EVALUATING THE EFFECTS OF THE SEED EXTRACT *MYRISTICA FRAGRANS* ON THE KIDNEYS OF THE ADULT WISTAR RATS

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CERTIFICATION

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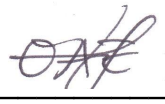

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DEDICATION

I dedicate this work to Jehovah for his guidance.

ACKNOWLEDGEMENT

I wish to express my heartfelt gratitude to all those who made these research work successful am grateful to my supervisor, Prof J.E Ataman whose invaluable advice, financial support saw to the successful completion of these work

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To all my wonderful friends who stood with me through my sojourn in the university of Benin, praise and Larry, it was a pleasure meeting u guys.

To my entire lecturer's, thanks for your support.

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ABSTRACT

The study was undertaken within a period of 4weeks to analyses the effects of *MYRISTICA FRAGRANS on* the kidneys of the adult Wister rats. A total of thirty rats weighing 180-190grams were used in the study. The rats were assigned into five groups of six rats per group. The animals allowed to acclimatize to the experimental procedure for 2 weeks and we're fed with grower mesh and water

Group A animals served as control and were fed with only water and fed with only water and feed. While group B, C, D, E animals were administered with nutmeg extract. Group B animals were fed with extract for 28 days, same routine were given to group, C, D and E.

Animals were sacrifices after the 28th days of administration , the kidneys were harvest weighed and further taken for histological results, also the blood of the animals was also been tested.

CHAPTER ONE

1.0 INTRODUCTION

Archaeological evidence indicates that the use of medicinal plants dates back to the Paleolithic age, approximately 60,000 years ago. Written evidence of herbal remedies dates back over 5,000 years to the Sumerians, who compiled lists of plants. Some ancient cultures wrote about plants and their medical uses in books called herbals. In ancient Egypt, herbs are mentioned in Egyptian medical papyri, depicted in tomb illustrations, or on rare occasions found in medical jars containing trace amounts of herbs. In ancient Egypt, the Ebers papyrus dates from about 1550 BC, and covers more than 700 compounds, mainly of plant origin. The earliest known Greek herbals came from Theophrastus of Eresos who, in the 4th century BC, wrote in Greek Historia Plantarum, from Diocles of Carystus who wrote during the 3rd century BC, and from Krateuas who wrote in the 1st century BC. Only a few fragments of these works have survived intact, but from what remains, scholars noted overlap with the Egyptian herbals. Seeds likely used for herbalism were found in archaeological sites of Bronze Age China dating from the Shang dynasty (1600–1046 BC). Over a hundred of the 224 compounds mentioned in the Huangdi Neijing, an early Chinese medical text, are herbs. Herbs were also commonly used in the traditional medicine of ancient India, where the principal treatment for diseases was diet.De Materia Medica, originally written in Greek by Pedanius Dioscorides (c. 40–90 AD) of Anazarbus, Cilicia, a physician and botanist, is one example of herbal writing used over centuries until the 1600s.

The World Health Organization (WHO) estimates that 80 percent of the population of some Asian and African countries presently uses herbal medicine for some aspect of primary health care. Some prescription drugs have a basis as herbal remedies, including artemisinin, digitalis, quinine and taxanes.

Regulatory review

In 2015, the Australian Government's Department of Health published the results of a review of alternative therapies that sought to determine if any were suitable for being covered by health insurance; herbalism was one of 17 topics evaluated for which no clear evidence of effectiveness was found. Establishing guidelines to assess safety and efficacy of herbal products, the European Medicines Agency

provided criteria in 2017 for evaluating and grading the quality of clinical research in preparing monographs about herbal products. In the United States, the National Center for Complementary and Integrative Health of the National Institutes of Health funds clinical trials on herbal compounds, provides fact sheets evaluating the safety, potential effectiveness and side effects of many plant sources and maintains a registry of clinical research conducted on herbal products.

According to Cancer Research UK as of 2015, "there is currently no strong evidence from studies in people that herbal remedies can treat, prevent or cure cancer".

Prevalence of use

The use of herbal remedies is more prevalent in people with chronic diseases, such as cancer, diabetes, asthma, and end-stage kidney disease. Multiple factors such as gender, age, ethnicity, education and social class are also shown to have association with prevalence of herbal remedies use. A good example of plant which that have such importance is nutmeg.

Nutmeg originated in the Banda Islands of Indonesia, and was discovered by the Portuguese in 1512. The importance of the nutmeg seed was propagated by the Dutch. The name nutmeg is derived from the Latin nux muscatus, meaning “musky nut.” In India, nutmeg is known as Jaiphal. According to the ethno-medical literature, nutmeg seed oil was used for intestinal disorders by Indians, in embalming by Egyptians, and to cure plague by Italians. In ancient times, nutmeg seeds were used in medicines as an aphrodisiac, abortifacient, and anti-flatulent, a narcotic, and as a means to induce menses. The effect of the nutmeg seeds on the central nervous system was first observed in the early 19th century. Traditional uses of nutmeg seeds include treatment of hemorrhoids, chronic vomiting, rheumatism, cholera, psychosis, stomach cramps, nausea, and anxiety. Nutmeg seed oil also has antiseptic, analgesic, and antirheumatic properties.

NUTMEG

Nutmeg is the seed, or the ground spice derived from that seed, of several tree species of the genus Myristica; fragrant nutmeg or true nutmeg (*myritica*. *fragrans*) is a dark-leaved evergreen tree cultivated for two spices derived from its fruit: nutmeg, from its seed, and mace, from the seed covering. It is also a commercial source of nutmeg essential oil and nutmeg butter. Indonesia is the main producer of nutmeg and mace, and the true nutmeg tree is native to its islands.

KIDNEY

In humans, the kidneys are two reddish-brown bean-shaped blood-filtering organs that are a multilobar multipapillary form of mammalian kidney, usually without signs of external lobulation. They are located on the left and right in the retroperitoneal space, and in adult humans are about 12 centimetres in length. They receive blood from the paired renal arteries; blood exits into the paired renal veins. Each kidney is attached to a ureter, a tube that carries excreted urine to the bladder.

WISTAR RATS

The Wistar rat is an outbred albino rat. This breed was developed at the Wistar Institute in 1906 for use in biological and medical research, and is notably the first rat developed to serve as a model organism at a time when laboratories primarily used the house mouse (Mus musculus).

CHAPTER 2

2.0 Literature review

Nutmeg is native to a small cluster of islands in Indonesia, the Banda Islands. It is the seed of a peach like fruit that grows from the tree *Myristica fragrans.* Europeans discovered nutmeg in the middle ages and it became quite valuable for its culinary and folk remedy uses. This led to conflict for conquest of the Banda Islands. By the late nineteenth century, nutmeg was becoming widespread and its purported uses continued to evolve. Reports in the medical literature began to appear of women ingesting large amounts of nutmeg to induce abortion. These ingestions are some of the first medical descriptions of the nutmeg toxicity. Ultimately, nutmeg was not effective as an abortifacient and the practice declined. After a lull in the medical literature, nutmeg reemerged as a drug of abuse in the 1960s. It continues to be rediscovered by individuals seeking a cheap and accessible high.

Quality issues and toxicity

Nutmeg and mace are classified by origin (East Indian nutmeg and West Indian nutmeg) and grade. Good quality has to be maintained for trade of nutmeg and mace. Whole nutmegs are grouped under three broad quality classifications:

- **Sound:** Nutmegs which are used mainly for grinding and, to a lesser extent, for oleoresin extraction.
- **Substandard:** Nutmegs which are used for grinding, oleoresin extraction and essential oil distillation.
- **Distilling:** Poor-quality nutmegs used for essential oil distillation.

In Indonesia, high-quality, sound, whole nutmegs are traded in grades which refer to their size in numbers of nutmeg per pound: 80s, 110s and 130s or ‘ABCD’ which is an assortment of various sizes. Substandard nutmegs are traded as ‘sound, shriveled’, which in general have a higher volatile content than mature sound nutmegs and are used for grinding, oleoresin extraction and oil distillation, and ‘BWP’ (broken, wormy and punky) which are used mainly for grinding as volatile oil generally does not exceed 8 %. Distilling grades of nutmeg are of poor quality: ‘BIA’ or ‘ETEZ’ with a volatile oil content of 8–10 % and BSL or ‘AZWI’ which has less shell material and a volatile oil content of 12–13 %.

In Grenada, sound nutmegs are sold as sound unassorted which corresponds to the Indonesian grade 'ABCD'. Substandard nutmegs are classified as floats and as defective, the latter being similar to the Indonesian BWP grade but considered of high quality.

Distilling grades of nutmegs are primarily exported to the USA and consist of floats.

Mace is classified as whole pale mace, the main broken mace, selected, unassorted or siftings (Indonesia) and as whole, broken blades or siftings (Grenada).

The international standard applicable for trade in spices of nutmeg and mace is ISO 6577:2002. Although national standards are available for maintaining quality, European traders prefer the American Society of Travel Advisors (ASTA) cleanliness specifications as they are more rigid than the national standards. The Quarantine System and Plant Protection Law and the Food Sanitation Act set the quality standard in Japan. Aflatoxin (the Netherlands, Japan) and salmonella (UK) are the common complaints regarding imported nutmeg. The presence of insects is a major complaint for US importers.

The essential oil has often been extracted before marketing; such nuts can be detected by their lightweight and are more subject to insect attacks. *Myristica fragrans(nutmeg)* is adulterated with *Myristica argentea*, *Myristica malabarica* and *Myristica otaba* which can be identified by their poor quality.

Mace from *Myristica argentea* is imported as Papuan nutmeg from Papua New Guinea, *Myristica malabarica* is traded as Bombay nutmeg from India and *Myristica otaba* as Otaba nutmeg. Trade in wild nutmegs exists, and these are marketed as long, female, Macassar, Papua, Guinea or Norse nutmeg. All these have been traced to *Myricatica argentea* of New Guinea from where they enter into the market as Macassar nutmegs. *Myristica malabarica*, *Myristica otaba* and *Myristica argentea* are devoid of any aroma of *Myristica fragrans*.

Toxicity

Both nutmeg and mace contain the active ingredient myristicin which possesses narcotic properties. Nutmeg butter contains elemicin and myristicin which are also

narcotic and cause psychotropic effects. Ingestion in large quantities produces narcosis, delirium, drowsiness, epileptic convulsions and even death. It also causes

Temporary constipation and difficulty in urination and increased fat deposition in liver. Powdered nutmeg is used occasionally as a hallucinogenic drug, but such use is dangerous as excessive dose of mace has a narcotic effect and symptoms of delirium and epileptic convulsions 1–6 hours after consumption.

Nutmeg includes two distinct spices: nutmeg (seed) and mace (aril). Native of Indonesia (Moluccas Islands), the nutmeg tree grows there abundantly and is now naturalized in West Indies, Sri Lanka, India, Philippines, Tropical America, and Pacific Islands. It is also grown in a small scale in Sri Lanka, Trinidad, China, India, Tobago, Zanzibar, and Mauritius.

Nutmeg is an evergreen aromatic tree usually 10–20m tall with spreading branches. The fruit is pyriform and yellow in color. The pericarp is fleshy when the fruit matures; it splits into two, exposing the scarlet-colored net like aril covering the dark brown seed (Purse glove et al., 1981; Verghese, 2000; Krishnamoorthy, 2000). The principal constituents of nutmeg are fixed oil (fat), volatile oil, and starch. The flavor and therapeutic action are due to the volatile oil whose content varies from 6% to 16% based on the origin and quality of nutmeg (Lewis et al., 1966). Nutmeg fat contains eight fatty acids, the most important of them being myristic acid. Nutmeg and mace are used as a stimulant, carminative, astringent, aphrodisiac, and hallucinogen. Oil of nutmeg or mace is employed for flavoring food products and liquors, soaps, tobacco, dental creams and perfumery products. The fleshy pericarp of the fruit is used for making pickles and jelly.

The genus *Myristica* consists of approximately 120 species. A high amount of variability has been reported in growth rate, productivity, size and shape of the leaf, flower size and shape and size of the fruit and nut, and the amount of mace in nutmeg (Krishnamoorthy and Rema, 1991; Haldankar et al., 1999). Crop improvement in nutmeg is confined to selection and multiplication of elite lines. So far, three improved varieties were released in nutmeg.

The general method of propagation of nutmeg is through seeds collected from regular-bearing and high-yielding trees. The seeds have low viability and hence are to be sown immediately after collection. Germination commences from about the 40th day and lasts for up to 90 days after sowing. The germinated sprouts must be transferred to polythene bags (30 cm×15 cm) containing a potting mixture. After 1

year in a nursery the seedlings can be field planted. Nutmeg, being a dioeciously crop, requires that the proper ratio of female and male plants be maintained in the

plantation. Vegetative propagation techniques, such as approach grafting, softwood grafting, epicotyl grafting, budding, and top was found working in nutmeg are commonly used, with epicotyls grafting being the best (Mathew, 1985; Haldankar et al., 1999; Rema et al., 2000).

Nutmeg requires a hot humid climate with no pronounced dry season and is a shade-loving plant. It can be grown up to approximately 900 m above sea level. In India, nutmeg is planted as an intercrop in coconut plantations. Fruiting commences from 6 to 9 years depending on the climate and planting materials used. Optimum productivity is attained in approximately 15 years. Fruits are collected periodically from the tree by hand or with hooked sticks or allowed to fall naturally on the ground. All three parts of the fruit, viz. the pericarp, aril (mace), and seed (nutmeg) are separated carefully and sun dried or are dried by mechanical means. Aflatoxin is a serious post-harvest problem in the mace and nuts.

Nutmeg is abused for its narcotic and hallucinogenic properties. One to three seeds or 5–30g of the ground nut are used to attain psychogenic effects. One tablespoon of ground nutmeg or one grated nutmeg yields ~7g. A fatality was reported in an 8year old who ate two nutmegs. A 55 years old was also suspected to have died from acute nutmeg poisoning an to have a blood level of 4.0 μg ml−1. Nutmeg may produce symptoms similar to those of an anticholinergic poisoning. The reported initial neurological effects include giddiness, tingling, euphoria, and hallucinations that may include distortion of time and space, detachment from reality, sensation of separation from one's limbs, and fear of impending death. This is followed by alternating delirium and extreme drowsiness or stupor. However, common unpleasant side effects occur and include headache, nausea, vomiting, abdominal pain, dizziness, chest pain, flushing, tremor, and tachycardia. The blood pressure may slightly increase, but a marked decrease with cyanosis and shock has been reported. Palpitations, agitation, anxiety, dry mouth, chest tightening, and blurred vision were reported in a pregnant woman in her third trimester who ingested one tablespoon of nutmeg. The fetal heartbeat was increased for 12h. Levels for myristicin and elemicin are not generally available. Myristicin has been isolated from nutmeg using high-performance liquid chromatography. Other laboratory values have been reported to be normal.

THE kIDNEYS

The kidneys are two reddish-brown bean-shaped organs found in vertebrates. They are located on the left and right in the retroperitoneal space, and in adult humans are about 12 centimeters in length. They receive blood from the paired renal arteries; blood exits into the paired renal veins. Each kidney is attached to a ureter, a tube that carries excreted urine to the bladder. The kidney participates in the control of the volume of various body fluids, fluid osmolality, acid–base balance, various electrolyte concentrations, and removal of toxins. Filtration occurs in the glomerulus: one-fifth of the blood volume that enters the kidneys is filtered. Examples of substances reabsorbed are solute-free water, sodium, bicarbonate, glucose, and amino acids. Examples of substances secreted are hydrogen, ammonium, potassium and uric acid .the left, and being placed slightly more to the middle than the left kidney. The left kidney is approximately at the vertebral level and the right is slightly lower. The right kidney sits just below the diaphragm and posterior to the liver. The left kidney sits below the diaphragm and posterior to the spleen. On top of each kidney is an adrenal gland. The upper parts of the kidneys are partially protected by the 11th and 12th ribs. Each kidney, with its adrenal gland is surrounded by two layers of fat: the perirenal fat present between renal fascia and renal capsule and pararenal fat superior to the renal fascia.

The human kidney is a bean-shaped structure with a convex and a concave border. A recessed area on the concave border is the renal hilum, where the renal artery enters the kidney and the renal vein and ureter leave. The kidney is surrounded by tough fibrous tissue, the renal capsule, which is itself surrounded by perirenal fat, renal fascia, and pararenal fat. The anterior (front) surface of these tissues is the peritoneum, while the posterior (rear) surface is the transversalis fascia. The superior pole of the right kidney is adjacent to the liver. For the left kidney, it is next to the spleen. Both, therefore, move down upon inhalation.

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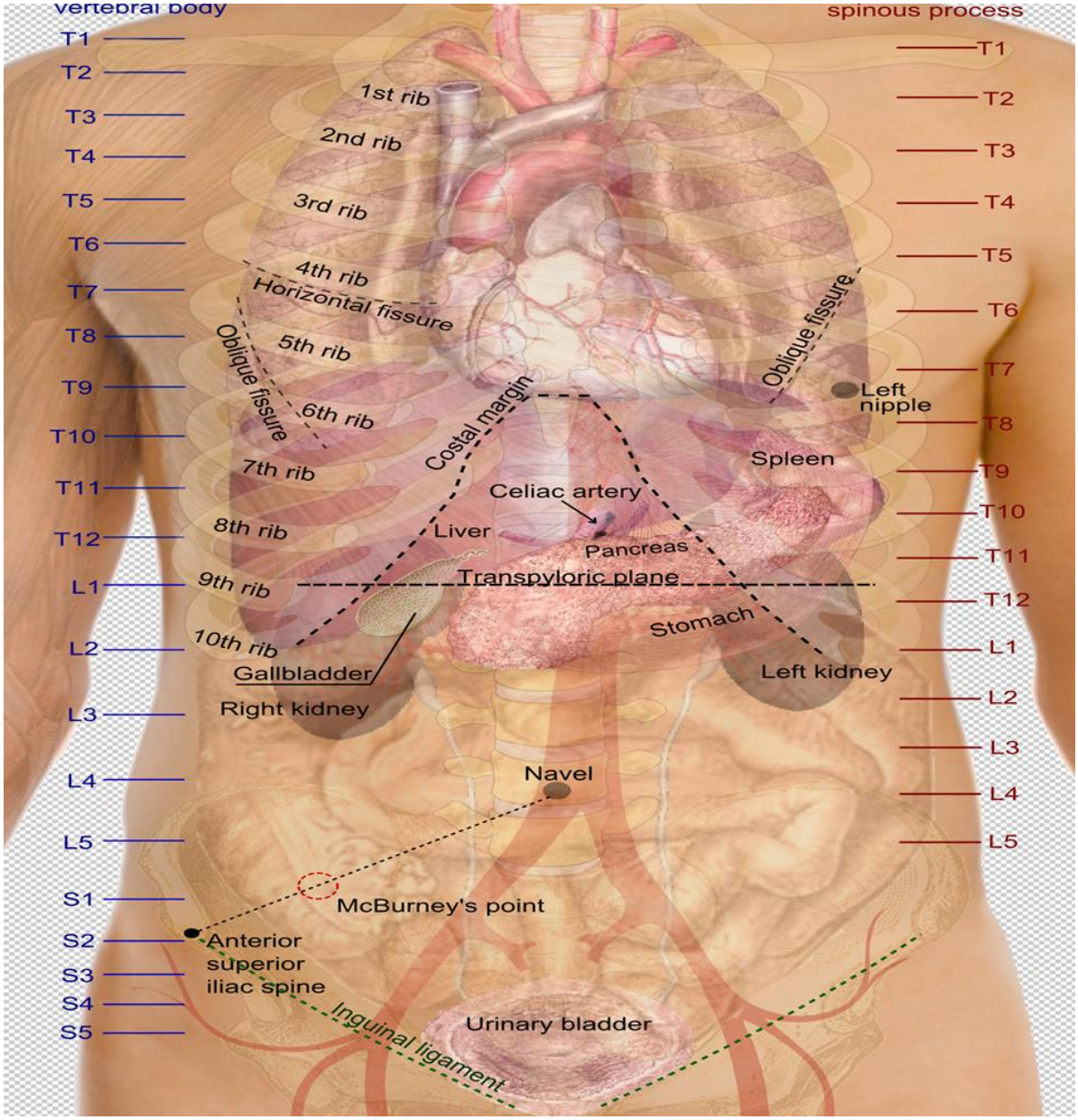

DIAGRAM SHOWING THE LOCATION OF THE NUTMEG

Nerve supply

The kidney and nervous system communicate via the renal plexus, whose fibers course along the renal arteries to reach each kidney. Input from the sympathetic nervous system triggers vasoconstriction in the kidney, thereby reducing renal blood flow. The kidney also receives input from the parasympathetic nervous system, by way of the renal branches of the vagus nerve; the function of this is yet unclear. Sensory input from the kidney travels to the T10–11 levels of the spinal cord and is sensed in the corresponding dermatome. Thus, pain in the flank region may be referred from corresponding kidney.

EMBRYLOGY

The development of the urinary tract begins with the formation of the nephrogenic cord in week four, along which the pronephros, mesonephros and metanephros form. Although the metanephric kidneys act as functional excretory units as early as week eleven, nephrogenesis is not complete until week thirty-two when multiple branching events have formed one to three million collecting tubules. Complex orchestrated interactions between various embryonic tissues, the mesonephric duct, ureteric bud, and metanephric blastema ensure the correct development of the urinary tract. Disruptions to these intricate signaling pathways, either genetic or environmental, result in congenital abnormalities of the kidney and urinary tract (CAKUT) including renal agenesis and dysplasia, multicystic dysplastic kidney disease and polycystic kidney disease.

Embryonic folding during the fourth week of development marks the beginning of the urinary tract with the formation of a longitudinal mass known as the urogenital ridge. The ridge can divide into parts depending on the system it forms; the nephrogenic cord will form the urinary tract while the gonadal ridge will develop into the reproductive system. Beginning rostrally and progressing caudally, three kidneys will form over a few weeks within the nephrogenic cord: pronephros, mesonephros, and metanephros. Pronephros development begins in the fourth week; however, they will not form functioning kidneys in humans. Pronephric ducts develop in the cervical region of the nephrogenic cord before extending and fusing with the cloaca. Adjacent to the pronephric ducts, the intermediate mesoderm will condense to form non-functional nephron units, known as pronephroi, which will regress by day 25.

The mesonephric duct, also known as the Wolffian duct, now begins development in the next most caudal region of the nephrogenic cord. Similarly, the adjacent intermediate mesoderm condenses to form mesonephro. Although approximately 40 pairs of mesonephro form, only those located between L1-L3 continue to differentiate to form functional excretory units. Thus, approximately twenty nephrons form capable of excreting small amounts of fluid into the amnion between the sixth and tenth week of development. Similar to the pronephric duct, the mesonephros and mesonephric duct will later degenerate in females; however, in males, these embryonic structures persist and develop into the epididymis, vas deferens, seminal vesicles, and the ejaculatory duct.

The third and final kidney, the metanephric kidney, begins development during the fifth week and will continue to differentiate to form the permanent kidneys. The mesonephric duct extends to fuse with the cloaca, thus inducing the sacral intermediate mesoderm to form an aggregate known as the metanephric blastema. At the beginning of week five, the metanephric blastema secretes a protein known as glial-cell derived neurotropic Factor (Gdnf), thus inducing an outgrowth in the mesonephric duct known as the ureteric bud; Gdnf acts as a ligand for cell surface receptor RET and on its co-receptor, Gdnf Family Receptor alpha 1 (Gfr-alpha1) which are both strongly expressed in the mesonephric duct.

During the sixth week of development, the ureteric bud begins a branching cascade which will subsequently create collecting tubules and the basic renal architecture. The first bifurcation occurs during the sixth week and forms the renal pelvis as well as the cranial and caudal lobes of the kidney. The next four bifurcations coalesce to form the major calyces, while the following four bifurcations coalesce in the seventh-week form the minor calyces. Branching gets induced by Gdnf acting on the RET expressing cells in the tips of the ureteric bud; each individual branch acquires a blastemal cap from which Gdnf gets secreted. This cascade continues until week 32, thus producing approximately 1 million to 3 million collecting tubules.

Functional nephrons begin to develop when the tip of each collecting tubule induces the blastemal caps to form nephric vesicles. These will then develop into nephric tubules consisting of an S-shaped Bowman’s capsule, proximal and distal tubules, and the loop of Henle. Development of glomerulus begins when podocyte

precursors lining the S-shaped body secrete VEGF2, thus attracting endothelial cells and generating a primitive vascular tuft. This activity will form the afferent

and efferent arterioles of the glomerulus. Contact between the podocyte precursors and the endothelial cells stimulate differentiation of podocytes, with the glomerular basement membrane forming at the boundary between the two. The distal end of the nephric tubule, the distal convoluted tubule, fuses with the collecting duct to form a uriniferous tubule. During early development, the kidneys lie close together in the sacral region of the embryo. However, as the abdomen enlarges, the kidneys are drawn apart and ascend to their final position in the lumbar region between weeks six to nine. The kidneys receive vascular supply from branches of the dorsal aorta called renal arteries; during their ascent, the caudal branches degenerate, and the kidneys receive their blood from successively higher branches.

Bladder and Ureter Development

The development of the bladder begins during week four when the urogenital septum divides the cloaca into two parts, the rectum posteriorly and the urogenital sinus anteriorly. The urogenital sinus will continue to grow to form the bladder, with the inferior end forming the urethra. As the mesonephric duct fuses with the cloaca, part of the duct gets incorporated into the posterior wall of the bladder. Although the ureteric bud is an outgrowth from the mesonephric duct, it has a separate opening into the urinary bladder. As the kidneys ascend, the ureters elongate and open into the bladder superiorly, while the roots of the mesonephric ducts are carried inferiorly, before fusing to form the trigone region. Endodermal cells from the urogenital sinus soon replace the mesodermal cells epithelium of the trigone region, thus completing development.

HISTOLOGY OF KIDNEY

The kidney parenchyma consists of two layers; an outer and inner medulla. They comprise around one million urine-producing nephrons. Urine is collected into a system of distinctive chambers within a kidney. Calyces gradually increase in size, starting with the minor calyces, which open into larger major calyces, which empty into the renal pelvis from the renal pelvis, the urine passes into the ureter. The portion of kidney which contains the calyces, renal pelvis, ureter and renal vessels is called the renal sinus.

CORTEX AND MEDULLA

The renal cortex is the outer layer of the kidney tissue. It is darker than its underlying renal medulla because it receives over 90% of the kidney blood supply. The cortex has a grainy appearance, as it mostly contains ovoid and coiled parts of the nephrons (renal corpuscles and convoluted tubules).

The renal medulla appears striped, as it contains vertical nephron structures (tubules, collecting ducts). It consists of renal (medullary) pyramids separated by projections of the renal cortex (renal columns). The apices of the pyramids project towards the renal pelvis and open into the minor calyces via perforated plates on their surfaces (area cribrosa). Each renal pyramid, with its surrounding cortical tissue, forms a renal lobe. Renal lobes are further divided into renal lobules. Each lobule consists of a group of nephrons emptying into one collecting duct. These structures can be observed in a coronal section of the kidney.

The nephron is the functional unit of the kidney. It produces concentrated urine by creating an ultrafiltrate from blood. A nephron consists of two main parts: a renal corpuscle and its associated renal tubule system.

Renal corpuscles are located in the renal cortex, while their tubular systems extend into the medulla. Depending on their distribution and morphology, there are two main types of nephrons in the kidney; cortical and juxtamedullary. Cortical nephrons have their corpuscles close to the kidney capsule. Their tubules are very short, extending only into the upper medulla. The corpuscles of the juxtamedullary nephrons are located close to the corticomedullary border. Their tubular systems are much longer, extending deep into the medulla.

Each nephron is surrounded by a network of capillaries. Branches from the renal interlobular arteries enter a nephron as the afferent arteriole, form a capillary tuft (glomerulus) then exit the nephron as the efferent arteriole. The capillary network then continues to surround the nephrons renal tubule system as peritubular capillaries, forming the vasa recta around the nephron loop.

Renal corpuscle

The renal corpuscle is the filtration apparatus of the nephron. Each corpuscle consists of two main elements; the glomerulus and glomerular (Bowman's) capsule. The glomerulus is a network of capillaries formed by branches of the renal artery (afferent and efferent arterioles).

The glomerular capsule surrounds the glomerulus. It consists of two layers (parietal and visceral), which bound a cavity called the glomerular capsular space (Bowman's / urinary space). The inner visceral layer is made of special cells called podocytes. Podocytes cover the walls of glomerular capillaries, interdigitating with each other and forming narrow slits between their projections. The outer parietal layer is made of simple squamous epithelium and is continuous with the nephron tubules. The afferent and efferent arterioles enter the renal corpuscle at the vascular pole, while the site where the glomerular capsule narrows and continues as the proximal thick segment of the nephron is called the urinary pole.

The renal corpuscle is the starting point of urine formation. Systemic blood passes through the glomerular capillary system, and is filtered to form primary urine (ultrafiltrate). It does this via a special filtration barrier which selectively filters water and solutes from the blood passing through the glomerular capillaries. The glomerular ultrafiltrate is collected by the glomerular space, and passes into the kidney tubules.

The kidney filtration apparatus is formed by three layers of tissue; endothelium of the glomerular capillaries, glomerular basement membrane (GBM) and podocytes (visceral layer of renal capsule). Glomerular capillaries are composed of fenestrated endothelium. Fenestrations function as pores. The GBM is more complex than other epithelial basal membranes. It consists of three layers; a thick central lamina densa and two thinner layers (lamina rara interna and lamina rara externa).

Podocytes cover the walls of the glomerular capillaries. Their finger like projections (pedicles) interdigitate, with narrow filtration slits (filtration slit diaphragm) forming between the projections. Together, these three layers function as a selective filter, allowing only molecules below a certain size, and of a certain charge, to pass from the blood and enter the renal tubular system. For example, blood cells, platelets, some proteins and some anions are prevented from leaving the glomerular capillaries, while water and solutes pass through. The remaining

unfiltered blood is carried out of the glomerulus by the efferent arteriole, and passes back into the venous system.

Renal tubule system

The tubule system is the part of the nephron which processes glomerular ultrafiltrate into urine by reabsorbing necessary molecules and secreting the unnecessary and waste substances. It consists of three parts;

Proximal tubule; convoluted proximal tubules and straight proximal tubule

Nephron loop; descending and ascending limbs

Distal tubule; straight distal tubule and convoluted distal tubule PROXIMAL TUBULE

The proximal tubule is the first part of the tubular system. It consists of convoluted and straight segments. The proximal convoluted tubule is located within the renal cortex and is continuous with the capsular space.

The straight proximal tubule (or thick descending limb) extends down into the medulla. Both parts are composed of simple cuboidal epithelium, rich in mitochondria and microvilli (brush border). This morphology is adapted to the proximal tubule function of absorption and secretion. More than half of the previously filtered water and molecules are returned to the blood (reabsorption) by the proximal tubules.

NEPHRON LOOP

The nephron loop is the U-shaped bend of a nephron which extends through the medulla of the kidney. Histologically, it consists of two parts; thin descending and thin ascending limbs.

Both limbs are composed of simple squamous epithelium. The cells have few organelles, little to no microvilli and low secretion abilities. The two limbs work in parallel, with the surrounding vasa recta capillaries, to adjust the filtrate's salt (example; sodium, chloride, potassium) and water levels. More specifically, the descending limb is highly permeable to water, less permeable to solutes, while the ascending limb is the opposite. Some authors consider the nephron loop to be synonymous with the loop of Henle, while other authors include the proximal straight tubule, nephron loop and distal straight tubule in this term.

DISTAL TUBULE

The distal tubule also consists of straight and convoluted segments. The straight distal tubule (thick ascending limb) continues on from the thin ascending limb of the nephron loop at the level between the inner and outer medulla. The convoluted distal tubule projects into the cortex. Both parts of the distal tubule are composed of simple cuboidal epithelium, similar in morphology to the proximal tubule.

COLLECTING SYSTEM

The collecting system of the kidney is a series of tubes that moves urine from the nephrons into the minor calyces. Several distal convoluted tubules from neighboring nephrons drain into a collecting duct via connecting/collecting tubules. Collecting ducts then travel through the kidney medulla, converging at the apex of each renal pyramid. Here, several ducts merge to form a single large papillary duct (of Bellini), which opens into the minor calyx through the area cribrosa.

Collecting ducts are termed cortical or medullary, depending on which part of the kidney parenchyma that part of the duct is located. They are made of epithelial cells, which get progressively taller as the ducts get larger.

Cortical collecting ducts - simple cuboidal epithelium

Medullary collecting ducts - simple columnar epithelium

Papillary ducts - simple columnar epithelium

Two additional types of cells are distinguishable in these ducts. The principal cells, which are pale staining and play a role in ion transport. Darker staining intercalated cells are scattered amongst the principal cells and are responsible for acid-base balance. Collecting ducts are the last chance site for water and electrolyte reabsorption from the filtrate further concentrating the urine, particularly under the influence of antidiuretic hormone (ADH). No more reabsorption takes place past the medullary collecting ducts.

Juxtaglomerular apparatus

Nestled into the vascular pole of the nephron is a collection of cells called the juxtaglomerular apparatus (JGA). It is formed by 3 types of cells; macula densa, juxtaglomerular granular (JG) cells and extraglomerular mesangial (Lacis) cells.

The macula densa are located in the wall of the distal tubule, at the point where the tubule comes in contact with the glomerulus. Here the regular cuboidal epithelium of the distal tubule crowd together and become columnar in shape. The juxtaglomerular granular (JG) cells are modified smooth muscle cells found surrounding the afferent, and sometimes efferent, arteriole. The third cell type of the JGA is the extraglomerular mesangial (Lacis) cells. These are located in the triangular space between the afferent and efferent arterioles.

The juxtaglomerular apparatus has two key functions;

Regulates glomerular blood flow and filtration rate

Regulates systemic blood pressure

Glomerular blood flow is regulated by a feedback mechanism, whereby the macula desnsa responds to high sodium chloride levels in the filtrate by releasing vasoconstrictor chemicals. These chemicals cause the afferent arteriole to vasoconstrict, thus lowering glomerular pressure and, in turn, filtration rate. This system maintains a mostly constant pressure within the nephrons. Systemic blood pressure is regulated through the renin-angiotensin-aldosterone system. Low systemic blood pressure, recognized by baroreceptors, triggers the juxtaglomerular granular cells to secrete an enzyme called renin. Renin, in turn, activates the renin-angiotensin-aldosterone system, raising systemic blood pressure through the actions of angiotensin and aldosterone.

Secretion and reabsorption

The nephron function is to maintain homeostasis of the body fluids, by excreting unwanted products in urine. Nephron anatomy is specialized to create urine from the blood through four key activities; filtration, reabsorption, secretion and excretion.

Filtration occurs in the renal corpuscle of the nephron, and is described above. Reabsorption and secretion are activities that occur in the nephrons renal tubular system. These processes fine tune what substances are excreted and what are kept, by the body. Reabsorption is the process by which water and molecules, lost from the blood during filtration, are reabsorbed back into the capillaries surrounding the nephron. Secretion is where water and molecules leave the peritubular capillaries

and enter (or re-enter) the urine filtrate. The remaining product, urine, is then excreted from the kidney via the ureters.

Reabsorption and secretion are finely controlled processes, whereby the epithelial cells of each segment of the tubular system reabsorbs and secretes different substances in order to achieve maximum control over the urine concentration. Regulation of these processes includes; passive (countercurrent exchange system), nervous (sympathetic nervous system) and hormonal (angiotensin, aldosterone and antidiuretic hormone) mechanisms. The result of this process is urine, a fluid highly concentrated with body metabolic waste and excess substances. In healthy individuals, urine normally contains ions, urea, creatinine and variable amounts of water. Healthy urine is free of microorganisms, glucose, blood cells and blood proteins.

General Objective

The main objective of this study is to investigate the effects of nutmeg on adult Wistar rats.

Aim and Objectives

- To evaluate the effects of nutmeg on the body and organ wieght of Wistar rats.
- To assess the histological effect of nutmeg on the kidneys of the wistar rats
- To assess the serum and creatinine level

CHAPTER THREE

3.0 MATERIALS AND METHOD

Harvesting and preparation of extract:

The nutmeg seed (*Myristica fragransHoutt*) we're gotten from the market in July 2023 (uselu market Benin city, Edo state), 500g of of nutmeg were bought from the market ,these seed were allow to dry properly and then grinded. These 500g of nutmeg is disolve in 1000ml of water which form the nutmeg extract. This extract is further kept in the fridge for preservation.

Experimental animals:

Thirty Wister rats were used for the study .The rats were obtained from the animal house of Anatomy department, college of medical sciences University of Benin. They we're kept in cages and allowed to acclimatize for weeks, during which they were fed with livestock growers mash and water before the commencement of experiment. After acclimatisation, the rats were categorized into four treatment groups and one control group. The control groups was fed on fed mash only and water throughout the period .The treatment group receive mash and nutmeg extract according to their body weight. Illustration is shown in the table below

TABLE 1: showing treatment regimen

GROUPS

TREATMENT REGIMEN

Groups	**DESIGN**
A	Control group (water and feed only)
B	1.5mg of nutmeg administered for 28 days
C	2.5mg of nutmeg administered for 28 days
D	3.5mg of nutmeg administered for 28 days
E	4.5mg of nutmeg administered for. 28 days

Histopathology

After the required day of administration, the animals were sacrificed by anesthetizing and with the aid of surgical blade and forceps. The kidneys were harvested .The harvested organs were fixed in 70% buffered formal saline for 24 hours of fixation, the fixed tissues were passed through ascending grade of alcohol starting from 50%, 70%, 70%, 90%, and 100% (absolute).The tissues were cooled and allowed to solidify. The embedded tissue block were trimmed and mounted and a wooden chack in preparation for sectioning. The tissue blocks were sectioned using a rotary microtome. The tissues were sectioned at 5 microns.

Hematoxylin and Eosin were used in staining the tissues following the following steps. Clearing with xylene (3-5mins) hydration by passing tissues through descending grades of alcohol starting from 100%, 90%,and 50%, for 3-5mins respectively Tissues were rinsed in distilled water. Tissues were stained with heamoxylin (5-15mins) after which they were differentiated in 1% acid alci for 30secs and rinsed in running water (30secs). The tissues were rinsed in bluing reagent for 1min and rinsed in running tap water for 1min before counter staining with eosin (3minutes).The stained microscope a slide after which they were cleared by xylene The stained were mounted with Canada balsam and cover slipped, ready for microscopy.

CHAPTER FOUR

4.0 RESULTS

Physical findings

The initial and final weights of animals in group A were as follows respectively; 197g and 198g for Am1, 198g and 199g for Am2,199g and 200g for Am3 ,196g and 200g for Am4 ,198g and 200g for Am5.For the group the initial and final mean weight values are 199g and 200g respectively. In group B, Bm1 was 201g and 203g, Bm2 was 199g and 203g, Bm3 was 199g and 203g, BM4 was198g and 203g,Bm5 was 198g and 204g, the initial and final weight values are 199g and 203g respectively. In group C,Cm1 was 201g and 204g ,CM2 was 202g and 203g ,Cm3 was 202g and 203g,Cm3 was 203g and 206g,Cm4 was 203g and 206g,Cm5 was 204 and 206g .The inertial and final weight are 203g and 206 respectively. In group D, Dm1 was 203g and 207g ,Dm2 was 204g and 206g,Dm3 was 204g and 206g,Dm4 was 205g and 206g,Dm5 was 204g and 207g .In this group the initial and final weight values are are 204g and 206g respectively .In group of E,Em1 was 205g and 207g,Em2 was 206 and 208,Em3 was 205g and 207g ,Em4 was 204g and 208g,Em5 was 205g and 207g.The initial and final weight are 205g and 207g.

EXPERIMENTAL DESIGN

Groups/Tests	Group A	Group B	Group C	Group D	Group E	P-value
Initial Weight	139.0±6.285	155.0±6.595	141.8±5.452	154.8±10.38	173.5±11.51	0.0741
Final Weight	185.8±5.721*	179.5±10.04	179.0±5.583*	170.0±13.63	197.0±2.677	0.2840
Weight Change	46.75±8.390	24.50±5.723	37.25±8.645	15.25±9.877	23.50±9.314	0.1253
Renal Weight	1.100±0.1291	1.175±0.1702	1.050±0.1848	1.075±0.1250	1.375±0.1315	0.5625
Reno-Somatic Index	0.5930±0.07237	0.6616±0.09851	0.5850±0.09824	0.6364±0.07123	0.7008±0.07474	0.8535
Sodium Ion	141.0±0.9129	139.5±0.6455	139.5±0.5000	144.0±0.4082*	139.0±0.4082*	0.0002
Potassium Ion	6.375±0.4661	5.500±0.2483*	5.325±0.2056*	5.875±0.2097	5.200±0.1780*	0.0607
Bicarbonate Ion	21.00±1.354	19.50±0.6455	20.75±0.7500	20.00±1.080	20.50±0.6455	0.7971
Chloride	103.5±1.041	103.0±0.4082	100.3±1.109*	104.3±0.2500	100.8±0.8539*	0.0116
Urea	37.25±0.8539	40.25±1.702	39.25±2.462	47.00±1.871*	39.25±0.6292	0.0082
Creatinine	0.6750±0.02500	0.7250±0.02500	0.7500±0.05000	0.8000±0.05774*	0.6500±0.02887	0.1123

Table 4.1 Showing Results of Biochemical analysis

*Represents Statistically Significant Difference ($p<0.05$)

There was a statistically significant increase ($p<0.05$) in the final weights of rats in groups A and C when compared with the initial weight.

There was a statistically significant increase ($p<0.05$) in the Sodium Ion level of rats in groups D and a statistically significant decrease ($p<0.05$) in the Sodium Ion level of rats in groups C when compared with the Group A (Control).

There was a statistically significant decrease ($p<0.05$) in the Potassium Ion level of rats in groups B, C and E when compared with the Group A (Control).

There was a statistically significant decrease ($p<0.05$) in the Chloride level of rats in groups C and E when compared with the Group A (Control).

There was a statistically significant increase ($p<0.05$) in the Urea level of rats in groups D when compared with the Group A (Control).

There was a statistically significant increase ($p<0.05$) in the Creatinine level of rats in groups D when compared with the Group A (Control).

*Represents Statistically Significant Difference ($p<0.05$)

There was a statistically significant increase ($p<0.05$) in the final weights of rats in groups A and C when compared with the initial weight.

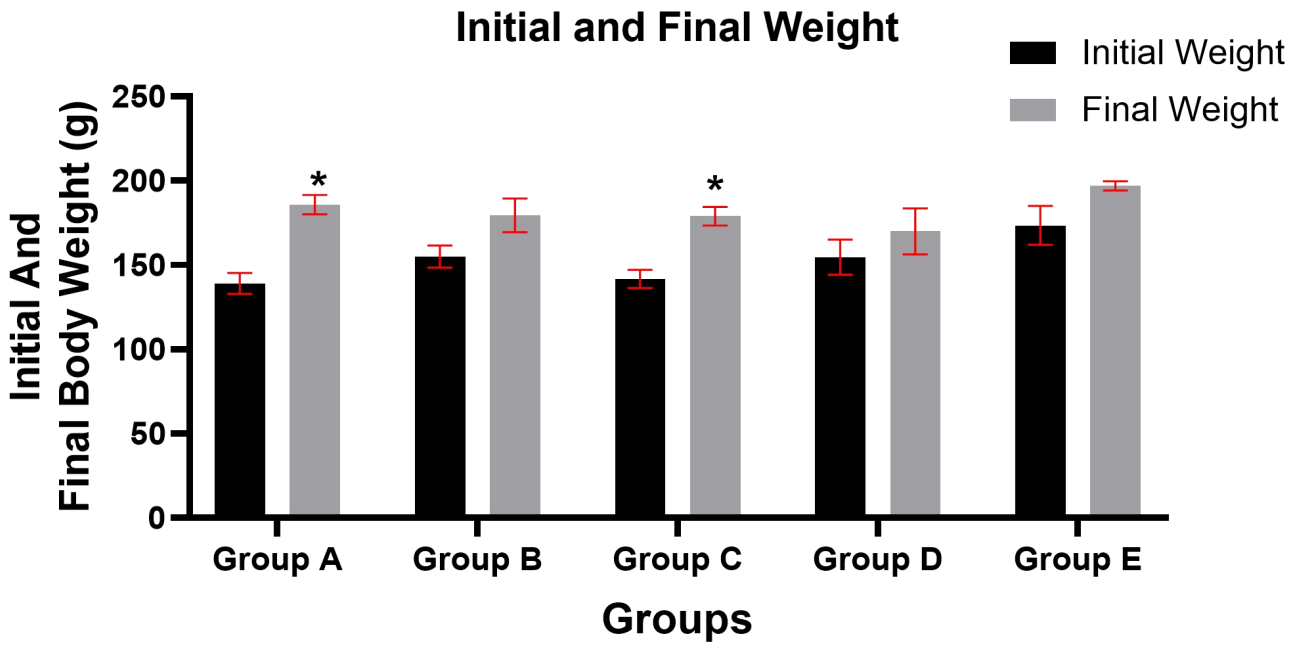

Chart 1: Showing initial and final weight Difference

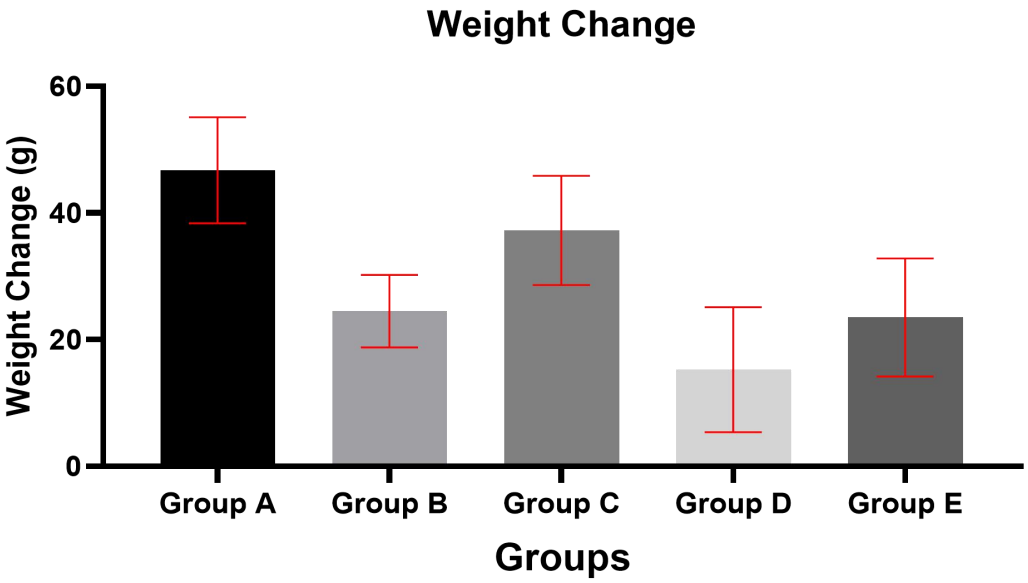

Chart 2: Showing weights change

There was no statistically significant Difference ($p<0.05$) in the weights change of rats across groups when compared with the initial weight.

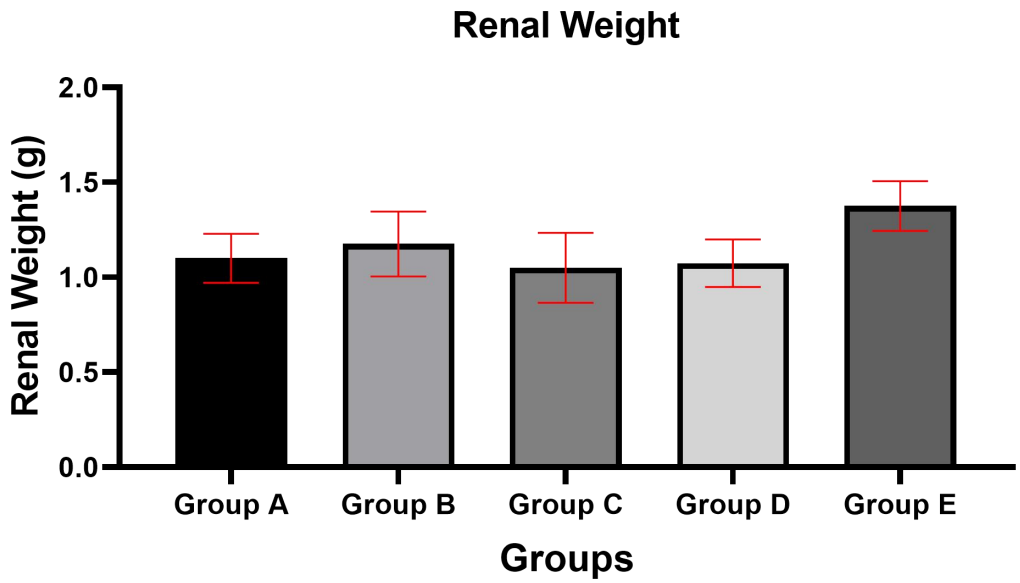

Chart 3: Showing renal weight

There was no statistically significant Difference ($p<0.05$) in the renal weight of rats across groups when compared with the initial weight.

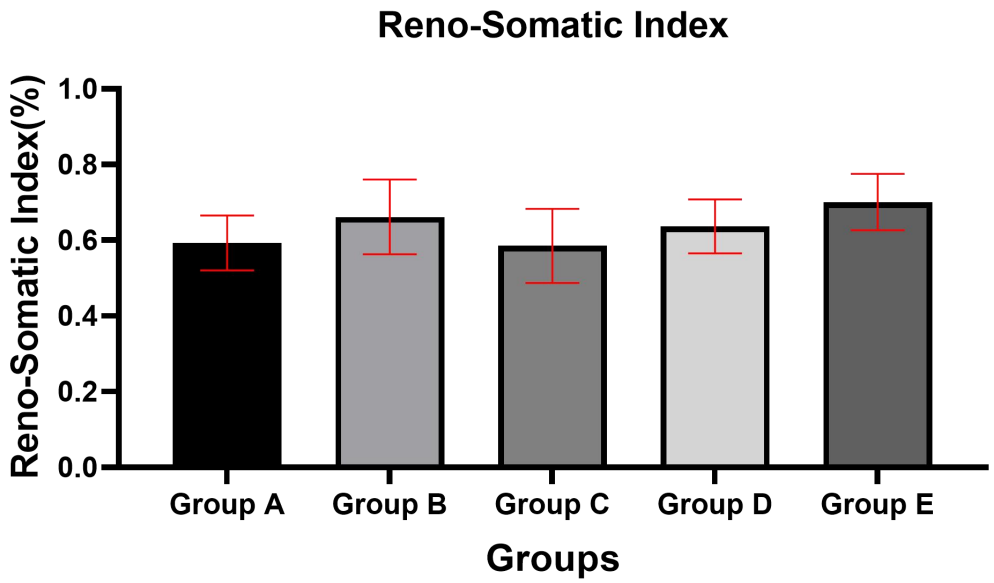

Chart 4: Showing Reno-somatic index

There was no statistically significant Difference ($p<0.05$) in the Reno-somatic index of rats across groups when compared with the initial weight.

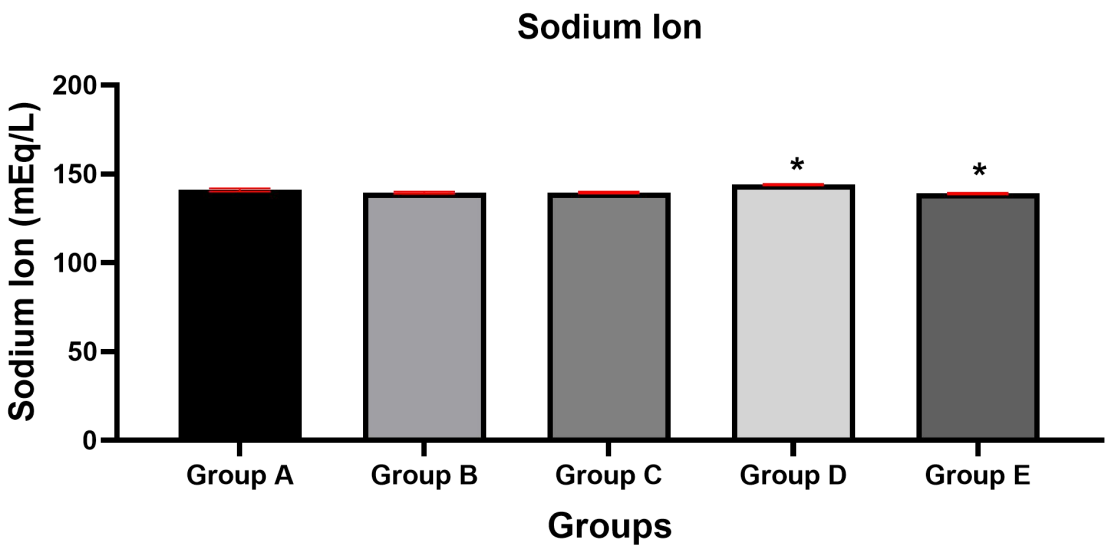

Chart 5: Showing Sodium Ion level

*Represents Statistically Significant Difference ($p<0.05$)

There was a statistically significant increase ($p<0.05$) in the Sodium Ion level of rats in groups D and a statistically significant decrease ($p<0.05$) in the Sodium Ion level of rats in groups C when compared with the Group A (Control).

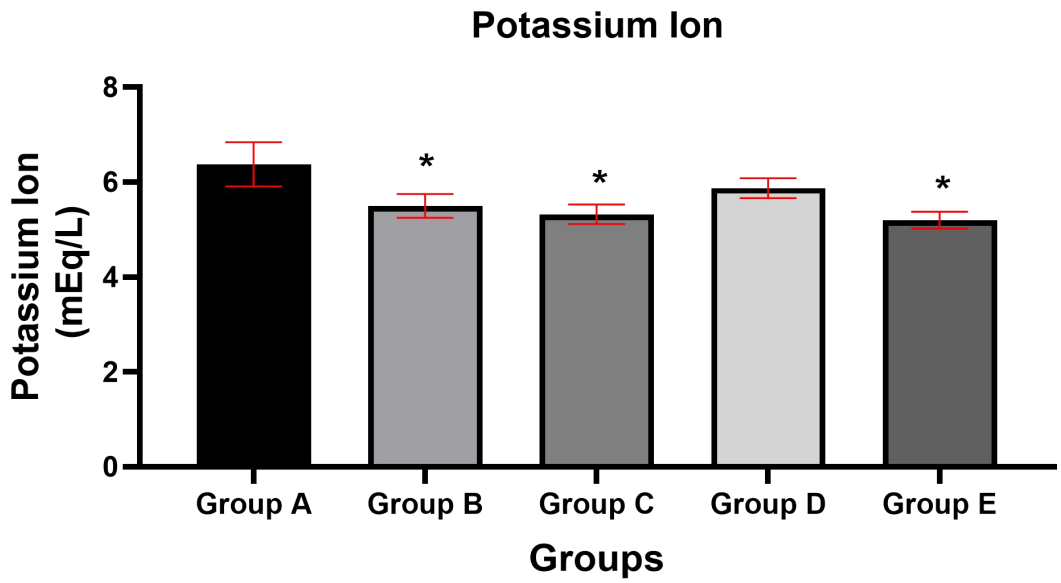

Chart 6: Showing Potassium Ion level

*Represents Statistically Significant Difference ($p<0.05$)

There was a statistically significant decrease ($p<0.05$) in the Potassium Ion level of rats in groups B, C and E when compared with the Group A (Control).

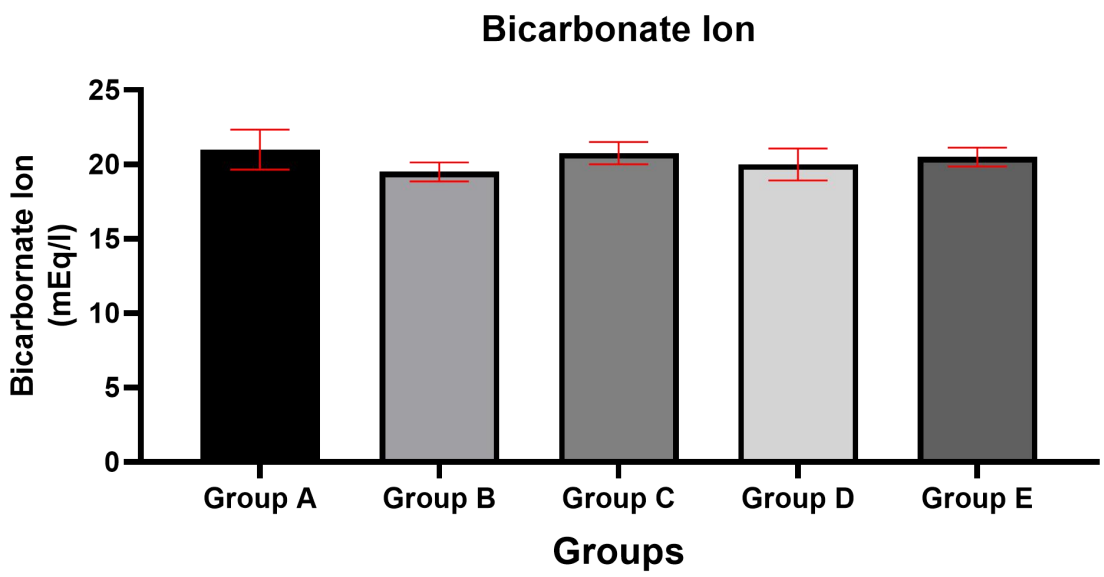

Chart 7: Showing Bicarbonate ion levels

There was no statistically significant Difference ($p<0.05$) in the Bicarbonate ion levels of rats across groups when compared with the initial weight.

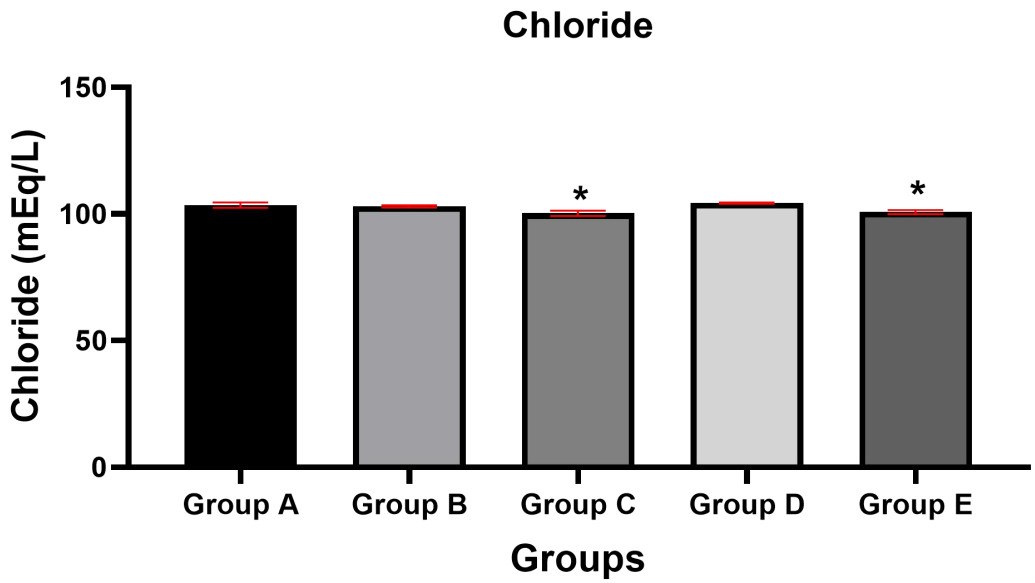

Chart 8: Showing Chloride levels

*Represents Statistically Significant Difference ($p<0.05$)

There was a statistically significant decrease ($p<0.05$) in the Chloride level of rats in groups C and E when compared with the Group A (Control).

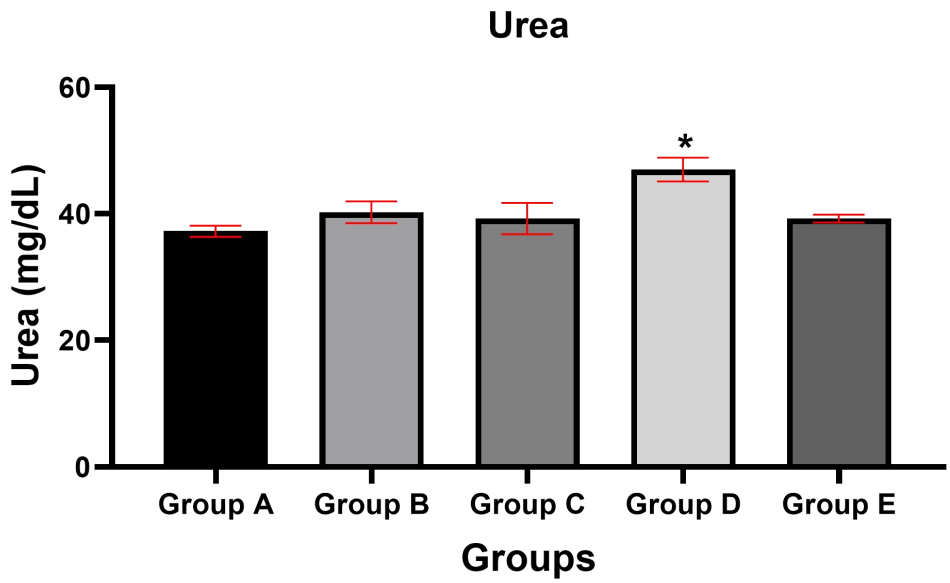

Chart 9: Showing Urea levels

*Represents Statistically Significant Difference ($p<0.05$)

There was a statistically significant increase ($p<0.05$) in the Urea level of rats in groups D when compared with the Group A (Control).

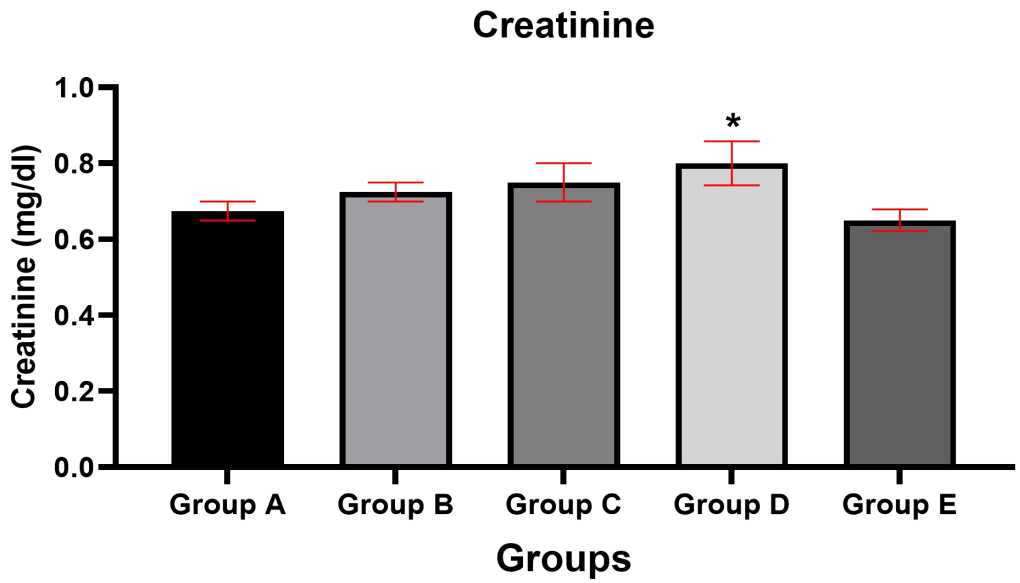

Chart 10: Showing Creatinine levels

*Represents Statistically Significant Difference ($p<0.05$)

There was a statistically significant increase ($p<0.05$) in the Creatinine level of rats in groups D when compared with the Group A (Control).

HISTOPATHOLOGY RESULT

TU Plate 1. Rat kidneys. Control. Composed of normal tissue architecture:

Tubules (TU), glomeruli (GL), interstitial space (IS): H&E x 40

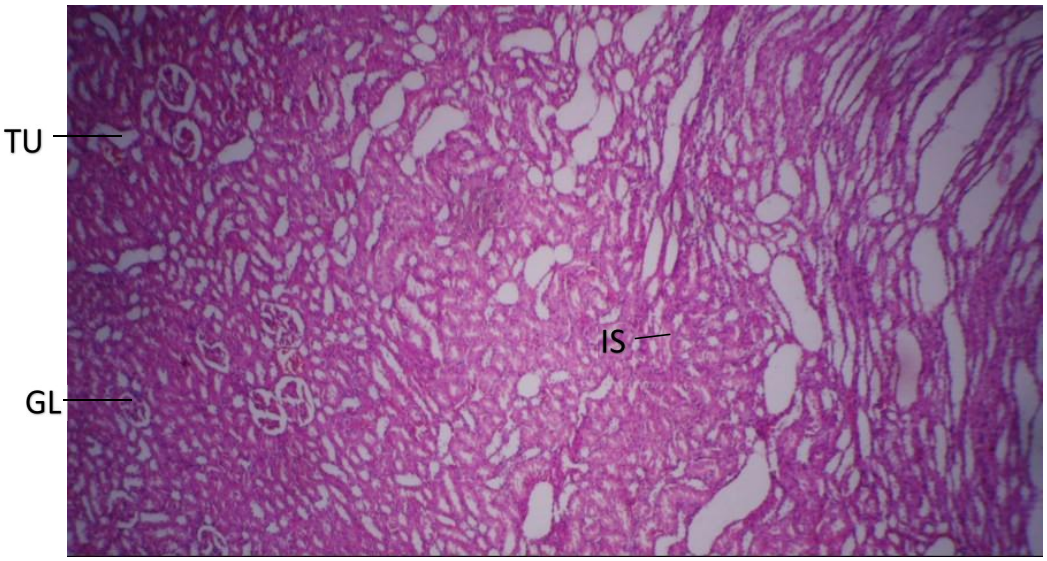

Plate 1. Rat kidneys. Control. Composed of normal tissue architecture:

Tubules (TU), glomeruli (GL), interstitial space (IS): H&E x 40

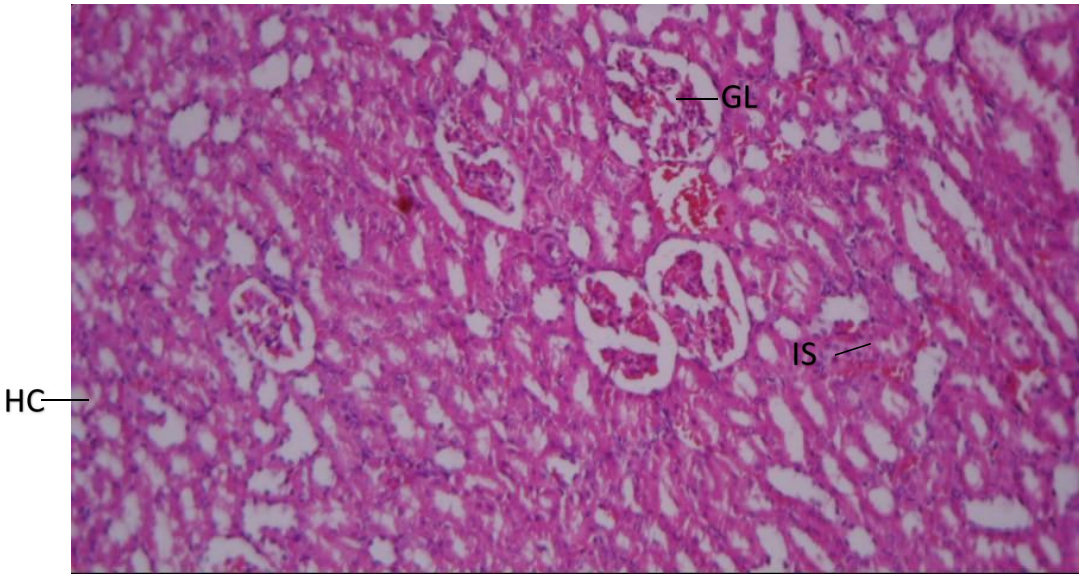

Plate 2. Rat kidneys. Control. Composed of normal tissue architecture: tubules (TU), glomeruli (GL), interstitial space (IS): H&E x 100

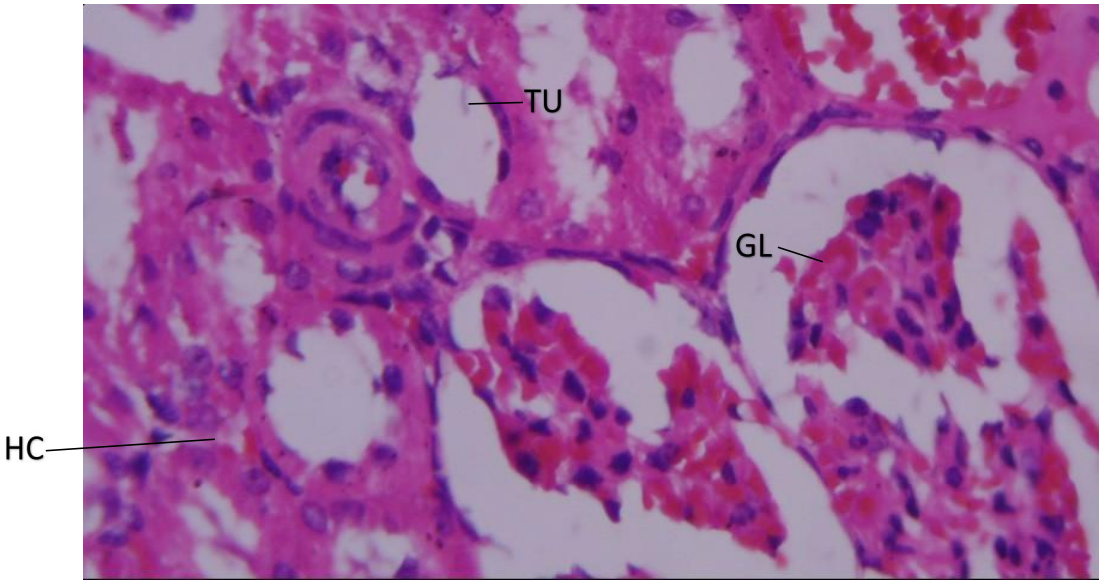

Plate 3. Rat kidneys. Control. Composed of normal tissue architecture: tubules (TU), glomeruli (GL), interstitial space (IS): H&E x 400)

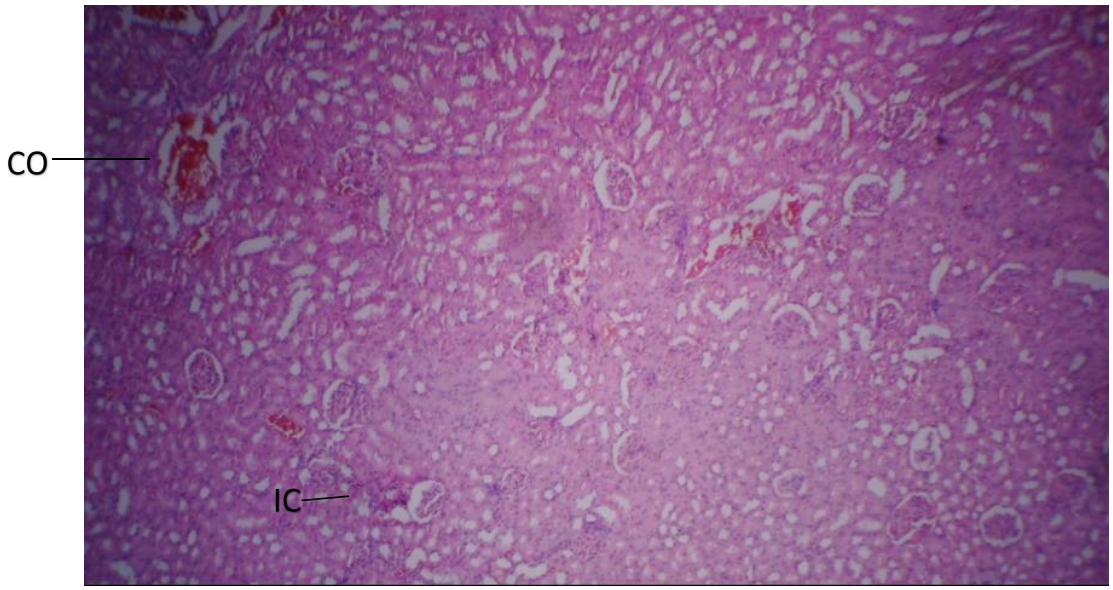

Plate 4. Rat kidneys given 1.5mg Nutmeg showing: interstitial congestion (CO) and interstitial infiltrates of inflammatory cells (IC): H&E x 40

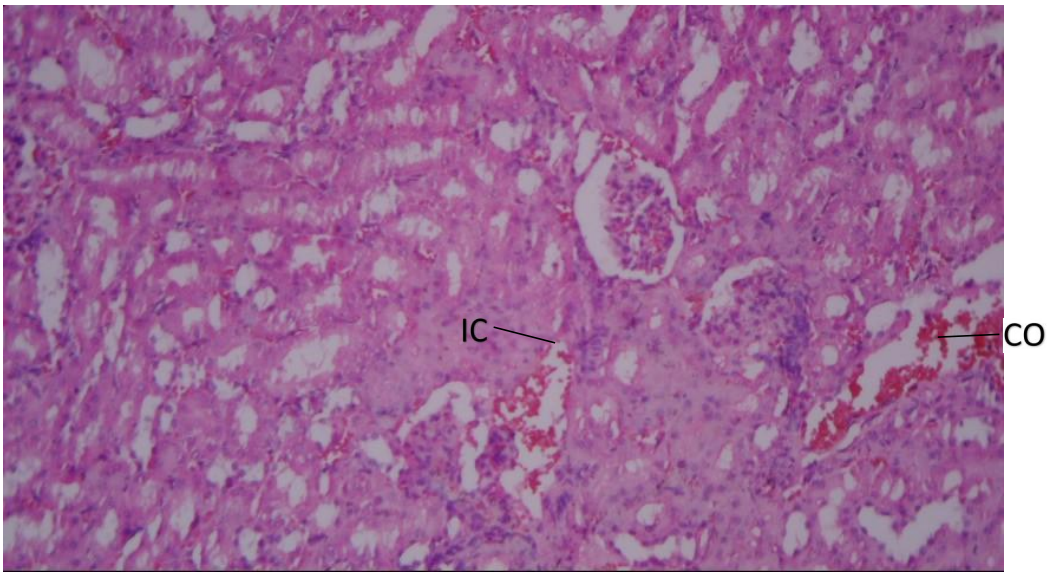

Plate 5. Rat kidneys given 1.5mg Nutmeg showing: interstitial congestion (CO) and interstitial infiltrates of inflammatory cells (IC) : H&E x 100

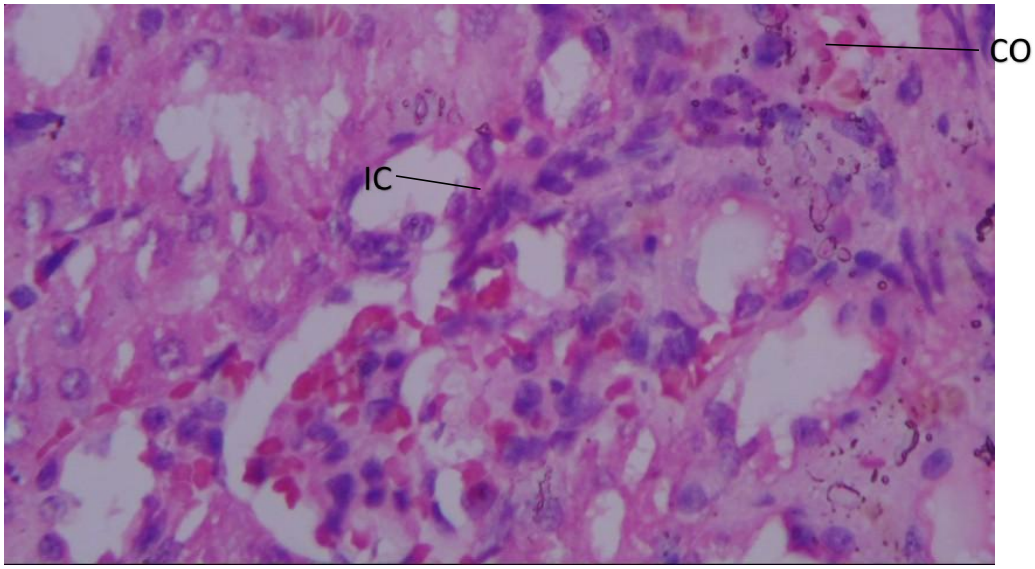

Plate 6. Rat kidneys given 1.5mg Nutmeg showing: interstitial congestion (CO) and interstitial infiltrates of inflammatory cells (IC) : H&E x 400

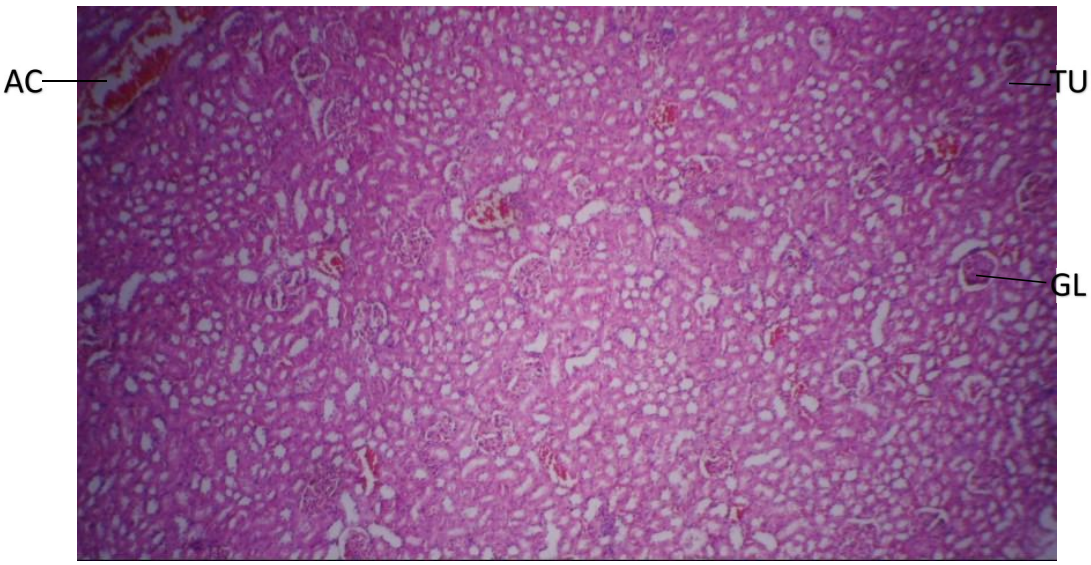

Plate 7. Rat kidneys given 2.5mg Nutmeg showing normal architecture: tubules (TU), active interstitial congestion (AC), glomeruli (GL): H&E x 40

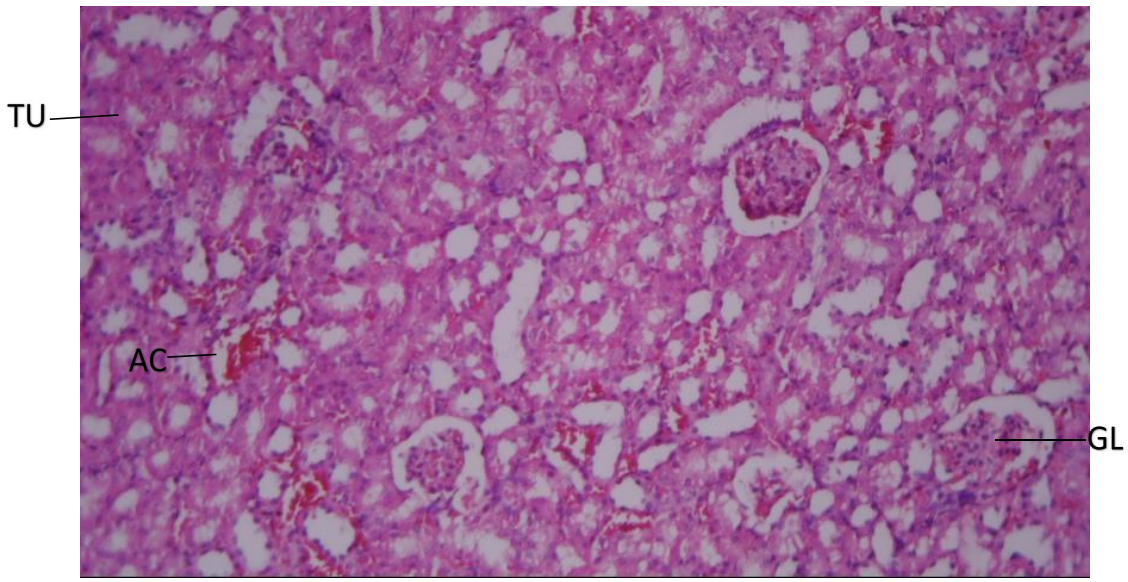

Plate 8. Rat kidneys given 2.5mg Nutmeg showing normal architecture: tubules (TU), active interstitial congestion (AC), glomeruli (GL): H&E x 100

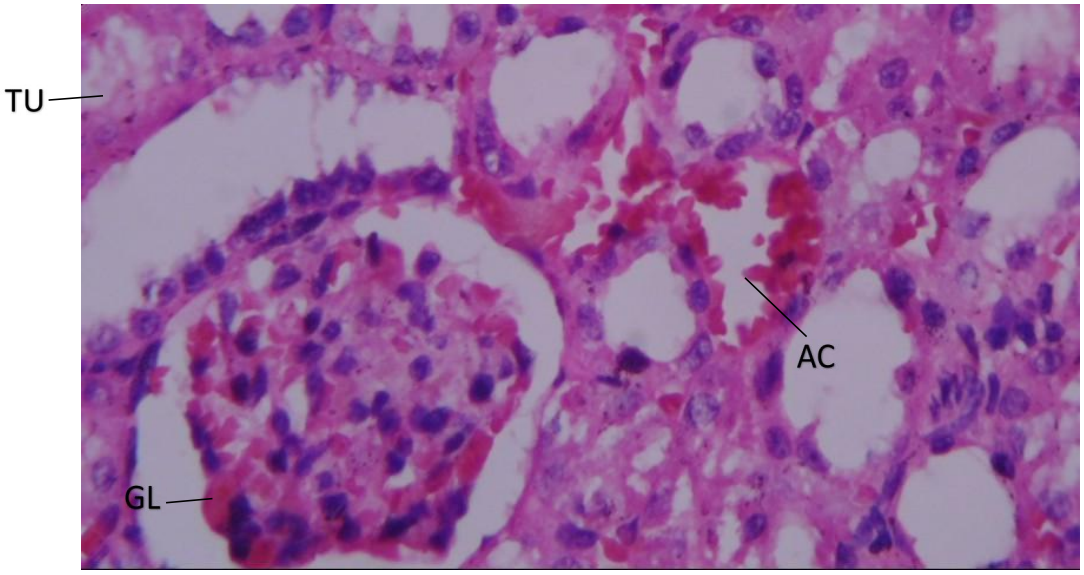

Plate 9. Rat kidneys given 2.5mg Nutmeg showing normal architecture: tubules (TU), active interstitial congestion (AC), glomeruli (GL) : H&E x 400

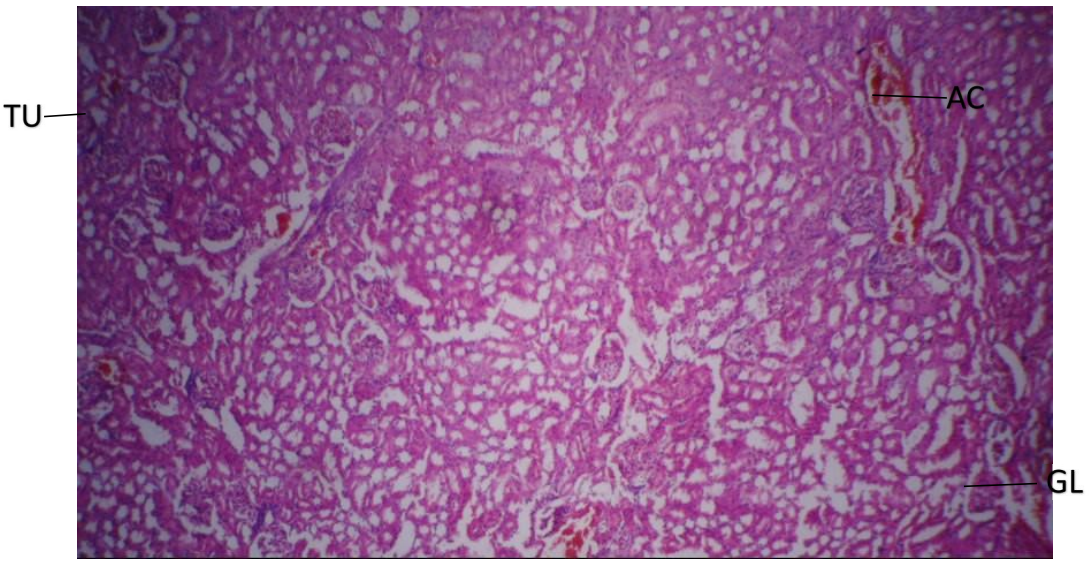

Plate 10. Rat kidneys given 3.5mg Nutmeg showing normal architecture: active interstitial congestion (AC), tubules (TU), glomeruli (GL): H&E x 40

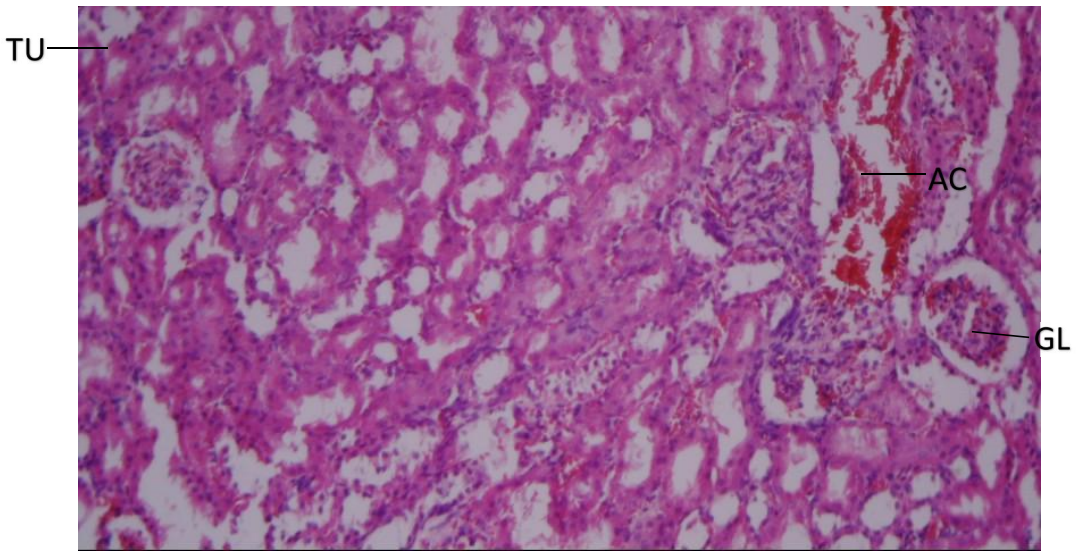

Plate 11. Rat kidneys given 3.5mg Nutmeg showing normal architecture: active interstitial congestion (AC), tubules (TU), glomeruli (GL) : H&E x 100

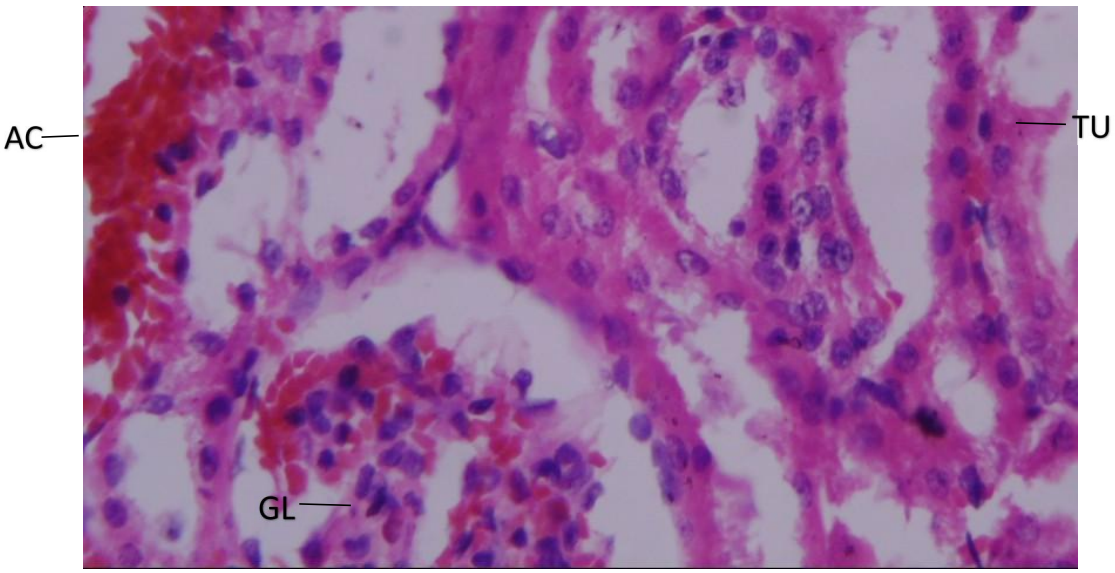

Plate 12. Rat kidneys given 3.5mg Nutmeg showing normal architecture: active interstitial congestion (AC), tubules (TU), glomeruli (GL): H&E x 400

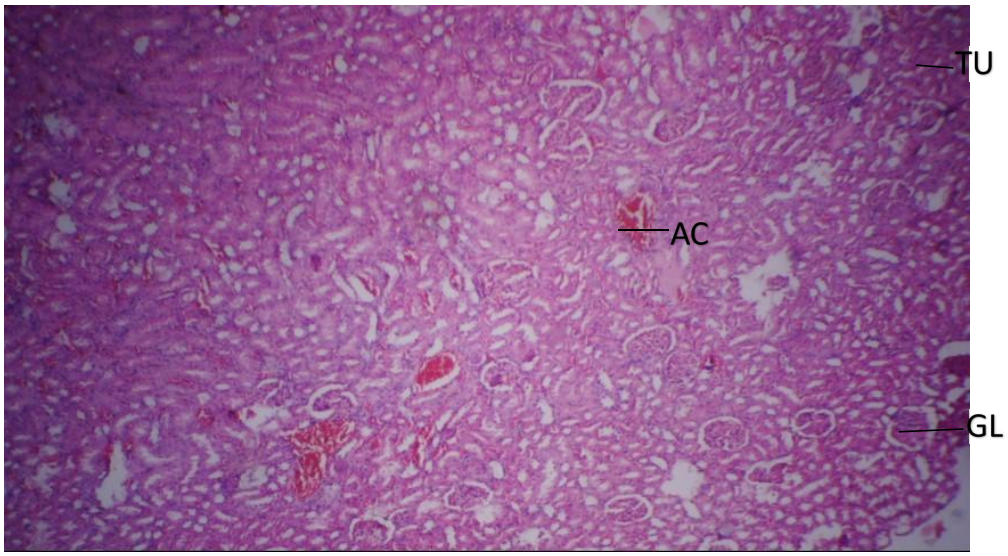

Plate 13. Rat kidneys given 4.5mg Nutmeg showing normal architecture: tubules (TU), glomeruli (GL), active interstitial congestion (AC): H&E x 40

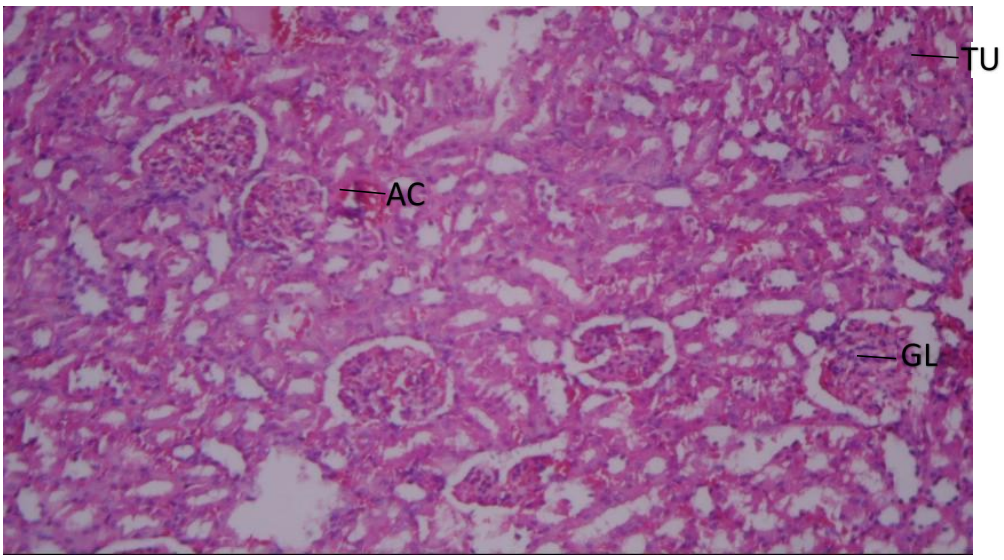

Plate 14. Rat kidneys given 4.5mg Nutmeg showing normal architecture: tubules (TU), glomeruli (GL), active interstitial congestion (AC) : H&E x 100

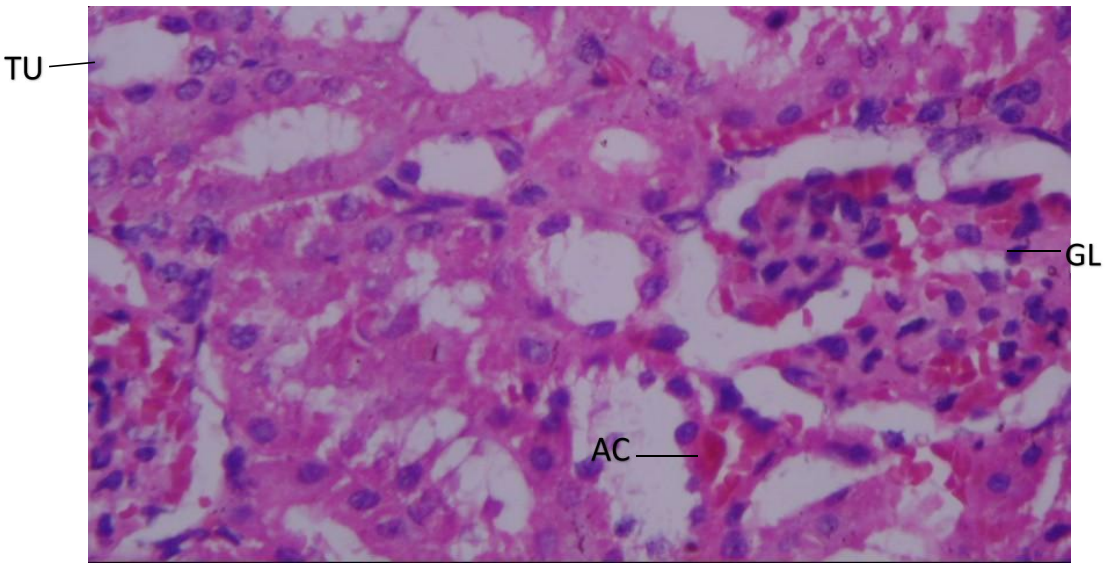

Plate 15. Rat kidneys given 4.5mg Nutmeg showing normal architecture: tubules (TU), glomeruli (GL), active interstitial congestion (AC) : H&E x 400

CHAPTER 5

The results (H & E) reactions revealed that chronic consumption of nutmeg does not greatly affect the cyto-architectural, increase number of renal corpuscle in the treated groups compared to the control group. There were several increase in vasodilatation in the kidneys of the treated animals. The weight of the experimented animals appears to be increase in weight

It may be inferred from the present results that higher doses of nutmeg consumption may have resulted in increase in body weight.

conclusion

The results obtained in this study revealed that Nutmeg consumption increase the blood flow of the kidney of adult Wistar rat; the cyto-architecture of the kidneys appear normal, Sparsely distribution of the Bowman's spaces. These results suggest that the functions of the kidney may have been appears to be functionally normal .

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