EFFECT OF LEVOFLOXACIN ON THE CARDIOVASCULAR AND RENAL SYSTEMS IN ALBINO RATS

OJIMBA PROMISE UCHECHUKWU
GOU/U22/BCH/318

BIOCHEMISTRY PROGRAMME
DEPARTMENT OF CHEMICAL SCIENCES
FACULTY OF NATURAL SCIENCES AND ENVIRONMENTAL STUDIES, GODFREY OKOYE UNIVERSITY, ENUGU
NIGERIA

JULY, 2026

EFFECT OF LEVOFLOXACIN ON THE CARDIOVASCULAR AND RENAL SYSTEMS IN ALBINO RATS

OJIMBA PROMISE UCHECHUKWU
GOU/U22/BCH/318

BIOCHEMISTRY PROGRAMME
DEPARTMENT OF CHEMICAL SCIENCES
FACULTY OF NATURAL SCIENCES AND ENVIRONMENTAL STUDIES
GODFREY OKOYE UNIVERSITY, ENUGU
NIGERIA

A PROJECT RESEARCH SUBMITTED TO THE DEPARTMENT OF CHEMICAL SCIENCES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc.) DEGREE IN BIOCHEMISTRY

SUPERVISOR: DISTINGUISHED PROF. K. N. AGBAFOR

JULY, 2026

APPROVAL

This research titled "**Effect of Levofloxacinon the Cardiovascular and Renal Systems in Albino Rats**" has been assessed and approved by the Committees of Department of Chemical Sciences and Faculty of Natural Sciences and Environmental Studies, Godfrey Okoye University, Enugu, Nigeria.

Distinguished Prof. K.N. Agbafor	.....	.....
Supervisor	Signature	Date
Dr. Anosike Joy .C.	.....	.....
HOD, Department of Chemical Sciences	Signature	Date
Prof. Chidi Uhuegbu	.....	.....
Dean, FNSES	Signature	Date
	.....	.....
External Examiner	Signature	Date

CERTIFICATION

This is to certify that this research titled "**Effect of Levofloxacin on the Cardiovascular and Renal Systems in Albino Rats**" was carried out by **Ojimba Promise Uchechukwu** with Registration Number GOU/U22/BCH/318, under the supervision in the Department of Chemical Sciences, Godfrey Okoye University, Enugu, Nigeria.

Ojimba Promise Uchechukwu	.....	.....
GOU/U22/BCH/318	Signature	Date

Dr. Anosike Joy .C.	.....	.....
HOD,	Signature	Date
Department of Chemical Sciences		

DEDICATION

I dedicate this work with all my heart to Almighty God, whose wisdom, grace and infinite favor have provided this work to full completion. All this would not have happened without His input in terms of direction, power, and blessings.

ACKNOWLEDGEMENTS

My profound appreciation goes to my supervisor Distinguished Prof. K. N. Agbafor, for his exceptional guidance, insightful corrections, and patient encouragement throughout the duration of this research.

I am equally grateful to the HOD Dr (Mrs) Joy Ansoike and Dean of the Faculty of Natural Sciences and Environmental Studies, and to the Vice Chancellor of Godfrey Okoye University, Enugu, for his constant advice, unwavering support, and dedication to fostering academic excellence.

I express my deep appreciation to my mentor, Dr. F. N. Olisaka, whose words of wisdom, motivation, and guidance have continually inspired me to strive for excellence.

I extend my heartfelt gratitude to all my lecturers in the Department of Chemical Sciences for their invaluable contributions to my academic growth. I deeply appreciate Prof. Engwa, Dr. Obiudu Kenneth, Dr. Ayuk, Dr. Enechi, Dr. Ejikeme, Dr. Uhuo, Dr. Chidimma, Dr. Agbara, Dr. Chukwuemeka Anorue, Mrs. Ugwuonah Linda, Mrs. Amara, and Mr. Jachike. Your commitment to teaching and mentorship has left an indelible mark on me.

To my wonderful parents Late HRH and Ugoeze Maria Ojimba, uncle Mr Samuel Ndema and my handsome brothers Ochudo III, Kaka, Ojimba 03, Fresh, thank you for your unwavering love, finances, sacrifices, and steadfast encouragement. Your prayers and support have been my bedrock.

May God richly bless you all.

I return all glory and honor to Almighty God, whose infinite wisdom, grace, and unfailing love have guided me through every step of this academic journey. His blessings and favor have made the completion of this project a reality.

ABSTRACT

Antibiotics are a class of drug used for the treatment and management of various infections. There have been several reports of toxicity of antibiotics on different organs of the body. Hence, the present study investigated the effect of LEBAL-500 (an antibiotic) on the cardiovascular system and kidney using Lipid profile, Creatine Kinase activity, Cardiac Troponin I, Creatinine, Uric acid, Urea, bicarbonate as biomarkers. The research was conducted with twenty-five (25) adult albino rats randomly distributed into five (5) groups (A, B, C, D and E), five rats in each. Groups C, D and E were administered (by oral intubation) 7.14, 14.29 and 21.43 mg/kg body weight respectively of LEBAL-500 solution for five consecutive days. Group B(standard), received an intraperitoneal single dose of 7.5 mg/kg of doxorobicin on the first day because of it known effect on the cardiovascular and renal system. Group A (normal control) was given distilled water. There was a decrease in feed and water intake as well as physical activities and average body weight in group B,C, D, and E relative to the control (group A). Creatine Kinase activity and levels of cardiac troponin I, total cholesterol, triglycerides and low density lipoprotein were significantly ($P<0.05$) higher while HDL was significantly($P<0.05$) lower in groups B, C, D&E than in the control (group A). The concentration of creatinine, urea, uric acid and bicarbonate recorded in groups B and E were significantly($P<0.05$) higher than in group A.The difference in these renal parameters between group A and groups C and D was not significant ($P>0.05$). Further, the effects of doxorubicin were significantly($P<0.05$) higher than that of LEBAL-500. These effects were found to be dose dependent. The findings of this study are indicative that the antibiotic (LEBAL-500) may be toxic to the cardiovascular system and renal system, thus, LEBAL-500, should not be recommended for a person with a cardiovascular disease, even at doses lower than the manufacturer's dose while persons with renal disease may take the drug but with caution.

Keywords: Antibiotics, LEBAL-500,Lipid profile, Cardiac Troponin I, Creatine Kinase, Creatinine, Urea, Uric acid, Bicarbonate,Doxorubicin,Albino rats.

TABLE OF CONTENTS

LIST OF TABLE

LIST OF FIGURES

LIST OF APPENDIX

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Antibiotics are specialized medications created to fight bacterial infections that have completely changed modern medicine since their discovery. Based on their range of activity, they are divided into two categories: narrow-spectrum antibiotics, which are effective against particular bacterial species, and broad-spectrum antibiotics, which target a variety of bacteria (Kucers *et al*., 2017). A significant turning point in healthcare was the introduction of penicillin, the first commonly used antibiotic, which significantly decreased the death rates from infectious diseases and revolutionized clinical procedures all over the world (Lindsay and e, 2017). Effective management of bacterial infections still depends on the continuous research and application of antibiotics (Taylor and Francis, 2019).

In the treatment of several bacterial illnesses, antibiotics are mostly used, it impacts several organ systems, such as infections of the skin, digestive system, urinary tract, or respiratory system and sepsis (Kucers *et al*, 2017).

In order to reduce the incidence of postoperative infections, they are also used preventively prior to surgical procedures (Taylor and Francis, 2019). Antibiotic resistance, a serious global health concern that reduces the efficacy of currently available medications and makes infection care more difficult, has alarmingly increased despite the effectiveness of antibiotics due to over prescription and overuse (Lindsay and Suzanne, 2017).

By interfering with vital bacterial functions, antibiotics can have bactericidal or bacteriostatic effects. As with penicillins and cephalosporins, they work by inhibiting the formation of cell walls, which keeps bacteria from preserving their structural integrity (microbeonline.com), they also use

drugs that target bacterial ribosomes, such as macrolides and tetracyclines, to obstruct protein synthesis (microbeonline.com). Other types, such as fluoroquinolones, stop nucleic acid production by attacking DNA gyrase and topoisomerase IV, necessary for DNA replication and repair (microbeonline.com). Lastly, medications such as sulfonamides block bacterial growth-promoting metabolic pathways, particularly folate production (microbeonline.com). Because bacteria and human cells have different biological structures and pathways, these mechanisms exclusively target bacteria, guaranteeing the safety and specificity of antibiotics (Zinner *et al*., 2006).

Levofloxacin is a frequently used fluoroquinolone antibiotic which is ofloxacin, the active L-isomer. Its mechanism includes topoisomerase IV and bacterial DNA gyrase inhibition, necessary in bacterial DNA transcription, replication, and repair (DrugBank, 2023). Levofloxacin is effective against gastrointestinal, cutaneous, urinary, and respiratory infections because of its broad-spectrum activity (NCBI, 2023). Pharmacokinetically, it has good tissue penetration and renal excretion, as well as a high oral bioavailability that makes oral administration convenient (DrugBank, 2023). Levofloxacin has clinical advantages, but there are dangers associated with its use, including tendinopathy, neuropathy, and cardiovascular problems. These risks should be taken into account when managing patients and doing research (NCBI, 2023).

Therefore, the intent of this investigation was to methodically evaluate the toxicity profile of a Levofloxacin tablet (400mg), using Albino rats, with a particular emphasis on its effect on the cardiovascular and renal systems using some specific biomarkers.

1.1.1 Justification of the problem

It is justifiable research effort to evaluate cardiovascular and renal function in albino rats given the antibiotic LEBAL-500 for the following reasons:

i) Assessment of cardiovascular function because the performance of the heart and blood vessels enables early detection of cardiovascular diseases.

ii) Assessment of renal function because the kidneys regulate the body's electrolyte levels, filter waste products, and preserve fluid balance.

iii) Monitoring cardiovascular and renal function is essential for determining an organism's general health and wellbeing.

iv) Understanding the potential cardiotoxic and nephrotoxic effects of this antibiotic and its safety profile by examining the effects of LEBAL-500 on cardiovascular and renal function in albino rats.

Relevance to medication safety

Both human and veterinary medicine make extensive use of antibiotics. However, some instances of cardiovascular and renal toxicity have been linked to several antibiotics, including LEBAL-500. Thus, evaluating the drug's safety profile and spotting any potential cardiovascular and kidney side effects can be aided by knowing how LEBAL-500 affects cardiovascular and renal function in albino rats. Both preclinical and clinical analyses of the medicine benefit from such knowledge. Due to their physiology and genetic resemblance to humans, as well as their long history of usage in biomedical research, albino rats are frequently utilized as experimental models. Researchers may replicate and assess the effect of LEBAL-500 on renal function in this study using albino rats, providing information that can be cautiously extended to people.

Specific antibiotic research: LEBAL-500 is a component of the extensively used fluoroquinolones category of antibiotics that are utilized to deal with a broad spectrum of bacterial diseases. On the effects of LEBAL-500 especially on the kidneys, heart and blood vessels, there is, however, little information. This knowledge gap can be filled and a thorough grasp of the effect

of the antibiotic on cardiovascular and renal health can be obtained by measuring the cardiovascular and renal function in albino rats given LEBAL-500.

Potential clinical repercussions: If the study demonstrates severe cardiotoxic and nephrotoxic effects of LEBAL-500 in albino rats, it may stimulate additional research in humans to assess the potential danger to patients receiving this antibiotic. The research could influence clinical recommendations, assist medical practitioners in keeping track of cardiovascular and renal function when patients take LEBAL-500, and aid in the creation of less dangerous antibiotics or different types of therapeutic approaches.

In conclusion, the evaluation of cardiovascular and renal function in albino rats given LEBAL-500 is justified given the significance of this evaluation, its relevance to medication safety, the use of an acceptable animal model, the demand for further research into this particular antibiotic, and potential clinical consequences. The investigation may shed light on LEBAL-500 cardiotoxic and nephrotoxic effects and advance our knowledge of the antibiotic's safety profile.

1.2 Statement of the Problem

The purpose of this research is to address the problem of incomplete knowledge regarding the potential effects of the antibiotic LEBAL-500 on cardiovascular system and renal function in albino rats. Although antibiotics are frequently used to treat infections, there is some concern about their potential cardiotoxic and nephrotoxic effects. The renal function of albino rats given LEBAL-500 must therefore be assessed in order to ascertain whether the antibiotic poses any dangers to cardiovascular and kidney health. This project intends to shed light on the possible cardiotoxicity and nephrotoxicity of LEBAL-500 and offer insightful information on its safety profile by measuring cardiovascular and renal function parameters.

1.3 Aim of the Study

The research's main purpose was to assess the potential effects and toxicity of Levofloxacin, an antibiotic, on the cardiovascular and renal system in Albino rats using cardiac troponin I, lipid profile and creatine kinase activity for cardiovascular system and creatinine, Urea, uric acid and bicarbonate for renal system as biomarkers.

1.4 Objectives of the Study

The objectives of the study were:

1. Determination of Creatine Kinase activity in the levofloxacin treated rats
2. Determination of Cardiac Troponin levels in the levofloxacin treated rats
3. Estimation of Lipid Profile in the levofloxacin treated rats
4. Measurement of Urea level in the levofloxacin treated rats
5. Determination of creatinine level in the levofloxacin treated rats
6. Estimation of Uric acid level in the levofloxacin treated rats
7. Determination of Bicarbonate level in the levofloxacin treated rats

1.5 Significance of the Study

Due to these following factors, it is important to examine cardiovascular and renal function in albino rats receiving LEBAL-500.

Patient Safety: Since antibiotics are often prescribed drugs, it is essential to comprehend any potential cardiotoxic and nephrotoxic effects. This investigation can offer important insights into the antibiotic's safety profile by analyzing the cardiovascular and renal function of albino rats exposed to LEBAL-500, assisting physicians in making knowledgeable choices when administering it to patients. The best way to use antibiotics is to avoid cardiotoxicity and nephrotoxicity, which can cause cardiovascular diseases and acute kidney injury or contribute to

the onset of chronic kidney disease. This endeavor can advance our understanding of the potential cardiotoxic and nephrotoxic consequences of LEBAL-500 by evaluating the cardiovascular and renal function parameters in albino rats. The potential for renal impairment and cardiovascular disorders in patients using this antibiotic can be reduced by using this information to create the best dosing strategies and guidelines.

Understanding cardiotoxicity and Nephrotoxicity Mechanisms: Albino rats are a useful animal model for researching cardiotoxicity and nephrotoxicity brought on by drugs. This project can shed light on the underlying processes of cardiotoxicity and nephrotoxicity associated with this antibiotic class by examining the effects of LEBAL-500 on the kidneys, heart and blood vessels. This information can help create focused therapies or different types of treatments that lower the chance of acute incidents like stroke or myocardial infarction and also lessen kidney damage while preserving the effectiveness of antibiotic therapy.

The knowledge obtained from examining LEBAL-500's effect on cardiovascular and renal function in albino rats can be extrapolated to other antibiotics within the same class or with similar mechanisms of action. This can enhance our understanding of cardiotoxicity and nephrotoxicity risks associated with antibiotics in general, facilitating the development of new treatments for the condition.

CHAPTER TWO

LITERATURE REVIEW

Antibiotics are some of the most important medications ever discovered and developed, and have played a crucial role in helping us to treat infectious diseases in recent years. In 1928 Alexander Fleming discovered penicillin and in 1929 he shared his discovery as the first true antibiotic and revolutionized treatment for bacterial infections (Fleming, 1928). In the early 1940s, penicillin was formulated as a therapeutic agent after Fleming noticed a substance in a mold (*Penicillium notatum*) that killed bacteria. (Lax, 2004) This led to the introduction of commonly used antibiotics in medical practice and its use led to a significant decline in bacterial infection deaths including pneumonia, syphilis and meningitis.

After penicillin, other classes of antibiotics emerged in the mid-20th century with different targets and functions of the bacteria (Cohen, 2000). Another important development was the identification of the fluoroquinolones in the 1980s, which inhibit two essential enzymes in topoisomerase IV, bacterial replication and DNA gyrase, making them more efficient against resistant bacteria (Nicolau, 2001). Following these, subsequent classes such as carbapenems, cephalosporins and glycopeptides were developed, and have allowed resistant infections to be treated, and importantly have had a significant impact on worldwide public health by decreasing deaths from infectious diseases (Davies and Davies, 2010).

Appropriate and rational antibiotic use (prescribing antibiotics when necessary, selecting the right antibiotic, right dose, and right duration) is essential to reduce the build-up of resistance (WHO, 2015). Additionally, the continued monitoring, research for new antibiotics and antibiotic stewardship initiatives play a critical role in the fight against resistance and ensuring that current medicines continue to work.

There is an alarming increase in anti-microbial resistance (AMR), a problem on a global ranking due to the indiscriminate use of antibiotics (Lax and Gilbert, 2015). Bacteria can develop evasive strategies to thwart the effects of antibiotics, including gene mutation, enzymatic drug degradation, efflux pumps, and biofilm formation (Lax and Gilbert, 2015). Resistance makes treatment more difficult, extends the course of disease, makes treatment more expensive and contributes to greater mortality rates, particularly in low resource areas.

Antibiotics are antimicrobial drugs that act specifically on bacteria, either by killing them (bactericidal) or preventing their growth (bacteriostatic). These are either natural, created by organisms like bacteria and fungi (e.g. *Penicillium spp.* and *Streptomyces spp*.) or synthetically engineered in the laboratory for a more broadened spectrum and increased efficacy (Davies and Davies, 2010). They are significant because they are effective at fighting off bacterial infections and prevent complications, death and complex medical procedures such as cancer therapy or surgeries, which require immune suppression.

Antibiotics have revolutionized medicine by checking infectious diseases that were once frequently deadly. They have also been instrumental in controlling pandemics, alleviating the burden of bacterial diseases throughout the world and enhancing life expectancy (Lax, 2004). This is because antibiotics are continually being discovered and there is a growing issue with antimicrobial resistance.

2.1 Mechanisms of Selective Toxicity

Antibiotics have selective toxicity, that is, they are more likely to affect bacteria than human cells, so that they do the least amount of harm to the body (Levy, 2002). These are the mechanisms of activity:

i) **Inhibition of cell wall production**: The action of many antibiotics including β-lactams (penicillins, cephalosporins) is to inhibit the production of peptidoglycan in bacterial cell walls causing bacterial lysis (Hahn, 2012).

Human cells do not have cell walls, therefore these drugs have little effect on human tissues (Hahn, 2012).

ii) **Protein Synthesis Disrupters**: Agents such as tetracyclines and macrolides bind to the ribosome subunit(s) of bacteria, and disrupt protein synthesis. The difference in the base sequences of the ribosomal RNA of bacteria and that of the human mitochondria or cytosomes make it possible for these antibiotics to distinguish between the two and specifically target the bacterial ribosomes. (Vázquez *et al*., 2011)

iii) **Nucleic Acid Synthesis Inhibition**: Fluoroquinolones are inhibitors of topoisomerase IV and DNA gyrase, enzymes that are not present or markedly different in human cells, and that inhibit bacterial DNA replication (Nicolau, 2001).

iv) **Metabolic Pathway Blockade:** Sulfonamides act by blocking the ability of bacteria to compete with para-aminobenzoic acid (pABA) for the production of folic acid (Levy 2002). This pathway offers a selectable target because humans can obtain folic acid through their diet (Levy 2002).

Fluoroquinolones (FQs) are one of the many classes of antibiotics that have been increasingly recognized as having pharmacokinetic properties and broad spectrum activity which makes them clinically important, especially levofloxacin (Hooper and Jacoby, 2015). While frequently used to deal with skin, urinary tract, and respiratory infections, there is emerging evidence of adverse effects such as cardiovascular as well as renal effects, although these are less well documented (Davis and Wallace, 2017). These issues highlight the requirement for broader studies of its pathogenicity, mechanism and safety.

The selection of levofloxacin, which also includes other antibiotics, is driven by the common use of the antibiotic in clinical practice, and the complex safety issues surrounding fluoroquinolones. The comparison of its mechanisms and toxic effects with other classes of antibiotics also reveals a mechanism of action that can shed light on organ toxic effects associated with drugs for clinical decision making, which can benefit patient outcomes (Zhao *et al*., 2019).

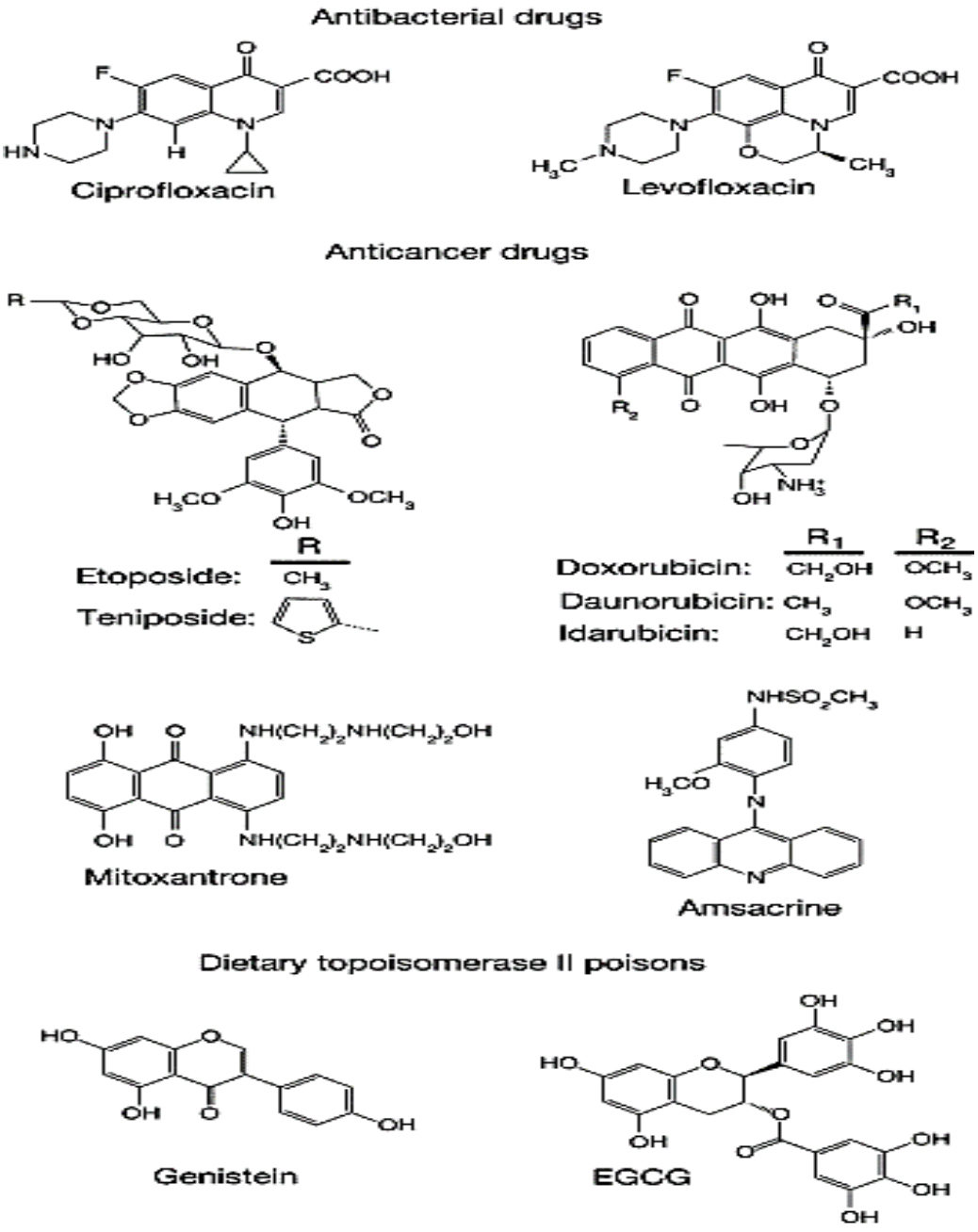

Figure 1 Chemical Structure and Classification of antibiotic

2.1.1 LEBAL-500 (Levofloxacin)

LEBAL-500 (Levofloxacin) is a broad-spectrum fluoroquinolone antibiotic which has a favorable PK profile and is widely used in clinical practice. The following detailed description, a summary of which forms the basis of the appended claims, provides a description of its chemical structure, mechanism of action, pharmacokinetics, spectrum of activity, indications, and resistance.

2.1.2 Chemical Structure and Classification.

Antibiotics ofloxacin and levofloxacin belong to the fluoroquinolone class of antibiotics, which have a core quinolone structure with a fluorine atom at position 6, increasing their potency and spectrum (Li *et al*., 2018)

2.1.3 Mechanism of Action

The antibacterial activity of levofloxacin is primarily due to its inhibitors of DNA replication and transcription depend on bacterial DNA gyrase and topoisomerase IV (Hooper, 2001). Topoisomerase IV (Gram-positive bacteria) while DNA gyrase (Gram-negative bacteria) (Zhanel *et al*., 2002).

The inhibition results in the stabilization of DNA-enzyme complexes which causes the breakage of DNA and ultimately bacterial cell death (Zhanel *et al*., 2002).

2.2 Topoisomerase IV and DNA gyrase inhibitor

These target enzymes are involved in controlling DNA topology during replication. DNA gyrase adds negative supercoils to DNA to help the replication forks move forward, and topoisomerase IV untangles interlinked daughter chromosomes during cell division. Levofloxacin is bound to these enzyme complexes, which is known to inhibit the activity of these enzymes (Hooper, 2001). Binding affinity is different between bacteria (Gram-negative and Gram-positive) and influences the spectrum of action.

2.2.1 The disruption of the replication of bacterial DNA.

Levofloxacin blocks these topoisomerases, inhibiting relaxation and decatenation of supercoiled DNA, thereby blocking DNA transcription and replication. Due to the accumulation of DNA damage, this interference produces bacteriostatic effects at lower concentrations and bactericidal effects at greater concentrations (Heevess *et al*., 2015).

2.2.2 Pharmacokinetics and Pharmacodynamics

Levofloxacin has a high bioavailability of ~99% and achieves peak plasma levels following oral treatment for one to two hours (McKinnon *et al*., 2018). It has a volume of distribution between 1-2L/kg, with excellent tissue penetrations including into the lungs, prostate and bone. Mainly eliminated through the kidneys, and has a half-life of about 6-8 hours, thus it could be dosed once or twice daily (Li *et al*., 2018). Pharmacodynamically, levofloxacin demonstrates concentration-dependent killing and the pharmacodynamic parameter most associated with its efficacy is AUC/MIC (Madel *et al*., 2016).

2.3 Spectrum of Activity

Levofloxacin is useful on a variety of Gram-positive and Gram-negative bacteria, comprising *Pseudomonas aeruginosa*, *Escherichia coli*, *Haemophilus influenza*, as well as *Streptococcus pneumoniae* and *Staphylococcus aureus (*Zhanel *et al*., 2002). It is effective against unusual microorganisms including *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae*. It is also effective against certain organisms that form biofilms because of its capacity to break through biofilms.

The Common indications and resistance are:

Levofloxacin is used to treat prostatitis, skin tissue infection, soft tissue infection, urinary tract infections (UTI), acute sinusitis, and respiratory infections (pneumonia) (Madel *et al*., 2016).

However, the emergence of resistance is a significant problem. Resistance usually results from modifications in the Quinolone Resistance Determining Regions (QRDRs) (gyrA and parC genes) that encode the DNA gyrase and DNA topoisomerase IV subunits, accordingly (Hooper, 2001). Resistance rates to fluoroquinolones have been rising, particularly among E. coli isolates, and this is partly attributed to the overuse and misuse of these antibiotics; caution must be exercised in using fluoroquinolones in these instances.

Common Adverse Effects

a) Gastrointestinal Disturbances

Common gastrointestinal (GI) side effects of antibiotics are diarrhea, nausea, vomiting and abdominal discomfort. Such effects are mainly caused by the disorder of the typical GI flora, causing dysbiosis, which is a risk factor for subsequent symptoms such as overgrowth of pathogenic bacteria such as *Clostridioides difficile* (Sakaguchi *et al*., 2010). The broad-spectrum antibiotics have a special association with GI side effects, such as fluoroquinolones and cephalosporins (Bryan and Smith, 2014).

b) Allergic Reactions

Antibiotic allergic hypersensitivity reactions can be mild, such as skin rashes or severe, such as anaphylaxis. Sulphonamides and penicillins are well-known to produce allergic reactions which may be cross-reactive between structurally related drugs (Fitzgerald *et al*., 2016). The immune mechanisms involved in allergic reactions include IgE-mediated hypersensitivity, which should be promptly recognized and managed (Korenblat and Chandler, 2018).

c) Changes in Microbiota and secondary infections

Antibiotics disrupt the balance of the normal flora and can cause an overgrowth of resistant bacteria and fungi, causing secondary infections like Clostridioides difficile colitis, fungal

infections (candidiasis), and other opportunistic infections (Dethlefsen, Huse, Sogin, and Relman, 2008). Changes in microbiota diversity can extend beyond the gastrointestinal tract and influence the host's health, such as metabolic and immune regulation (Dickson, 2018).

Although antibiotics are essential to the treatment of infections, they may have direct toxic effects on a number of organs or affect cellular processes, causing organ dysfunction. The mechanisms are complex and diverse, and can involve cellular and molecular mechanisms. They include:

1. Hepatic Toxicity

The hepatotoxicity induced by an antibiotic is most commonly due to a hypersensitivity reaction or direct hepatotoxic effect on the hepatocyte. For instance, macrolides such as erythromycin can result in cholestatic hepatitis, while tetracyclines can lead to hepatocellular injury (García-Bustos *et al*., 2008). The molecular mechanism involves the generation of metabolites that cause oxidative stress (Andrade and Chalasani, 2000), mitochondrial dysfunction and lipid peroxidation, thereby inducing hepatocyte apoptosis or necrosis. A mitochondrial dysfunction may lead to a decrease in ATP production, which can cause oxidative damage inside mitochondria and cause cell death (Zhang *et al*., 2014).

This hepatocellular damage is seen clinically as liver enzyme elevation, cholestasis or acute liver failure in severe instances (García-Bustos *et al*., 2008).

2. Nephrotoxicity

Aminoglycosides such as gentamicin are nephro-toxic by binding to mitochondria in the proximal tubular cells, generating reactive oxygen species (ROS) production, apoptotic pathway activation, and mitochondrial damage (Nielsen and Jørgensen, 2011). Aminoglycosides bind to the phospholipids in the lysosomal membrane, leading to phospholipidosis (Kier, 2010) which results in disruption of normal lysosomal function, cell injury, and cell death. The mechanism of

vancomycin associated nephrotoxicity includes oxidative stress and dysfunction of the renal tubular epithelial cell, which occurs through mitochondrial dysfunction and increase in ROS production (Poluzzi *et al*., 2014).

The clinical manifestation is acute kidney injury with reduced glomerular filtration rate, oliguria and electrolyte disturbances (Nielsen and Jørgensen, 2011).

3. Cardiotoxicity

Some antibiotics, including fluoroquinolones and macrolides, may have a molecular-level effect on heart-related ion channels. The potassium-related human ether-à-go-go gene (hERG) channels are blocked by fluoroquinolones, leading to delayed repolarization of cardiac myocytes, prolonged QT interval and risk of torsades de pointes (TdP) (Gelen *et al*., 2020). Molecularly, this is a blockade of potassium efflux channels that have a significant part in the process of repolarization during the action potential (Gelen *et al*., 2020). Other macrolides such as erythromycin, which also prolong QT, do so in similar ways (Gelen *et al*., 2020).

If not carefully monitored, arrhythmogenic potential can cause life-threatening arrhythmia and sudden cardiac death.

4. Hematological Toxicity

Antibiotics like chloramphenicol directly supress bone marrow through cytotoxic activity or by causing the immune system to destroy the hematopoietic cells, causing aplastic anemia (Rajan and Singh, 2020). Molecular mechanisms include the production of reactive metabolites that react with cellular macromolecules and trigger immune responses or direct damage to DNA and disruption in hematopoiesis (Rajan and Singh 2020). Hemolytic anemia can be caused in G6PD deficient individuals (Beutler, 2016) by oxidative stress which causes damage to red blood cell membranes due to the effect of sulfonamides.

As a result, the body's total blood cell count decreases (pancytopenia) or hemoglobin (anemia), increasing susceptibility to infections and bleeding tendency (Rajan and Singh, 2020).

5. Dysfunction of mitochondria and cell death via apoptosis.

Mitochondrial impairment is a common molecular pathway involved in many toxic effects. Antibiotics with similar structure to prokaryotic ribosomes (including tetracyclines and aminoglycosides) can also disrupt mitochondrial protein synthesis (Zhang *et al*., 2014). This results in reduced ATP production, ROS generation and activation of intrinsic apoptotic pathways, which finally triggers cell death in various tissues (Zhang *et al*., 2014).

2.4 The Cardiovascular System

The heart pumps blood through the circulatory system like a muscle pump, delivering nutrients and oxygen to tissues and eliminating waste products from metabolism. It operates via coordinated contractions of its chambers, regulated by the conduction system which ensures synchronized heartbeat (Guyton and Hall, 2016). After entering (the right atrium) and being forced into the ventricle (right), the artery (pulmonary) transports blood(deoxygenated) from the systemic circulation to the lungs (Sherwood, 2018).

Oxygenated blood is pumped into the left ventricle and then transported to systemic tissues by the aorta after returning to the left atrium (Sherwood, 2018).

The heart, blood vessels, and blood itself make up the cardiovascular system, which cooperates to eliminate waste products from metabolism and provide tissues with oxygen and nutrients. By controlling blood pressure and promoting immunological responses, it is essential for preserving homeostasis (Shepherd and Vanhoutte, 1979). Cardiovascular health can be greatly impacted by a number of medications that affect heart rhythm, contractility, or vascular tone, necessitating close

monitoring during treatment(Shepherd and Vanhoutte, 1979). When assessing the effects of drugs, it is essential to comprehend this system, particularly when studying cardiotoxicity.

Diseases affecting the heart or blood arteries are known as cardiovascular disease (CVD) (Mendis *et al*., 2011). Cardiovascular diseases encompass aortic aneurysms, ischaemic heart disorder (IHD), fibrillation (atrial), coronary heart disease, rheumatic heart disease (RHD), peripheral artery infection (Mendis *et al*., 2011). Heart cells are harmed by toxic Reactive Oxygen Species (ROS), like hydroxyl radicals, superoxide anion and hydrogen peroxide (Anderson, 2011). Two significant risk factors for cardiovascular disease are increased levels of total cholesterol (TChol), low density lipoprotein cholesterol (LDL-C)(Anderson, 2011). Assessment of cardiovascular toxicity often involves measuring specific biomarkers that provide insights into cardiac injury or functional alterations. They include:

a) Creatine Kinase (CK)

Recently, CK (creatine kinase), an enzyme that supports salt retention and ATP-dependent vascular contractility, has stood specified as a new risk factor for hypertension (Salomons and Wyss ,2010). Consequently, CK maintains a crucial role in pressor responses, but little is known about the haemodynamics of this process (The contractility of human resistance arteries is largely dependent on CK, and there is a substantial correlation between clinical blood pressure and microvascular CK mRNA expression (Salomons and Wyss,2010). Because CK is attached to cytosolic ATPases such Ca2+-ATPase, myosin ATPase, and Na+/K+-ATPase that are applied in cardiovascular contractility and sodium retention, it quickly regenerates ATP in the following reaction: Phosphocreatine+ MgADP ↔ Creatine + MgATP

The contractility of human resistance arteries is largely dependent on CK, and there is a substantial correlation between clinical blood pressure and microvascular CK mRNA expression (Salomons

and Wyss,2010) Furthermore, increased sodium retention in humans is associated with resting plasma CK because CK biologically stimulates Na+/K+-ATPase, the kidney's sodium retention-promoting enzyme The contractility of human resistance arteries is largely dependent on CK, and there is a substantial correlation between clinical blood pressure and microvascular CK mRNA expression (Salomons and Wyss,2010). Crucially, elevated CK predicts hypertension and rises in tandem with blood pressure, while CK inhibition lowers blood pressure and human resistant arterial contractility in animal models. The idea that CK is linked to blood pressure is thus supported by the collective data, most likely as a result of elevated SVR and SV.

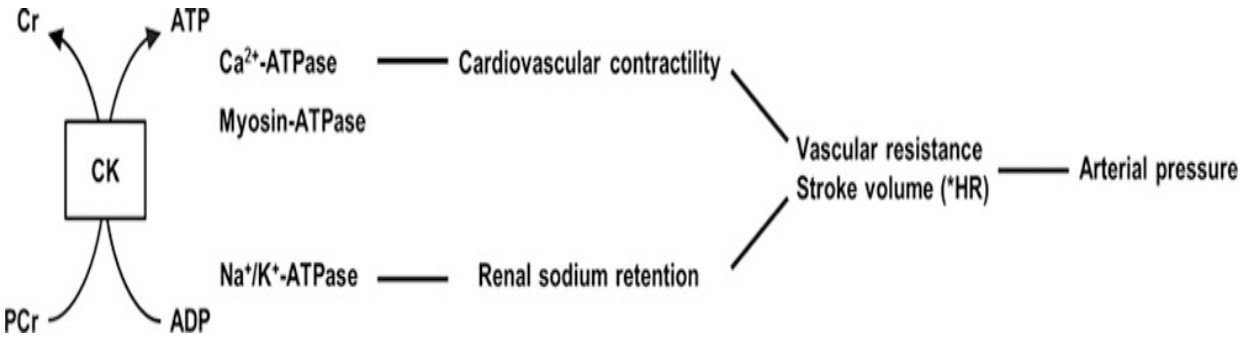

Figure 2. CK mechanism (creatine [Cr] kinase)-induced hypertension.
Source (Salomons and Wyss,2010).

ATPases are biologically supported by cytoplasmic CK because it quickly produces ATP from phosphocreatine (PCr) in the vicinity of these enzymes. Blood pressure is believed to rise as a result of increased vascular resistance and stroke volume brought on by increased cardiovascular contractility and sodium retention. The symbol for heart rate is HR (Salomons and Wyss, 2010).

By catalysing the conversion(reversible) of ATP and creatine into ADP and phosphocreatine, an enzyme known as creatine kinase (CK) aids muscle cells in storing and transporting energy (Aujla and Singh, 2020). In clinical settings, high levels of CK, particularly the CK-MB isoenzyme, are sensitive markers of myocardial damage, including infarction (Martínez *et al*., 2021). CK level monitoring is also useful in research settings to evaluate drug-induced cardiac or muscle toxicity, offering information about possible side effects of pharmaceutical drugs.

b) Cardiac Troponin 1 (cTn1)

A protein called troponin is present in some muscles, including the heart muscle. It is essential for heart muscle contraction, that is, it aids in heartbeat (Thygesen *et al*., 2018).

Normally, the blood contains very little troponin. However, this is altered if the heart muscle is damaged. A portion of the troponin is then released and starts to circulate in the blood. Troponin is released in proportion to the degree of heart injury (Katz *et al*., 2018).

Oxygen-rich blood cannot get to the heart during a heart attack. The heart muscle is harmed as a result. Troponin is then released into the blood as a result (Thygesen *et al*., 2018).

There are three different kinds of troponins

.i) Troponin I (TnI) is unique to the heart muscle. After the heart is damaged, TnI level will stay elevated for four to seven days (Thygesen *et al*., 2018).

ii) The heart muscle contains troponin T (TnT), which is also present in other muscles in trace amounts. However, the heart's TnT structure is a little different from other parts of the body. This enables medical professionals to identify the source and determine the location of the harm.

iii) Troponin C (TnC) is present in the heart muscle and other muscles, thus TnT level will stay elevated for at least a few days, possibly up to three weeks (Katz *et al*., 2018). A heart attack can only be diagnosed using the first two, TnI and TnT. This is due to the fact that it is impossible to determine whether TnC originated in the heart muscle or another muscle (Thygesen *et al*., 2018).

Cardiac Troponin I (cTnI) is an essential part of the troponin complex that controls the contractility of the heart. Elevated troponin I levels are regarded as the gold standard biomarker for myocardial damage because of their great specificity for cardiac tissue (Rifai, 2022). In clinical diagnostics, elevated troponin levels are frequently used to detect acute coronary syndromes, including heart attacks (Rifai, 2022) . Increased troponin I levels can also indicate drug-induced cardiac stress or toxicity, according to research investigations, offering important information about the cardiotoxic potential of pharmacological drugs.

c) Lipid profile Component

Triglycerides, high density lipoprotein (HDL), total cholesterol(TChol) and Low density lipoprotein (LDL), are among the measurements that make up a lipid profile, which gives a complete picture of a person's lipid health(Sharma *et al*., 2020). Elevated LDL, triglycerides, and lower HDL are linked with a great danger of cardiovascular disease and atherosclerosis (Sharma *et al*., 2020). In order to guide therapeutic measures targeted at lipid management, lipid profiles are helpful for evaluating the metabolic effects of medications and measuring overall cardiovascular risk (Sharma *et al*., 2020).

2.5 Cholesterol

To meet the body's demands, the liver produces adequate cholesterol, it accounts for around 80% of the body's total cholesterol, the remainder is gotten from the food eaten (Sherwood, 2018). Foods heavy in trans fat, such as packaged confectionery and fried foods, or saturated fat, such as whole-fat dairy and red meat, are the main sources. Usually, the body is able to filter out unnecessary cholesterol. However, a variety of factors can impact the amount of cholesterol that remains in the body, these include age and inherited genes (Sherwood, 2018).

All of the body's cells contain cholesterol. It also moves through the blood, but due to the way it is formed, it is unable to do so on its own (Sherwood, 2018). Molecules of cholesterol must be bonded to other molecules. Thus, cholesterol combines with proteins and triglycerides, another kind of lipid (Sherwood, 2018). A lipoprotein is a particle that is created when these molecules combine. The body uses lipoproteins to move cholesterol, they pass through the bloodstream like little boats. They transport cholesterol to the tissues in the body so they can function correctly. Reverse cholesterol transfer is the process by which some lipoproteins carry excess cholesterol to the liver, which is later broken down and eliminated (Sherwood, 2018). It aids in maintaining normal blood cholesterol levels.,

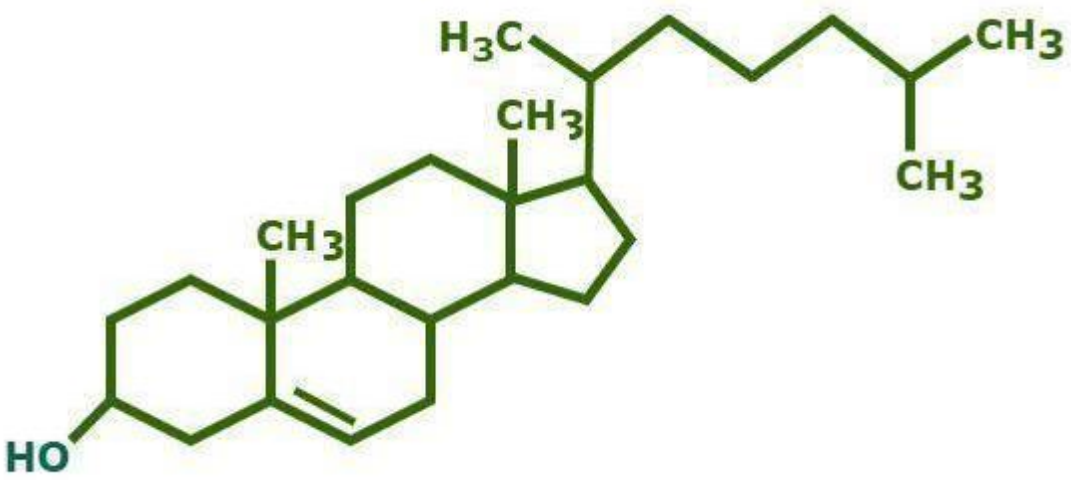

Figure 3: Structure of Cholesterol
Unnumbered 12p350b
Biochemistry, seventh edition
Source: Huang and Freter (2016)

Cholesterol chemical formula $C_{27}H_{46}O$, it is composed of 46 hydrogenatoms, one(1) oxygen atom and 27 carbon atoms(Sharma *et al*.,2020)

Cholesterol types

Excessive LDL cholesterol may increase the risk of heart disease, thus, regarded as harmful cholesterol, High HDL cholesterol is regarded as cholesterol(good) because of its benefit to the body (Sharma *et al*., 2020).

High density Lipoprotein (HDL)

By returning cholesterol to the liver and preventing it from building up in arteries, HDL lowers the amount of blood cholesterol. Therefore, high HDL lowers the chance of heart attack and disorder (Sharma *et al*., 2020). HDL range (more than 40 mg/dL) (Sharma *et al*., 2020).

Low-density Lipoprotein (LDL)

LDL makes up most of the cholesterol in the body. LDL accumulates in the walls (blood vessels), resulting in them to stiffen, narrow, and blocking blood flow (Sharma *et al*., 2020).

This raises blood LDL levels and raises the danger of a heart attack or stroke. LDL cholesterol should not exceed 100 mg/dL (Sharma *et al*., 2020).

2.6 Triglycerides (TGs)

TGs are lipid molecules that have been esterified to glycerol varying lengths and compositions consisting of three fatty acid chains (Akivis *et al*., 2024). Each of these chains of fatty acids has a unique chemical composition and may be unsaturated or saturated. Each chain is composed of hydrogen and carbon atoms with distinct chains (single or double-bonded), depending on the level (saturation or unsaturation) (Akivis *et al*., 2024). Structural comparisons between mixed chains of TGs reveal heterogeneity (Akivis *et al*., 2024). The form in which the body stores energy is called TG, and it is the most prevalent dietary lipid molecule. During the initial digestion of dietary TGs, pancreatic lipase hydrolyses one(1) fatty acid chain at a time, resulting in one 2-monoglyceride (2MG) molecule and two free fatty acid (FFA) chains for each TG(Akivis *et al*., 2024). When fatty molecules are present in the ingesta, the duodenum releases bile salts in response to the production of cholecystokinin. Bile salts promote lipid micelles, which are composed of lipid molecules,

including FFA, with a hydrophilic surface and a hydrophobic interior (Akivis *et al*., 2024). Lipid molecules can be absorbed into the enterocyte (metabolic usage) through both diffusion across the cellular membrane and lipid transporters (luminal side) of the enterocyte (Akivis Y., *et al*., 2024). 2MG compounds and FFA chains are transported to the endoplasmic reticulum after entering the enterocyte. There, they are re-esterified into TGs and packaged into chylomicrons, which are the biggest and least dense lipoproteins. The Golgi apparatus then receives apolipoproteins unique to chylomicrons, for example, apo B48, a marker for TG chylomicron. (Akivis Y., *et al*., 2024). The lymphatic system carries these freshly created chylomicrons to circulation once they are released from the enterocyte, avoiding the portal vein (Akivis Y., *et al*., 2024). The TG-rich chylomicrons move through the vasculature in the blood and go through a complicated protein exchange process that is mediated by HDL. Depending on the outcome of this process, either the liver absorbs the chylomicrons for further processing and packing, or lipoprotein lipase (LPL) delipidates them at the arterial endothelial surface (Akivis Y., *et al*., 2024). The majority of chylomicrons that contain dietary TGs are absorbed by the liver, where they are transported to peripheral tissues as very-low-density lipoprotein (VLDL) (Akivis *et al*., 2024). Endogenous synthesis of TGs occurs in the liver via *de novo* lipogenesis or glycerol esterification with fatty acids from dietary intake. These TGs are incorporated into VLDL particles carrying a unique marker apolipoprotein B-100 on their surface as they enter systemic circulation (Akivis Y., *et al*., 2024).

Both chylomicrons and VLDLs are the major TG-rich lipoproteins and act as carriers of TGs in serum, transporting them to peripheral adipose tissue and skeletal muscle, for energy production or storage (Akivis Y., *et al*., 2024).

A smaller portion of FFA is transported as an unesterified form that is complexed to albumin (Akivis Y., *et al*., 2024). TG is eliminated at several LPL receptor sites by LPL throughout the

endothelium, including adipose and muscle tissue, after the VLDL enters the peripheral tissues and goes through a delipidation cascade (Akivis Y., *et al*., 2024).After delipidation, the majority of the TG that was initially packed is liberated into a VLDL remnant (intermediate density lipoprotein), which is either eliminated by the liver or transformed into lipoprotein(LDL) by the process of protein exchange(serum) (Akivis Y., *et al*., 2024). TG provides 9 Kcal/g of FFA, making it a significant high-energy molecule for energy storage. Apart from store-specific transmembrane proteins that help muscle tissue and adipocytes produce lipid droplets for later usage as an energy source, LPL recognises and removes lipids meant for storage from VLDL (Akivis Y., *et al*., 2024). Apart from store-specific transmembrane proteins that help muscle tissue and adipocytes create lipid droplets to benefit as a source of energy later on, LPL recognises and removes lipids that are meant for storage from VLDL (Akivis Y., *et al*., 2024). When the circulating systemic nutrition supply is insufficient to fulfil the metabolic energy demand, TGs are released from lipid reserves under metabolic stresses. Adipose TG lipase, hormone-sensitive lipase, and monoacylglycerol lipase are activated by cAMP-independent pathways and cyclic adenosine monophosphate (cAMP)-mediated that regulate the enzymes required for lipolysis (Brockerhoff, 2012). These enzymes hydrolyse the bonds (ester) of stored TG to produce and FFA chains and glycerol (Brockerhoff, 2012). After being generated from adipocytes for use in energy generation, FFAs can be metabolised into signaling molecules, delivered to serum, or esterified (Brockerhoff, 2012). These lipid molecules proceed through fatty acid oxidation, which yields substrates for oxidative phosphorylation and acetyl-coenzyme A for hepatic ketogenesis. FFA and TGs have been linked to the development of atherogenic lipoprotein levels that contain TG and FFA (Brockerhoff and Jensen, 1974). As mentioned previously, an increase in TGs can be a sign of insulin resistance when low HDL and high LDL are present. Due to their elevated circulating

chylomicron levels, high VLDLs, and small LDL and HDL particles, patients with this lipid profile are at risk for coronary heart disease (Brockerhoff and Jensen, 1974). The US Preventive Services Task Force recommends lipid screening for women who are 45 years of age or older and are at a higher chance of coronary heart infection, and for men and women who are 35 years of age or older (Brockerhoff and Jensen, 1974).

The Agency for Healthcare Research and Quality offers similar suggestions to begin routine screening at age 35 or for people with heart disease risk factors who are between the ages of 20 and 35. There are currently no recommendations for routine lipid screening in young individuals (20–35 years old) who do not have a risk of coronary heart disease. Similarly, there is insufficient data to warrant routine lipid screening in children, and as a result, there is no recommendation. When non-fasting TGs surpass 440 mg/dL (5 mmol/L), repeating a fasting sample is recommended. Abnormal TGs on screening lipid panels may be caused by other causes of hypertriglyceridemia; hence, clinical evaluation should focus on identifying and treating suspected secondary causes. Secondary hypertriglyceridemia can be induced by a number of medical conditions, including diabetes (uncontrolled), hypothyroidism, nephrotic syndrome, renal disease (end stage), metabolic syndrome, obesity, systemic lupus erythematosus, sepsis, paraproteinemia, pregnancy (particularly in the third trimester), and HIV.. Corticosteroids, oral oestrogens, anti-depressants, protease inhibitors, non-cardioselective β-blockers, thiazide diuretics, bile-binding resins, oral oestrogens, progestins, tamoxifen, cyclophosphamide, cyclosporine, protease inhibitors, clozapine, antiretroviral therapy, and other common drugs and treatments can all raise TG levels (Li, 2015)..

Patients with malnutrition, hyperthyroidism, and malabsorption syndrome have low serum TG levels(Li, 2015).

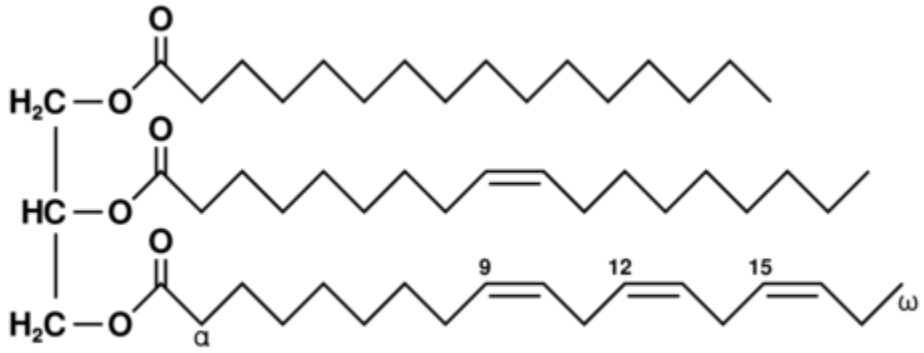

Figure 4: Triglyceride structure composed of three fatty acids (palmitic, oleic and linolenic acids)

2.7 The Renal System (Kidney)

The kidneys are paired, retroperitoneal organs characterized by a complex structure designed to facilitate their primary functions in filtration, reabsorption, and secretion (Guyton and Hall, 2016). Each kidney consists of approximately one million nephrons, the fundamental functional units, which comprise the glomeruli, tubules, and associated vasculature. The glomeruli are specialized capillary networks responsible for filtering blood plasma into the Bowman's capsule, initiating urine formation. Subsequently, In order to maintain homeostasis, waste and surplus ions are secreted by the renal tubules into the tubular fluid while reabsorbing vital nutrients, electrolytes, and water back into the blood (Brenner and Rector, 2015). This intricate process maintains systemic fluid, electrolyte, and acid-base balance, as well as removing metabolic waste products. The kidney's capacity to carry out these functions is important in preserving homeostasis, the internal environment of the body. A quarter of the resting cardiac output is transferred to the kidney vascular bed. As a result, glomerular, tubular, and interstitial cells regularly come into contact with high concentrations of harmful substances, such as drugs and their metabolites, which can alter the function and structure of the renal system (Brenner and Rector, 2015). The kidneys, ureters, and urethra are all part of the renal system. Its immediate role is to sift two hundred (200) litres of liquid from kidney flow (blood) per day. It keeps vital elements in the bloodstream while eliminating toxins, excess ions, and metabolic waste products (Brenner and Rector, 2015). The renal regulates plasma osmolarity by modifying the solute, electrolyte levels, and blood's fluid (Brenner and Rector, 2015). They assist in sustaining acid-base equilibrium, produce erythropoietin (long term), and promotes formation of blood cells (red). They produce renin, control blood pressure, and transforms vitamin D into its active form (Brenner and Rector, 2015).

Change in blood flow, direct cell and tissue damage, inflammatory tissue damage, or complications with renal excretion can all lead to renal toxicity (Brenner and Rector, 2015).

Glomerular Filtration (GFR)

Glomerular filtration (GFR) is the foremost phase in the synthesis of urine (Gottschalk *et al*., 2013). Fluids and solutes without the need for energy are passively forced across a membrane (hydrostatic pressure) (Gottschalk *et al*., 2013). The membranes include three (3) layers namely:

i) The podocytes' foot processes (glomerular capsule), which create a filtration system that is more selective (Gottschalk *et al*., 2013).

ii) the glomerular capillaries' fenestrated endothelium, allows blood components to pass through them.

iii) The basement membrane; functions as a charged barrier, stopping the movement of proteins ((Gottschalk *et al*., 2013). The movement of water and other substances inside and outside the capillaries is determined by a force.

The main force for filtration comes from the hydrostatic pressure (glomerular capillaries), which is 55 mmHg (Gottschalk *et al*., 2013). Permeability of the filtration membrane, total surface area, net filtration pressure available for filtration, all affect GFR, as well as measures how much fluid is filtered each minute. Normally, GFR (120 to 125 ml/min) (Gottschalk *et al*., 2013). Both intrinsic and extrinsic mechanisms work together to regulate GFR. The intrinsic control system uses tubuloglomerular and myogenic feedback to modify its own blood flow resistance. The myogenic process narrows the afferent arteriole in response to high blood pressure, which helps maintain GFR; conversely, a low afferent arteriole pressure causes the vascular smooth muscle to relax, increasing the flow of blood. The feedback mechanism (tubuloglomerular) regulates the quantity of sodium chloride (NaCl) in the tubule to hold a constant glomerular filtration (GFR).

NaCl levels along the ascending limb of the nephron loop are detected by macula densa cells (Gottschalk *et al*., 2013). Higher GFR from elevated blood pressure shortens the time for salt reabsorption and raises the sodium content in the tubule. Conversely, when pressure is low, the macula densa produces fewer vasoconstricting substances, which promotes Na reabsorption and lowers its concentration in the tubule (Gottschalk *et al*., 2013). Extrinsic control keeps systemic blood pressure and GFR constant through the sympathetic nervous system (SNS) and the renin-angiotensin-aldosterone pathway (RAAS). When the extracellular fluid volume falls too much, norepinephrine and adrenaline are released(Gottschalk *et al*., 2013). This encourages vasoconstriction, which reduces blood flow and the GFR of the kidney (Gottschalk *et al*., 2013). Additionally, as blood pressure falls, the renin-angiotensin-aldosterone (RAAS) axis is triggered through three (3) different processes. First, the kidney's granular cells release renin when the beta-1 adrenergic receptor is activated. The cells (macula densa), which detect low sodium chloride concentrations during reduced renal flow of blood, are the second mechanism, renin is produced by the granular cells as a result. According to (Gottschalk *et al*., 2013), the stretch receptor that envelops the granular cells is known as the third mechanism. Renin, which regulates glomerular filtration, is released by the kidney in response to a decline in blood low.

2.7.2 Reabsorption through tubules

According to Holstein-Rathlou *et al*. (2011), the absorptive properties of each of the four tubular segments are unique, the major capacity for absorption is found in PCT cells. Usually, the PCT reabsorbs 65% of Sodium and water in addition to glucose and amino acids (Johnson *et al*., 2023). A basolateral Na-K pump is the main active transport mechanism the PCT uses to reabsorb sodium ions. An electrochemical gradient is what propels passive paracellular diffusion, while secondary active transport with Na facilitates the reabsorption of glucose, amino acids and vitamins. Solute

reabsorption drives osmosis, the process by which water is reabsorbed by the PCT (Johnson *et al*., 2023). Moreover, passive lipid-soluble solutes are reabsorbed by passive diffusion; it is activated by the concentration gradient produced by water reabsorption. According to Johnson *et al*., (2023), the PCT reabsorbs urea driven by a chemical gradient through inactive paracellular diffusion .The loop of the nephron receives the non-reabsorbed filtrates from the PCT. The loop of the nephron has descending and ascending branches that serve different purposes. Osmosis reabsorbs water in the descending limb. Aquaporins enable this process. In this case, solutes are not taken up again (Johnson *et al*., 2023). Conversely, In the upper limb, a symporter reabsorbs potassium, chloride, and sodium collectively in the thick segment while sodium passively travels in the thin segment along its concentration gradient (Johnson *et al*., (2023). In the basolateral membrane the Na-K ATPase keeps the symporter's functionality by generating an ionic gradient, the passive paracellular diffusion that reabsorbs calcium and magnesium ions in the ascending limb is driven by electrochemical gradients. Water is not reabsorbed by the ascending leg (Johnson *et al*., (2023). The second tubular segment involved in reabsorption is known as the distal convoluted tubule (DCT), primary active sodium transport (basolateral membrane), secondary active sodium transport is enabled by Na-Cl symporters and channels (apical membrane). Aldosterone controls the distal portion of this process. On the other hand, parathyroid hormone controls calcium's passive absorption. Aldosterone stimulates the synthesis of Na-K ATPase and the growth and maintenance of apical Na and K channels in the cells of the distal part of the DCT. In a collecting tubule, the last phase of reabsorption occurs right after the DCT. These reabsorptions include; primary active sodium transport (basolateral membrane), secondary active transport (apical membrane) via the Na-Cl symporter and channels controlled by aldosterone, passive calcium

uptake through PTH-modulated channels (apical membrane), and primary and secondary active transport (basolateral membrane)(Johnson *et al*., (2023)

2.7.3 Tubular Secretion

Tubular secretion removes metabolites and drugs that attach to plasma proteins (Zhang *et al*., 2020b).Moreover, tubular secretion eliminates undesirable substances like uric acids and urea that were passively reabsorbed. According to Zhang *et al*.,(2020b) , tubular secretion activities include the elimination of potassium (excess) through the regulation of aldosterone hormone at the distal DCT and collecting duct.

When pH of the blood drops below normal, hydrogen ions are removed. Bicarbonic acid is then removed and chloride ions are reabsorbed (elevation of the blood pH above the normal range), they secrete ammonia, creatinine, essentials and many other organic acids (Zhang *et al*., 2020b).

2.7.4 Storage of Urine

According to Zhang *et al*., (2020b), urine is produced, passes through the ureter, a structure, and is subsequently deposited in the bladder. There are 2 ureters in the human body: one on the left and one on the right. These are slender tubes with three wall layers, specifically:

i) The ureter's exterior is covered in adventitia, a fibrous connective tissue.

ii) Muscular is, which is composed of an external circular layer and internal longitudinal layer.

iii) Mucosa, which comprises of epithelial tissue (transitional)(Zhang *et al*., 2020b).

Peristaltic contractile waves are produced when the smooth muscle of the ureters flexes, facilitating the passage of urine into the bladder (Zhang *et al*., 2020b). Due to the slanted insertion at the posterior bladder wall of the ureters, urine cannot flow backward. The bladder's special structure enables effective pee storage once the urine is within. The bladder (muscular sac) consists

of three layers with the exception of the layer(muscular), which has muscle fibres dispersed in internal and external longitudinal layers as well as central circular layer, its three layers resemble those of the ureter (Johnson *et al*., 2023). The detrusor muscle is also known as the muscular layer. Although the bladder's normal functional capacity is between 300 and 400 millilitres, its distensibility allows it to hold up to 1000 millilitres (Johnson *et al*., 2023). The smooth, triangular section, trigone of the bladder, has three openings, two (2) of the holes are located at the distal portions of the ureters insert, and the urethra orifice is one of them (Johnson *et al*., 2023). The urethra is a muscular tube with thin walls that drains the bladder of urine. Pseudostratified columnar epithelium makes up most of the mucosa lining, with transitional epithelial tissue found in the proximal area. The muscle (detrusor) thickens to produce the internal urethral sphincter at the bladder-urethra site. Which is regulated by the autonomic nervous system(Johnson *et al*., 2023) Urethra has a second role for men: it transfers semen. Spongy urethra, prostatic urethra, and membranous urethra make up the urethra for the male, which is approximately 22.3 cm long. Females, on the other hand, have an external urethral aperture that is between 3.8 and 5.1 cm long and situated posterior to the clitoris and before the vaginal opening (Johnson *et al*., 2023).

2.7.5 Micturition Process

The muscle (detrusor) contracts while the external and internal urethral sphincters relax during the micturition process (Zhang *et al*., 2020c). The procedure varies slightly according to age. The micturition process is governed by the spinal reflex in children under three. The detrusor muscle is stretched by the buildup of urine in the bladder, which activates receptors (stretch). The stretch sensation travels to the region (sacral) of the spinal cord by the afferent (visceral), it synapses with an interneuron that stimulates (parasympathetic neurons) and suppresses (sympathetic neurons) (Zhang *et al*., 2020c). The visceral afferent impulse simultaneously reduces the somatic efferent,

which normally maintains the urethral sphincter (external) closed to allow reflexive urine output (Zhang *et al*., 2020c). However, beyond the age of three, the urethral sphincter (external) can be consciously controlled, overriding reflexive urination (Zhang *et al*., 2020c). Pontine micturition center is activated by elevated bladder volume, suppresses (sympathetic nervous system), activates (parasympathetic nervous system), and causes consciousness of a full bladder (Zhang *et al*., 2020c). When the bladder is prepared to void, this causes the internal sphincter to relax and the exterior urethral sphincter to relax as well. Urine may build up in the bladder because reduced bladder capacity triggers the pontine depository center, which in turn stimulates the sympathetic nervous system and impedes the parasympathetic nervous system (Zhang *et al*., 2020c).

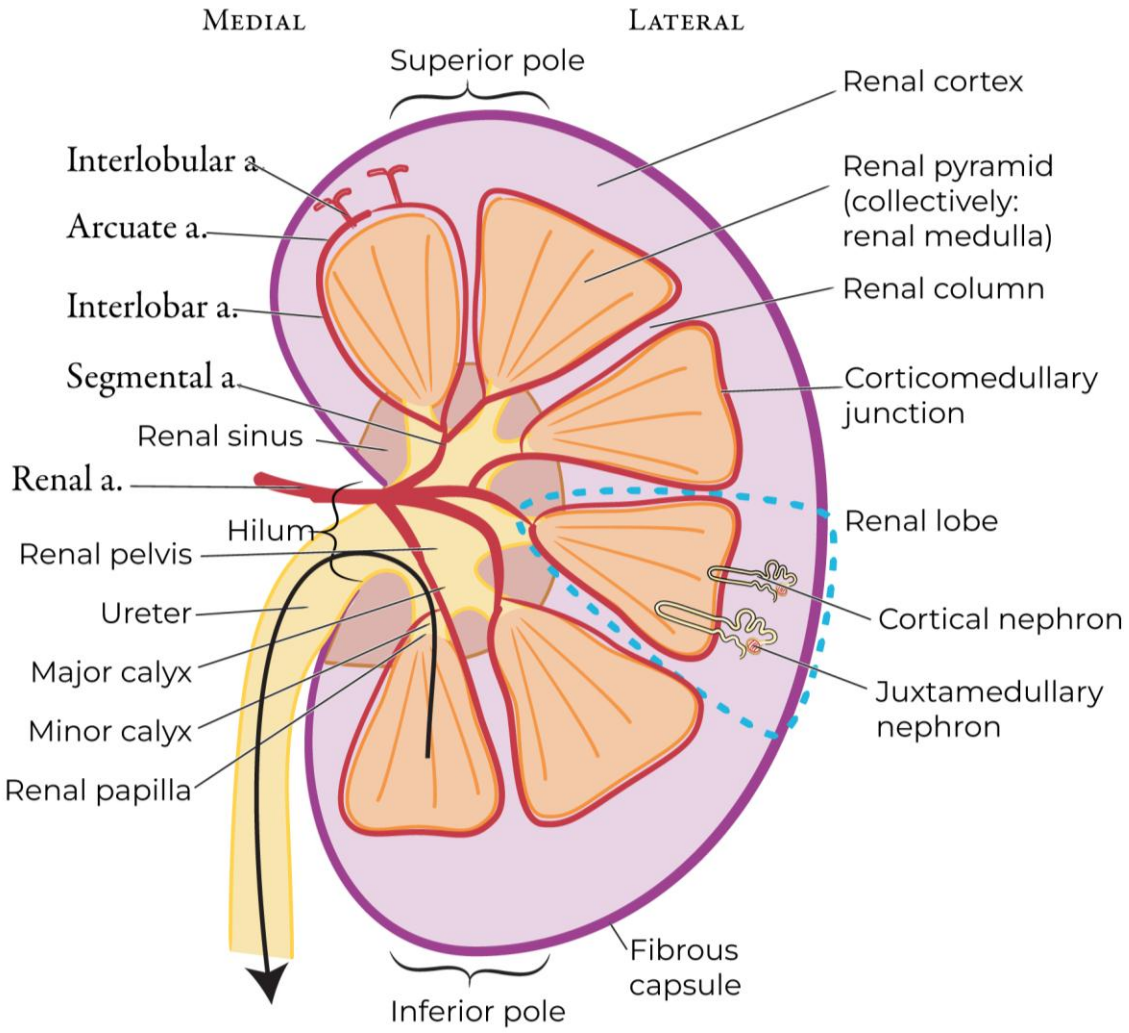

Figure 5: Kidney Left; Coronal Section
Source: https://ditki.com/course/gross-anatomy/urinary-system/drawing-highlights/1803/kidney/video

Renal Biomarkers

Creatinine,

The breakdown of creatine phosphate in the muscles produces a waste product. It is freely filtered by the glomeruli and is produced at a comparatively steady rate proportional to the muscle mass while being minimally reabsorbed, making it an ideal marker of GFR (Levey *et al*., 2006). Serum creatinine is used to calculate estimated GFR (eGFR) which is a quantitative estimation of renal function and also used to interpret and observe established kidney disease (CKD)(National Kidney Foundation, 2002).

Mechanism of Creatinine Excretion

It is the metabolism of muscle mass and is independent of age, sex, exercise, and diet. Due to their smaller muscular mass, women excrete less creatinine than men. The generation of creatinine is independent of protein catabolism and other external influences, and its daily excretion is ±15% per person. Protein metabolism and other outside variables have no effect on the excretion of creatinine. Thus, the best indicator of glomerular function (filtration) is serum creatinine. Only when 50% of renal function is lost does creatinine increase. Creatinine from tubular secretion is present in the urine in trace amounts. When the amount of creatinine in the blood grows, so does the amount of creatinine in the urine. Creatinine is directly correlated with kidney function since it is completely eliminated by the kidneys.

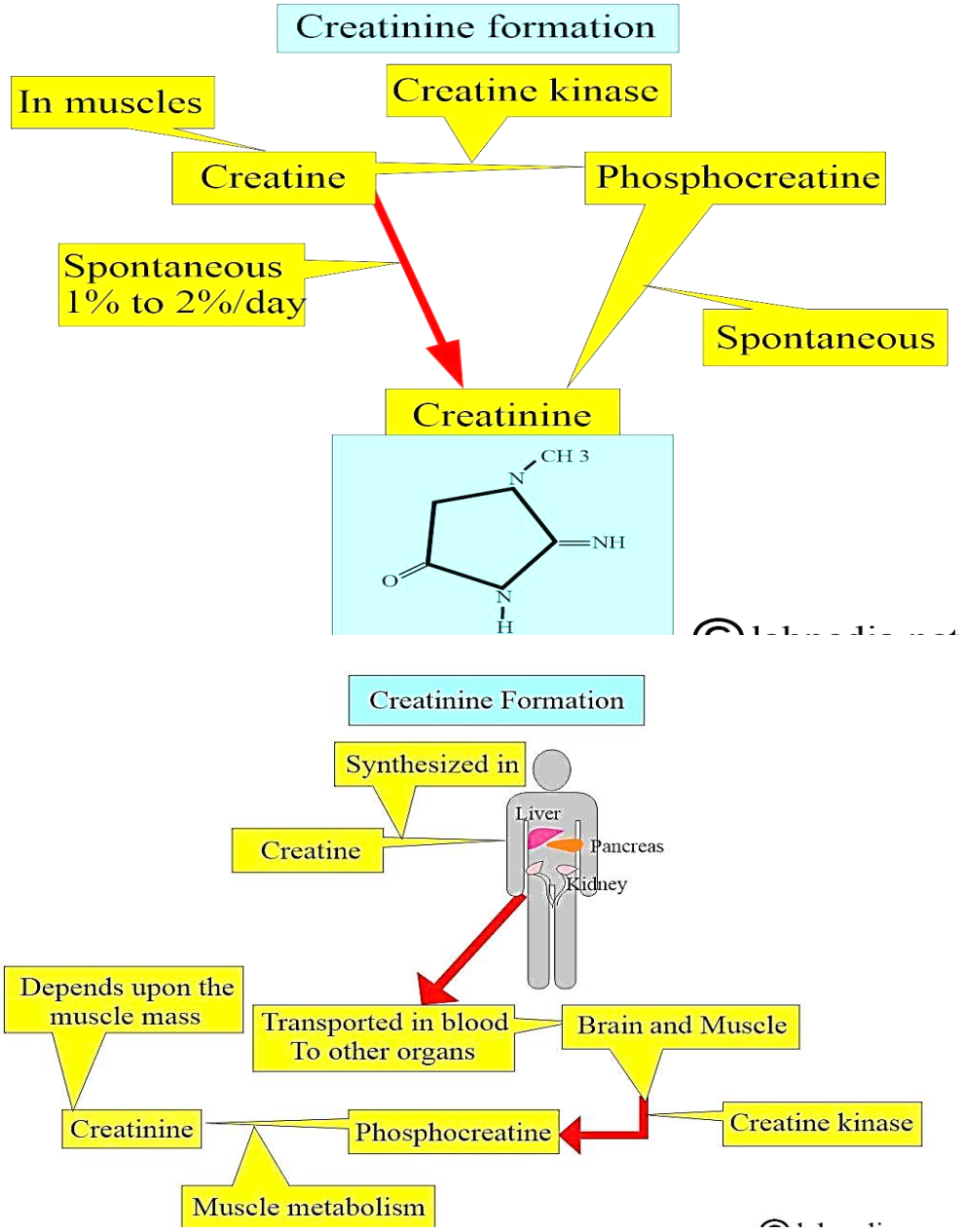

Figure 6: Creatinine Formation in Muscle and liver
Source: © labpedia.net

2.8 Uric Acid

Purine metabolism ends with Uric Acid synthesis. Blood containing a higher uric acid level in the blood is a hallmark of gout and may also signal renal impairment as a result of less excretion (Feig *et al*., 2013). Recent studies indicate that uric acid is also involved in oxidative stress and cardiovascular diseases, affecting endothelial dysfunction, and hypertension (Kang *et al*., 2015).

The Biochemistry of Uric Acid

The intestinal mucosa and liver produce uric acid. Through the GI tract, the kidneys eliminate 2/3 and 1/3. Uric acid is produced when nucleic acid is broken down. The final result of the catabolism of purine nucleosides, adenosine, and guanosine is uric acid (Feig *et al*., 2013).

sources of purines:

1) Present in a lot of diets.

2) It could result from the body's catabolism of guanosine and adenosine.

3) Uric acid is directly produced from purine derived from dietary nucleic acid (Kang *et al*., 2015).

4) Uric acid yields 400 mg per day in the body.

5) 300 mg per day is an additional dietary source.

The majority of purines in the body are eliminated in the urine as uric acid (Kang *et al*., 2015).

Monosodium urates are a kind of uric acid present in plasma. Urine, synovial fluid, and plasma all have higher uric acid solubility than water solutions. Urate levels in gout patients range from 18,000 to 30,000 mg. Increased purine precursor synthesis is the cause of this overproduction. Uric acid mostly forms in the liver (Kang *et al*., 2015).

The following factors determine blood uric acid level:

I) The hepatic rate of synthesis.

ii) The kidneys' rate of elimination.

iii) Removal of uric acid

The blood carries uric acid from the liver to the kidneys, 75% is filtered out of the kidney. In the GI tract, the remaining 25% is eliminated and broken down. Urates, which are soluble in urine, are the most common type of uric acid, with pH values greater than 5.57. The body accumulates uric acid when the uricase enzyme is deficient. Excessive cell breakdown raises the quantity of uric acid. Nucleic acid catabolism and inability to excrete, such as in renal failure (Feig *et al*., 2013). The levels of uric acid vary daily and are highly labile. Elevated by weight gain, fasting, and mental stress (Kang *et al*., 2015).

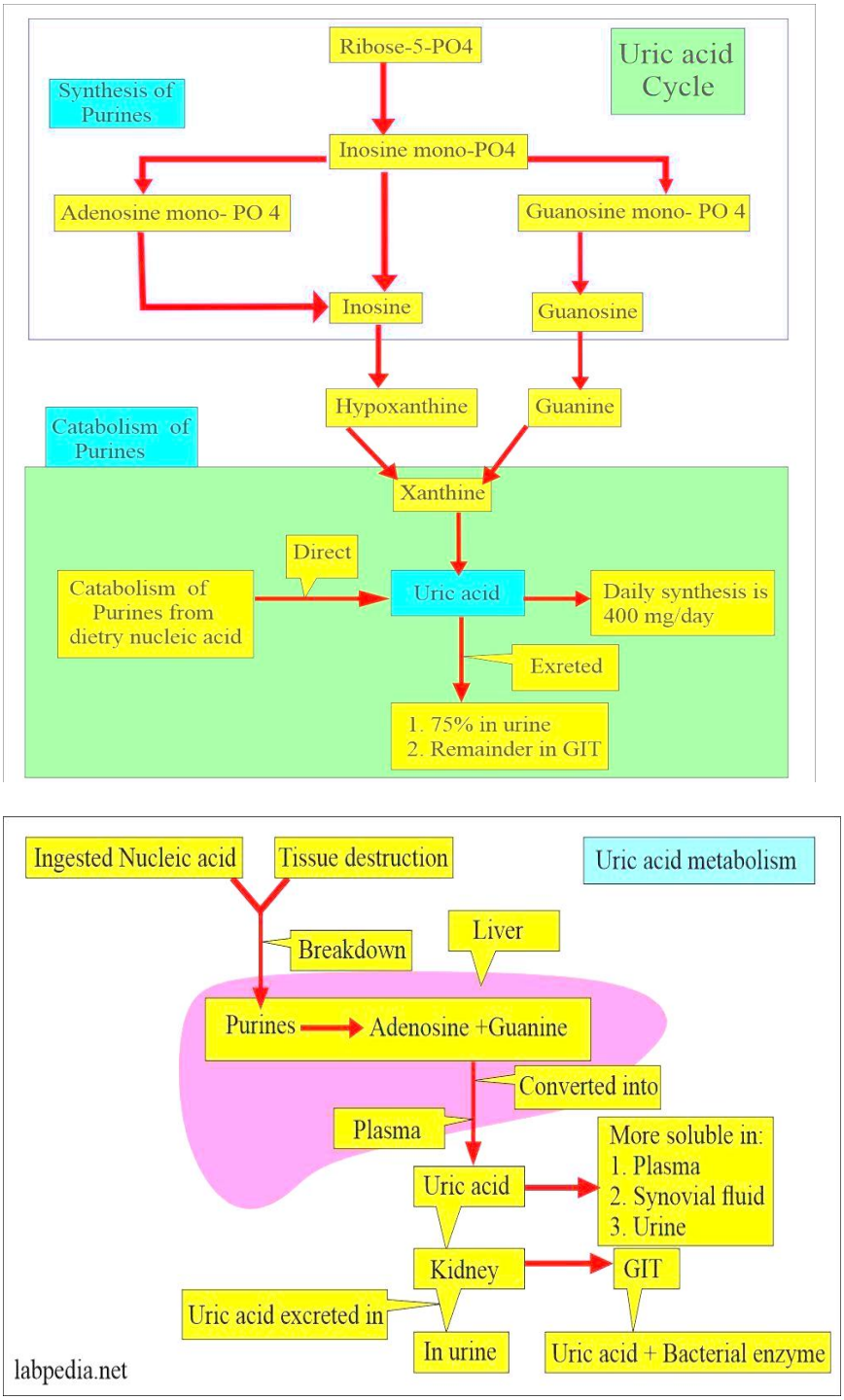

Figure 7: Synthesis of purine, urine acid cycle, Catabolism of purine and Uric acid metabolism
Source: labpedia.net

2.9 Urea

Most **urea** is formed during protein catabolism in the liver and is known as blood urea nitrogen (BUN). It's level in the blood is indicative of renal clearance and hydration. High BUN is common in renal failure, but can also happen when someone is dehydrated or has too much protein in their diet (Lloyd, 2019). So BUN is used in conjunction with serum creatinine to determine overall renal function.

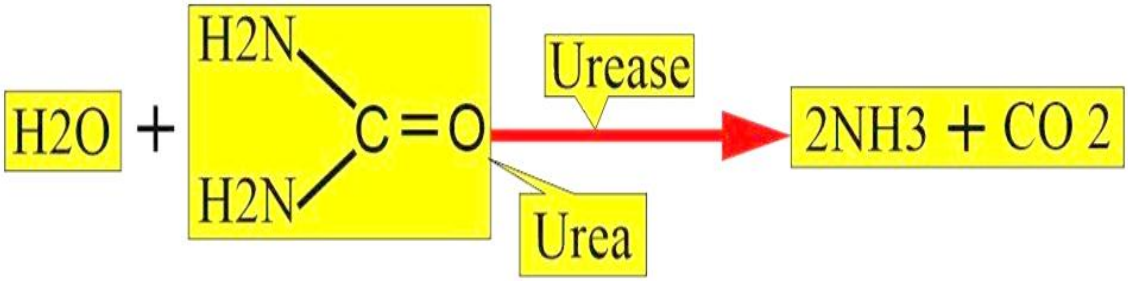

Figure 8: Blood urea and the action of the urease enzyme
Source: ©labpedia.net

2.10 Bicarbonate (HCO_3^-) ion as an electrolyte

Bicarbonate (HCO3-) is a significant component in the systemic acid-base balance. The proximal tubules reabsorb bicarbonate, whereas the distal tubules produce it. These processes are crucial for controlling acid-base disorders (Kasper *et al*., 2015). A key component of our bodies is bicarbonate. It is required for digestion and is secreted by the stomach. For instance, when consumed with mineral water, it lowers the acidity of food ingredients, helps buffer lactic acid produced during exercise, and prevents dental cavities (Kessler and Hesse, 2000). All bodily fluids and organs contain bicarbonate, which is essential to the human body's acid-base balances.The stomach is the first organ in our bodies where food, drink, and water are stored. Thirty million glands in the human stomach's mucous membrane create gastric juice that contains both acids and bicarbonate (Kessler and Hesse, 2000).The stomach can produce up to 1,200 μmol of bicarbonate every hour (73.2 mg/h), with a baseline output of 400 μmol (24.4 mg/h). Accordingly, at least 0.5 grams of bicarbonate are stored daily by the tummy (Pommier *et al*., 2010). The range of bicarbonate secretion rate (gastric) is 2 to 10% of the highest acid secretion level. Bicarbonate is a component of the stomach's mucus-bicarbonate barrier, which is known as the body's first cord of defence and healing. Sodium bicarbonate (6.17g) exits the stomach along with the fluid of the food as acid neutralises bicarbonate to form carbon dioxide (Kessler and Hesse, 2000). Bicarbonate anomalies are a reflection of the body's acid-base balance and renal tubular function and are commonly observed in acidosis of renal tubules.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Apparatus\equipment

The following equipment and instruments were used:

Colorimeter (by Louis J Duboscq 1870)

Centrifuge (Antonin Prandtl, 1864)

Incubator (Lyman Byce, 1879)

Cuvettes (James Franklin Hyde, 1934)

2UV- VIS Spectrophotometer

3.1.2 Reagents and chemicals

The following chemicals and reagents used were of analytical standard

Spectrum biochemical kits

Micropipette

3.1.3 Biological materials

Twenty five (25) adult albino rats

3.2 Methods

3.2.1 Collection of samples

Ocular Puncture

3.2.2 Collection of drugs

LEBAL-500 was bought from Manni pharmaceutical shop, Odumegwu Ojukwu Street, Thinkers Corner, Enugu state.

3.2.3 Collection of rats

The animals were brought to Godfrey Okoye University's animal house from the zoology department's animal home at University Of Nigeria (NSUKKA).

3.3 Preparation of drug solution

To make the stock solution, 1000mg of (10 tablets) were dissolved in 50mls of distilled water.

3.3.1 Animal handling and treatment

3.3.1 Animal Grouping

Twenty five (25) healthy adult albino rats were divided into five groups, A, B, C, D, and E each of which had five rats (n=5 rats). They were kept in iron cages under conventional settings (12-h light/dark cycle; 25-3°C; 35-60% humidity), as well as under stringent hygiene guidelines, and fed with standard commercial pallet meal and water. To reduce any unnecessary stress, the animals were accustomed to laboratory scenarios for fourteen days before the experiment.

3.3.2 Measurement of weight of animals

The weights of animals were taken on a daily basis using a weighing balance and the sample volume of the drug to be administered were calculated based on their body weight.

3.3.3 Drug administrations

The preparations were administered by oral intubation to each of the rats in groups C, D, and E respectively, after fourteen (14) days of acclimatization. Group A served as the control while Group B served as Positive control and was administered intraperitoneal single dose. The experimental design were summarized as follows:

Group A: Normal control (distilled water).

Group B: Administered 7.5mg/kg DOX once according to their body weight.

Group C: Administered 7.14mg/kg Lebal-500 each according to their body weight.

Group D: Administered 14.29mg/kg Lebal-500 each according to their body weight.

Group E: Administered 21.43mg/kg Lebal-500 each according to their body weight.

3.3.4 Collection of Samples from Animals

After five days of administration of the sample to the albino rats, they were starved overnight, under a mild anesthesia using chloroform, blood samples were collected from the albino rat by ocular puncture method. The blood samples were put into a plain sterile bottle free of anticoagulant.

3.3.5 Preparation of Sample

Five mills (5mls) of blood were collected from the animal (albino rat) in a sterile specimen bottle and allowed to cloth. It was centrifuged at 3000rpm for 5minutes and the serum separated from the plasma with the aid of a Pasteur pipette.

3.4 Determination of Serum Total Cholesterol

Principle

Total cholesterol (TC) was determined by the enzymatic end-point methods using cholesterol-oxidase peroxidase (CHOD-PAP) regent for hydrolysis and oxidation (Tietz, 1990). Indicator was quinoneimine formed from hydrogen peroxide and 4-aminoatipyrine in the presence of phenol and peroxidase.

Cholesterol ester $+H_2O \xrightarrow{\text{cholesterol esterase}}$ Cholesterol + fatty acid

Cholesterol $+ O_2 \xrightarrow{\text{cholesterol oxidase}}$ Cho-3one $+ H_2O_2$

$2H_2O_2$ + phenol + 4 – Aminoantipyrine $\xrightarrow{\text{peroxidase}}$ Quinoneimine $+ 4H_20$

Procedure

Distilled water (10ul), standard solution (10ul) and 10ul of samples were placed and labeled in different test tubes. Cholesterol determination regent (1000ul) was added to each test-tubes and

the content was gently mixed and then incubated for 10minutes at 25^{o}C. The spectrophotometer was adjusted to zero absorbance with the blank before the sample and standard were measured against the regent blank was measured at the wavelength of 500nm.

Calculations:

Cholesterol conc = $\frac{\Delta\, A\ sample}{\Delta\, A\ standard}\ x\ standard\ conc\ \left(\frac{mg}{dl}\right)$

Where $\triangle$A sample = Absorbance of sample

$\triangle$ A standard = Absorbance of standard

3.5 Determination of HDL Cholesterol

Principle:

Low density lipoprotein (LDL), very low density lipoprotein (VLDL) and chylomicrons fractions were precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation the cholesterol concentration on the HDL (High density lipoprotein) fraction, which remains in the supernatant, was determined (Tietz, 1990).

Procedure:

Test-tubes labeled reagent blank, standard and sample were set up, 100ul of distilled water was added to the reagent blank test tube only, 100ul of supernatant was added to the sample test tube, also 100ul of standard supernatant was added to standard test tube and then 1000ul of the regent was added to all the test tubes, mixed and incubated at 25^{o}C for 10 minutes. The spectrophotometer was adjusted to zero absorbance before the sample and standard against the regent blank was measured at the wavelength of 500nm.

Calculation:

Concentrations of HDL in supernatant = $\frac{\Delta\, A\ sample}{\Delta\, A\ standard}\ x\ standard\ conc\ \left(\frac{mg}{dl}\right)$

Where A sample = Absorbance of sample

A standard =Absorbance of standard

Conc of standard (mg/dl)= 206mg/dl

3.6 Estimation of Triglycerides

Principle

Triacylglycerols were determined after enzymatics hydrolysis with lipase by glyceride -3-phosphate oxidase – peroxidase (GPO-PAP) method (Tietz, (1990).

Indicator was quinoneimine formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase.

Triglycerides + H_20 $\xrightarrow{\text{Lipase}}$ glycerol + fatty acid

Glycerol -3-phosphate + ATP $\xrightarrow{\text{GPO}}$ Dihydroxyacetate + phosphate + H_20_2

$2H_20_2$ + 4- aminophenazone + 4- chlorophenol $\xrightarrow{\text{POD}}$ quinoneimine + HCl + $4H_2O$

Procedure:

Test tubes labeled reagent blank, standard and sample were set up, 10ul of standard solution was added to the standard test tube and then 1000ul of regents (R1a= buffer and R1b = enzyme) was at 25^{o}c for 10 minutes before the absorbance of the sample and standard against the regent blank was taken using a spectrophotometer at the wavelength of 500nm.

$$\text{Triglyceride conc} = \frac{\triangle \text{A sample}}{\triangle \text{A standard}} \times \text{standard conc (mg/dl)}$$

Where A sample = absorbance of sample

A standard = absorbance standard

Standard conc (mg/dl) = 191mg/dl.

3.7 Determination of LDL- Cholesterol in serum

Low density lipoprotein (LDL) concentration in the blood was calculated according to (Friedewald *et al.*, 1972).

LDL – cholesterol (mg/dl) = Total cholesterol - HDL-c - TG/5

3.8 Determination of serum bicarbonate ion concentration (Kaplan and Pesce, 1984).

Principle: Phosphoenol pyruvate carboxylase catalyses the reaction between phosphoenol pyruvate and bicarbornate to form oxaloacetate and phosphate ion. Oxaloacetate is reduced to malate with simultaneous oxidation of an equimolar amount of reduced nicotinamide, adenine dinucleotide to NAD; the reaction is catalysed by malate dehydrogenase. This result in a decrease in absorbance at 340nm that is directly proportional to CO_2 concentration in the sample.

Procedure

- CO_2 reagent was Prepared according to the reagent preparation.
- Tubes were labeled respectively with blank, standard, and samples.
- 1.0 ml of CO_2 reagent was Pipette into each tube.
- All the test tubes were incubated for 3mins at 37^{o}C.
- The spectrophotometer wavelength was set at 340nm, temperature to 37^{o}C.
- 5µl of water, standard and sample were pipetted into the cuvette and were labeled blank, standard, and sample respectively.
- They were mixed gently by inversion and incubated for 5mins.
- The absorbance was read at 340nm.
- Bicarbonate concentration was calculated.

Determination of creatine kinase (Steen *et al*., 2010)

Principle

ADP+ Creatine Phosphate $\xrightarrow{CK}$ Creatine + ATP

ATP + Glucose $\xrightarrow{HK}$ ADP+ Glucose-6-Phosphate

G6P + NAD $\xrightarrow{G6PDH}$ 6-Phosphogluconate + NADH + H+

CK catalyzes the reversible phosphorylation of ADP, in the presence of creatine phosphate, to form ATP and creatine. The auxiliary enzyme hexokinase (HK) catalyzes the phosphorylation of glucose by the ATP formed, to produce ADP and glucose-6-phosphate (G6P). The G6P is oxidizes to 6-phosphogluconate with the concomitant production of NADH. The rate of NADH formation, measured at 340nm, is directly proportional to serum CK activity.

Procedure

1. Reagents were prepared according to laboratory instructions.

2. The reagents were collected 1.0 ml each and dispensed into appropriate tubes and pre-warmed at 37°C for five minutes.

3. The Spectrophotometer was zeroed with water at 340nm.

4. Transferred 0.025ml of sample to the reagent, mixed and incubated at 37°C for two minutes.

5. After two minutes, the reading and the records of the absorbance were collected. The reading was repeated for two minutes.

6. The average absorbance was calculated per minute AAbs./min.

3.9 Determination of Cardiac Troponin I (Etievent *et al*., 1995)

Principle

The i-STAT cTnI test cartridge uses a two-site enzyme-linked immunosorbent assay (ELISA) method. Antibodies specific for human cardiac troponin I (cTnl) are located on an electrochemical sensor fabricated on a silicon chip. Also deposited in another location on the sensor silicon chip

are an antibody/alkaline phosphatase enzyme conjugate specific to a separate portion of the cTnI molecule. The whole blood or plasma sample is brought into contact with the sensors allowing the enzyme conjugate to dissolve into the sample. The cTnI within the sample becomes labeled with alkaline phosphatase and is captured onto the surface of the electrochemical sensor during an incubation period of approximately seven minutes. The sample, as well as excess enzyme conjugate, is washed off the sensors. Within the wash fluid is a substrate for the alkaline phosphatase enzyme. The enzyme bound to the antibody/antigen/antibody sandwich cleaves the substrate releasing an electrochemically detectable product. The electrochemical (amperometric) sensor measures this enzyme product which is proportional to the concentration of cTnI within the sample

Procedure

1. The desired number of coated wells was secured in the holder.
2. Standards, specimens, and controls were dispensed into the appropriate wells at 100µl each and were gently mixed for 10 seconds.
3. Enzyme Conjugate Reagent (100µl) were dispensed into each well and was thoroughly mixed for 30 seconds.
4. They were incubated at room temperature (18-25°C) for 90 minutes. The incubation mixture was removed by flicking plate contents into a waste container.
5. The microliter wells were rinsed and flicked 5 times with distilled or deionized water.
6. All residual water droplets were removed using paper towels and was sharply striked onto absorbent paper
7. T.M.B Reagent 100µl each was dispensed of into each well gently mixed for 5 seconds and Incubated at room temperature for 20 minutes.

8. The reaction was stopped by adding 100 µl of Stop Solution into each well and gently mixed for 30 seconds. Measures were taken to ensure that all the blue color changes to yellow color completely.
9. The absorbance was read at 450nm with a microtiter reader within 15 minutes.

Doxorubicin (DOX), an anthracycline antibiotic, is an excellent drug for the treatment of a wide variety of human solid tumors and leukemias. However, its clinical uses are limited by high incidence of toxicity (Eman *et al*., 2011). An initial acute effect includes hypotension and transient electrocardiographic abnormalities. Doxorubicin is converted in the tissues into its semiquinone form, which is a toxic, short-lived metabolite that interacts with molecular oxygen and initiates a cascade of reaction leading to ROS generation (Vikas *et al*., 2015).

CHAPTER FOUR

RESULT

4.1 Physical observation

There was a decrease in feed and water intake in the group administered levofloxacin & doxorubicin during the period of administration while no significant change was observed in the control which continued to thrive.

Physical activity of the groups that received levofloxacin reduced significantly during the period of administration.

4.2: Average weight of the rat during administration

The changes in average weight of the rats during administration are presented in table 4.1

There was a linear decrease in the average body weight of the rat administered the Levofloxacin & doxorubicin while the weight of the normal control increased. This change in average body weight was linearly dependent on the dose.

Table 4.1: The effect of LEVO and DOX on body weights of rats after five days of administration

Days	Group A (g)	Group B (g)	Group C (g)	Group D (g)	Group E (g)
1	87.20 ± 3.63	92.50 ± 4.32	123.01 ± 11.85	113.20 ± 3.56	96.20 ± 5.02
2	87.40 ± 3.65	90.40 ± 4.28	118.80 ± 11.90	108.80 ± 4.49	95.40 ± 4.39
3	93.60 ± 5.41	86.20 ± 4.10	112.80 ± 10.28	104.80 ± 4.96	93.40 ± 4.33
4	93.20 ± 5.80	87.10 ± 4.01	103.00 ± 9.59	101.00 ± 5.83	91.20 ± 4.60
5	96.80 ± 4.71	81.10 ± 2.92	99.20 ± 11.62	96.20 ± 4.14	83.20 ± 3.11

4.3 Cardiovascular parameters of the animals after administration of levofloxacin & doxorobicin.

The values of some Cardiovascular Parameters (C.K. activity, CTnI Concentration and Lipid profile) recorded in the animals after 5 days of administration as Shown in figures 9, 10, 11, 12, 13, 14.

The total cholesterol, triglyceride, Low density lipoprotein (LDL), CTn I & CK activity recorded in the treated groups (B, C, D, E) was Significantly ($P<0.05$) higher than in the normal Control (group A) on the Other hands, high density Lipoprotein (HDL) of the rats in the treated groups was Significantly ($P<0.05$) lower than in the normal Control.

This effect of Levofloxacin on the parameters was found to be dose dependent (I.e Increased with the doses)

Further, the effect of doxorubicin was significantly ($P <0.05$) higher than the highest dose of the drug (group E)

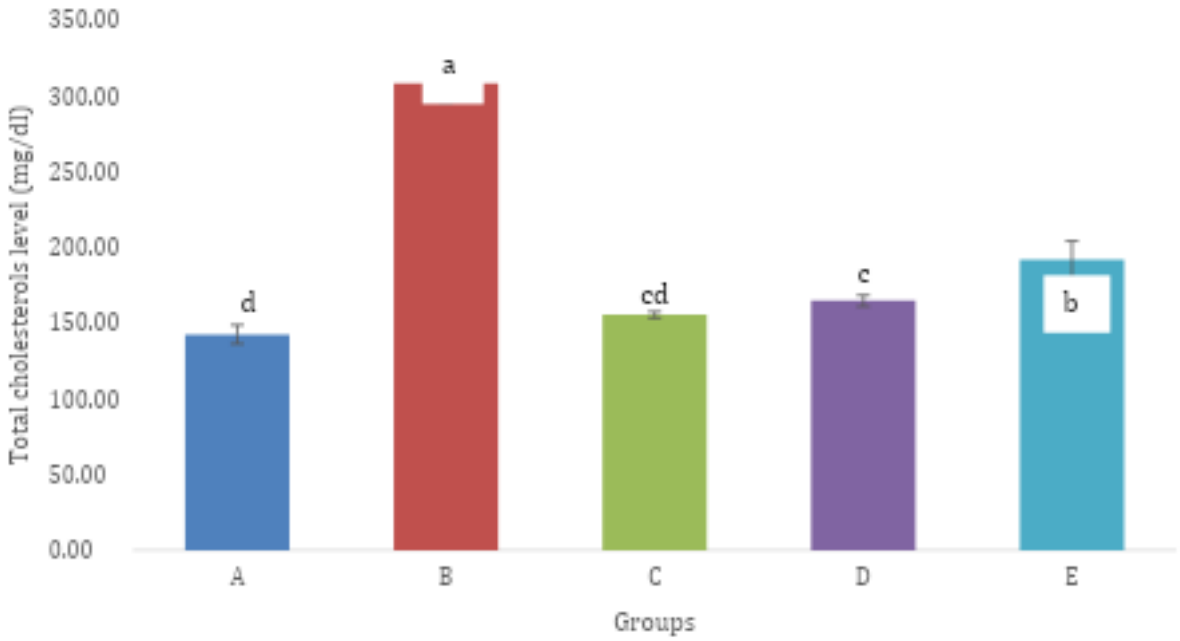

Figure 9: Effect of Lebal-500 on total cholesterols level (mg/dl) after administration
Values and mean ± SD: n = 5. Means with different alphabets differ significantly ($p<0.05$)
Key: group A = 0.2mls/kg distilled water (normal control). Group B = 7.5 mg/kg doxorobicin (DOX), Group C = 7.14 mg/kg levofloxacin (Levo), Group D = 14.29mg/kg Levofloxacin (Levo) Group E = 21.43 mg/kg Levofloxacin (Levo)

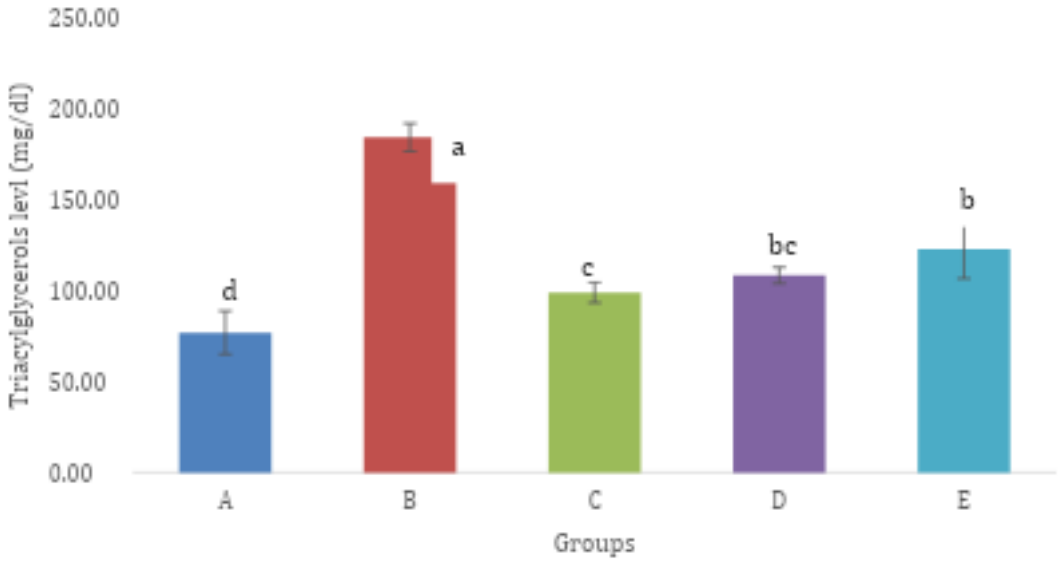

Figure 10: Effect of Lebal-500 on Triacylgycerols level (mg/dl) after administration
Values and mean ± SD: n = 5. Means with different alphabets differ significantly ($p<0.05$)
Key: group A = 0.2mls/kg distilled water (normal control). Group B = 7.5 mg/kg doxorobicin (DOX), Group C = 7.14 mg/kg levofloxacin (Levo), Group D = 14.29mg/kg Levofloxacin (Levo) Group E = 21.43 mg/kg Levofloxacin (Levo)

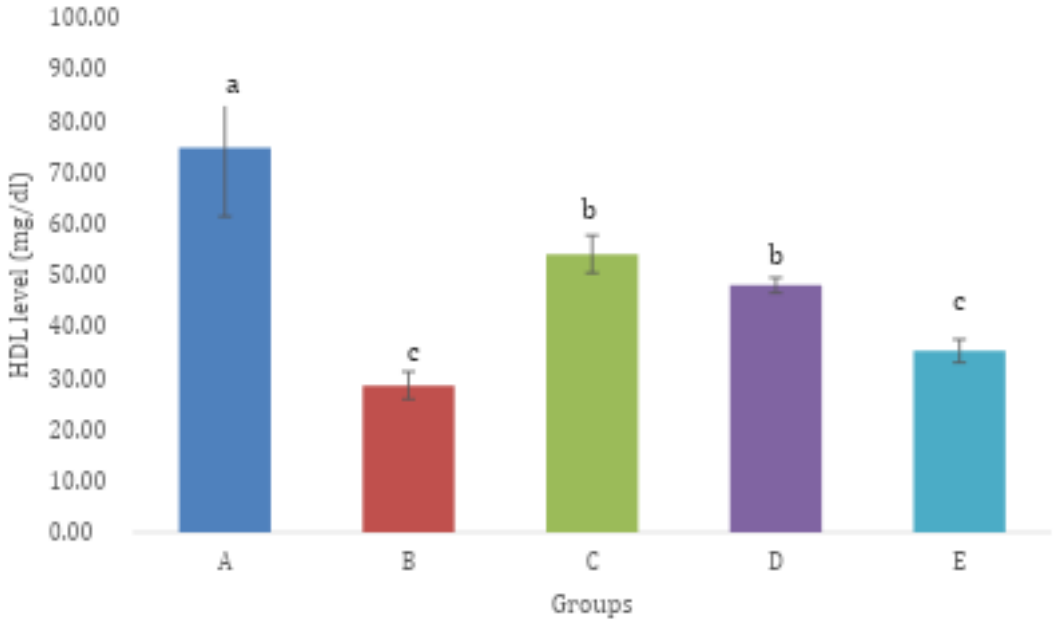

Figure 11: Effect of Lebal-500 on HDL level (mg/dl) after administration
Values and mean ± SD: n = 5. Means with different alphabets differ significantly ($p<0.05$)
Key: group A = 0.2mls/kg distilled water (normal control). Group B = 7.5 mg/kg doxorobicin (DOX), Group C = 7.14 mg/kg levofloxacin (Levo), Group D = 14.29mg/kg Levofloxacin (Levo) Group E = 21.43mg/kg Levofloxacin (Levo)

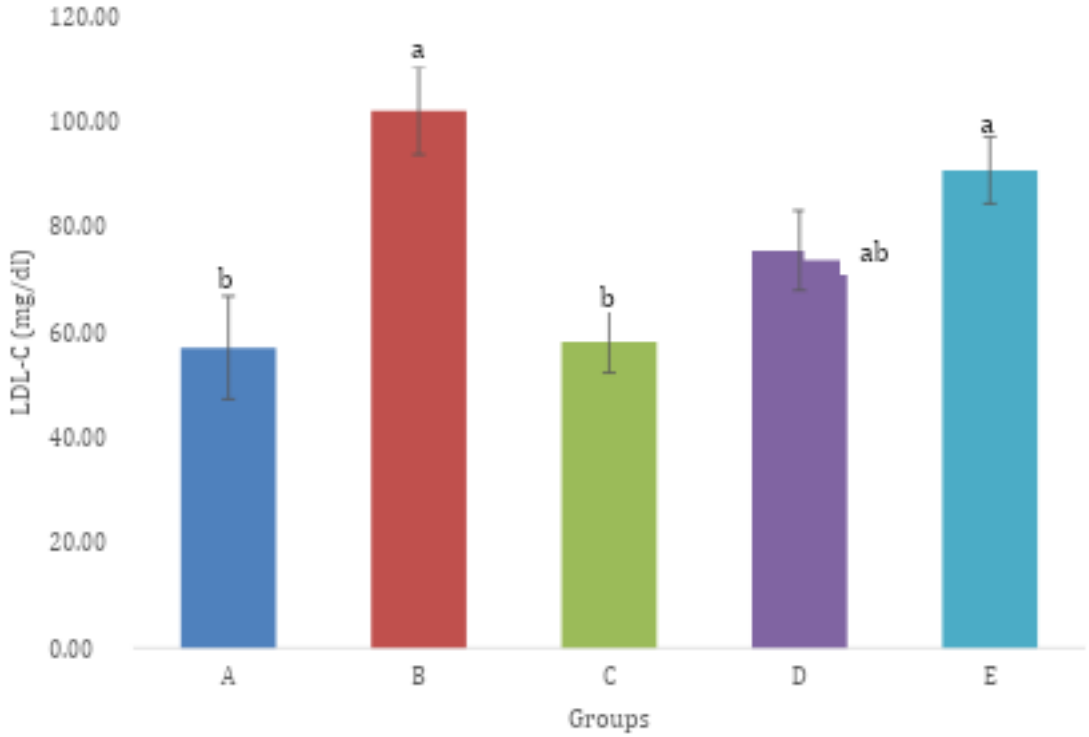

Figure 12: Effect of Lebal-500 on LDL-C level (mg/dl) after administration
Values and mean ± SD: n = 5. Means with different alphabets differ significantly ($p<0.05$)
Key: group A = 0.2mls/kg distilled water (normal control). Group B = 7.5 mg/kg doxorobicin (DOX), Group C = 7.14 mg/kg levofloxacin (Levo), Group D = 14.29mg/kg Levofloxacin (Levo) Group E = 21.43 mg/kg Levofloxacin (Levo)

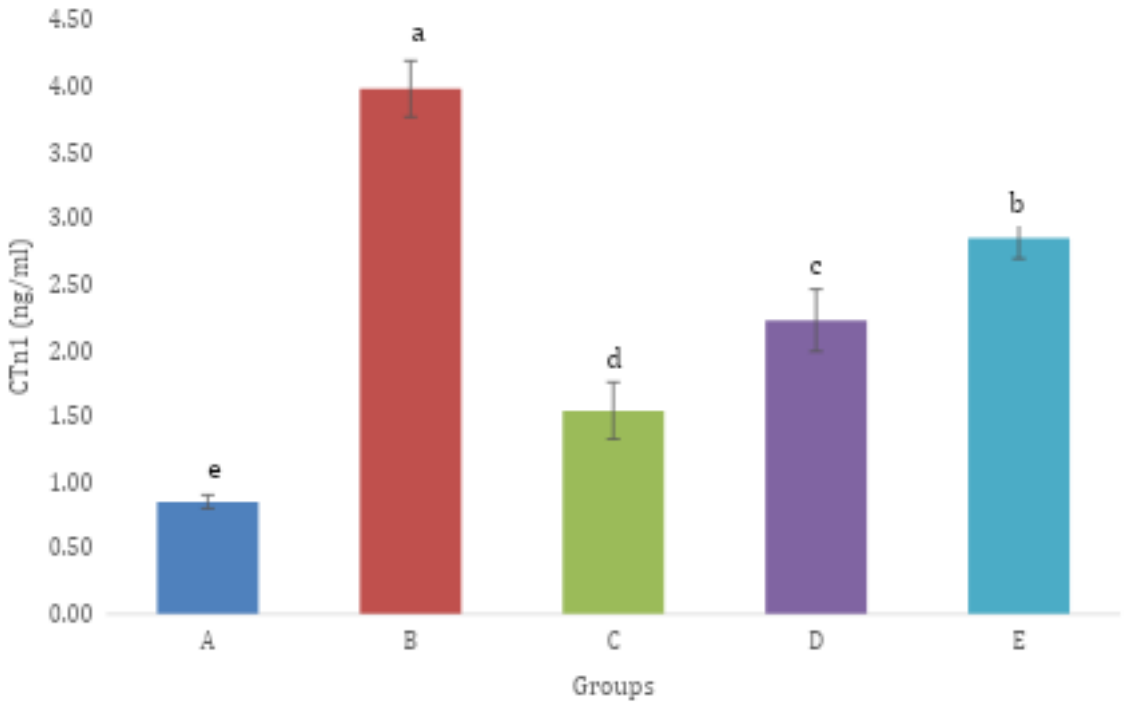

Figure 13: Effect of Lebal-500 on CTnI level (ng/dl) after administration
Values and mean ± SD: n = 5. Means with different alphabets differ significantly ($p<0.05$)
Key: group A = 0.2mls/kg distilled water (normal control). Group B = 7.5 mg/kg doxorobicin (DOX), Group C = 7.14 mg/kg levofloxacin (Levo), Group D = 14.29mg/kg Levofloxacin (Levo) Group E = 21.43 mg/kg Levofloxacin (Levo)

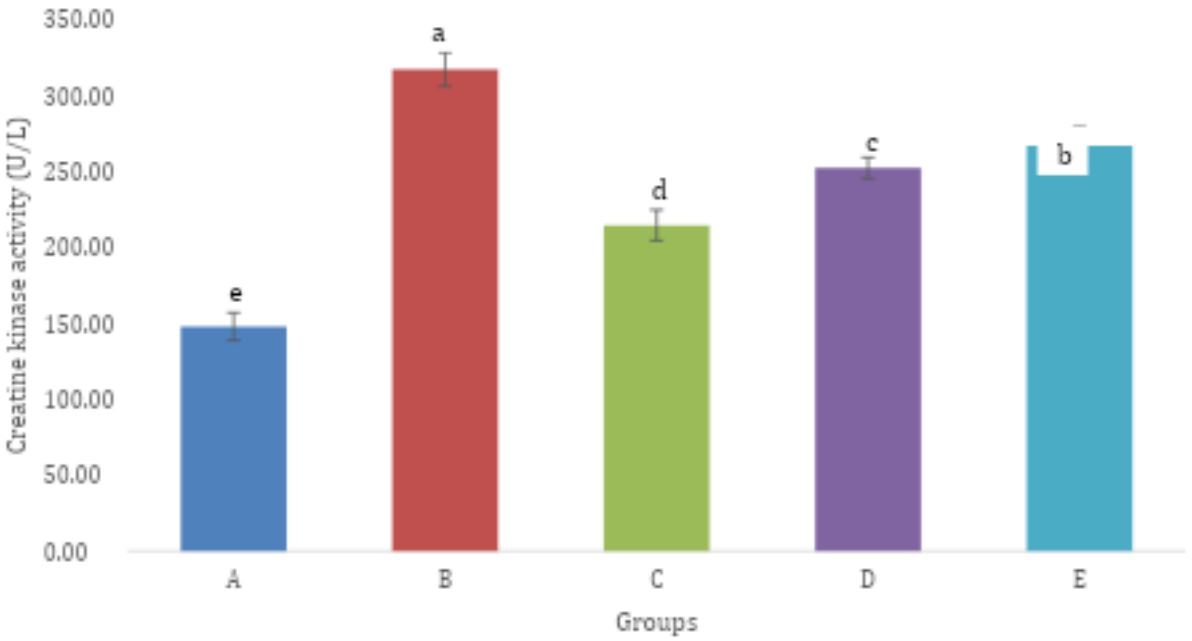

Figure 14: Effect of Lebal-500 on Creatine Kinase activity (U/L) after administration
Values and mean ± SD: n = 5. Means with different alphabets differ significantly ($p<0.05$)
Key: group A = 0.2mls/kg distilled water (normal control). Group B = 7.5 mg/kg doxorobicin (DOX), Group C = 7.14 mg/kg levofloxacin (Levo), Group D = 14.29mg/kg Levofloxacin (Levo) Group E = 21.43 mg/kg Levofloxacin (Levo)

4.4 The effect of Levofloxacin and Doxorubicin on the Renal System Parameters of the rats after administration.

The values of some Cardiovascular Parameters (Creatinine level, Urea Level, Bicarbonate Level and Uric Acid Level) recorded in the animals after 5 days of administration.

The creatinine, urea, uric acid and bicarbonate levels recorded in the group administered the highest dose of the levofloxacin (group E) and doxorubicin (group B) were significantly ($p<0.05$) higher than the control.

On the other hand, the difference between the groups administered lower doses of levofloxacin (group C and D) and the normal control was not significant ($p>0.05$).

The significant ($p>0.05$) increase in the renal parameters by doxorubicin was significantly ($p<0.05$) higher than that of the highest dose of the drug.

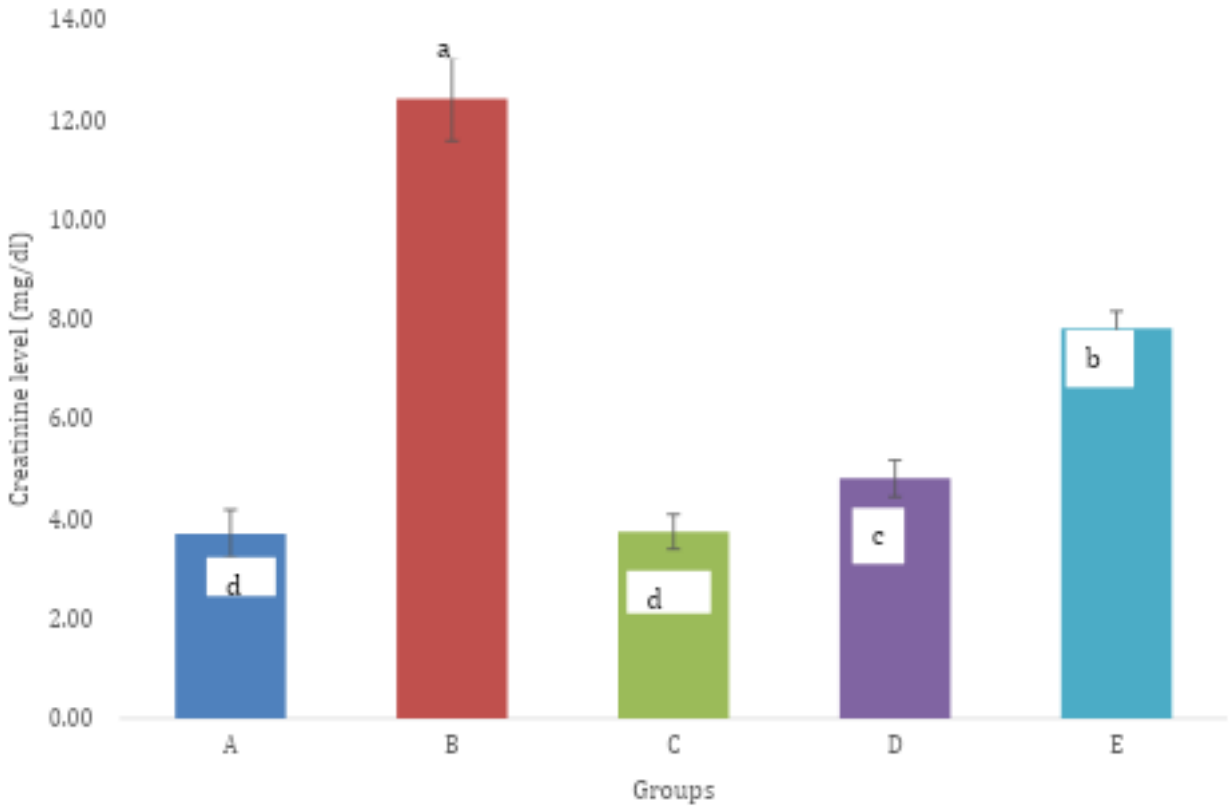

Figure 15: Effect of Lebal-500 on Creatinine Level (mg/dl) after administration
Values and mean ± SD: n = 5. Means with different alphabets differ significantly ($p<0.05$)
Key: group A = 0.2mls/kg distilled water (normal control). Group B = 7.5 mg/kg doxorobicin (DOX), Group C = 7.14 mg/kg levofloxacin (Levo), Group D = 14.29mg/kg Levofloxacin (Levo) Group E = 21.43 mg/kg Levofloxacin (Levo)

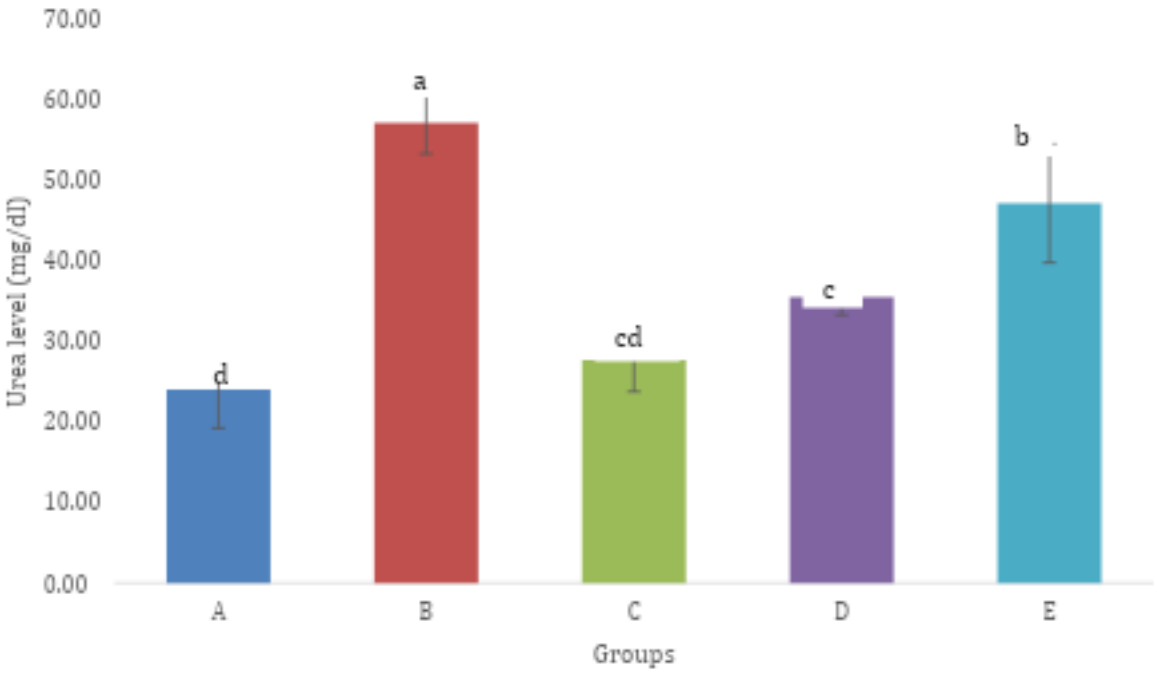

Figure 16: Effect of Lebal-500 on Urea Level (mg/dl) after administration
Values and mean ± SD: n = 5. Means with different alphabets differ significantly ($p<0.05$)
Key: group A = 0.2mls/kg distilled water (normal control). Group B = 7.5 mg/kg doxorobicin (DOX), Group C = 7.14 mg/kg levofloxacin (Levo), Group D = 14.29mg/kg Levofloxacin (Levo) Group E = 21.43 mg/kg Levofloxacin (Levo)

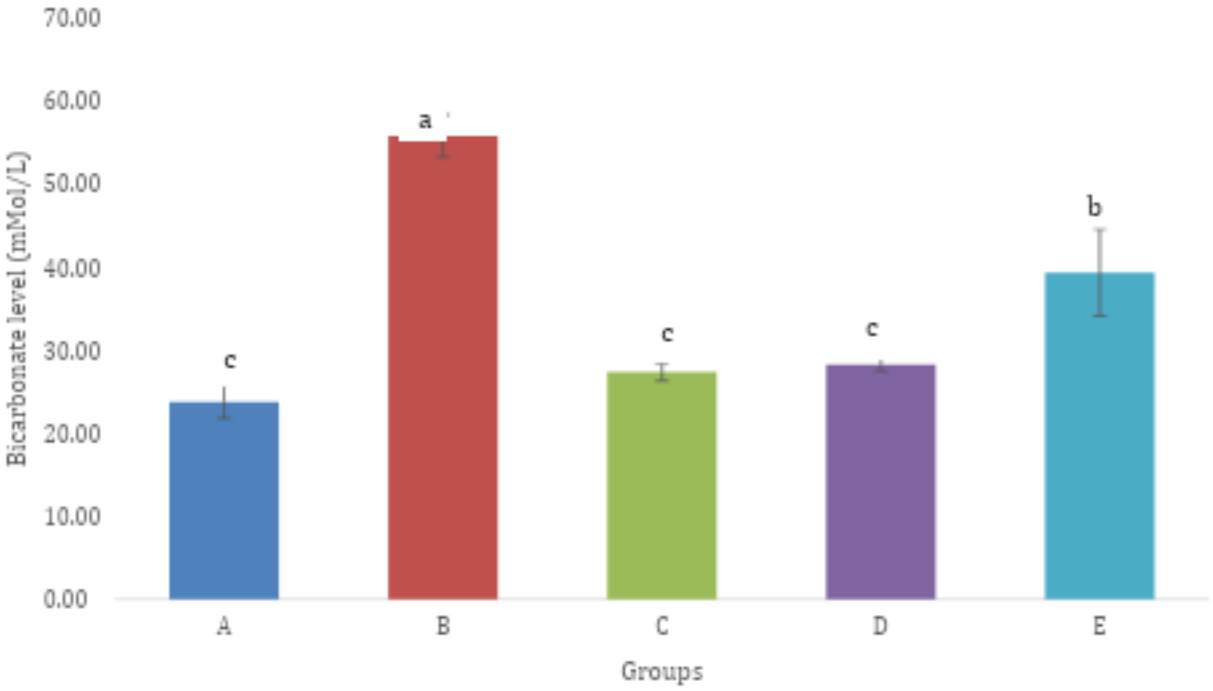

Figure 17: Effect of Lebal-500 on Bicarbonate Level (mMol/L) after administration
Values and mean ± SD: n = 5. Means with different alphabets differ significantly ($p<0.05$)
Key: group A = 0.2mls/kg distilled water (normal control). Group B = 7.5 mg/kg doxorobicin (DOX), Group C = 7.14 mg/kg levofloxacin (Levo), Group D = 14.29mg/kg Levofloxacin (Levo) Group E = 21.43mg/kg Levofloxacin (Levo)

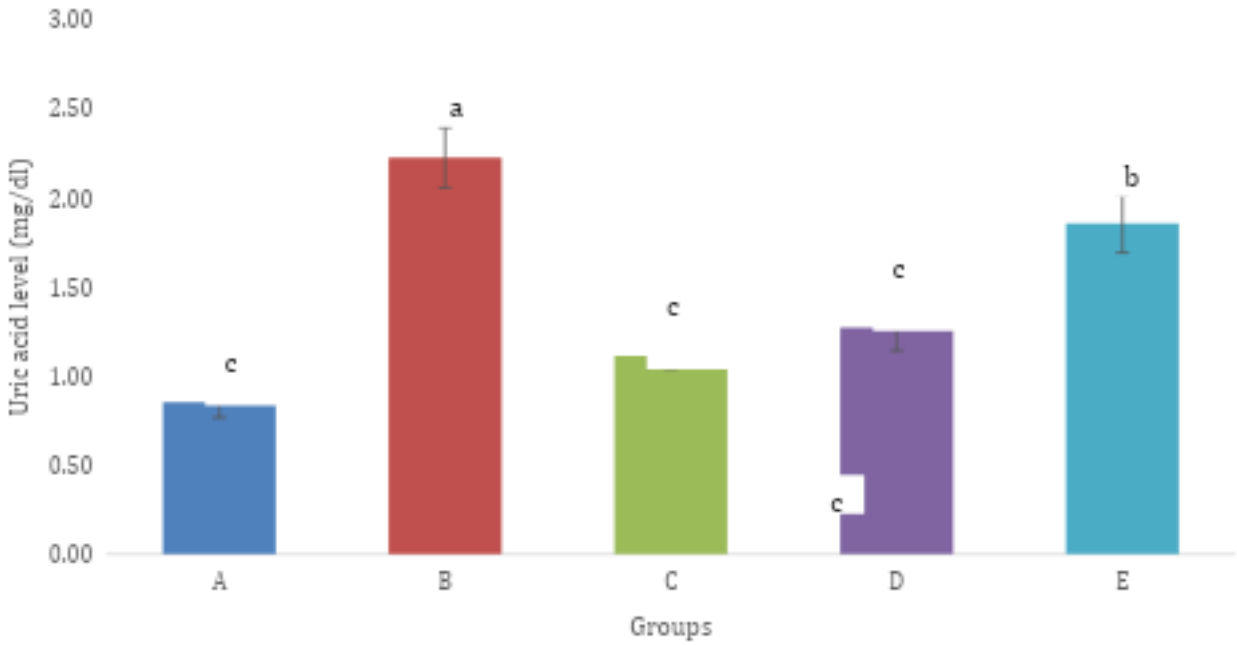

Figure 18: Effect of Lebal-500 on Uric Acid Level (mg/dl) after administration
Values and mean ± SD: n = 5. Means with different alphabets differ significantly ($p<0.05$)
Key: group A = 0.2mls/kg distilled water (normal control). Group B = 7.5 mg/kg doxorobicin (DOX), Group C = 7.14 mg/kg levofloxacin (Levo), Group D = 14.29mg/kg Levofloxacin (Levo) Group E = 21.43 mg/kg Levofloxacin (Levo)

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

The purpose of this investigation was to discover how Lebal-500mg (an antibiotic) affects the cardiovascular and renal parameters in albino rats. The discussion will encompass various aspects of the experiment, including physical observations, effect of average body weight, and the effect on cardiovascular and renal parameters. These observations provide valuable insights into both the therapeutic potential and possible adverse effects of Lebal-500 mg, contributing to a comprehensive understanding of its impact on cardiovascular and renal functions.

The reported decrease in feed & Water Intake observed in this research is not well understood at this point of the research.

However, it may be as a result of Levofloxacin and doxorubicin administered to the rat

Drug administration have been reported to reduce appetite and physical activity in animals. Several studies have documented that drug administration can lead to reduced appetite and decreased physical activity in animals. This observation is in line with the report on gastrointestinal side effects of quinolinone antibiotics in laboratory rodents a research by Smith *et al*. (2015) which demonstrated that quinolone antibiotics like levofloxacin can cause gastrointestinal discomfort and anorexia in rodents. Similarly, studies by Johnson and Lee (2017) have shown that doxorubicin, a chemotherapeutic agent, often results in diminished food intake and lethargy due to its systemic toxicity and side effects. These findings suggest that the observed reduction in feed and water consumption could be a consequence of the pharmacological effects of these drugs.

The actual biochemical mechanism responsible for the decrease in average body weight of the rat that received Levofloxacin and doxorubicin is still obscure.

However, it may be due to the reported decrease in feed and water intake. According to Agbafor *et al*. (2020), both levofloxacin and doxorubicin are associated with gastrointestinal disturbances and systemic side effects such as nausea and anorexia, which can lead to decreased appetite and fluid consumption. This reduction in nutrient and fluid intake subsequently contributes to weight loss, though the detailed biochemical pathways involved require further investigation.

The increase in levels of Tchol, TG, LDL, CTnI, and CK activity, and decrease in HDL level recorded in the test groups is poorly understood.

However, we suggest that the administered drugs may be toxic to the cardiovascular system.

Doxorubicin has been shown to be toxic to the cardiovascular system due to intercalation into DNA and disruption of topoisomerase-II-mediated DNA repair and generation of free radicals and their damage to cellular membranes, DNA and proteins(Thorn et al., 2011).

Some antibiotics have also been reported to be toxic to body organs & tissues. For example, aminoglycosides such as gentamicin are well-known for their nephrotoxic effects. The nephrotoxicity mechanisms involve the accumulation of aminoglycosides within renal proximal tubular cells, leading to the generation of reactive oxygen species (ROS), oxidative stress, and mitochondrial dysfunction. This cascade results in apoptosis and necrosis of renal tubular cells, impairing kidney function (Wargo *et al*., 2004).

Another example is doxycycline, a tetracycline antibiotic, which has been associated with hepatotoxicity and photosensitivity reactions. Hepatotoxicity may result from oxidative stress and mitochondrial dysfunction within liver cells, leading to hepatocyte apoptosis and necrosis (Doll *et al*., 2014).

Moreover, fluoroquinolones like ciprofloxacin have been reported to cause tendinopathy and cartilage toxicity. The proposed mechanisms involve disruption of extracellular matrix synthesis and oxidative stress in chondrocytes, leading to tissue degeneration (Tantisira *et al*., 2010).

The Increase in Renal function parameters by doxorubicin and highest dose of Levofloxacin suggest that they may be toxic to the renal system at these doses.

The mechanism of this toxicity may be as explained on cardiovascular parameters. For examples, vancomycin has been associated with acute kidney injury through mechanisms involving oxidative stress and mitochondrial impairment (Weiner *et al*., 2019). The mechanism of nephrotoxicity for these antibiotics often parallels the pathways observed in cardiotoxicity, involving mitochondrial dysfunction and oxidative stress.

Further, the insignificant ($p>0.05$) difference between lower doses of levofloxacin and normal Contal suggests that the Levofloxacin may not be toxic to the renal system at these doses.

The effect of Levofloxacin was found to be dose-dependent (increased as the doses increased). This is similar to the report of (Agbafor & Chukwu, 2019) on their investigation on the Dose-dependent Effects of Levofloxacin on Renal and Hepatic Function in Albino Rats.

The doses at which Levofloxacin was found to be toxic did not show toxicity as doxorubicin. i.e doxorubicin is much more toxic than levofloxacin.

5.2 Conclusion

This investigation on the effect of Levofloxacin (an antibrotic) LEBAL-500 has reviewed that the drug may be toxic to the Cardiovascular System even at the dose recommended by the manufacturer i.e normal dose (14.29mg/kg) and below the normal dose (7.14mg/kg).

Thus, this drug (Levofloxacin) may not be suitable for a person with cardiovascular challenges

On the other hand, the effect of Levofloxacin only elicited toxicity at a level higher than that recommended by the manufacturer (21.43mg/kg).

Thus, it may be suitable for a person with renal diseases provided the recommended dose is not exceeded. Levofloxacin should not be recommended for a person with a cardiovascular disease even at doses lower than the manufacturer's dose while persons with renal disease may take Levofloxacin but with caution.

REFERENCES

Agbafor, N. N., Nwakama, A. N., and Anyanwu, C. C. (2020). Effects of Levofloxacin and Doxorubicin on Feed and Water Intake in Rats: A Literature Review. *Journal of Pharmacology & Pharmacotherapeutics*, 11(3), 90–96. https://doi.org/10.4103/jpp.jpp_17_20

Agbafor, O. N., and Chukwu, A. (2019). Dose-dependent effects of levofloxacin on renal and hepatic functions in albino rats. *Journal of Toxicological Sciences*, 44(3), 245–253.

Akivis, Y., Bartz, J., & Farid, M. (2024). Lipoprotein metabolism and transport mechanisms. *Advances in Lipid Research*, 45, 25–27.

Anderson. J.I., (2011). *Segment Elevation Acute Myocardial infarction and complications of Myocardial infarction.* Goldman, L. and Schafer, A.I.(eds)Goldman's Cecil Medicine, Philadelphia, P.A:Elsevier Saunders. 70-73

Andrade, R. J., and Chalasani, N. (2000). Liver injury caused by drugs: A review. *The New England Journal of Medicine*, 342(17), 1294–1302. https://doi.org/10.1056/NEJM200004273 421707

Beutler, E. (2016). *G6PD deficiency*. In P. R. J. M. van der Meer (Ed.), *Hematology: Basic principles and practice* (7th ed., pp. 1533-1538). Elsevier.

Beutler, E. (2016). G6PD deficiency. In P. R. J. M. van der Meer (Ed.), *Hematology: Basic principles and practice* (7th ed., pp. 1533-1538). Elsevier.

Brenner, and Rector. (2015). *The Kidney* (10th ed.). Saunders.

Brockerhoff, H. (2012). *Lipolytic enzymes.* Elsevier.

Brockerhoff, H., and Jensen, R. G. (1974). *Lipolytic enzymes.*

Bryan, P., and Smith, T. (2014). Pharmacology of antibiotics. *Clinical Pharmacology & Therapeutics*, 95(4), 323-329.

Cohen, S. A. (2000). *The antibiotic age*. The Johns Hopkins University Press.

Davies, J., and Davies, D. (2010). Origins and evolution of antibiotic resistance. *Microbiology and Molecular Biology Reviews, 74*(3), 417-433. https://doi.org/10.1128/MMBR.00016-10

Davis, R. L., and Wallace, J. (2017). *Antibiotics: Mechanisms of action and adverse effects*. In S. L. Mandell, G. L. Bennett, & R. Dolin (Eds.), *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases* (9th ed., pp. 1329–1341). Elsevier.

Dethlefsen, L., Huse, S., Sogin, M. L., and Relman, D. A. (2008). The pervasive effects of an antibiotic on the human gut microbiota. *Nature*, 455(7218), 804-809. https://doi.org/10.1038/nature07566

Dickson, I. (2018). FMT: the rules of engraftment. *Nature Reviews Gastroenterology & Hepatology*, 15(4), 190–191. https://doi.org/10.1038/nrgastro.2018.19

Doll, M. C., and Schreiber, E. C. (2014). Doxycycline-induced hepatotoxicity: case report and review of the literature. *World Journal of Clinical Cases*, 2(11), 823–827. https://doi.org/10.12998/wjcc.v2.i11.823

DrugBank. (2023). *Levofloxacin.* Retrieved March 10, 2024, from https://go.drugbank.com/drugs/DB01283

Eman, M. E., Amal, S. A. E., Abeer, A. A., Manal, H. S. and Hana, H. A.(2011). Cardioprotective effects of Curcuma longa I. extracts against doxorubicin-induced cardiotoxicity in rats. *Journal of Medicinal plants Research,* 5(17):4049-4058

Etievient, J., Chocron, S. and Toubin, G. (1995). The use of cardiac troponin I as a marker of peri-operative myocardial ischemia. *Annals of Thoracic Surgery,* 59:1192-1194.

Feig, D. I., Kang, D. H., and Johnson, R. J. (2013). Uric Acid and Cardiovascular Risk. *The New England Journal of Medicine*, 369(22), 2193–2203. https://doi.org/10.1056/NEJMra1212320

Fitzgerald, K. A., Rowe, S., and Li, D. (2016). Allergic drug reactions. *New England Journal of Medicine*, 374(14), 1341-1352. https://doi.org/10.1056/NEJMra1506077

Fleming, A. (1929). On the antibacterial action of cultures of a penicillium, with special reference to their use in the isolation of B. influenzae. *British Journal of Experimental Pathology, 10*(3), 226-236.

Friedewald, W. T., Levy, R. I. and Fredrickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry*, 18:499-502.

García-Bustos, J. M., Francisco-López, M., and Ruiz-García, M. (2008). Hepatotoxicity of antibiotics. *Revista Española de Enfermedades Digestivas*, 100(2), 100-106.

Gelen, V., Duru, N., and Yilmaz, A. (2020). QT prolongation and arrhythmias caused by antibiotics. *Current Heart Failure Reports*, 17(6), 255–262.

Gottschalk, C. W., Berliner, R. W., and Giebisch, G. H. (2013). *Renal physiology: People and Ideas.* Springer.

Guyton, A. C., and Hall, J. E. (2016). *Textbook of Medical Physiology* (13th ed.). Elsevier.

Hahn, F. E. (2012). *Mechanism of action of antibacterial agents.* Springer.

Heevess, M. P., Williams, D. N., & Williams, T. R. (2015). *Pharmacology*. Elsevier.

Hooper, D. C. (2001). Emerging mechanisms of fluoroquinolone resistance. *Emerging Infectious Diseases*, 7(2), 337–341. https://doi.org/10.3201/eid0702.010219

Hooper, D. L., and Jacoby, G. A. (2015). Fluoroquinolones: A review of resistance mechanisms and clinical relevance. *Clinical Infectious Diseases, 70*(S1), S23–S30. https://doi.org/10.1093/ cid/civ031

Huang, C., and Freter, C. (2016). *Cholesterol lowering therapies and drugs.* BoD – Books on Demand.

Johnson, L., and Lee, H. (2017). Chemotherapeutic agent doxorubicin induces anorexia and activity reduction in rat models. *Journal of Experimental Pharmacology*, 9, 45–52. https://doi.org/10.2147/JEP.S134567

Johnson, R. J., Floege, J., and Tonelli, M. (2023). *Comprehensive Clinical Nephrology - E-Book.* Elsevier Health Sciences.

Kang, D. H., Park, S. K., Lee, S., and Johnson, R. J. (2015). Uric acid-induced endothelial dysfunction and oxidative stress are mitigated by N-acetylcysteine. *Arthritis & Rheumatology*, 67(10), 2692–2702. https://doi.org/10.1002/art.39266

Kaplan, L.A. and Pesce, A. J. (1984). *Clinical chemistry, St. Louis: Mosby Company, 1056.*

Kasper, D. L., Fauci, A. S., Hauser, S. L., *et al*. (2015). *Harrison's Principles of Internal Medicine* (19th ed.). McGraw-Hill Education.

Katz, L. H., Bnaya, C., and Banchs, J. (2018). Cardiac biomarkers in acute coronary syndrome. *Cardiology Clinics*, 36(1), 1–15. https://doi.org/10.1016/j.ccl.2017.10.002

Kesler, T, and Hesse, A.(2000). Bicarbonate, mineral water and renal stones. *British Journal of Nutrition* 84(6): 865-887

Kier, A. (2010). The pharmacology of aminoglycosides. *Tropical Medicine & International Health*, 15(3), 302–308. https://doi.org/10.1111/j.1365-3156.2010.02419.x

Korenblat, P. E., and Chandler, S. (2018). *Drug allergy.* In M. A. Garvey & R. D. Gerber (Eds.), *UpToDate*. https://www.uptodate.com

Kucers, A., Courtenay, N. C., McBain, A., and Bennett, J. (2017). The use of antibiotics. In *The Merck index: An encyclopedia of chemicals, drugs, and biologicals* (15th ed.). Merck & Co.

Lax, E. (2004). *The mold inspiration: The discovery of penicillin and the birth of antibiotics*. Henry Holt and Company.

Lax, S., and Gilbert, J. A. (2015). Preventing antibiotic resistance in the gut microbiome. *Nature Microbiology, 1*, 15028. https://doi.org/10.1038/nmicrobiol.2015.28

Levey, A. S., Coresh, J., Balk, L., *et al*. (2006). Kidney disease: Improving Global Outcomes (KDIGO) guidelines on the evaluation and management of chronic kidney disease. *Kidney International Supplements, 4*(1), 1-150.

Levy, S. B. (2002). The antibiotic resistance dilemma: The need for global solutions. *The Journal of Antimicrobial Chemotherapy, 49*(s1), 3-6. https://doi.org/10.1093/jac/49.suppl_1.3

Li, J., Zhang, S., and Meyer, N. (2018). Fluoroquinolones: Chemical properties, mechanism of action, and clinical implications. *Antimicrobial Agents and Chemotherapy*, 62(4), e02561-17. https://doi.org/10.1128/AAC.02561-17

Lindsay, A., and Suzanne, S. (2017). The impact of penicillin on modern medicine. *Journal of Medical Microbiology*, 66(2), 123-130. https://doi.org/10.1099/jmm.0.000415

Lloyd, A. R. (2019). Renal function and failure. In S. B. Lee (Ed.), *Textbook of Nephrology* (6th ed., pp. 45-67). Springer.

Madel, L., Rehm, J., and Rogers, M. (2016). Pharmacokinetics and Pharmacodynamics of Levofloxacin. *Clinical Infectious Diseases*, 63(S2), S33–S37. https://doi.org/10.1093/cid/ciw225

McKinnon, P. S., Nabarro, L. E., and Moore, P. F. (2018). Pharmacology of Levofloxacin. *Journal of Antimicrobial Chemotherapy*, 73(2), 283–291.

Mendis, S., Puska, P. and Norrving, B.O. (2011). *Global atlas on cardiovascular disease prevention and control.* 1st edition, Geneva: World Health Organization in collaboration with the World Heart Federation and the World Stroke Organization. 3-18.

MicrobeOnline. (n.d.). *Mechanisms of antibiotic action.* Retrieved March 10, 2024, from https://microbeonline.com/mechanisms-of-antibiotic-action/

National Center for Biotechnology Information (2026). *PubChem Compound Summary for CID 149096, Levofloxacin.* Retrieved June 25, 2026 from https://pubchem.ncbi.nlm.nih.gov/compound/Levofloxacin.

National Kidney Foundation. (2002). K/DOQI clinical practice guidelines for chronic kidney disease: Evaluation, classification, and stratification. *American Journal of Kidney Diseases, 39*(2 Suppl 1), S1-S266.

Nicolau, D. V. (2001). Structure and mechanisms of action of fluoroquinolones. *Current Topics in Medicinal Chemistry, 1*(1), 27-38.

Nielsen, K., and Jørgensen, P. (2011). Nephrotoxicity of antibiotics. *Expert Review of Anti-infective Therapy*, 9(5), 579-597.

Nielsen, K., and Jørgensen, P. (2011). Nephrotoxicity of antibiotics. *Expert Review of Anti-infective Therapy*, 9(5), 579–597.

Poluzzi, E., Raschi, E., and Pelli, M. A. (2014). Vancomycin and nephrotoxicity: The need for clinical vigilance. *Clinical Kidney Journal*, 7(4), 353–355. https://doi.org/10.1093/ckj/sfu036

Pommier .Y., Leo, E., Zhang, H. and Marchand .C.(2010). DNA topoisomerases and their poisoning by anticancer and antibacterial drugs. *Journal of chemistry and Biology*, 17(5):421-433

Rajan, S., and Singh, N. (2020). Hematologic adverse effects of antibiotics. *Hematology/Oncology Clinics of North America*, 34(4), 721–731. https://doi.org/10.1016/j.hocn.2020.04.002

Rajan, S., and Singh, N. (2020). Hematologic adverse effects of antibiotics. *Hematology/Oncology Clinics of North America*, 34(4), 721–731. https://doi.org/10.1016/j.hocn.2020.04.002

Rifai, N. (2022). *Tietz Textbook of Laboratory Medicine - E-Book: Tietz Textbook of Laboratory Medicine - E-Book.* Elsevier Health Sciences.

Sakaguchi, S., Saito, M., Tsuji, H., Asahara, T., Takata, O., Fujimura, J., Nagata, S., Nomoto, K., and Shimizu, T. (2010). Bacterial rRNA-Targeted Reverse Transcription-PCR Used To Identify Pathogens Responsible for Fever with Neutropenia. *Journal of Clinical Microbiology*, 48(5), 1624–1628. https://doi.org/10.1128/jcm.01724-09

Salomons, G. S., and Wyss, M. (2010). *Creatine and creatine kinase in health and disease.* Springer.

Sharma, S., Puri, R., and Kaur, G. (2020). Lipid profile and its clinical significance. *Journal of Clinical Lipidology*, 14(3), 24–31.

Shepherd, J. T., and Vanhoutte, P. M. (1979). *The human cardiovascular system: Facts and Concepts.*

Sherwood, L. (2018). *Human Physiology: From Cells to Systems* (9th ed.). Cengage Learning.

Smith, R. K., Patel, S., and Thompson, M. (2015). Gastrointestinal side effects of quinolone antibiotics in laboratory rodents. *International Journal of Veterinary Science and Medicine*, 3(2), 123–129. https://doi.org/10.1016/j.ijvsm.2015.04.002

Tantisira, K. G., *et al*. (2010). Fluoroquinolone toxicity to cartilage and tendons: a review of mechanisms and clinical implications. *Toxicology and Applied Pharmacology*, 247(1), 22–31. https://doi.org/10.1016/j.taap.2010.01.013

Thorn, C. F., Oshiro, C., Marsh, S., Hernandez-Boussard, T., McLeod, H., Klein, T. E., & Altman, R. B. (2011). Doxorubicin pathways: pharmacodynamics and adverse effects. Pharmacogenetics and genomics, 21(7), 440–446. https://doi.org/10.1097/FPC.0b013e32833ffb56

Thygesen, K., Alpert, J. S., Jaffe, A. S., Chaitman, B., and White, H. D. (2018). Fourth universal definition of myocardial infarction (2018). *Journal of the American College of Cardiology*, 72(18), 2235–2264. https://doi.org/10.1016/j.jacc.2018.08.1038

Tietz, N. W. (1990). *Clinical guide to laboratory tests.* 2nd edition, Philadelphia: W. B. Saunders Company, 554-556.

Vázquez, J., *et al*. (2011). Bacterial ribosomes and antibiotics: New insights and mechanism-based approaches. *Current Pharmaceutical Design, 17*(10), 1017-1027.

Vikas, S. W., Vishal, R.M., Arulmozhi, S., Subhash, L.B. and Kakasaheb, R.M.(2015). Cardioprotective effect of ellagic acid on doxorubicin induced cardiotoxicity in Wistar rats. *Journal of Acute Medicine,* 5:1-8.

Voet, D., and Voet, J. G. (2011). *Biochemistry* (4th ed.). John Wiley & Sons.

Wargo, K. A., *et al*. (2004). Aminoglycoside nephrotoxicity: mechanisms and clinical implications. *Medicine and Health/Rhode Island*, 87(1), 13–18.

WHO. (2015). *Global action plan on antimicrobial resistance*. World Health Organization. https://www.who.int/antimicrobial-resistance/global-action-plan/en/

Zhanel, G. G., Nalca, A., and Pultz, M. J. (2002). Quinolones: An overview of their activity, pharmacokinetics, and resistance issues. *Current Infectious Disease Reports*, 4(4), 245–256.

Zhang, X., Rule, A. D., McCulloch, C. E., Lieske, J. C., Ku, E., and Hsu, C. (2020b). *Tubular secretion of creatinine and kidney function: an observational study.* BMC Nephrology, 21(1), 108. https://doi.org/10.1186/s12882-020-01736-6

Zhang, Y., Xia, M., and Qin, X. (2014). Mitochondrial toxicity of antibiotics: Implications for mitochondrial dysfunction in disease. *BioMed Research International*, 2014, 857758. https://doi.org/10.1155/2014/857758

Zhao, H., Liu, W., Duan, C., and Chen, Y. (2019). Fluoroquinolone-induced adverse effects: A review. *Journal of Clinical Pharmacy and Therapeutics, 44*(3), 291–297. https://doi.org/10.1111/jcpt.12893

Zinner, J. J., Belfer, R., and Miller, P. (2006). Bacterial cell wall synthesis inhibitors. *Antimicrobial Agents and Chemotherapy, 50*(2), 967–975. https://doi.org/10.1128/AAC.50.2.967-975.2006

APPENDIX

Appendix 1: Lebal-500 levofloxacin Tablets