)
3. The **true fibrous capsule**, which peels easily from a healthy kidney but adheres tightly in cases of inflammation [Moore *et al.*, 2014].

Together, the **renal fascia, collagen bundles, perinephric and paranephric fat**, as well as the anchoring effect of the renal vessels and ureter, help secure the kidneys in place. Despite this, the kidneys are not completely immobile; they shift during breathing and positional changes, with normal renal movement being about the height of one vertebral body [Moore *et al.*, 2014].

2.9.2 GROSS ANATOMY

2.9.2.1 Relations:

Posterior relations: The kidneys are positioned in front of the diaphragm (which separates them from the pleura), quadratus lumborum, psoas, and transversus abdominis muscles, as well as the 12th rib. They are also closely associated with three nerves: the subcostal (T12), iliohypogastric, and ilioinguinal (L1).

Anterior relations: The right kidney is in contact with the liver, the second part of the duodenum (which can be accidentally opened during a right nephrectomy), and the ascending colon. The left kidney lies adjacent to the stomach, pancreas and its vessels, spleen, and the

descending colon. The adrenal glands sit like caps on the upper poles of both kidneys [Ellis, 2006].

2.9.2.2 Surface Markings:

The kidney measures approximately 11 × 5 cm and can be marked both posteriorly and anteriorly.

Posterior surface: The kidney is located within Morris' I parallelogram. This is outlined by two horizontal lines at the levels of the 11th thoracic and 3rd lumbar vertebrae, and two vertical lines at 2.5 cm and 9 cm from the midline. The hilum lies opposite the lower border of the first lumbar vertebra, slightly lower on the right side.

Anterior surface: The bean-shaped kidney has the following relations:

Right kidney: The hilum lies about 5 cm from the midline, slightly below the transpyloric plane. The upper pole is 4–5 cm from the midline, halfway between the xiphisternum and the transpyloric plane, slightly lower than the left. The lower pole is 6–7 cm from the midline at the umbilical plane.

Left kidney: The hilum is 5 cm from the midline, slightly above the transpyloric plane, near the tip of the 9th costal cartilage. The lower pole aligns with the subcostal plane [Chaurasia, 2006].

2.9.2.3 Structure:

Each kidney has a concave medial border called the renal hilum, which opens into the renal sinus. The renal hilum allows entry and exit of vessels, nerves, and urine-draining structures.

The left hilum is near the transpyloric plane, about 5 cm from the midline. The superior pole of the right kidney lies slightly lower (2.5 cm) than the left due to the liver. Posteriorly, the upper parts lie deep to the 11th and 12th ribs. Kidney positions vary with respiration and posture, moving 2–3 cm vertically during deep breathing. Surgically, the right kidney's inferior pole is roughly a finger's breadth above the iliac crest. Adult kidneys are reddish-brown, roughly 10 cm long, 5 cm wide, and 2.5 cm thick. Posteriorly, they relate to the diaphragm, psoas major, and quadratus lumborum muscles. The subcostal, iliohypogastric, and ilioinguinal nerves cross their posterior surfaces.

Anterior relations:

Right kidney: liver, duodenum, ascending colon; separated from the liver by the hepatorenal recess.

Left kidney: stomach, spleen, pancreas, jejunum, descending colon.

Hilum arrangement: The renal vein is anterior, the artery is behind it, and the pelvis lies posteriorly. The renal sinus contains the renal pelvis, calyces, vessels, nerves, and variable fat. Each kidney has anterior and posterior surfaces, superior and inferior poles, and medial and lateral borders. The medial border is concave due to the renal sinus, giving the kidney a bean-shaped appearance. Oblique placement over the lumbar vertebrae shortens the transverse diameter in anterior and AP radiographs.

Renal pelvis and calyces: The renal pelvis is a funnel-shaped expansion at the ureter's superior end, receiving 2–3 major calyces, each dividing into 2–3 minor calyces. Minor calices surround the renal papillae at the apex of renal pyramids, through which urine drains.

In living individuals, the renal pelvis and calices are typically collapsed. The pyramids and cortex form kidney lobes, which are prominent in fetuses and may remain visible after birth [Moore *et al.*, 2014].

2.9.2.4 Arterial Supply

Each kidney is typically supplied by a single renal artery originating from the abdominal aorta. However, accessory renal arteries are found in approximately 30% of individuals. These usually arise from the aorta, run parallel to the main renal artery, and enter the kidney at the hilum or at one of its poles. Near the hilum, the renal artery divides into anterior and posterior branches, which further branch into segmental arteries supplying distinct vascular segments of the kidney. Five segments are recognized: apical, upper, middle, lower, and posterior. Segmental arteries are end arteries, meaning each vascular segment functions independently. Each segmental artery branches into lobar arteries, typically one for each renal pyramid. Lobar arteries then divide into interlobar arteries that run along the sides of the pyramids. At the corticomedullary junction, interlobar arteries bifurcate into arcuate arteries, which arch over the pyramid bases at right angles. The arcuate arteries give rise to interlobular arteries that extend radially into the cortex. Neither arcuate nor interlobular arteries form anastomoses with their neighbors, so they function as end arteries. Small branches from interlobular arteries penetrate the fibrous capsule to connect with a capsular plexus, allowing potential limited collateral circulation with suprarenal, phrenic, testicular or ovarian, or lumbar vessels [Chaurasia, 2006]. Glomerular circulation: Most afferent arterioles arise from interlobular arteries, though some originate directly from arcuate or interlobar arteries. The efferent arteriole from each glomerulus quickly branches into the peritubular

capillary plexus surrounding the proximal and distal convoluted tubules, forming a renal portal system with two capillary networks: glomerular and peritubular. The medullary blood supply mainly comes from efferent arterioles of juxtamedullary glomeruli, supplemented by a few a glomerular arterioles. Each arteriole descends into the renal pyramid, forming 12–24 descending vasa recta, which create capillary networks in the inner medulla around loops of Henle and collecting ducts. Venous return occurs through ascending vasa recta, which drain into interlobular or arcuate veins. In the outer medulla, the close association between descending vasa recta, venules, and medullary tubules supports the countercurrent exchange and multiplier system.

Two main patterns of renal circulation are recognized:

Free circulation: Primarily cortical, involving glomeruli; considered the normal pattern.

Restricted circulation: Mainly medullary, bypassing cortical glomeruli; blood flows through juxtamedullary glomeruli and vasa recta, with faster medullary circulation [Chaurasia, 2006].

2.9.2.5 Venous Drainage

Blood from the peritubular capillary plexus drains into interlobular veins, which empty into arcuate veins, then into interlobar veins, and finally converge at the renal sinus to form the renal vein, draining into the inferior vena cava. The vasa recta also contribute to the arcuate veins (Chaurasia, 2006).

2.9.2.6 Nervous Supply

The kidneys receive innervation from the renal plexus, a branch of the coeliac plexus. It contains sympathetic fibers (T10–L1) that mainly regulate vasomotor activity. Afferent sensory fibers correspond to spinal segments T10–T12 [Chaurasia, 2006].

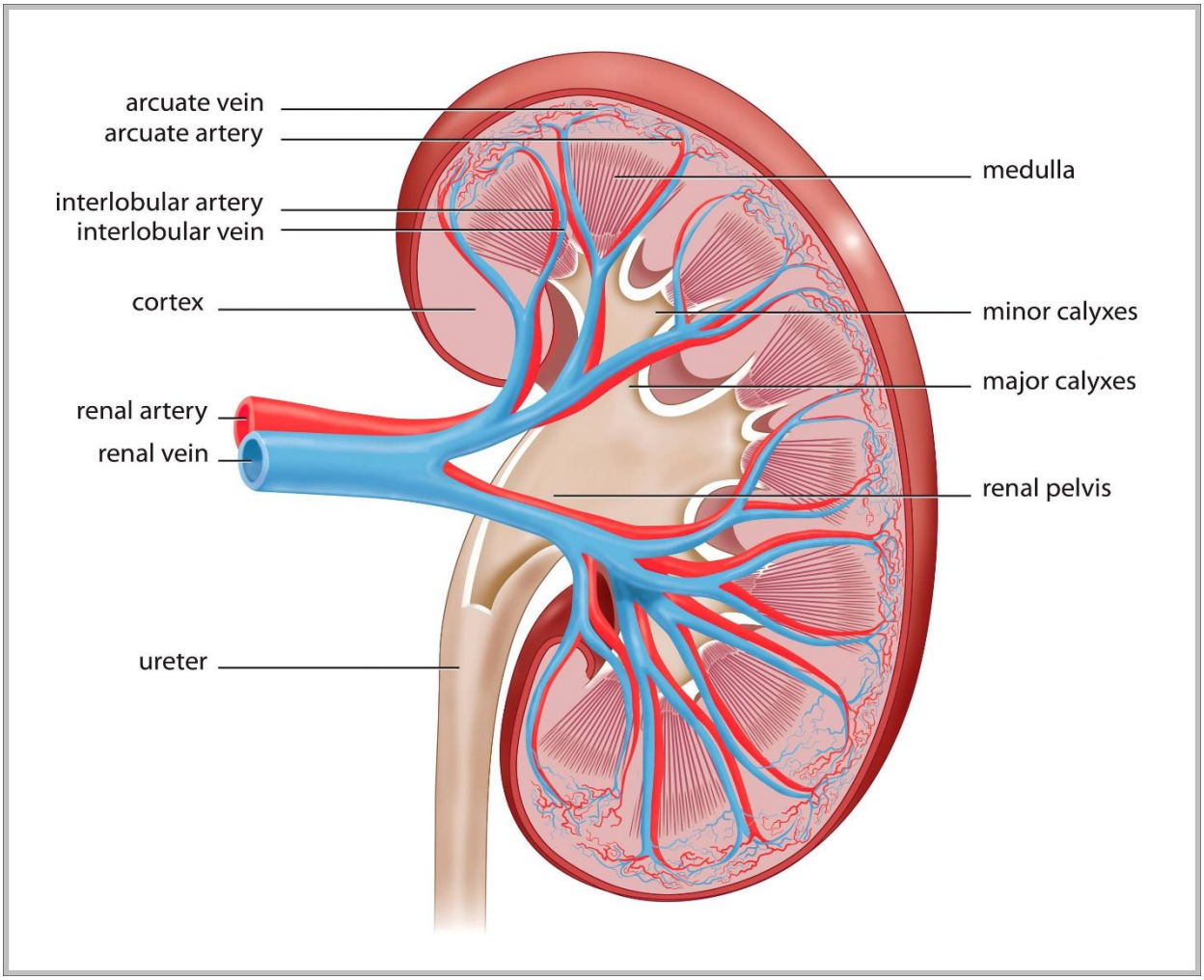

Fig 2.5 Internal structure of the kidney

2.9.3 Development of the Kidney

The kidneys are derived from the **intermediate mesoderm**, which lies between the paraxial and lateral plate mesoderm. Their development, called **nephrogenesis**, occurs in three successive stages:

1. **Pronephros** – the earliest and most rudimentary form of the kidney. It appears in the cervical region during early embryonic life but is non-functional in humans. It quickly regresses.

2. **Mesonephros** – develops after the pronephros and functions temporarily during early fetal life. It consists of mesonephric tubules and the mesonephric (Wolffian) duct. Although it degenerates later, some of its structures give rise to parts of the male reproductive system.

3. **Metanephros** – this is the final and permanent kidney, appearing around the 5th week of development. The metanephros gives rise to the fully functional kidney that persists throughout life (Singh, 2011; Moore *et al.*, 2014).

2.9.4 Functions of the Kidney

The kidneys play a crucial role in eliminating metabolic waste products from the body by excreting them in urine. The **nephron**, the microscopic structural and functional unit of the kidney, processes blood through **filtration, reabsorption, secretion, and excretion**, ultimately leading to urine formation. The wastes removed include nitrogenous by-products such as **urea** (from protein metabolism) and **uric acid** (from nucleic acid breakdown).A unique feature of mammals and some birds is their ability to concentrate urine, producing a

volume much smaller than the blood filtered. This process depends on the **countercurrent multiplication mechanism**, which relies on:

- the hairpin-shaped arrangement of the nephron tubules,
- selective permeability of the descending limb to water and ions,
- impermeability of the ascending limb to water, and
- active ion transport in the thick ascending limb.

Additionally, **countercurrent exchange** by blood vessels surrounding the nephron supports this concentration process.

Beyond excretion, the kidneys are essential for maintaining overall **homeostasis**, which includes regulating **acid–base balance, electrolyte levels, extracellular fluid volume, and blood pressure**. These roles are achieved both directly and through interactions with the **endocrine system**. Hormones such as **renin, angiotensin II, aldosterone, antidiuretic hormone (ADH), and atrial natriuretic peptide (ANP)** play key roles in coordinating these regulatory functions (Singh, 2011; Moore *et al.*, 2014).

2.9.5 Histology of the Kidney

2.9.5.1 Renal Corpuscle

The renal corpuscle is the functional unit responsible for plasma filtration. It consists of Bowman's capsule and the glomerulus. Here, water and small molecules from plasma pass through the glomerular capillaries into Bowman's space, forming the glomerular ultrafiltrate, which then enters the renal tubules [Young *et al*.].

2.9.5.2 Glomerular Filtration Barrier

Within the renal corpuscle, water and small molecules from the blood in the glomerular capillaries are filtered into Bowman's space, forming the initial urine. Filtration occurs across a specialized barrier that consists of three main components:

1. **Capillary endothelium:** The endothelial cells contain numerous pores (fenestrae) that are larger than those in most other capillaries and are not covered by a membrane. This allows filtrate to pass through easily, so the endothelium itself does not act as a major barrier.

2. **Podocytes:** These cells have a star-like shape due to a few primary processes that wrap around the capillaries. Each primary process divides into many secondary processes called **pedicels**, which rest on the glomerular basement membrane (GBM). The spaces between pedicels, known as **filtration slits**, are covered by a thin **slit diaphragm**. Because filtrate passes through these slits rather than through podocyte cytoplasm, podocytes provide structural support but are not the primary barrier to filtration.

3. **Glomerular basement membrane (GBM):** The GBM lies between the endothelium and podocytes and is the main barrier to filtration. It is thickened at the filtration slits by the slit diaphragm and carries a strong negative charge that repels large molecules such as proteins. Damage to this charge, as seen in some kidney diseases, can result in excessive protein loss in the urine [Singh, 2011].

2.9.5.3 Glomerular Basement Membrane (GBM):

The glomerular basement membrane (GBM) is much thicker than most other biological membranes, measuring approximately 300 nm. It is composed of three layers: a central dense layer called the **lamina densa**, and two outer, electron-lucent layers known as **lamina rara interna** and **lamina rara externa**. The lamina densa contains a network of type IV collagen fibers, providing a **physical barrier**, while the electron-lucent layers contain **heparan sulfate**, a negatively charged glycosaminoglycan that contributes an **electrical barrier**, preventing the passage of large molecules. The GBM, along with the podocyte cytoplasm above it, does not completely surround the glomerular capillaries. Gaps are filled by the **mesangium**, which supports the capillaries (described later). A thin membrane continuous with the lamina rara interna may separate the endothelial cytoplasm from the mesangium, and in spaces between capillaries, the GBM comes into direct contact with mesangial tissue.Damage to the GBM can lead to **nephrotic syndrome**, characterized by excessive protein loss in the urine. In this condition, the normally orderly arrangement of podocyte processes is also disrupted [Singh, 2011].

2.9.5.4 Mesangium

When the afferent arteriole enters the glomerulus, it usually splits into five branches, each supplying a separate capillary network. This organization allows the glomerular circulation to be divided into distinct **lobules or segments**. The capillaries within each segment are supported by the **mesangium**, which consists of mesangial cells embedded in a non-cellular **mesangial matrix**. The mesangium forms a fold around the capillary loops, similar in structure to the mesentery of the small intestine. Mesangial cells extend processes through

the matrix, helping to maintain the structure between capillaries.Mesangial cells contain contractile filaments resembling **myosin** and possess **angiotensin II receptors**. When stimulated by angiotensin II, these filaments contract, suggesting that mesangial cells help regulate blood flow within the glomerulus. Additionally, they perform **phagocytosis** and contribute to the maintenance of the glomerular basement membrane. In certain diseases, such as **glomerulonephritis**, the mesangium becomes enlarged and more prominent [Singh, 2011].

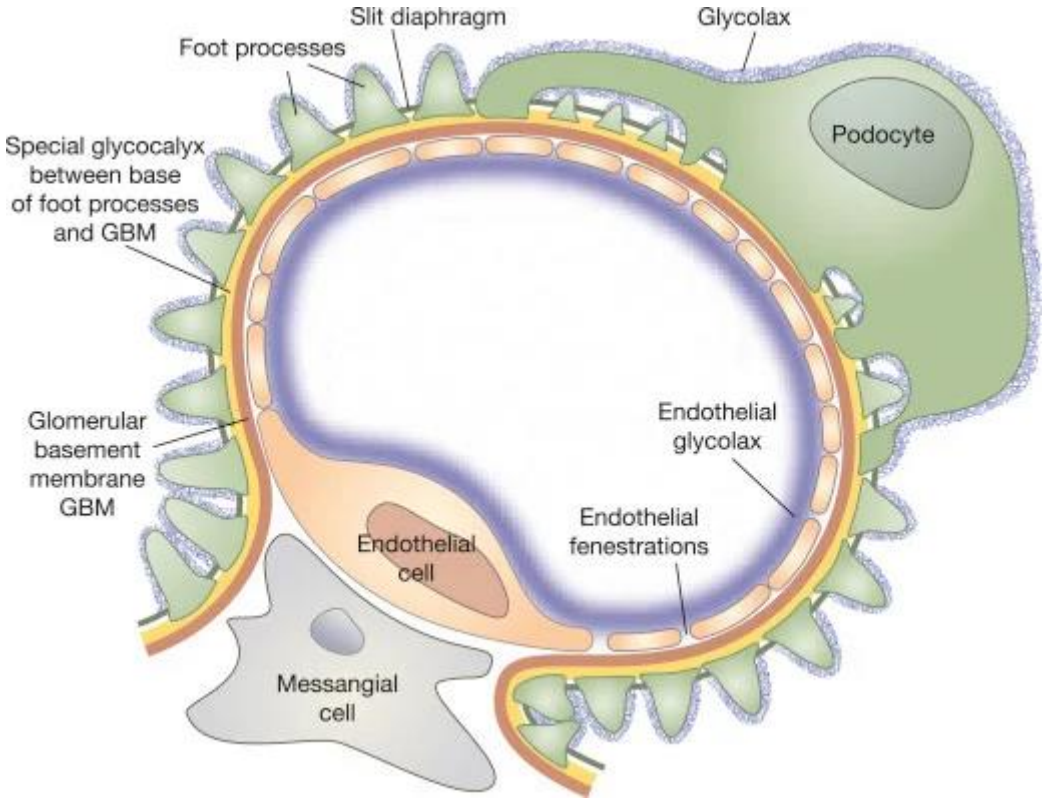

Fig 2.6 Glomerular Basement Membrane Layers

2.9.5.5 Renal Tubule

The renal tubule consists of, in order from proximal to distal, the **proximal convoluted tubule (PCT)**, the **loop of Henle**, and the **distal convoluted tubule (DCT)**. The distal convoluted tubule ultimately opens into a **collecting tubule**. Key features include:

a) The junction between the proximal convoluted tubule and Bowman's capsule is narrow and is called the **neck**.

b) The proximal convoluted tubule has two parts: an initial highly coiled segment located in the cortex, and a straight portion that descends into the medulla, becoming continuous with the **descending limb of the loop of Henle**.

c) The **descending limb**, the loop, and part of the **ascending limb** are narrow and thin-walled, forming the **thin segment** of the loop. The upper portion of the ascending limb is wider and thicker-walled, referred to as the **thick segment** [Singh, 2011].

d) The distal convoluted tubule consists of a straight part continuous with the ascending limb of the loop of Henle, and a convoluted segment located in the cortex. At the junction of these parts, the distal tubule lies close to its own renal corpuscle. The terminal straight portion is called the **connecting tubule** or **junctional tubule**, which eventually joins a collecting duct [Singh, 2011].

2.9.5.6 Epithelium Lining the Renal Tubule

The renal tubule is lined throughout its length by a **single layer of epithelial cells** resting on a basal lamina. The characteristics of these cells vary in different segments:

A. **Neck:** Lined by **simple squamous epithelium**, continuous with that of Bowman's capsule.

B. **Proximal convoluted tubule (PCT):** Measures 40–60 μm in diameter with a relatively narrow lumen. Lined by **cuboidal or columnar cells** with a prominent **brush border**. The nuclei are centrally located and euchromatic, and the cytoplasm stains pink with hematoxylin and eosin. The basal part of the cell shows vertical striations.

C. **Loop of Henle:** The thin segment is 15–30 μm in diameter, lined by **low cuboidal or squamous cells**. The thick segment has **cuboidal cells**.

D. **Distal convoluted tubule (DCT):** Measures 20–50 μm in diameter and can be distinguished from the PCT by a **larger lumen**, cuboidal cells **without a brush border**, and lighter eosin staining.

E. **Collecting tubules:** Vary from 40–50 μm (smallest) to 200 μm (largest). Lined by **simple cuboidal or columnar epithelium**, they are easily distinguished from convoluted tubules because they have **larger, circular lumina**, lightly staining cytoplasm, distinct cell outlines, and **no brush border** [Singh, 2011].

2.10 Clinical Correlations of the Kidney

1. **Renal Calculi (Kidney Stones):** Solid masses made up of crystals, usually calcium oxalate or uric acid, can form in the kidney. These stones may obstruct urine flow, leading to **colicky flank pain, hematuria, and hydronephrosis** if not passed

naturally. Larger stones may require surgical or lithotripsy intervention (Moore *et al.*, 2014; Singh, 2011).

2. **Polycystic Kidney Disease (PKD):** A **genetic disorder** where multiple cysts progressively enlarge within the renal parenchyma, compressing and replacing functional tissue. Over time, this leads to **hypertension, hematuria, and eventually chronic kidney disease**. Autosomal dominant PKD is the most common form (Standring, 2011).

3. **Hydronephrosis:**

   Refers to **dilation of the renal pelvis and calyces** caused by urinary tract obstruction, which may occur due to calculi, congenital narrowing of the ureteropelvic junction, tumors, or prostatic hypertrophy. Persistent obstruction can cause **renal atrophy and impaired renal function** (Ellis, 2006).

4. **Pyelonephritis:**

   An infection of the kidney, usually ascending from the bladder, caused commonly by *E. coli*. Patients present with **fever, flank tenderness, dysuria, and pyuria**. Recurrent or chronic cases may cause **renal scarring and functional loss** (Young *et al.*, 2014).

5. **Glomerulonephritis:**

   Inflammation of the glomeruli often due to immune-mediated mechanisms. It can present with **proteinuria, hematuria, edema, and hypertension**. Severe cases may

progress to **nephrotic or nephritic syndrome**, ultimately leading to end-stage renal disease (Singh, 2011).

6. **Renal Hypertension (Renovascular Hypertension):** Narrowing of the renal artery (renal artery stenosis) decreases blood supply to the kidney. This activates the **renin–angiotensin–aldosterone system (RAAS)**, causing systemic **secondary hypertension**. It is a reversible cause of hypertension if corrected surgically or with stenting (Chaurasia, 2006; Moore *et al.*, 2014).

7. **Renal Trauma:** The kidneys are **vulnerable to injury** due to their retroperitoneal location and limited bony protection, especially during road traffic accidents, falls, or penetrating trauma. Injuries range from minor contusions to **lacerations or vascular pedicle avulsion**, which may require nephrectomy (Ellis, 2006).

8. **Renal Tumors:** The most common malignant tumor of the kidney is **renal cell carcinoma (hypernephroma)**. Classic symptoms include **hematuria, flank mass, and dull flank pain**, but they often present late. Wilms' tumor (nephroblastoma) is a significant pediatric malignancy (Standring, 2011).

9. **Chronic Kidney Disease (CKD):** Progressive loss of kidney function due to conditions like diabetes, hypertension, or chronic glomerulonephritis. Clinical consequences include **uremia, anemia, electrolyte imbalance, and bone disease**. Ultimately, CKD may require **dialysis or renal transplantation** (Moore *et al.*, 2014).

10. **Congenital Anomalies:**

a. **Horseshoe Kidney:** Fusion of the lower poles of the kidneys, which remain low in the abdomen as they get trapped under the inferior mesenteric artery.

b. **Ectopic Kidney:** Kidney located in an abnormal position (e.g., pelvic kidney).

c. **Polycystic Kidney (congenital form):** Multiple cysts present from birth, leading to early renal failure. These anomalies may remain asymptomatic or predispose to **obstruction and infection** (Singh, 2011 and Standring, 2011).

CHAPTER THREE

MATERIALS AND METHOD

3.1 MATERIALS

Animals:20 Adult wistar rats; Feed: Growers mash; Instruments: Plastic cages, ceramic plates, Waltman filter paper, funnel, conical flask, surgical blade, forceps, 5ml syringe, laptop, weighing balance, microtome, slide tray, tissue embedding station, microscope, specimen bottles, cotton wool, orogastric tube, disposable gloves, measuring cylinder, pestle and mortar; Reagents:10% formal saline, chloroform, distilled water, eosin, hematoxylin, paraffin wax, xylene.

3.2 COLLECTION OF PLANT SAMPLE /EXTRACT

The plant sample, seeds of *Myristica fragrans*, were gotten from a market place in Mile12 Lagos and it was then identified by a plant taxonomist from the Department of Plant Biology and Biotechnology, Dr. Akinnibosun, *as Myristica fragrans Houtt*. Aqueous extraction of *Myristica fragrans* seeds was performed using a maceration technique. Fresh seeds were first air-dried at room temperature, then milled into a fine powder using an electric blender. Five hundred grams (500g) of the powdered sample were soaked in 2 liters of distilled water for 24 hours. The mixture was filtered using white filter paper to separate the residue from the liquid extract. The resulting filtrate was concentrated in the medical laboratory of the University of Benin, Benin City

3.3 METHOD

3.3.1 EXPERIMENTAL ANIMAL

Animal Care and management

Twenty adult Wistar rats were used as experimental animals in this study. Their weight ranged between 130g and 160g.The rats were purchased and maintained at the animal House in the Department of Anatomy, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Edo State. The rats were then put in their cages. Before transferring the rats into their cages, cages were cleaned and disinfected. The rats were left to acclimatize for a period of two weeks in their cages. They were fed with livestock's growers marsh manufactured by Top Feed limited, Sapele, Delta State, Nigeria throughout the acclimatization period as much as they were allowed access to water. The cages were made of plastic and wire gauze at the top to allow proper ventilation and the cages were cleaned daily and disinfected at intervals.

Each animal procedure was carried out in accord with approved protocols and in compliance with the recommendation for the proper management and utilization of laboratory animals used for research (Buzek and Chastel, 2010).

3.3.2 EXPERIMENTAL DESIGN

Animals were exposed to natural room temperature with a 12 hour day/night cycle. The animals were acclimatized for two weeks and then randomized (based on weight) into four groups; A, B, C and D with each group having five Wistar rats. Their individual weights were noted prior to administration. Group A was the control group and each rat herein was given

normal feed and water only, while groups B, C and D were given various doses of 200mg/kg, 750mg/kg and 1000mg/kg weight respectively and administration was done via an orogastric tube. The animals were weighed weekly using an electronic scale at the temporal animal house. Acute toxicity testing has shown that the aqueous extract of nutmeg seed caused no mortality at doses up to 1000 mg/kg body weight in rats, indicating that the LD50, if measured, is greater than 1000mg/kg (Kareem *et al.*, 2013).

GROUPS	DOSAGE
Group A	Served as control and will be fed with Animal feed and water ad libitum.
Group B	Will receive 200mg/kg of the *Myristica fragrans* aqueous extract daily for 28 days.
Group C	Will receive 750mg/kg of the *Myristica fragrans* aqueous extract daily for 28 days.
Group D	Will receive 1000mg/kg of the *Myristica fragrans* aqueous extract daily for 28 days.

TABLE 1.0: Experimental design

3.3.3METHOD OF SACRIFICE AND TISSUE COLLECTION

At the end of 29 days treatment, the rats were weighed using a weighing scale. Then animals were anesthetized with chloroform for about two minutes and sacrificed. In sacrificing, a midline incision was made through the ventral abdominal wall of each rat. The kidney of each rat was harvested immediately, and blotted dry on a filter paper. The rats were weighed and their standard weights calculated using the following formula: Standard weight = {organ

weight/(g)/body weight (g)} × 100. The kidney tissues were then fixed for about 24 hours in 10% buffered formalin for routine hematoxylin and eosin histological processing. Blood was directly collected from the abdominal aorta and heart. The samples were put into heparin bottles for renal function analysis. Both the harvested organ (kidney) and blood samples were then taken for tissue processing and functional analysis respectively.

3.4 HISTOLOGICAL TECHNIQUE

3.4.1 Paraffin Embedding

The kidney was excised and promptly transferred into 10% formal saline for fixation. Dehydration was carried out by passing the tissue through ascending grades of alcohol {70%, 90%, 95%, and 100% (absolute alcohol)} respectively for one hour. The tissue stayed in 70% alcohol for two hours, 90% alcohol for 18 hours (overnight) and 100% alcohol which was changed twice for two hours each. Clearing was carried out using xylene. The tissue was immersed in xylene for one hour so that alcohol will be completely removed. Infiltration of the tissue was carried out in an oven using molten paraffin wax at a temperature range of 30°C to 60°C for one hour. Three changes each at 15 minutes (twice) and 30 minutes (once) were carried out. Embedding was carried out using an embedding mould. The molten paraffin wax was poured into the embedding moulds and the infiltrated tissues were placed in it. The orientation of the tissue was such that both longitudinal and transverse sections were cut. The tissue block was formed by allowing the molten paraffin wax to cool. Before sectioning, the tissue blocks were trimmed and placed on a wooden block holder. Sectioning was carried out a rotatory microtome. The tissue was clipped to the microtome and sectioned at the thickness of five microns. Sections came out as ribbon and we're placed in 20% alcohol for spreading

of the tissue. In a 30%-temperature water bath, the ribbons were sliced and floated. The sectioned tissue was placed in xylene for 5 minutes to remove paraffin wax from the tissue. Hydration was carried out by passing the tissues through descending grades of alcohol (100%,95%,100%, and 70%) for 5 minutes each.

3.4.2 STAINING

Hematoxylin and Eosin staining method

The dyes utilized for staining were hematoxylin and eosin. The tissues were stained in hematoxylin for 30 minutes and washed in water for 10 minutes. They were differentiated in 1% acid alcohol briefly and then washed in water. They were subsequently counterstained in Eosin for 2 to 3 minutes, and then rinsed in 90% alcohol

Dehydration was done with the sections passed through ascending grades of alcohol 70%, 90%, 95% for 30 seconds and in absolute alcohol for 2 seconds. The sections were immersed in xylene for 1 minutes. They were mounted in Discrete Plasticizer and Xylene (DPX), covered with coverslip using Canada balsam. Sections were viewed under a microscope.

3.5 PHOTOMICROGRAPHY

The sections of the kidney were obtained and examined under Leica DM750 research microscope with a digital camera (Leica ICC50) attached and connected to a Hewlett-Packard laptop. Digital photomicrographs of the tissue were taken at X40, X100 and X400 objective magnifications. The photomicrographs were then analyzed.

3.6 STATISTICAL ANALYSIS

All collected disks were subjected to statistical analysis using IBM SPSS statistics software (Statistical package for social science) (version 25) and relevant statistical values were obtained. One-way analysis of variance (ANOVA) was carried out and data presented as mean ±SEM LSD post-hoc test was used. Values of $P<0.05$ were considered significant. The statistical values obtained were converted into graphical representations in form of bar charts.

CHAPTER FOUR

RESULTS

4.1 Weight

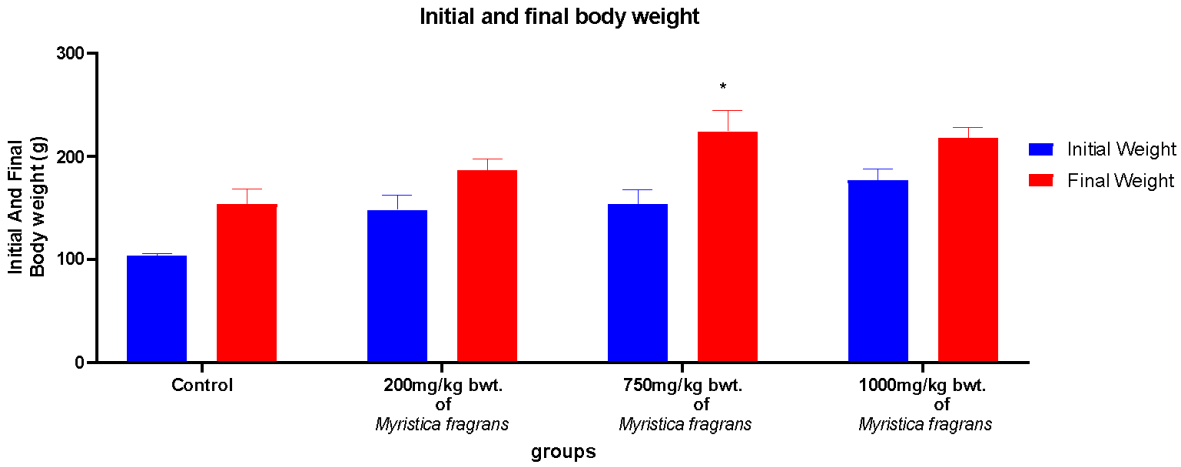

Chart 1: Initial and Final weight after 28 days of administration Values are given as mean ± SEM. * $p < 0.05$ compared with the initial weight.

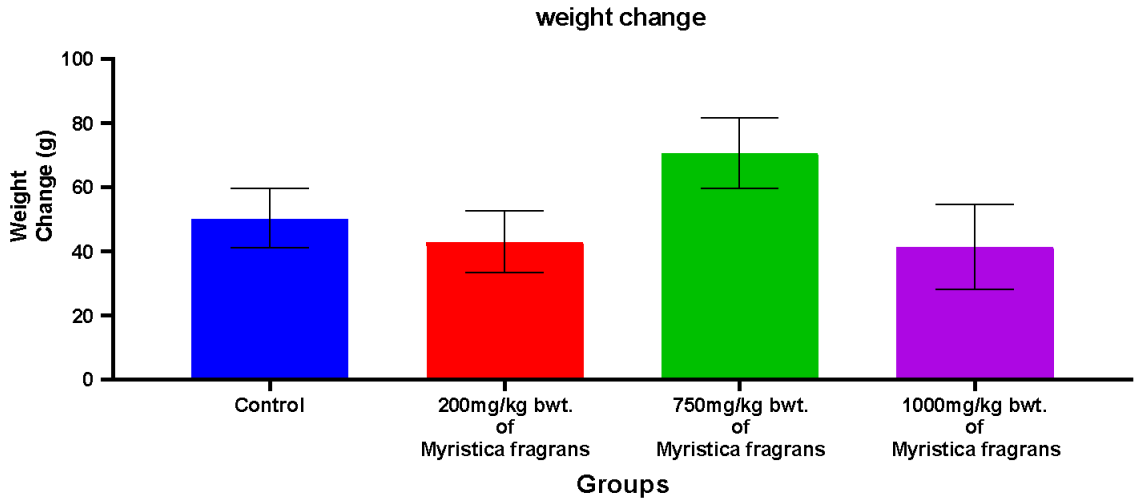

Chart 2: weight change after 28 days. Values are given as mean ± SEM. * $p < 0.05$ compared with the control group.

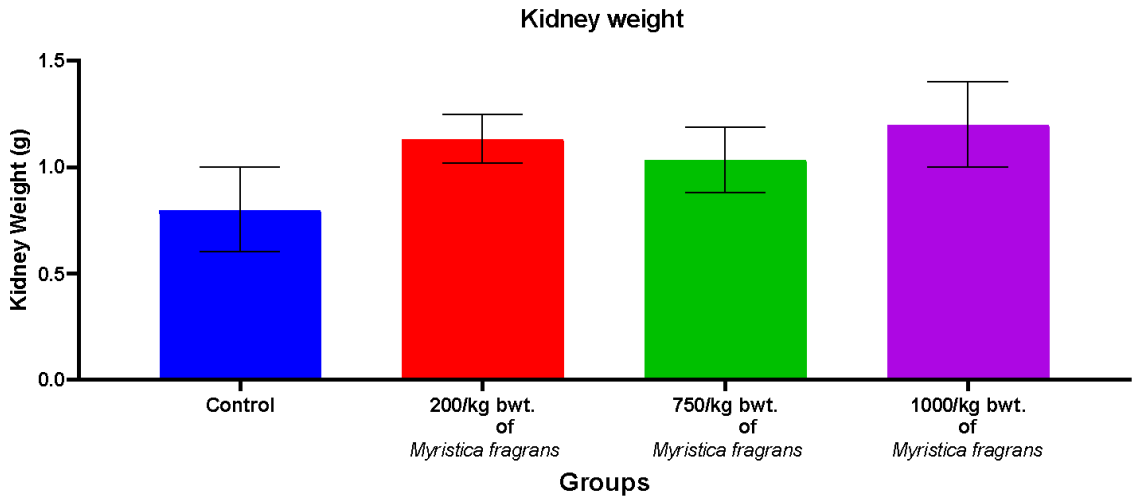

Chart 3: Kidney weight after 28 days. Values are given as mean ± SEM. * $p < 0.05$ compared with the control group.

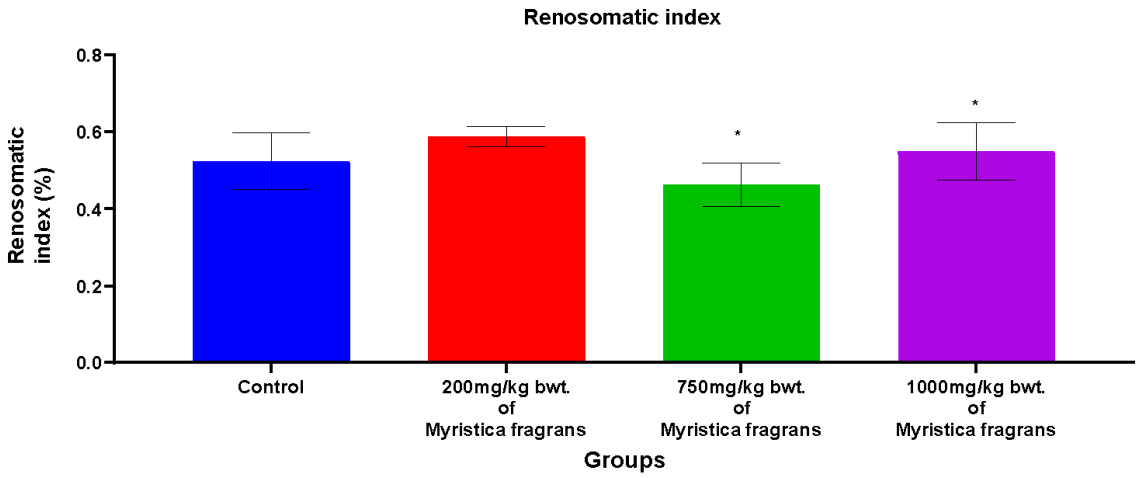

Chart 4: Reno-somatic index of control and treatment groups after 28 days. Values are given as mean ± SEM. * $p < 0.05$ compared with the control group.

4.2 Urea and Creatinine

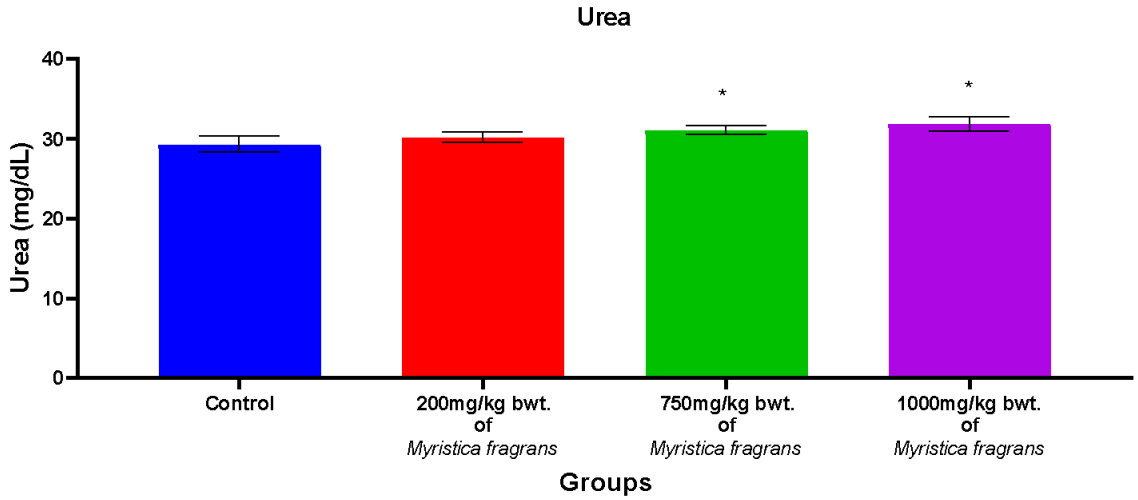

Chart 5: Activity of Urea in the Kidney of control and treatment groups after 28 days. Values are given as mean ± SEM. $^{*}p< 0.05$ compared with the control group

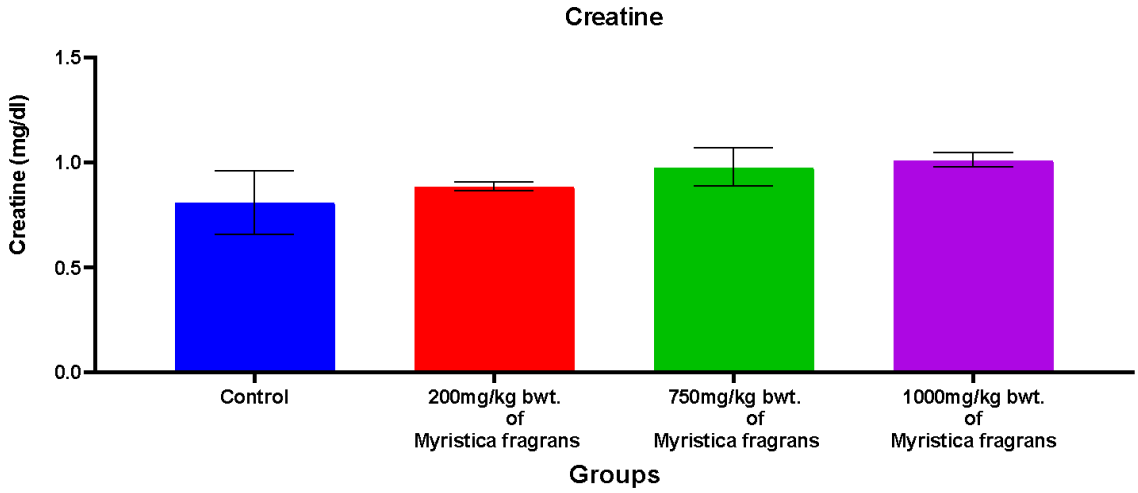

Chart 6: Activity creatinine in the kidney of control and treatment groups after 28 days. Values are given as mean ± SEM. $^{*}p< 0.05$ compared with the control group.

4.3 Oxidative stress

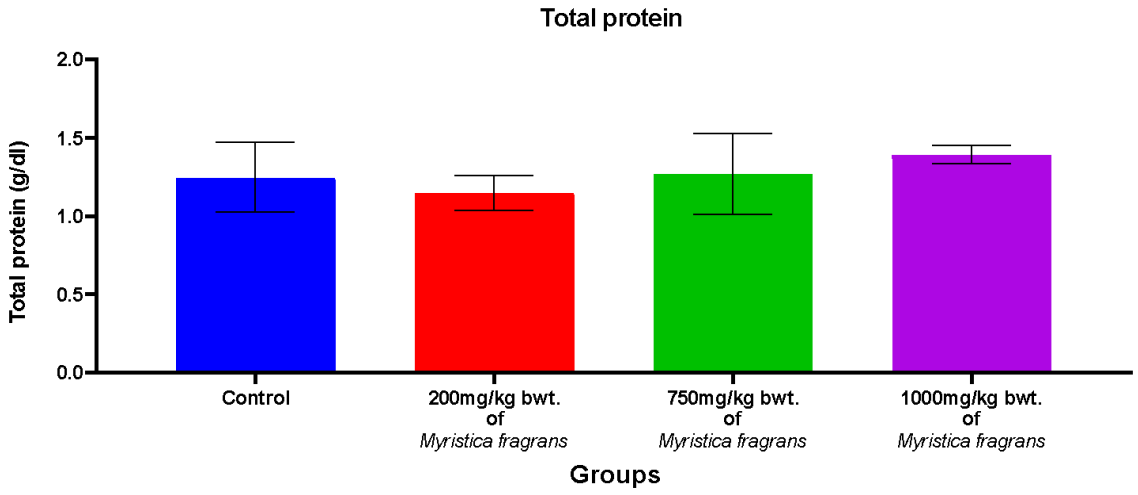

Chart 7: Total protein activity in the Kidney of control and treatment groups after 28 days. Values are given as mean ± SEM. $^{*}p< 0.05$ compared with the control group

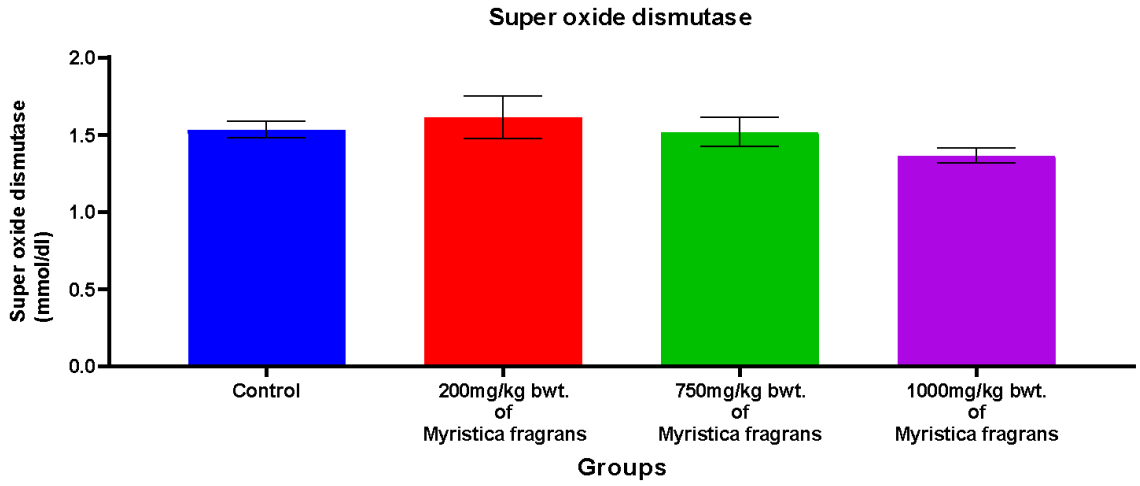

Chart 8: Superoxide dismutase activity in the Kidney of control and treatment groups after 28 days. Values are given as mean ± SEM. $^{*}p< 0.05$ compared with the control group.

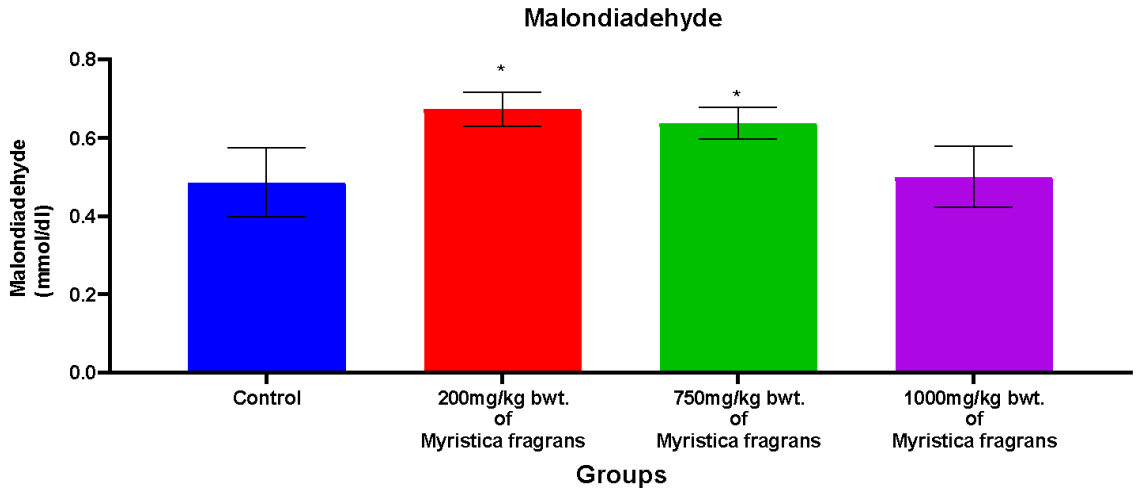

Chart 9: Lipid peroxidation activity in the Kidney of control and treatment groups after 28 days. Values are given as mean ± SEM. $^{*}p< 0.05$ compared with the control group;

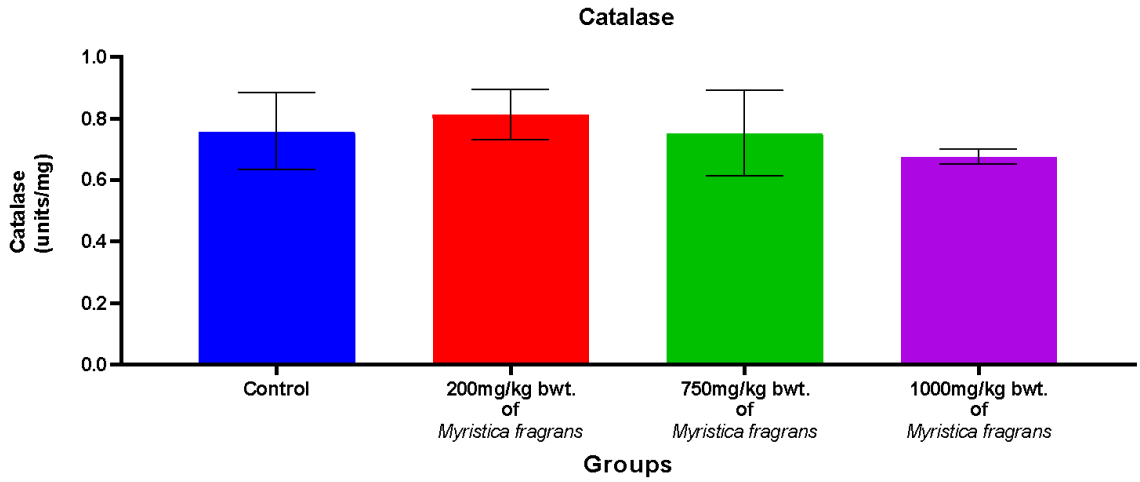

Chart 10: Catalase activity in the Kidney of control and treatment groups after 28 days. Values are given as mean ± SEM. $^{*}p< 0.05$ compared with the control group.

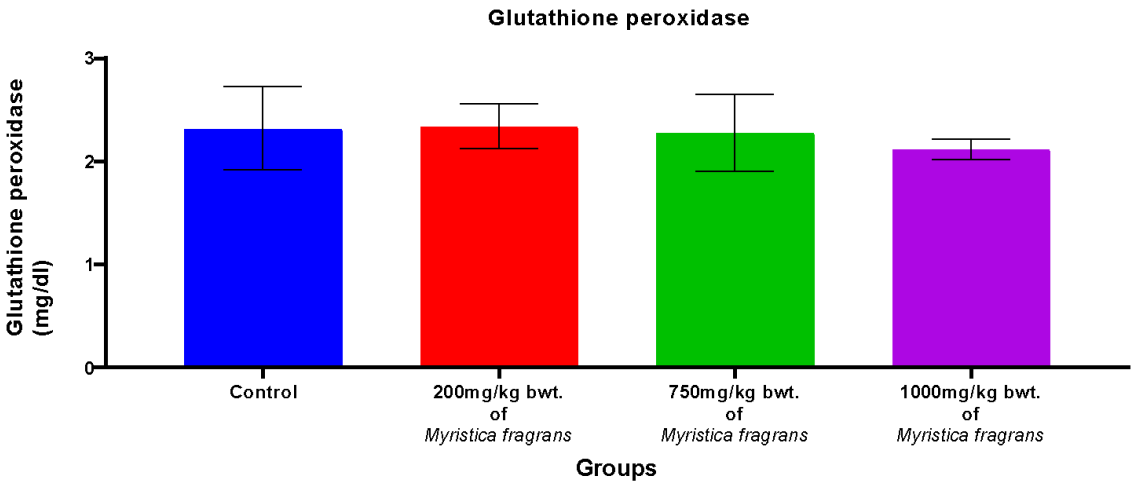

Chart 11: Glutathione Peroxidase activity in the Kidney of control and treatment groups after 28 days. Values are given as mean ± SEM. * $p < 0.05$ compared with the control group.

4.4 Histology

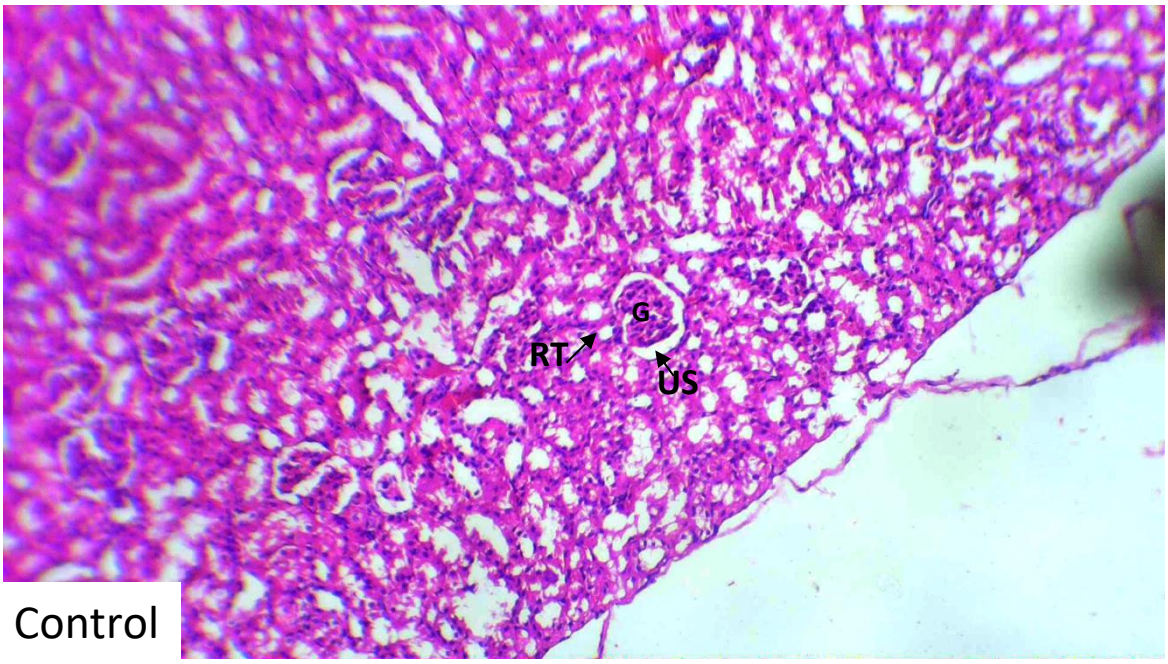

Plate 1: Control group shows normal histological features with glomerulus (G), urinary space (US) and renal tubules (RT): H&E 100 X

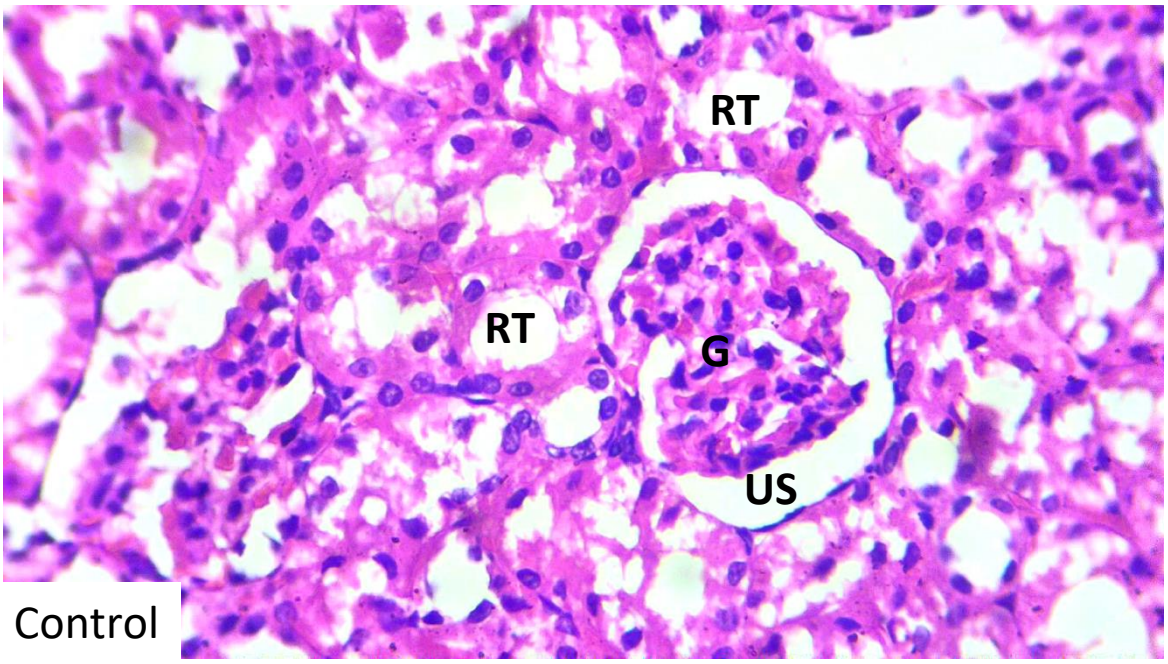

Plate 2: Control group shows normal histological features with glomerulus (G), urinary space (US) and renal tubules (RT): H&E 400 X

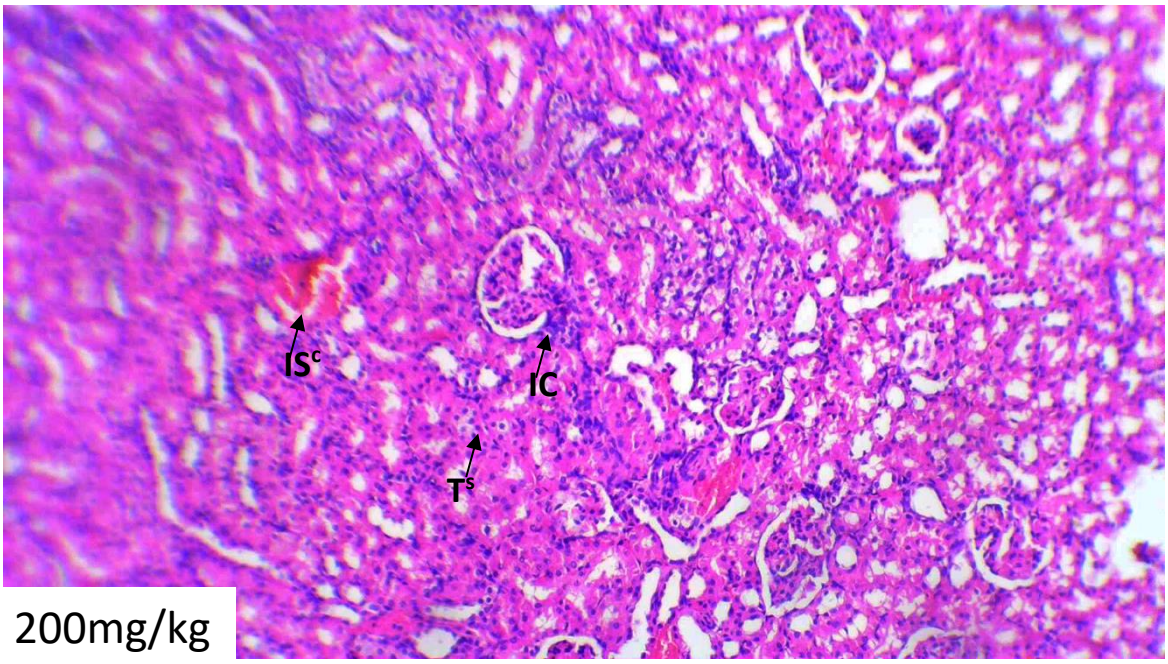

Plate 3:200 mg/kg group show mild tubular swelling (T^s), interstitial congestion (IC) and mild periglomerular infiltrates of inflammatory cells (IC): H&E 100 X

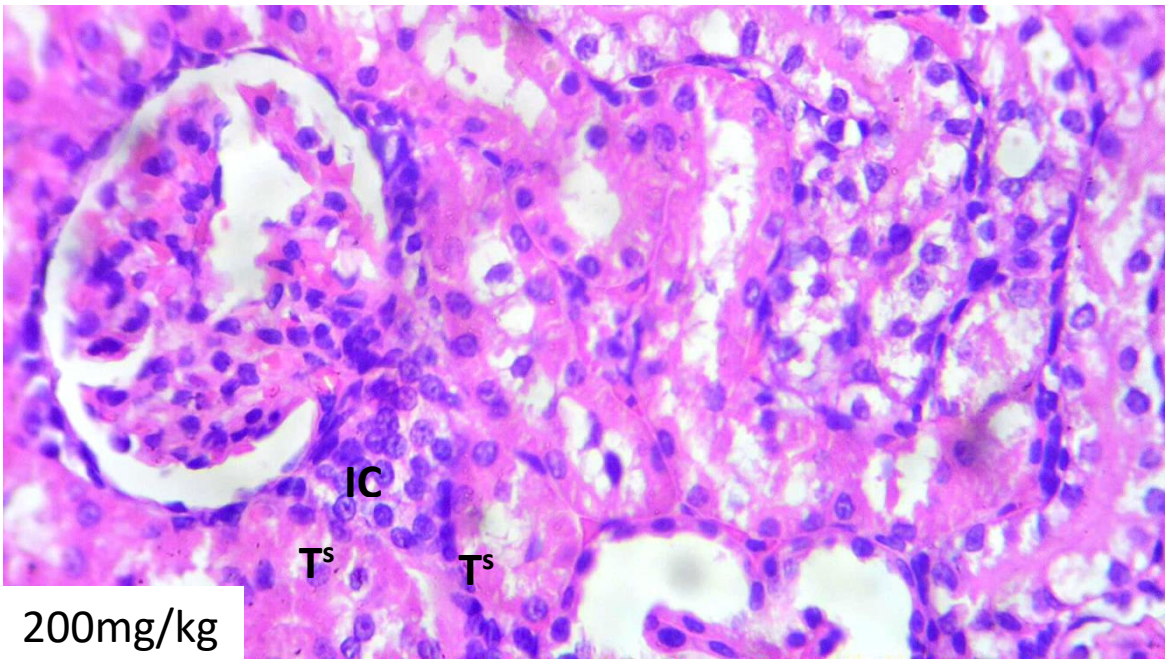

Plate 4: 200 mg/kg group show mild tubular swelling (T^s) and mild periglomerular infiltrates of inflammatory cells (IC): H&E 400 X

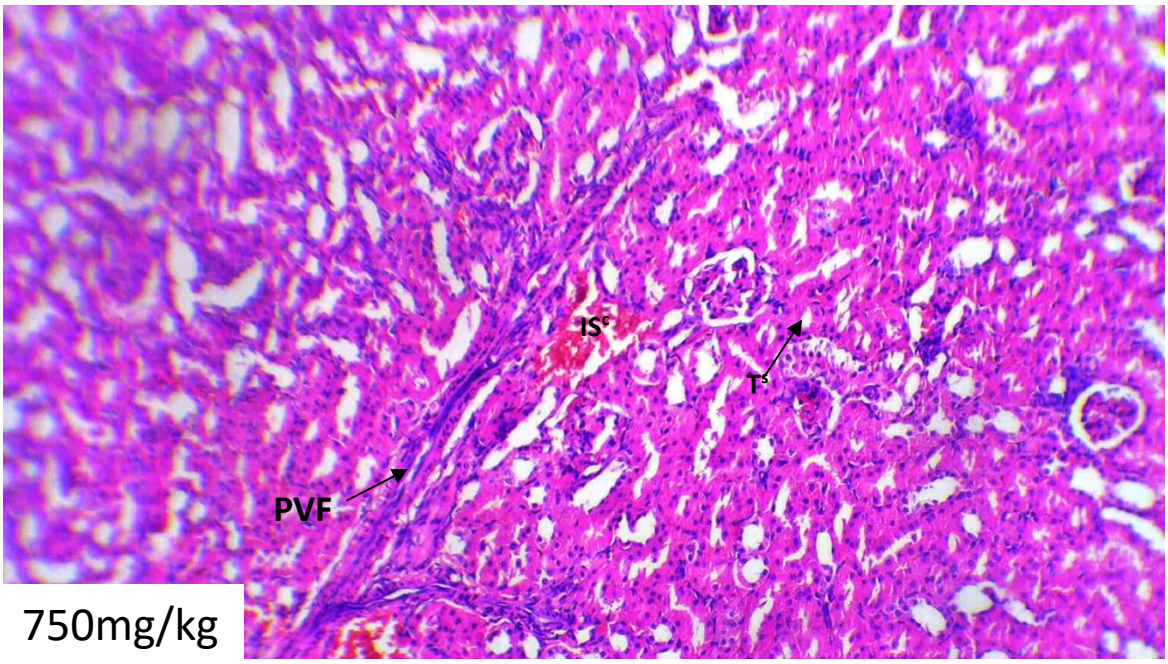

Plate 5: 750 mg/kg group shows mild tubular swelling (T^s), perivascular fibrosis and severe interstitial congection (Is^c): H&E 100 X

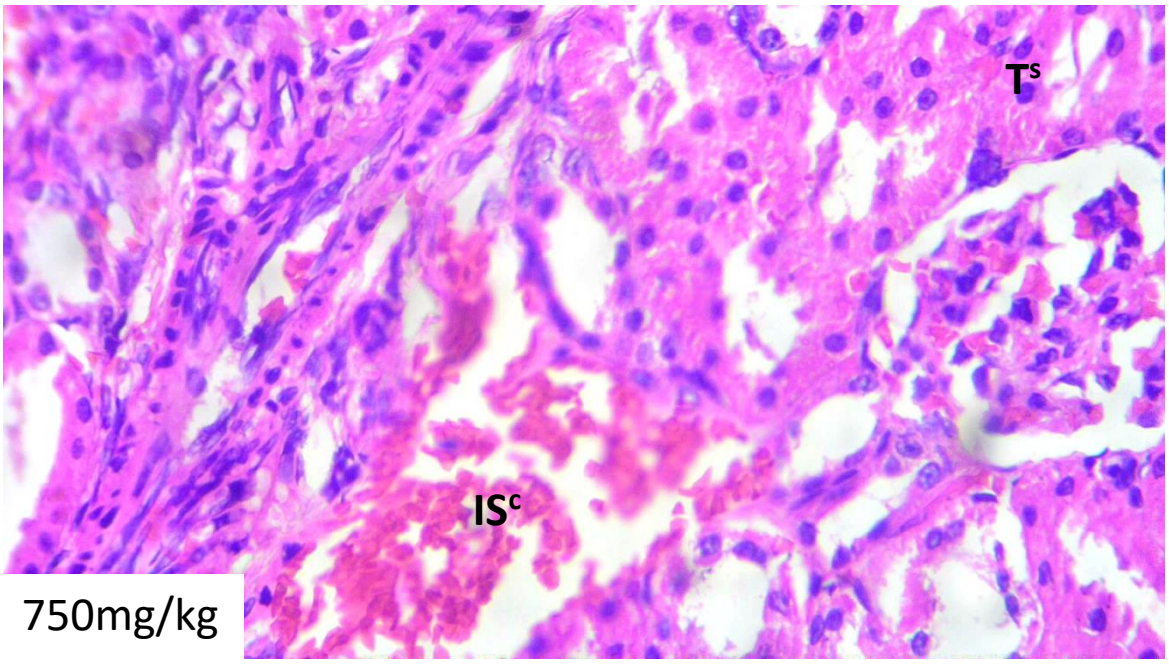

Plate 6: 750 mg/kg group shows mild tubular swelling (T^s) and severe interstitial congestion (Is^c): H&E 400 X

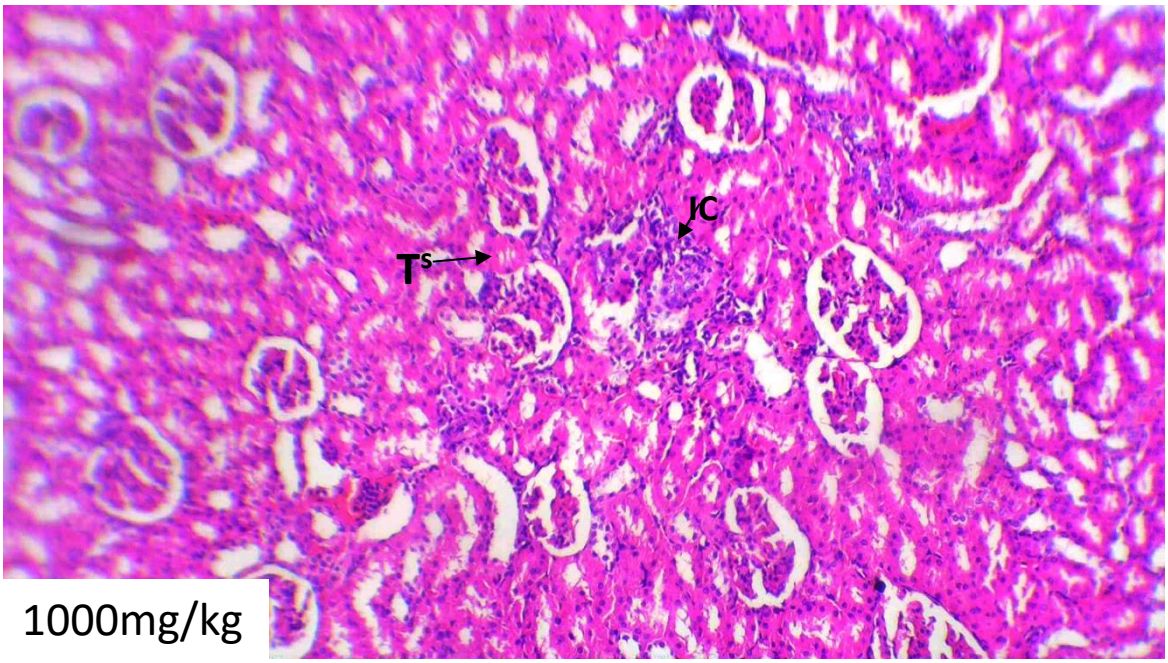

Plate 7: 1000 mg/kg group shows mild tubular swelling (T^s) and peritubular infiltrates of inflammatory cells (IC): H&E 100 X

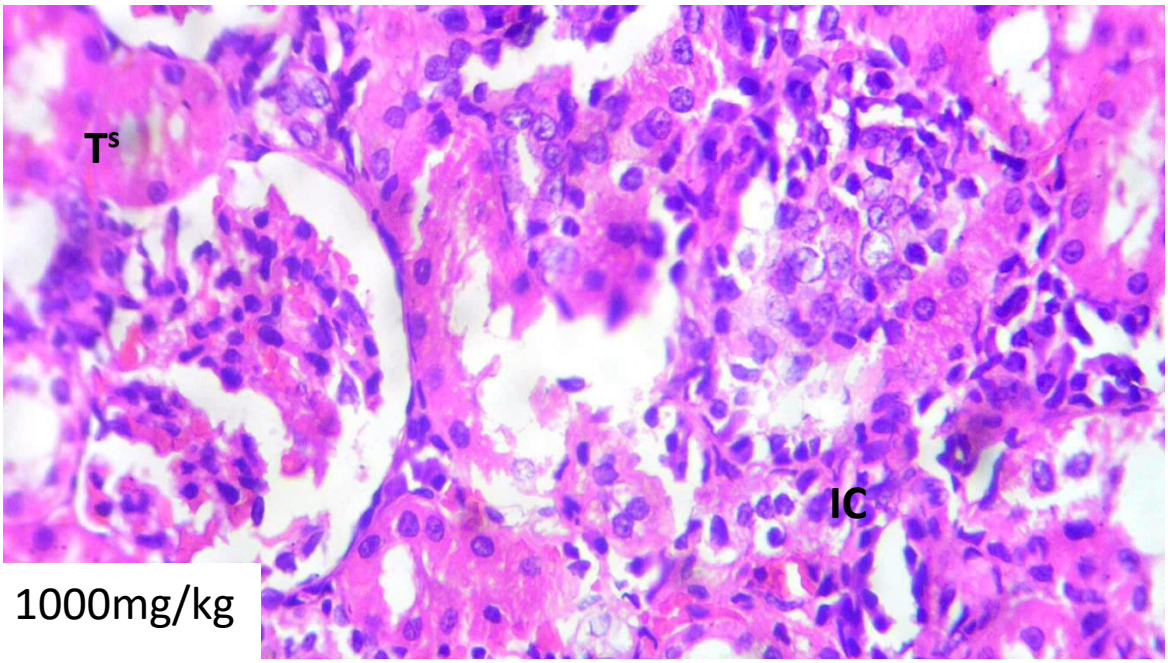

Plate 8: 1000 mg/kg group shows mild tubular swelling (T^s) and peritubular infiltrates of inflammatory cells (IC): H&E 400 X

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 DISCUSSION

The present study investigated the effect of aqueous *Myristica fragrans* (nutmeg) extract on renal function, oxidative stress parameters, and histological architecture in adult Wistar rats. The findings demonstrate that the extract exerts a dose-dependent influence on renal physiology and structure, with both biochemical and morphological evidence of oxidative stress–mediated nephrotoxicity. Variations in body weight, renal indices, biochemical markers, antioxidant enzymes, and histopathological features collectively indicate that while moderate exposure may induce adaptive changes, higher doses trigger oxidative damage and inflammatory responses in renal tissues.

Body weight changes are often used as indicators of general toxicity and metabolic adaptation. In this study, all groups showed progressive body-weight gain, but rats treated with 750 mg/kg exhibited a statistically significant increase ($p < 0.05$) compared with baseline, whereas the high dose (1000 mg/kg) produced a marginal but non-significant change. The moderate weight gain at 750 mg/kg suggests improved metabolic efficiency, possibly due to mild stimulation of digestive and hepatic functions by the phytochemical constituents of *Myristica fragrans*, which include myristicin, sabinene, eugenol, and elemicin as described by Al-Rawi *et al.* (2024) in *Frontiers in Pharmacology*. However, the lack of further increase at the highest dose implies metabolic disturbance caused by accumulating reactive intermediates or mild systemic toxicity, aligning with the findings of Anaduaka *et*

al. (2022), who reported reduced weight gain and altered metabolic activity following high-dose nutmeg exposure.

Assessment of kidney weight and the reno-somatic index (RSI) provides valuable insight into renal hypertrophy, atrophy, and organ adaptation to xenobiotic stress. In the present study, kidney weights increased slightly in all treatment groups without statistical significance, indicating the absence of overt hypertrophy. The RSI, however, showed a significant decrease at 750 mg/kg and a significant increase at 1000 mg/kg relative to the control. The initial reduction may correspond to the disproportionate body-weight gain observed at moderate doses, while the subsequent elevation at higher doses likely reflects renal edema, inflammatory infiltration, or hypertrophic adaptation secondary to tissue injury. Elfia and Susilo (2023) described similar biphasic RSI changes in nutmeg-treated animals, suggesting that the organ's initial adaptive response can transition to pathological enlargement at higher concentrations due to oxidative and inflammatory stress. Such variations have also been attributed to compensatory glomerular hypertrophy and vascular congestion as previously documented by Anaduaka *et al.* (2022).

Serum urea and creatinine are classical biochemical markers of renal function, and alterations in their levels often indicate impaired filtration or tubular reabsorption. In the current study, urea levels were significantly elevated at 750 mg/kg and 1000 mg/kg ($p < 0.05$), while creatinine showed a mild, non-significant increase across all treated groups. The urea elevation reflects early nephrotoxicity, suggesting compromised excretory function, while the marginal creatinine change implies partial preservation of glomerular filtration. Pashapoor *et al.* (2022) reported similar findings in nutmeg-induced nephrotoxicity, linking

urea elevation to tubular degeneration and oxidative disruption of nitrogen metabolism. Elfia and Susilo (2023) further associated such biochemical trends with endothelial and epithelial damage caused by reactive oxygen species generated from nutmeg's active constituents. Together, these results suggest that prolonged or high-dose nutmeg exposure leads to subclinical renal stress that can progress to overt nephrotoxicity if exposure continues.

The pattern of oxidative stress markers in this study provides mechanistic support for the observed biochemical alterations. Aqueous *Myristica fragrans* extract caused dose-dependent suppression of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), accompanied by significant increases in malondialdehyde (MDA) levels across all treated groups, even at the lowest dose (200 mg/kg). The significant rise in MDA, an index of lipid peroxidation, signifies enhanced free-radical generation and membrane lipid degradation. Poorbagher *et al.* (2022) and Pashapoor *et al.* (2022) similarly observed elevated MDA and reduced antioxidant activity following nutmeg exposure, indicating that oxidative stress represents the core mechanism underlying renal injury. The depletion of enzymatic antioxidants indicates exhaustion of the kidney's redox defense system, while MDA accumulation marks irreversible peroxidative damage to cellular membranes and organelles. This redox imbalance may impair mitochondrial respiration, promote inflammatory signaling, and exacerbate tissue degeneration, consistent with the oxidative model of nephrotoxicity proposed by Götz *et al.* (2021) in *Food and Chemical Toxicology*.

Histopathological examination provided direct morphological evidence supporting the biochemical findings. The kidneys of control rats exhibited normal glomeruli, open urinary

spaces, and intact renal tubules, indicating physiological architecture. In contrast, the 200 mg/kg group showed mild tubular epithelial swelling, interstitial congestion, and focal periglomular inflammatory cell infiltration, signifying the onset of degenerative changes and vascular stress. The 750 mg/kg group displayed more pronounced tubular swelling, perivascular fibrosis, and severe interstitial congestion, reflecting progressive inflammation and early fibrotic remodeling. At the highest dose (1000 mg/kg), the kidneys exhibited tubular swelling and peritubular inflammatory infiltrates, confirming sustained oxidative and inflammatory injury. These histological features correspond with those described by Anaduaka *et al.* (2022), who reported vacuolar degeneration and epithelial desquamation in nutmeg-treated rats, and by Elfia and Susilo (2023), who observed perivascular fibrosis and collagen deposition following chronic nutmeg administration. The presence of inflammatory cell infiltrates and fibrosis further suggests that lipid peroxidation products and reactive aldehydes contribute to secondary tissue remodeling and glomerulotubular damage.

Integrating the biochemical, oxidative, and histological data reveals that aqueous *M. fragrans* extract induces dose-dependent nephrotoxicity primarily via oxidative stress mechanisms. The early rise in MDA levels, accompanied by reduced SOD, CAT, and GPx activity, indicates an imbalance between free-radical production and antioxidant defense. This imbalance disrupts membrane integrity, leading to tubular swelling, vascular congestion, and inflammatory infiltration, as confirmed histologically. The progressive nature of these lesions, from mild vacuolar changes at 200 mg/kg to perivascular fibrosis at 750 mg/kg and persistent inflammation at 1000 mg/kg, indicates a continuum from reversible oxidative damage to chronic structural injury. These findings are in agreement with Götz *et al.* (2021)

and Anaduaka *et al.* (2022), who identified reactive oxygen species generation and mitochondrial dysfunction as key drivers of nutmeg-induced renal pathology. The results therefore suggest that while moderate exposure to *Myristica fragrans* may elicit adaptive responses, prolonged or high-dose use overwhelms endogenous antioxidant defenses, resulting in lipid peroxidation, cellular degeneration, and functional impairment. Consequently, the nephrotoxic potential of *Myristica fragrans* appears closely linked to its pro-oxidant constituents, and caution should be exercised in its medicinal or dietary application, especially under chronic or high-dose conditions.

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