EFFECT OF CO-ADMINISTRATION OF METFORMIN AND CEFIXIME ON SOME BIOMARKERS IN DIABETIC RATS

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M.Sc/Pharm-Sci/11855/2007-08

A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL AHMADU BELLO UNIVERSITY, IN PARTIAL FUFILLMENT OF THE REQUIRMENTS FOR THE AWARD OF MASTERS IN PHARMACOLOGY

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS

FACULTY OF MEDICINE ,

AHMADU BELLO UNIVERSITY,

ZARIA, NIGERIA

MAY, 2011

DECLARATION

I declare that the work in the thesis entitled 'Effect of co-administration of Metformin and Cefixime on some biomarkers in diabetic rats has been performed by me in the Department of Pharmacology and Therapeutics under the supervision of Dr (Mrs) O.A. Salawu and Dr. A.U. Zezi.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

Olurishe, Comfort Omoigemete

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Name of Student	Signature	Date

CERTIFICATION

This thesis entitled **"EFFECT OF CO-ADMINISTRATION OF METFORMIN AND CEFIXIME ON SOME BIOMARKERS IN DIABETIC RATS"** by Olurishe, Comfort Omoigemete meets the regulations governing the award of the degree of Masters in Pharmacology in Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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ACKNOWLEDGEMENT

All Glory and thanks to the Lord God Almighty the author and giver of knowledge and wisdom for the grace, willpower and resources he gave to me to execute this project. I pledge my allegiance to you forever O Lord, Amen.

I also acknowledge the immense contribution and encouragement of my supervisors Dr (Mrs) O.A. Salawu and Dr A.U. Zezi, thank you for being there all through. My sincere gratitude goes to Dr B.O. Olayinka, for supervising the microbiological aspect of this work. My parents Mr and Mrs A.A. Alufohai who sponsored me in my academic carrier to first degree level without which there would have been no second degree, the Lord God Almighty shall preserve your lives in good health in Jesus name Amen. To my parents in-law Elder and Mrs R.D. Olurishe, for their constant support and prayers, may God bless you richly and fulfil the desires of your heart Amen. It is impossible to mention everyone who has contributed to the success of this project. Nevertheless my sincere gratitude to my colleagues Pharm (Mrs) M.E. Ukaegbu, Pharm T.T. Shekarau and my friends Mr and Mrs J. Odoh, Dr and Mrs A.G. Olayemi and Dr and Mrs S.B. Danborno you will always be remembered by God.

A special thanks to my husband Dr T.O. Olurishe for his support and tutorship throughout the programme. To my children Ireolubori, Olutimayin and Opemiato who have earned themselves honorary Masters degree by being very patient and understanding, the Lord God bless you and cause you to do greater than this in Jesus name, Amen.

ABSTRACT

The incidence of infections in Type 2 Diabetes Mellitus (T2DM) continues to be on the increase, necessitating the combined use of antibiotics and hypoglycaemic agents. Metformin, a biguanide is the first drug of choice in the management of T2DM and is most frequently employed with antibiotics when infections exist as co morbid state. This study investigated the effect of Metformin (200 mg/kg) and Cefixime (400 mg/kg) in alloxan (140 mg/kg) induced diabetic rats. Metformin was administered orally for 28 days alone and 14 days co-administration with Cefixime and the effect on some metabolic biomarkers were recorded. The effect of combination of Metformin 4µg/ml, 2µg/ml and 1µg/ml on *in vitro* antimicrobial activities of Cefixime 5µg/ml against *K. species, P. mirabilis, H. influenza* and *E.coli* was also investigated. There was a statistically significant reduction ($p<0.05$) in Fasting Blood Glucose (FBG) on Day 28 and Random Blood Glucose (RBG) ($p<0.01$) on day 21 of diabetic rats in Metformin/Cefixime group compared to Metformin alone group. There were no statistically significant changes in the haematological (WBC, RBC and PCV) and biochemical (urea, creatinine and electrolytes) parameters studied. There was no significant difference in body weight and relative weights of selected organs in the drug treated groups and the control. Histopathological findings revealed various degrees of pathological changes in the liver and kidney of the diabetic rats in Metformin and Metformin/ Cefixime group. The pancreas showed areas of pancreatic acini with less cellular endocrine portion in Metformin alone group. Metformin did not significantly alter the *in vitro* antimicrobial activities of Cefixime against the selected Microrganism used in this study. The study showed that Cefixime may slightly increase the hypoglycaemic activity of Metformin when co-administered.

This effect may not appear to be of much clinical significance when both drugs are administered at therapeutic doses, but will call for caution when used for a longer period or in other situations predisposing patients to possible accumulation of Metformin.

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ABBREVIATIONS

ABUTH	Ahmadu Bello University Teaching Hospital
DM	Diabetes Mellitus
FBG	Fasting Blood Glucose
H&E	Haematoxylin and Eosin
IZD	Inhibition Zone Diameter
NI	No Inhibition
OGTT	Oral Glucose Tolerance Test
PCV	Packed Cell Volume
RBG	Random Blood Glucose
RBC	Red Blood Count
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
WBC	White Blood Count

Chapter One

INTRODUCTION

1.1 General Introduction

Drugs generally exert effects other than their primary actions in other parts of the body. This increases the possibility that two concomitantly administered drugs will affect same systems in the body leading to drug-drug interactions. The potential for interactions between drugs is not only concerned with their primary effect but also the secondary activities that they possess which are very often overlooked. By implication, the greater the number of drugs a patient is exposed to, the higher the risk of drug-drug interaction (Rhoades, 2000). An example of drug combinations frequently employed in therapy is the use of chlorpromazine, trihexyphenidyl and amitriptyline in psychiatric disease. These agents have considerably different primary effect, but all possess anticholinergic activities characterized by symptoms such as dry mouth, blurring of vision, constipation e.t.c. Even though the anticholinergic effects of any one of these drugs may be slight there is generalized additive increase in their anticholinergic effects when used concurrently and sometimes serious intensification occurs (Stockley, 2002).

Drug interactions have also been frequently encountered with the use of prescription drugs alongside non-prescription drugs (e.g. antacids, decongestants, haematinics) and herbal preparations. Some non-prescription or over the counter drugs are being taken in such a routine manner and for long periods that patients do no longer consider them as drugs. When patients are therefore asked about medications they are taking, they neglect to mention these drugs including herbal preparations and a lot of other health promotion

products e.g. Golden Neo-Life Dynamite (GNLD) products, that have recently saturated the market. Also, in a system where proper patients medication profiles are not kept and where it is not uncommon for a patient to see one or more specialists in addition to a family physician, it is frequently difficult for one physician to know completely what medications have been prescribed by another physician. An example is one physician may prescribe an antihistamine for a patient for whom another physician has prescribed an anxiolytic e.g. a benzodiazepine, with the possible consequence of an excessive depressant effect.

The risk of drug interaction is also common amongst the elderly who do not take medications as directed by the physician either because they have forgotten, or are confused about the directions given especially as regards spacing of administrations. Some of these circumstances lead to excessive use of medications thereby increasing the possibility of drug interactions e.g. some patients on realizing they have forgotten a dose of medication doubles the next dose to make up for it. While others subscribe to the philosophy that if the one tablet that has been prescribed provides some relief of symptoms two or more tablets will be more effective.

The concept of drug interaction is often extended to include situations in which,

i. Food or dietary items influence the activity of a drug i.e. (drug-nutrient interactions)

ii. Environmental chemicals or smoking influences the activity of a drug

iii. A drug causes alterations of diagnostic laboratory test results i.e. (drug- laboratory test interactions)

iv. Alteration of a drug effect by concurrent use with a herbal preparation i.e. (drug- herb interaction)

v. A drug causes undesirable effect in patients with certain disease states i.e. (drug-disease interaction)

There are multiple factors that can influence either favorably, or unfavorably the outcome of a drug interaction. These factors include:

i. Drug-related factors i.e. Properties of the object drug and the precipitating drug which includes the dose, pharmacokinetic sequence, route of administration and dosage form of the drugs being used.

ii. Patient-related factors such as age, renal and hepatic function, pregnancy, severity of disease, presence of other co morbid states and pharmacogenetic factors.

iii. Other factors include serum protein levels, urinary pH, polypharmacy, dietary and environmental factors (Herman, 1999).

Drug interactions also occur at varying rates in patients. The causes for variability include genetics, diseases, diet/nutrition, environment, smoking, and alcohol use. Patients who metabolize drugs faster are at higher risk for drug-drug interactions e.g. all humans do not express the isoenzyme CYP2D6, those who express the gene are referred to as extensive metabolizers and those without the gene are poor metabolizers. Diseases also have an effect on drug interactions and drug transporters, e.g. cytokines are modified during the infectious process and may facilitate drug interactions.

Drug interactions usually manifests as enhanced efficacy, increased toxicity, or lack of efficacy of either drug. Interactions can be avoided by identifying patients at risk of developing interactions as well as medications with high interaction potential. Overall, the best means to manage a drug interaction is to avoid the interaction, but when this is not possible one should attempt to minimize the impact of the interaction by adjusting the dose, route, or order of administration of the drugs. Drug interactions may also be beneficial in certain disease management; of significance is the administration of probenicid with penicillins to reduce the excretion of penicillin thereby prolonging their antibiotic effect (Tom-Revzon, 2006).

1.2 Reasons for Drug Interaction Studies

The development of new and unique medications designed to treat specific diseases is on the increase, this progress in science ultimately implies an increase in incidence of drug related side effects and interactions. Drug-drug interactions (DDIs) represent a critically important and widely under recognized outcome of medical errors. DDIs manifest in therapeutic failure, increased mortality and morbidity, unexpected life-threatening and even lethal toxicities. Also of concern, is the increased rate of hospitalization of patients and increased lengths of stay in hospitals due to DDIs with obvious increased costs on the part of the patient. Very hard to quantify, is the opportunity cost of lost work hours of patients and those who must care for them, as well as the decreased productivity that arises from the stress that these DDIs create among patients and caretakers. However the lives lost to DDIs give us more urgent reasons to find solutions than the tangible and intangible health care costs.

Due to the important clinical consequence of DDIs, it is desirable to carry out research and studies to identify, predict and prevent fatal potential interactions of a drug candidate with other drugs. The advances in the identification, isolation and characterization of different isoenzymes of CYP enzyme system has made it possible to correlate a specific isoenzyme activity with the metabolism of a specific drug. This has helped in prediction of *in vivo* drug interactions of new drugs based on their *in vitro* interaction data, which can help in early detection, refusal or approval of the drug or withdrawal of a drug from the market. Nevertheless it is worthy of note that DDIs can also have significant positive outcomes, these have been harnessed in the management of certain diseases e.g. hypertension (Triplitt, 2006).

Based on the knowledge of DDIs, physicians can also anticipate drug interactions. This will enhance rational drug therapy and better drug combinations. It is also important to become familiar with DDIs for drugs that are used most frequently, drugs with a low therapeutic index and to select agents with a low likelihood of producing DDIs.

Predictions of DDIs are also easier when a critical medical history is taken, this should identify factors such as hepatic or renal dysfunction, both prescription and non prescription medication and should be updated routinely.Drug interactions have also been observed to sometimes produce actions different from those of the interacting drugs. **S**ome drug-drug interactions may involve more than one mechanism which may be occurring simultaneously.

1.3 Statement of Research Problems

Diabetes Mellitus is a chronic disease affecting about 2.8% of the World's population in 2000 and is estimated to rise to 4.4% by 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 (Wild *et al*., 2004). The International Diabetes Federation (IDF) estimated that in 2003 the numbers of people with diabetes was 194 million and forecasts that this figure would have risen to 334 million by 2025. In Sub-Saharan Africa, the number of people age 20 to 79 years with diabetes accounts for over 7 million of the (194 million) for a population of more than 295 million, giving a prevalence rate of 2.4 percent, with Nigeria accounting for about 1.2 million (IDF, 2003).

People with diabetes are more adversely affected when they get an infection than someone without the disease, due to weakened immune defenses and diminished vascularization (Powers, 2006). Infections associated with either increased frequency or severity among individuals with diabetes include mucomycosis, cystitis, complicated urinary tract infections (e.g. pyelonephritis and intra renal abscesses). Others include pneumonia, lower-extremity soft tissue infection, polymicrobial gangrene, emphysematous cholecystitis and malignant otitis externa (Joshi *et al.*, 1999).

When patients are diagnosed with diabetes, a large number of medications become appropriate therapy, due to most often presence of other co morbid states e.g. hypertension, atherosclerosis, obesity, myocardial infarction and other cardiovascular diseases. Low dose aspirin (75mg) for example, is recommended in diabetes as

prophylaxis against thromboembolism. Long standing Type 2 Diabetes Mellitus (T2DM) and improperly managed diabetes predisposes patients to a number of infections and development of microvascular and macrovascular complications. Medications in addition to oral hypoglycemic agents are therefore needed for rational and effective management of T2DM patients.

The sometimes mandatory and unavoidable need for polypharmacy in these patients makes them vulnerable to drug interactions which may result in poor glycaemic control or therapeutic failure in other presenting or associated diseases/disorders. Problems of substandard and counterfeit drugs in African countries account for therapeutic failure in several clinical instances. It may thus be difficult to have accurate information about what may be responsible for therapeutic failure. Meanwhile some of these therapeutic failures or negative outcomes in pharmacological actions may be direct consequences of Drug/Drug or Drug/Disease interactions which may necessitate modification or alteration in therapeutic drug regimens.

The study and knowledge of drug/disease state and drug/drug interactions of medications commonly used in diabetes and co morbid diseases is of utmost importance in drug therapy because interactions can result in significant morbidity and mortality.

1.4 Significance of the study/Justification

The incidence of type 2 Diabetes Mellitus continues to grow rapidly in the developing and developed world as a result of high calorie and excess saturated fatty diet, genetic

factors and the obesity epidemic. Oral hypoglycaemic agents have remained effective in the treatment of T2DM and the risk of development of complications associated with hyperglycaemia.

Metformin, a biguanide is currently the first drug of choice in the treatment of uncomplicated T2DM, for its effect on glucose control, absence of weight gain and is not associated with hypoglycaemia when used as monotherapy (Luna and Feinglos, 2001). It also has generally low level of side effects, high level of acceptance, and relatively low cost (Nathan *et al.*, 2006; Powers, 2006).

Diabetic patients have increased incidence and severity of complications especially of infections. This is coupled with the ever evolving resistant strains of microorganisms. Cefixime, a third generation oral cephalosporin was introduced into Nigerian market in january 2008 and documented to have efficacy comparable with ceftriaxone an injectable third generation cephalosporin. Cefixime is used in the treatment of Lower Respiratory Tract infections (LRTIs), including acute exacerbations of chronic bronchitis. It is also employed in Urinary Tract Infections (UTIs), otitis media, cystitis, uncomplicated cervical and urethral gonorrhoea. It is used in Multi Drug Resistant and quinolone resistant thyphoid fever (Santillan *et al.*, 2000). Most of these infections occur with a greater incidence in the diabetic population. Cephalexin, a first generation cephalosporin has been shown to exhibit moderate drug\drug interactions when co administered with Metformin necessitating close monitoring of patients, (Jayasagar *et al*., 2002).

Based on the aforementioned reasons, it is important that potential interactions between Cefixime, the only currently orally available third generation cephalosporin antibiotic and Metformin which are administered concurrently in real life clinical situations be investigated.

1.5 Aim of the Study

The aim of the study is to investigate possible modifications in therapeutic effects of Metformin and/or Cefixime or possible changes in some biochemical parameters and histological features due to co-administration of the drugs using specific *in vitro* and *in vivo* models.

1.6 Specific Objectives

The specific objectives of the study are as follows

1. To investigate the possible effect of drug-drug interaction between Metformin and Cefixime on glycaemic control
2. To investigate the possible effect of Metformin and Cefixime co administration on antimicrobial activity of Cefixime.
3. To investigate the possible effect of co-administration of the two drugs on haematological indices, body electrolytes, urea and creatinine values.
4. To investigate the possible effect of Metformin and Cefixime co- administration on the weights and histological features of the rat pancreas, liver, kidney and heart.

1.7 Hypothesis

Drug-drug interaction occurs with the co-administration of Metformin and Cefixime necessitating modification of therapy when Diabetes Mellitus and infections exist as co morbid states.

Chapter Two

LITERATURE REVIEW

2.1 General introduction

Drug Interaction occurs when the effects of one drug are changed by the presence of another drug, food, drink or environmental chemical agents (Stockley, 2002). Drug interactions also occur with the administration of two or more drugs concurrently with the consequence of modification of pharmacological effects (Ojewole, 1996). Interactions may also result from prior administration of a drug as a result of accumulation or long half life. A drug interaction may also be defined as occurring whenever the effects of one drug are modified in or on the body by the prior or concurrent administration of another (or the same) drug (s) (Hartshorn, 2006). These modifications can be positive and sometimes form the basis of very important drug combinations. Synergism, additive effects, potentiation and antagonism are all possible outcomes of drug interactions and the magnitude of the effect seen (positive or negative) depends on the mechanism by which the interaction occurs.

2.2 Mechanism of Drug Interactions

Drug interactions occur through two broad mechanisms one is Pharmacokinetic interactions in which absorption, distribution, metabolism and excretion of interacting drugs are altered resulting in increase, decrease or delayed pharmacological actions. The second mechanism is Pharmacodynamic interactions which are considered more with actions of drug at various sites and will usually not have effect on timing of drug action

as much as pharmacokinetic interactions. Pharmacodynamic drug interactions occur, when concurrent administration of drugs brings about additive or antagonistic pharmacological effects.

2.2.1 Pharmacokinetic Drug Interactions

These are interactions which can affect the processes by which drugs are absorbed, distributed, metabolized and excreted.

2.2.1.1 Drug Absorption Interactions

When drugs are administered orally, they are intended for absorption through the mucous membrane of the gastrointestinal tract into the general circulation. Drug interactions that go on via alterations in mechanisms of absorption mostly results in reduced rather than increased absorption. Some interactions will lead to a decrease in the rate of absorption of drugs. For drugs given chronically as multiple dose regimens e.g. oral anticoagulants, the rate of absorption is usually not so important as long as the total amount of drug absorbed is not markedly altered. But for drugs given as single doses intended for rapid absorption where there is need to achieve high concentration rapidly then a reduction in the rate of absorption may result in failure to achieve an adequate effect e.g. hypnotics and analgesics.

Drug absorption interaction can be as a result of;

i. Effect of changes in GIT pH: Many drugs are weak acids or bases, and drugs are more absorbed in the unionized (more lipid soluble form) than the ionized form. Drugs such as

itraconazole, ketoconazole, glipizide, glyburide, cefpodoxime, and cefuroxime have pH dependent absorption. The amount of these drugs absorbed from the gut may be increased or decreased by drugs that increase stomach pH e.g. antacids and drugs that decrease stomach pH e.g. aspirin.

ii. Adsorption, chelation and other complexing mechanisms: These can lead to very significant drug- drug and drug- food interactions. Drugs that act as binding agents such as cholestyramine and colestipol when co administered with warfarin, cardiac glycosides and thiazide diuretics bind with these drugs reducing their absorption and hence bioavailability. This has resulted in a reduction in the therapeutic effect of the object drug and the effect can be profound with some combinations such as cholestyramine and furosemide. This interaction mechanism has also been successfully employed in the management of digitoxin and digoxin intoxication by interrupting their enterohepatic recycling and hastening their excretion. Some drugs such as fluoroquinolone antibiotics e.g. Ciprofloxacin and tetracyclines are susceptible to chelation with cations such as aluminum, magnesium, iron and calcium in milk and other dairy products. The complexes so formed are poorly absorbed reducing bioavailability.

iii. Changes in Gastrointestinal Tract (GIT) motility: Drugs that alter the rate at which the stomach empties its contents can affect absorption. When drugs that increase GIT motility like cathartics, are co administered with others they increase the rate at which the drugs pass through the GIT. These could result in decrease in absorption for drugs that are normally slowly absorbed and require prolong contact time with the absorbing

surface. Conversely drugs like propantheline or anticholinergics in general delays gastric emptying and the reduced peristalsis may retard dissolution and delay absorption from the small intestine, example of drug affected is acetaminophen (Stockley, 2002).

iv. Malabsorption caused by drugs: Drugs like Neomycin causes malabsorption syndrome and impairs the absorption of a number of drugs e.g digoxin and phenoxymethicillin.

2.2.1.2 Alteration of Distribution i.e. Drug Displacement (protein- binding) Interactions

Following absorption, drugs are rapidly distributed into body fluids and tissues by the circulation. Some drugs are totally dissolved in plasma water while many others are transported with some portion of their molecule in solution and the others bound to plasma proteins particularly the albumins. The extent of this binding varies, some drugs are highly bound e.g. Dicoumarol is about 96% bound.

The binding of drugs to plasma proteins is reversible and an equilibrium being established between those molecules that are bound and those that are not. Only the unbound molecules remain pharmacologically active while the bound molecules form a circulating but pharmacologically inactive reservoir. Depending on their concentration and relative affinity for their binding sites one drug may successfully competes with another and displace it from binding sites. The displaced and now active drug molecules pass into plasma where its concentration rises with a resultant increase in its effect. Example of highly bound drugs includes methotrexate, nalidixic acid, phenytoin and warfarin. When phenyl butazone and warfarin are co-administered, phenylbutazone

displaces warfarin from the binding site because of its greater affinity resulting in an increase anticoagulant effect with risk of hemorrhage.

2.2.1.3 Drug Metabolism Interaction: Drug metabolism is the modification or degradation of drugs. Metabolism can make drugs more toxic, less toxic, active, inactive, or more easily eliminated from the body. The primary organ involved in metabolism is the liver, although metabolism also occurs in the kidneys, lungs, gastrointestinal system, blood, and other tissues. The most extensively studied family of isoenzymes is the cytochrome P450 (CYP450) system, and they are responsible for biotransformation of many drugs via oxidation. The enzymes are heme-containing membrane proteins, which are located in the smooth endoplasmic reticulum of several tissues but the greatest proportion is carried out by enzymes that are found in the membranes of the endoplasmic reticulum of the liver cells commonly referred to as liver microsomal enzymes.

Drug metabolism via the cytochrome P450 system has emerged as an important determinant in the occurrence of several drug-drug interactions. A greater degree of interaction predictability has been achieved through the identification of P450 isoenzymes and some of the drugs that share them. The name "cytochrome P450" comes from the experimental techniques used to identify the isoenzymes. CYP2D6, for example, includes "2," the genetic family; "D," the genetic subfamily; and "6," the specific gene member. The nomenclature used to classify different subsets of the CYP system has no functional implications but clinically allows us to classify metabolism interactions.

To date, about 55 human isoforms (varieties) of CYP450 have been discovered, but six different P450 isoenzymes namely CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP2E1, and CYP3A4 that play important roles in drug metabolism have been identified. Out of these 6 isoenzymes, the CYP3A4 isoenzyme is predominant and has resulted in several clinically significant drug-drug interactions. Significant inactivation of some orally administered drugs is due to the extensive first-pass metabolism in the gastrointestinal tract by the CYP3A4 isoenzyme. The metabolic reactions that occurs in the liver is via two phases

i. Phase 1 metabolism: The reaction introduces or exposes a functional group (-OH, -NH2, -SH) to the parent compound, i.e. they are oxidation, reduction and hydroxylation reactions. Metabolites formed are inactive but in some instances active metabolites are also formed. The enzymes (CYPs) involved are located primarily in the endoplasmic reticulum. The CYPs catalyse a variety of phase one reactions including N-dealkylation, O- dealkylation, S-oxidation, epoxidation, and hydroxylation. Phase one reactions generally lead to loss of pharmacological activities e.g.morphine, propanolol and phenobarbitone metabolism. However prodrugs like levodopa and propanolol get bioactivated after phase one reaction. Other oxidative enzymes such as dehydrogenases and flavin containing monoxygenases are also capable of catalyzing the metabolism of specific drugs but in general such items are of minor overall importance (Kalra, 2007).

ii. Phase 2 Metabolism: This leads to the formation of a covalent linkage between a functional group on the parent compound or on the phase 1 metabolite with endogenously derived glucoronic acid, sulphate, glutathione, aminoacids or acetate i.e.(glucuronidation,

acetylation, sulfation, e.t.c). These lead to the formation of water soluble molecules, which aid elimination and detoxification of the drugs. The enzymes involved are located in the cytosol and phase 2 metabolism need not but often does occur after metabolism by the CYP450 system.

The phase 1 metabolic reactions by the CYP450 enzymes can be accelerated by drugs or environmental pollutants that stimulate the synthesis of more enzyme protein, enhancing the enzyme's metabolizing capacity. These drugs are referred to as enzyme inducers and their resultant effect is a reduction in plasma concentration of the unmetabolized drug. This is one of the most common mechanisms by which clinically important drug interactions occur. Since only a few different cytochrome P450 isoenzymes are involved in drug metabolism, competition between two drugs for these isoenzymes will occasionally occur.

Most drugs can exhibit decreased efficacy due to rapid metabolism, but drugs with active metabolites can display increased drug effect and/or toxicity due to enzyme induction. Examples of enzyme inducers include aminoglutethimide, barbiturates, carbamazepine, glutethimide, griseofulvin, phenytoin, primidone, rifabutin, rifampicin, and troglitazone. Drugs affected include haloperidol, theophylline, corticosteroids and oral contraceptives. Some drugs, such as ritonavir, may act as either an enzyme inhibitor or an enzyme inducer, depending on the situation. Drugs metabolized by CYP3A4 or CYP2C9 are particularly susceptible to enzyme induction. In some cases, especially for drugs that undergo extensive first-pass metabolism by CYP3A4 in the gut wall and liver, the

reduction in serum concentrations of the object drug can be profound. The analgesic drug acetaminophen is converted primarily to non-toxic metabolites, but a small amount is converted to a hepatotoxic metabolite. Enzyme inducers can increase the formation of the toxic metabolite and increase the risk of hepatotoxicity as well as damage to other organs.

Enzyme inhibition on the other hand occurs when two drugs sharing metabolism via the same isoenzyme compete for the same enzyme receptor site. The more potent inhibitor will predominate, resulting in decreased metabolism of the competing drug. For most drugs, this can lead to increased serum levels of the unmetabolized entity, leading to a greater potential for toxicity or effect. Inhibitors of CYP2C9 e.g. chloramphenicol and isoniazid can increase the risk of toxicity from phenytoin, tolbutamide, and oral anticoagulants such as warfarin. Inhibitors of CYP3A4 can increase the risk of toxicity from many drugs, including carbamazepine, cisapride, cyclosporine, ergot alkaloids, lovastatin, pimozide, protease inhibitors, rifabutin, simvastatin, tacrolimus, and vinca alkaloids.

For drugs whose pharmacological activity requires biotransformation from a pro-drug form, inhibition of the metabolism of these prodrugs may reduce the amount of active drug formed thus decreasing or eliminating their therapeutic effect. The analgesic codeine derives its pharmacological effect from bioactivation to morphine by CYP2D6 isoenzyme. Inhibitors of CYP2D6 can therefore impair the therapeutic effect of codeine. A drug may also be a substrate for i.e. metabolized by one or more of the enzyme subsets, and clinically, if an inhibitor or inducer affects that isoenzyme, it could affect the efficacy

of the drug. Drugs can have a complex profile, being a substrate for, or an inhibitor, or inducer of multiple subsets. For example, quinidine is a potent inhibitor of CYP2D6, but it is primarily metabolized by CYP3A4.

More than 50% of all drugs are metabolized at least in part by CYP3A4 or CYP2D6, and several important diabetes drugs are metabolized by these pathways (Triplitt, 2006). The two isozymes most affected by genetic control are also CYP2C19 and CYP2D6, (Belpaire and Bogaert 1996). Individuals lacking the gene for these isoenzymes are poor metabolizers and those possessing it are capable of normal drug metabolism and are considered extensive metabolizers. This genetically determined variations, for example is seen in 5–10% of Caucasians, but only 0–1% of Asians, have little CYP2D6 enzyme activity, making them poor metabolizers, the consequences of this are dependent on the drug and alternative pathways available for metabolism. (Guengerich *et al.*, 1998). Factors affecting drug metabolism include Age, Disease, Species Differences, Gender, Pregnancy, smoking and alcohol, Drug Dose, Enzyme Induction, Enzyme Inhibition, Diet and Heredity/Genetics.

Cytochrome P450 subfamilies and associated drug interactions

CYP3A: Members of the CYP3A subfamily are the most abundant cytochrome enzymes in humans. They account for 30 percent of the cytochrome P450 enzymes in the liver and are also substantially expressed in the intestines. CYP3A4 alone is responsible for more than 60% of the clinically prescribed drugs metabolized by the liver Terfenadine is a substrate of CYP3A4, high plasma concentrations of terfenadine have been associated

with torsade de pointes, a life-threatening cardiac arrhythmia characterized by altered cardiac repolarization and a prolonged QT interval. Terfenadine is a prodrug that undergoes complete first-pass metabolism to an active carboxymetabolite. It is therefore unusual to detect terfenadine in the plasma of patients who take this drug at the recommended dosage. Since it is terfenadine rather than its active metabolite that is cardiotoxic, arrhythmias occur when a build-up of parent terfenadine takes place as a result of CYP3A4 inhibition when co-administered with azole antifungal medications or macrolide antibiotics (Badyal and Dadhich, 2001).

Substrates, inhibitors and inducers of CYP3A are listed below

Substrates: Amitriptyline, Benzodiazepines, Alprazolam, Triazolam, Midazolam, Calcium blockers, Carbamazepine, Cisapride, Dexamethasone, Erythromycin, Ethinyl estradiol, Glyburide, Imipramine, Ketoconazole, Lovastatin, Terfenadine, Astemizole, Verapamil, Sertraline, Testosterone, Theophylline, Venlafaxine Ritonavir, Saquinavir, Indinavir , Nelfinavir .

Inhibitors: Nefazodone, Fluoxetine, Sertraline, Paroxetine, Venlafaxine, Ketoconazole, Itraconazole, Fluconazole, Cimetidine, Clarithromycin, Diltiazem, Erythromycin, Protease inhibitors.

Inducers: Carbamazepine, Dexamethasone, Phenobarbital, Phenytoin, Rifampin, Rimactane.

CYP2D6: This isoenzyme has been studied extensively because it exhibits genetic polymorphism, meaning that distinct population differences are apparent in its expression or activity. Approximately 5 to 10 percent of Caucasians and 1% of Asians are poor

metabolizers of drugs metabolized by CYP2D6 (Murray, 1992). Individuals with normal CYP2D6 activity are termed extensive metabolizers. Ethnic differences are indicated in this genetic polymorphism, since Asians and blacks are less likely than Caucasians to be poor metabolizer, (Benny and Adithan, 2001).

Poor metabolizers are at risk for drug accumulation and toxicity from drugs metabolized by this isoform. Poor metabolizers of CYP2D6 substrates are at risk for postural hypotension and antipsychotic side effects such as oversedation, because several antipsychotic agents are metabolized by CYP2D6. Conversely, when formation of an active metabolite is essential for drug action, poor metabolizers of CYP2D6 can exhibit less response to drug therapy compared with extensive metabolizers. Many antidepressants are also metabolized by CYP2D6, but other cytochrome P450 isoforms can also contribute to their metabolism.

Substrates: Amitriptyline, Clomipramine, Desipramine, Doxepin, Fluoxetine, Imipramine, Nortriptyline, Paroxetine. Haloperidol, Perphenazine, Risperidone, Thioridazine, Metoprolol, Penbutolol, Propranolol, Timolol, Codeine, Tramadol.

Inhibitors: Paroxetine, fluoxetinesertraline, fluvoxamine, Nefazodone, Venlafaxine Clomipramine, Amitriptyline, Haloperidol, Perphenazine, Fluphenazine and Cimetidine.

CYP2C9: This isoenzyme is involved in the metabolism of a few but very important drugs and inhibition of this isoform results in several clinically important drug interactions, an example of a substrate is S-Warfarin. Warfarin is produced as a racemic mixture of R-warfarin and S-warfarin, but the predominance of pharmacologic activity

resides in the S-enantiomer (Guttendoff, 1991). Most metabolism of S-warfarin is by means of CYP2C9 (Ngow *et al*., 2009) as such when given concomitantly with antifungals, metronidazole and amiodarone (CYP2C9 inhibitors) warfarin metabolism is profoundly inhibited producing marked increases in prothrombin time measurements. Phenytoin is also primarily metabolized via CYP2C9, although CYP2C19 may also play a small role (Levy, 1995).

Substrates: Non steroidal antiinflamatory drugs, Phenytoin, S-warfarin, Torsemide.

Inhibitors: Fluconazole, Ketoconazole, Metronidazole, Itraconazole, Ritonavir.

Inducers: Rifampin

CYP2C19: Like CYP2D6, CYP2C19 has been shown to exhibit genetic polymorphism (Kalra, 2007). This enzyme is completely absent in 3 percent of Caucasians and 20 percent of Japanese. Drugs metabolized by this isoform include the proton pump inhibitors such as omeprazole, lansoprazole and diazepam a benzodiazepine, (Badyal and Dadhich, 2001).

Substrate: Clomipramine, Diazepam, Imipramine, Omeprazole and Propanolol

Inhibitors: Fluoxetine, Sertraline, Omeprazole ,Ritonavir .

CYP1A2: It can be induced by exposure to polycyclic aromatic hydrocarbons, such as those found in charcoal grilled foods and cigarette smoke. This is the only P450 isoform affected by tobacco and cigarette smoking (Badyal and Dadhich, 2001) and can result in an increase of as much as threefold in CYP1A2 activity. Theophylline is metabolized in

part by CYP1A2 which explains why smokers require higher doses of theophylline than nonsmokers (Badyal and Dadhich, 2001). Certain quinolone antibiotics can also inhibit theophylline metabolism, although this effect is highly variable, additive inhibition of theophylline metabolism occurs when cimetidine an inhibitor of CYP1A2, is combined with a fluoroquinolone.

Substrates: Amitriptyline, Clomipramine, Clozapine, Imipramine, Propranolol, Theophylline. *Inducers:* Omeprazole, Phenobarbital, Phenytoin, Rifampin, Smoking. *Inhibitors:* Fluvoxamine, Grapefruitjuice, Quinolones, Ciprofloxacin, Enoxacin, Ofloxacin, Norfloxacin, lomefloxacin.

CYP2E1**:** This isoform is inducible by ethanol and isoniazid and is responsible in part for the metabolism of acetaminophen. The product of acetaminophen's cytochrome P450 metabolism is a highly reactive intermediate that must be detoxified by conjugation with glutathione. Patients with alcohol dependence are at increased risk for acetaminophen hepatotoxicity because ethanol induction of CYP2E1 increases formation of this reactive intermediate, and glutathione concentrations are decreased in these patients. Cimetidine exhibits only moderate affinity for this isoform and produces no significant inhibition of the production of acetaminophen's toxic metabolite. Substrates are Acetaminophen, Ethanol. Disulfiram is an inhibitor while Ethanol and Isoniazid are inducers.

2.2.1.4 Interactions due to Changes in Excretion.

Generally most drugs are excreted either in the bile or in the urine. Blood entering the kidneys along the renal arteries are delivered to the glomerulus of the tubules where

filtration occurs into the lumen of the tubules. The cells of the tubules possess active and passive transport system which enables further secretion and reabsorption of drug molecules as blood flows across the kidney. In the presence of other drugs or chemical moieties various factors come into play that influences or alters the normal functioning of the kidneys to excrete drugs as waste with a resultant effect in increasing or decreasing the plasma concentration of the drug. The following are the factors.

i. Altered Renal Elimination. For some drugs, active secretion into the renal tubules is an important route of elimination. For example, digoxin is eliminated primarily through renal excretion, and drugs such as amiodarone, clarithromycin, itraconazole, propafenone, and quinidine can inhibit this process resulting in elevated plasma levels of digoxin or digoxin toxicity.

ii. Interference with active kidney tubule secretion: Drugs that use the same active transport systems in the kidney tubules can compete with one another for excretion e.g. probenecid used clinically to reduce the excretion of penicillin thus prolonging its effect, which may also lead to toxicity.

iii. Changes in kidney blood flow: The flow of blood through the kidneys is partially controlled by the production of renal vasodilatory prostaglandins. If the synthesis of these prostaglandin is inhibited e.g. by indomethacin, renal excretion of drugs like lithium is reduced leading to a rise in serum levels.

iv. Alteration of urinary pH: Alteration of urinary pH either done intentionally or occurring unknowingly can influence the activities of certain drugs. The most important clinical implications of altering urinary pH involves the use of drugs that are excreted unchanged or in the form of an active metabolite. Alterations in urinary pH will influence ionization of weak acids and bases affecting the extent to which these agents are reabsorbed or excreted e.g. acidifying agents have been administered with methenamine to enhance its antibacterial actions

2.2.2 Pharmacodynamic Drug Interactions

These are interactions where the effects of one drug are changed by the presence of another drug at its site of action. Sometimes the drug directly compete for particular receptors e.g. beta-2 agonist such as salbutamol and beta- blockers such as propanolol but often the reaction is more indirect and involves physiological mechanisms.

2.2.2.1 Additive Pharmacodynamic Effects

When two or more drugs with similar pharmacodynamic effects are given, the additive effects may result in excessive response and toxicity e.g. Fluoxetine a Selective Serotonin Reuptake Inhibitor (SSRI) when administered with clomipramine (a tricyclic antidepressant with serotonergic activity) can cause serotonin syndrome in some patients.

2.2.2.2 Antagonistic Pharmacodynamic Effects

Drugs with opposing pharmacodynamic effects may reduce the response to one or both drugs, e.g. Non Steroidal Anti-inflammatory Drugs, such as ibuprofen or indomethacin,

may reduce the effectiveness of antihypertensives e.g. ACE inhibitors like captopril and lisinopril. Non-steroidal anti-inflammatory drug inhibit biosynthesis of prostaglandins, including renal vasodilator/natriuretic prostaglandins (PGE_2, PGI_2). If administered to patients receiving treatment for hypertension, they cause a variable but sometimes marked increase in blood pressure, and if given to patients being treated with diuretics for chronic heart failure can cause salt and water retention and hence cardiac decompensation.

2.2.2.3 Drugs with Opposing Pharmacologic Effects: Thiazide diuretics e.g bendrofluazide, hydrochlorthiazide may elevate blood glucose levels such that when prescribed for a diabetic who takes insulin or an oral hypoglycemic agent like tolbutamide, the hypoglycemic action of the antidiabetic drug may be partially counteracted by the diabetogenic agent necessitating a dosage adjustment.

2.2.2.4 Drugs with Similar Pharmacologic Effects: When anti-anxiety agents, antipsychotic agents, antihistamines, or other drugs having Central Nervous System (CNS) depressant effects are taken with alcoholic beverages there is likely to be an increased CNS-depressant effect. Combining drugs with CNS-depressant activity increases the risks of excessive sedation and dizziness.

2.2.2.5 Interactions at Receptor Sites: The enzyme Monoamine Oxidase (MAO) metabolizes catecholamines such as norepinephrine, this helps in balancing the production of norepinerphrine with the rate of destruction so that no accumulation results.

When MAO is inhibited, norepinephrine accumulates within the adrenergic neurons and can bring about exaggerated hypertensive responses characterized by severe headache, hypertension (possibly a hypertensive crisis), and cardiac arrhythmias. An interaction may occur between MAO inhibitors (e.g., isocarboxazid, phenelzine, tranylcypromine, pargyline) when taken with indirectly acting sympathomimetic amines. While most sympathomimetic amines (e.g., amphetamine) are available only by prescription, others (e.g., ephedrine, phenylephrine, phenylpropanolamine), known to interact with MAO inhibitors, are present in many popular Over the Counter (OTC) cold, allergy, and diet remedies. Certainly, it becomes imperative that patients being treated with MAO inhibitors avoid using these products. It is the duty of the clinician and pharmacist to take a good history of patients past and present medication in order to give counsel on the use of these agents to prevent hypertensive crisis.

2.2.2.6 Interactions due to Disturbances in Fluid and Electrolyte Balance: Interactions can occur as a result of alterations in the levels of fluid and electrolyte in particular tissues or organs. In the use of potassium depleting diuretics such as furosemide, there is a fall in plasma potassium causing an increase in the sensitivity of the myocardium to the effect of digitalis glycosides and resultant toxicity. There is also an increase in plasma lithium levels on concomitant administration with thiazide diuretics. This is because the clearance of the lithium by the kidney is changed probably as a result of changes in sodium excretion.

2.3 Diabetes Mellitus

Diabetes Mellitus (DM) is a chronic systemic disease caused by absolute or relative deficiency of insulin. It is a group of metabolic disorders of carbohydrates, lipids and protein metabolism in which glucose is underutilised, producing hyperglycaemia (WHO, 1999). The disease manifests as disorders of metabolism, macro-vascular and micro-vascular diseases involving the eyes, nerves, kidneys, and basement membrane lesions.

2.3.1 Classification of Diabetes Mellitus

DM has been classified (WHO, 1999) into three, based on the etiology of the disease

2.3.1.1 Type 1: Type 1 Diabetes Mellitus (T1DM) also referred to as juvenile onset or Insulin Dependent Diabetes Mellitus, is characterized by auto immune destruction of pancreatic beta-cell in which "insulin is required for survival" to prevent the development of ketoacidosis, coma and death. Type 1 indicates the processes of beta cell destruction and an individual with type 1process may be metabolically normal before the disease is clinically manifest, but the process of beta-cell destruction can be detected. Type 1 is usually characterized by the presence of, islet cell or insulin antibodies which identify the autoimmune processes that lead to beta-cell destruction. In some subjects with this clinical form of diabetes, particularly non-Caucasians, no evidence of an autoimmune disorder is demonstrable and these are classified as "Type 1 idiopathic (WHO, 1999).

2.3.1.2 Type 2: Type 2 Diabetes Mellitus (T2DM) also referred to as adult onset or Non Insulin Dependent Diabetes Mellitus is the most common form of diabetes. It is a heterogeneous group of disorders characterized by variable degrees of insulin resistance,

impaired insulin secretion and increased glucose production. It is preceded by a period of abnormal glucose homeostasis classified as impaired fasting glucose (IFG) or impaired glucose tolerance (IGT). Type 2 diabetes is becoming more prevalent owing to rising rates of obesity, physical inactivity and urbanization (Levitt, 2008) necessitating months of hospitalization for some patients within a single year.

2.3.1.3. Type 3/Gestational Hyperglycemia: Gestational hyperglycaemia is carbohydrate intolerance resulting in hyperglycemia of variable severity with onset or first recognition during pregnancy. It does not exclude the possibility that the glucose intolerance may antedate pregnancy but has been previously unrecognized. Women who become pregnant and who are known to have Diabetes Mellitus which antedates pregnancy do not have gestational diabetes but have "Diabetes Mellitus and pregnancy" and should be treated accordingly before, during, and after the pregnancy (WHO, 1999).

In the early part of pregnancy, (i.e. first trimester and first half of second trimester) fasting and postprandial glucose concentrations are normally lower than in normal non-pregnant women. Elevated fasting or postprandial plasma glucose levels at this time in pregnancy may well reflect the presence of diabetes which has antedated pregnancy.

The occurrence of higher than usual plasma glucose levels at this time in pregnancy mandates careful management and may be an indication for carrying out an Oral Glucose Tolerance Test (OGTT). Nevertheless, normal glucose tolerance in the early part of pregnancy does not itself establish that gestational diabetes may not develop later. Individuals at high risk for gestational diabetes include older women, those with previous

history of glucose intolerance, those with a history of large gestational age babies, women from certain high-risk ethnic groups, and any pregnant woman who has elevated fasting, or casual, blood glucose levels. It may be appropriate to screen pregnant women belonging to high-risk populations during the first trimester of pregnancy in order to detect previously undiagnosed Diabetes Mellitus. Formal systematic testing for gestational diabetes is usually done between 24 and 28 weeks of gestation.

2.3.1.4 Other Specific Types: These specific types are currently less common causes of Diabetes Mellitus, but are those in which the underlying defect or disease process can be identified in a relatively specific manner. They include for example, fibrocalculous pancreatopathy, a form of diabetes which was formerly classified as one type of malnutrition-related Diabetes Mellitus (WHO, 1999).

2.3.2 Factors Predisposing to Type 2 Diabetes Mellitus

The major risk factors for type 2 Diabetes Mellitus includes the following

Age: There is increase prevalence in individuals that are older than 45 years (though type 2 Diabetes Mellitus is occurring with increasing frequency in young individuals).

Obesity: All obese individuals have insulin resistance and persons with weight greater than 120% of desirable body weight. About 90% of type 2 diabetes is attributable to excess weight (Hossain *et al.*, 2007).

Genetic predisposition: Family history of type 2 diabetes in a first-degree relative (i.e. parent or sibling) and Hispanics, Native Americans, African Americans, Asian Americans, or Pacific Islander descents also have an increased risk of developing T2DM.

Past and present medical history: Example is history of previous impaired glucose tolerance (IGT) or impaired fasting blood glucose (IFG), Hypertension (>140/90 mm Hg) or dyslipidemia (low-density lipoprotein [LDL]), cholesterol level >40 mg/dl or triglyceride level >150 mg/dl). Also women who have history of GDM or of delivering a baby with a birth weight of >9 pounds, and Polycystic ovarian syndrome (which results in insulin resistance (Votey and Peters, 2008).

2.3.4 Complications of Diabetes Mellitus

The morbidity and mortality associated with diabetes are related to the short and long term complications. Complications include the following

Hyperglycaemia, resulting in (i) Diabetic ketoacidosis (DKA) in Type 1 Diabetes Mellitus and (ii) Nonketotic hyperosmolar coma in Type 2 Diabetes Mellitus.

Hypoglycemia due to excess insulin and body reacts to the stress of hypoglycemia, or treatment. Symptoms include tachycardia, perspiration, shakiness, and anxiety, progressively followed by confusion, seizure and coma. There is also an increased risk of both acute and chronic infections, microvascular complications e.g. retinopathy, nephropathy and neuropathy. Also seen are macrovascular diseases e.g. coronary artery disease and stroke (Votey and Peters, 2008).

2.3.5 Management of Diabetes Mellitus

Goals of therapy: Treatment of type 2 diabetes is aimed at lowering insulin resistance and increasing function of beta cells, the primary target being glycemic control, which is assessed by the following indisces; Glycosylated haemoglobin (Hb A1C) < 7%, Fasting

Plasma Glucose (FPG) < 126 mg/dl (7.0 mmol/l), Random Plasma Glucose (RPG) < 200 mg/dl (11.1 mmol/l) (Odili, 2010).

In addition to symptoms (polyuria, polyphagia, polydypsia) control, the target Blood pressure of a diabetic patient as against that of a non diabetic is BP < 130/80 and Cholesterol level < 200 mg/dl. It is very important that the blood pressure and cholesterol levels be well controlled because dyslipidaemia and hyperglycemia are among the major risk factors for development of cardiovascular disease (CVD) and dyslipidaemia might at least in part, explain the additional risk associated with hyperglycemia (Albert, 2008).
Others are to treat physiologic derangement when present, prevent complications of Diabetes Mellitus, maintain normal growth in children and to promote emotional well being of the patient.

Management of Type 2 Diabetic Mellitus aims at lowering plasma glucose levels with the use of oral glucose lowering agents that target different pathophysiologic processes in T2DM. Classes of oral hypoglycaemic agents are based on their mechanism of action and include:

i. Sulphonyl ureas e.g Glibenclamide: They increase insulin secretion directly and decrease glucagon release. They are useful only in patients with intact and functioning beta cells.

ii. Biguanides e.g. Metformin: They lower blood glucose by reducing hepatic glucose production and glycogen metabolism in the liver and enhancing insulin mediated glucose uptake by skeletal muscle.

iii. Alpha Glucosidase inhibitors e.g. Acarbose: They are particularly effective in patients who experience postprandial hyperglycaemia. They reduce the rate of complex carbohydrate digestion by preventing the hydrolysis of complex starches, oligosaccharides, trisaccharides into the readily digestible monosaccharide.

iv. Thiozolidinediones e.g. Rosiglitazone.: They are insulin sensitizers that lowers blood glucose by enhancing the action of insulin and reducing insulin resistance.

iv. Non-sulfonylurea secretagogues e.g. Nateglinide and repaglinide: Their action is similar to that of sulphopnyl ureas. They cause an increase in insulin secretion from the pancreas.

The guidelines and treatment algorithm in the management of T2DM as presented by (Nathan *et al*, 2006) emphasize achievement and maintenance of normal glycemic goals by instituting an initial therapy with lifestyle intervention and Metformin. This is followed by rapid addition of medications and transition to new regimens when target glycemic goals are not achieved or sustained. Finally, an early addition of insulin in patients who do not meet treatment goals.

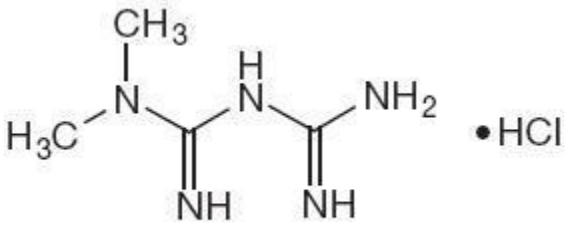
American Diabetes Association and the European Association for the Study of Diabetes, in a consensus statement in 2006, recognized that for most individuals with type 2 diabetes, when lifestyle interventions fail to achieve or maintain metabolic goals, Metformin is recommended as the initial pharmacologic therapy. This is in the absence of specific contraindications, for its effect on glycaemia, absence of weight gain and not associated with marked hypoglycaemia. Metformin also has low level of side effects,

high level of acceptance, and relatively low cost (Nathan *et al*., 2006; Powers, 2006). It improves insulin sensitivity in muscle tissues thereby improving a variety of other factors related to increased cardiovascular risk (Sheehan, 2003). Therefore, the consensus is that Metformin therapy be initiated concurrently with lifestyle intervention at diagnosis.

2.4 Metformin

Metformin hydrochloride is a biguanide oral hypoglycaemic agent used in the management of type 2 diabetes. Metformin hydrochloride (N,N-dimethylimidodicarbonimidic diamide monohydrochloride) is not chemically or pharmacologically related to sulfonylureas, thiazolidinediones, or α-glucosidase inhibitors. It is a white to off-white crystalline compound with a molecular formula of C4H12ClN5 (monohydrochloride) and a molecular weight of 165.63. Metformin hydrochloride is freely soluble in water and is practically insoluble in acetone, ether, and chloroform. The pKa of Metformin is 12.4. The pH of a 1% aqueous solution of Metformin hydrochloride is 6.68.

The structural formula is as shown:



Metformin Hydrochloride

2.4.1 Mechanisms of Action

Metformin lowers blood glucose by reducing hepatic glucose production and glycogen metabolism in the liver. It also enhances insulin mediated glucose uptake by skeletal muscle and increases glycolysis. Metformin is the only currently available oral antidiabetic/hypoglycemic agent that acts predominantly by inhibiting hepatic glucose release. This is very important because patients with type 2 diabetes often have excess hepatic glucose output. Use of Metformin is effective in lowering glycosylated hemoglobin (HbA_{1c}) by 1 to 2 percentage points when used as monotherapy or in combination with other blood glucose-lowering agents or insulin. Other metabolic variables (eg, dyslipidaemia, fibrinolysis) may be improved with the use of Metformin, while body weight is often maintained or slightly reduced from baseline. (Setters *et al.,* 2003)

2.4.2 Pharmacokinetics

Metformin hydrochloride is slowly and incompletely absorbed from the gastrointestinal tract. The absolute bioavailability of a single 500-mg dose is reported to be about 50 to 60%, although this is reduced somewhat if taken with food. Once absorbed plasma protein binding is negligible and is distributed into breast milk in small amounts.

Metformin does not undergo metabolism and is substantially excreted unchanged in the urine by tubular secretion and glomerular filtration. The plasma elimination half-life, range from about 2 to 6 hours after oral doses. Metformin is a cationic (positively charged) molecule and may compete with other cationic drugs for renal secretion through organic cation transporters in the kidneys. Procainamide, digoxin, quinidine,

trimethoprim, and vancomycin are all cationic drugs that have potential to interact with Metformin, but only cimetidine has been implicated in one case of Metformin associated lactic acidosis (MALA) (Somogyi *et al*., 1987). Lactic acidosis is a rare, but potentially severe consequence of therapy with Metformin occurring in cases of over dosage or impaired renal function. The risk of accumulation and lactic acidosis increases with the degree of impairment of renal function. Patients with renal function below the limit of normal for their age should not receive Metformin. In elderly patients, renal function should be monitored regularly and Metformin should not be used in any patient ≥80 years of age unless measurement of creatinine clearance verifies normal renal function. Use of concomitant medications that may affect renal function (i.e. tubular secretion) e.g cephalexin, may also affect Metformin disposition, leading to accumulation resulting in hypoglycaemia and increased toxicity.

2.4.3 Adverse Reactions

Metformin is well tolerated and is associated with few clinically deleterious adverse effects. The most important and potentially life-threatening adverse effect associated with its use is lactic acidosis. Lactic acidosis is to be suspected in any diabetic patient receiving Metformin who has evidence of acidosis when evidence of ketoacidosis is lacking. Lactic acidosis is characterized by elevated blood lactate levels (> 5 mmol/L), decreased blood pH, electrolyte disturbances with an increased anion gap, and an increased lactate/pyruvate ratio. When Metformin is implicated as the cause of lactic acidosis, Metformin plasma levels > 5 µg/mL are generally found. Metformin is to be discontinued in clinical situations predisposing to hypoxemia, including conditions such

as cardiovascular collapse, respiratory failure, acute myocardial infarction, acute congestive heart failure, acute or chronic metabolic acidosis with or without coma (including diabetic ketoacidosis) and septicemia. Metformin should be suspended in patients with dehydration and/or pre renal azotaemia.

Gastrointestinal side effects include Nausea, vomiting, diarrhea and flatulence. Central nervous system effects seen are headache, chills, dizziness and lightheadedness. Skin rash is also seen and hypoglycaemia as severe endocrine and metabolic effect. Other effects are muscular weakness, myalgia chest discomfort, flushing, and palpitations. On prolonged use vitamin B_{12} deficiency may also occur.

Drug interactions resulting from absorption, distribution, metabolism, or elimination, as well as pharmacodynamic factors, are present for many common medications given to people with diabetes (Dinesh *et al.,* 2007).

2.4.4 Mechanisms of Drug Interactions involving Metformin

2.4.4.1 Absorption Interactions. This does not only apply to oral administration of a medication and absorption from the gastrointestinal system, but it applies to all routes of administration, including injection, inhalation, topical, buccal, sublingual, and others. Drug-food interactions can affect the total amount of drug absorbed (bioavailability), but most often they only slow absorption. For example, the absorption of Metformin is delayed in the presence of food (Berelowitz *et al*., 1994). Metformin may cause malabsorption of vitamin B12, which may result in B12 deficiency and subsequent anemia (Kimura *et al.,* 2005). The mechanism by which metformin causes malabsorption

of B12 is not clearly defined, although oral or injected B12 (cyanocobalamin) or calcium supplementation may be effective for correction

2.4.4.2 Distribution Interactions: Distribution interactions can be significant for drugs that have extremely rapid distribution, narrow safety margins, and possibly nonlinear kinetics. No significant distribution interactions are pertinent for oral medications commonly used for diabetes (Benet and Hoener, 2002).

2.4.4.3 Metabolism Interactions: Drugs can inhibit (decrease) metabolism, induce (increase) metabolism, or have no effect on each CYP450 isoenzyme subset. Thus, inhibition of metabolism will likely increase the affected drug's systemic concentrations, whereas induction of metabolism often reduces systemic concentrations. Not all isoenzymes are inducible and only CYP2C9 and CY P3A4 induction is clinically relevant to people with diabetes. Metformin is not metabolized and is excreted renally as unchanged, by tubular secretion and glomerular filtration, it is thus unlikely that drug interactions would occur via this pathway (Triplitt, 2006).

2.4.4.4 Elimination Interactions: Renal drug-drug interactions are dependent on the pH of the urine and the pH of the drug or on competition for the same pathway of elimination. If the pH of the urine and the drug are the same, renal reabsorption of the drug will be increased. When two drugs compete for elimination through a single route, one drug may competitively inhibit the elimination of the other. Metformin and cimetidine, both cationic (positively charged) drugs, can compete for elimination through kidneys by renal tubular secretion, resulting in higher Metformin concentrations in the plasma (Somogyi *et al*, 1987). Organic Cation Transporter (OCTs) transports a number of

drugs including cimetidine, Metformin, procainamide, and triamterene from the plasma into hepatocytes and renal tubular cells. As with cytochromes, a variety of different Organic Anion Transporters (OAT) and OCT exist. Metformin's uptake into the liver, where it exerts its pharmacologic effect, is mediated by OCT1, while its elimination via the kidney is primarily due to OCT2. The capacity of OCT2 to transport Metformin is at least 10 times greater than OCT1. Thus, OCT2 and the renal tubular secretion of Metformin are primarily responsible for its pharmacologic properties. Cimetidine also is a substrate for OCT and can compete with Metformin for both OCT1 and OCT2. Because OCT2 is primarily responsible for Metformin's elimination, competition from cimetidine will result in reduced renal clearance of Metformin and elevated plasma concentrations (Wang, 2008).

Cephalexin, a first generation cephalosporin has been shown to exhibit a moderate drug\drug interaction when co administered with Metformin necessitating close monitoring of patients. The concomitant administration of cephalexin may increase plasma concentrations and adverse effects of Metformin. The mechanism is competitive inhibition of active renal tubular secretion. After administration of single doses of 500 mg cephalexin and Metformin, the mean maximum plasma concentration (Cmax) of Metformin increased by 34%, area under the concentration-time curve (AUC) increased by 24% and Metformin renal clearance decreased by 14% (Jayasagar *et al.*, 2002). Patients receiving this combination should be monitored for altered efficacy and safety of Metformin, which may necessitate dosage adjustments.

Another study clearly indicated that availability of Metformin can be altered in presence of most of the H2 receptor antagonists studied except in presence of famotidine at pH 4 where the drug concentration remains unaltered. The availability of Metformin was increased in simulated gastric juice, pH 7.4 and pH 9 (except ranitidine at pH 9) (Sultana *et al.,* 2006).

2.4.4.5 Pharmacodynamic interactions: Several documented studies revealed the drug drug interactions of Metformin with quite a number of medications ranging from simple cold medications to antihypertensives, oral contraceptives phenothiazines, phenytoin, corticosteroids and antibiotics e.g gratifloxacin and cephalexin leading to loss of glycaemic control. Gratifloxacin may also interfere with the therapeutic effects of other oral antidiabetic agents and insulin.

The use of various quinolones has been associated with disturbances in blood glucose homeostasis possibly by interfering with pancreatic beta cell ATP-sensitive potassium channels that regulate insulin secretion. However, hypoglycemia and hyperglycemia have been reported more frequently with gratifloxacin than with other quinolones. Gratifloxacin-induced hypoglycemic episodes have generally occurred within the first 3 days of therapy and sometimes even after the first dose, while hyperglycemia usually occurred 4 to 10 days after initiation of therapy. Death has been reported in severe cases. Elderly patients and patients with reduced renal function are particularly susceptible. As such the use of gratifloxacin is therefore contraindicated in patients with Diabetes Mellitus. Other quinolones may be safer alternatives in such patients, although all

quinolones should be used with caution. Blood glucose should be monitored closely whenever quinolones are prescribed to patients receiving oral antidiabetic agents and/or insulin, especially if they are elderly or have renal impairment. Patients should be counseled to recognize symptoms of hypoglycemia such as headache, dizziness, drowsiness, nausea, tremor, weakness, hunger, excessive perspiration, and palpitations. If hypo or hyperglycemia occurs during quinolone therapy, patients should initiate appropriate remedial therapy immediately and discontinue the antibiotic.

In a recent study conducted by (Anitha *et al*., 2008) in diabetic rats, the actions of Metformin enhanced the hypolipidaemic activity of atorvastatin and rosuvastatin. Similarly atorvastatin and rosuvastatin enhanced the hypoglycaemic effect of Metformin, due to pharmacodynamic interactions. The drug combinations also increased the body weight of the diabetic animals.

2.4.5 Drug Disease Interactions

Many co morbid diseases can affect metabolism in people with diabetes. Patients with diabetes have higher rates of infections, cardiovascular, renal, gastrointestinal, neurological, thyroid diseases and ophthalmological complications compared to individuals without diabetes. All may increase the chance of having drug-disease interactions. Metformin has many drug-disease interactions that can increase the risk of Metformin Associated Lactic Acidosis (MALA). Metformin and impaired renal function is a well-documented drug-disease interaction. Serum creatinine 1.4 mg/dl in women or

1.5 mg/dl in men warrants discontinuation of Metformin because systemic concentrations can be elevated, increasing the risk of lactic acidosis.

Metformin may increase lactic acid production from the splanchnic tissues slightly, but MALA occurs in the setting of co morbid diseases that increase systemic levels of lactic acid or reduce elimination of Metformin. Tissue hypoperfusion from congestive heart failure, hypoxic states, shock, or septicemia can increase lactic acid production, and alcohol or severe liver disease can reduce removal of lactic acid in the liver, increasing the risk of lactic acidosis.

2.5 Infection in the Diabetic

Individuals with DM have a greater frequency and severity of infections (Joshi *et al.*, 1999) leading to considerable morbidity and mortality in patients. The reasons for these include incompletely defined abnormalities in cell mediated immunity and phagocyte function associated with hyperglycaemia as well as diminished vascularisation (Powers, 2006). Hyperglycemia and acidemia also exacerbates impairments in humoral immunity, polymorphonuclear leukocyte and lymphocyte functions causing infections to thrive easily (Votey and Peters, 2008). These impairments are substantially, if not entirely reversed when pH and blood glucose levels return to normal. Although the exact level above which leukocyte function is impaired is not defined, *in vitro* evidence suggests that glucose levels greater than 200 mg/dL impair leukocytic function (Votey and Peters 2008).

Patients with long-standing diabetes tend to have microvascular and macrovascular disease with resulting poor tissue perfusion and increased risk of infection. The ability of the skin to act as a barrier to infection may be compromised when the diminished sensation of diabetic neuropathy results in unnoticed injury. Several immune function factors are related to this increased risk first, neutrophil function is depressed, affecting adherence to endothelium, chemotaxis, and phagocytosis (Deasy, 2005). The antioxidant systems involved in bacteriocidal activity may be compromised, and cell-mediated immunity is probably depressed.

Many common infections are more frequent and severe in the diabetic populations whereas several rare infections are seen almost exclusively in the diabetics e.g. are rhinocerebral mucomycosis, emphesematous infections of the gall bladder, malignant or invasive otitis media and a greater risk of post operative wound infection (Powers, 2006).

Acute infections in Diabetes Mellitus include:

Urinary tract infections: They are more common among patients with diabetes (Geerlings *et al.,* 2000). *E. coli* is the most common causative organism, followed by other gram-negative bacteria. Because of the high incidence of often unsuspected upper tract infection, a 7 to 14 days course of therapy has been recommended for treatment of cystitis among patients with diabetes (Stamm and Hooten, 1993). Stable infections of the UTI (acute pyelonephritis, exacerbation of chronic pyelonephritis e.t.c.) often results in a septic condition which is conducive to decompensation of Diabetes Mellitus and even the development of diabetic coma (Potemkin, 1981).

Respiratory tract infections: Pneumonia has traditionally been described as a major cause of morbidity and mortality among patients with diabetes. Pneumonia due to both gram-negative organisms and fungi has been described as being more frequent among diabetic patients (Koziel and Koziel, 1998). Organisms implicated are *Streptococcus pneumoniae* and influenza. The result of a study on pattern of DM in the savannah north of Nigeria reports that acute infection occurred in 20.9% of patients and contributed to the morbidity in those who presented with acute diabetic complications such as ketoacidosis and hyperosmolar non diabetic coma (Onyemelukwe and Bakare, 2005). There were also clinical challenges posed by typhoid fever and acute viral hepatitis.

Chronic infections include;

Renal infections: More than 70% of cases of emphysematous pyelonephritis occurred in patients with diabetes. Emphysematous pyelonephritis is an uncommon necrotizing renal infection caused by *Escherichia coli, Klebsiella pneumoniae,* or other organisms capable of fermenting glucose to carbon dioxide. Other renal infections that are more common in individuals with diabetes include renal carbuncles (intrarenal abscesses caused by the hematogenous spread of *S. aureus*), renal corticomedullary abscesses (intrarenal foci of infection associated with reflux and obstruction caused by the same organisms that typically cause pyelonephritis).

Skin and soft tissue infection: Soft tissue infections of the lower extremities and gangrene are among the most dreaded complications associated with diabetes. Patients with diabetes clearly have an elevated risk of infected lower-extremity ulceration and

subsequent amputation. Foot ulcers usually occur in patients with sensory polyneuropathy who develop skin breakdown after unrecognized trauma. Infection more often polymicrobial, then easily occurs in tissue with an inadequate microvascular or macrovascular blood supply.

Ear, nose, and throat infections: Two head and neck infections that are associated with high rates of morbidity and mortality are malignant otitis externa and rhinocerebral mucormycosis, these are seen almost exclusively in patients with diabetes. Malignant or necrotizing otitis externa principally occurs in patients with diabetes who are older than 35 years and is almost always due to *Pseudomonas aeruginosa*. Infection starts in the external auditory canal and spreads to adjacent soft tissue, cartilage, and bone. Patients typically present with severe ear pain and otorrhea. Although they often have preexisting otitis externa, progression to invasive disease is usually rapid. Intravenous antibiotics should be started immediately in patients with invasive disease. Patients with diabetes with severe otitis externa but no evidence of invasive disease can be treated with an otic antibiotic drop and oral ciprofloxacin.

Mucormycosis collectively refers to infections caused by various ubiquitous molds. Invasive disease occurs in patients with poorly controlled diabetes, especially with DKA. Organisms colonize the nose and paranasal sinuses, spreading to adjacent tissues by invading blood vessels and causing soft tissue necrosis and bony erosion. Patients usually present with periorbital or perinasal pain, swelling, and induration. Osteomyelitis, the

contiguous spread of a polymicrobial infection from a skin ulcer to adjacent bone is also common in patients with diabetes

2.5.1 Treatment of Infections in the Diabetic

In general, the selection of antibiotics in treating infections should be based on results from microbial culture and sensitivity test. Consideration is also given to the patient and factors which must be considered include disease states, history of allergy, renal and hepatic function, genetic factors, severity of sickness, age, and if female whether pregnant or breastfeeding. In the diabetic patient, it becomes important that antibiotics to be used should be highly effective and do not interact with hypoglycaemic agents. This is in order not to interfere with glycaemic control and alter other important biochemical parameters useful in the monitoring of therapeutic management of acute presentation of T2DM.

Antibiotic treatment failure is also a possible consequence of drug interactions. Various antibiotics have been employed in the treatment of infections in the diabetics depending on the susceptibility of the organism and the site and severity of the infection (Schaberg, 2002).

Cephalosporins are broad spectrum antibacterial agents, they are structurally similar to the penicillins but are not susceptible to the action of beta lactamase producing bacteria. Cephalosporins are also used in all age groups and presently constitute one of the life saving antibiotics in severe septicaemia. They are grouped into generations based on their

spectrum of antimicrobial activity with each newer generation having significantly greater Gram-negative antimicrobial properties than the preceding generation. Cefixime, a third generation cephalosporin was endorsed by the World Health Organization as an optimal therapy in Multi Drug Resistance treatment of uncomplicated typhoid fever in children (WHO, 2003). Cefixime, an oral cephalosporin which was introduced in Nigeria in 2008 is documented to have efficacy comparable with ceftriaxone another third generation cephalosporin available as injectable in the treatment of Lower Respiratory Tract infections including acute exacerbations of chronic bronchitis and is employed as a switch from intravenous ceftriaxone. It showed markedly better *in vitro* activity against certain major respiratory tract pathogens than the other orally administered antimicrobials commonly used against respiratory tract infections (Lehtonen and Huovinen, 1993).

It is also employed in uncomplicated gonorrhoea and Urinary Tract Infections (UTIs) which is a serious health problem affecting millions of people each year. It is used in Multi Drug Resistant and Quinolone resistant Thyphoid fever (Santillan *et al.,* 2000) and it shows excellent penetration into pancreatic and liver tissues, consequently making them effective in infections of susceptible organism in these organs.

2.6 Cefixime

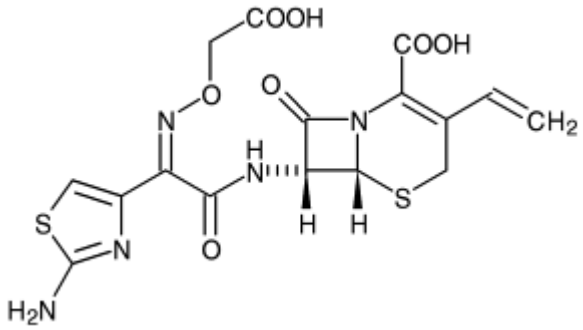
Cefixime is a semisynthetic third-generation cephalosporin antimicrobial formulated for oral administration only. It Inhibits bacterial cell wall synthesis by binding to one or more of the penicillin binding proteins (PBPs), which in turn inhibits the final transpeptidation step of peptidoglycan synthesis in bacterial cell walls thus inhibiting cell

wall biosynthesis. Bacteria eventually lyse due to ongoing activity of cell wall autolytic enzymes (autolysins and murein hydrolases) while cell wall assembly is arrested.

Cefixime has an aminothiazole group on the acyl side chain and is also extremely stable in the presence of B-lactamase enzymes. Its B-lactamase stability is greater than that of the aminopenicillins (e.g., ampicillin, amoxicillin) and the orally administered first-generation cephalosporins (e.g. cephalexin, cephadrine, cefadroxil). Therefore, many organisms that are resistant to penicillins and some cephalosporins may be susceptible to cefixime.

2.6.1 Chemical name and structure

The chemical name is [6*R*-[6α,7β(*Z*)]]-7-[[(2-Amino4thiazoyl)[(carboxymethoxy)imino]acetyl]amino]-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid. The Molecular weight is 507.50 as the trihydrate.



Cefixime

Chemical Formula is $C_{16}H_{15}N_5O_7S_2.3H_2O$

2.6.2 Pharmacokinetics

Only 40 to 50% of an oral dose of **C**efixime **i**s absorbed from the gastrointestinal tract, whether taken before or after meals, although the rate of absorption may be decreased in the presence of food. Cefixime is better absorbed from oral suspension than from tablets. Absorption is fairly slow, peak plasma concentrations of 2 to 3 micrograms/ml and 3.7 to 4.6 micrograms/ml have been reported between 2 and 6 hours after single doses of 200 and 400 mg, respectively. The serum elimination half-life of Cefixime in adults with normal renal function averages 2.4–4 hours, and serum half-life of Cefixime is independent of dosage form (Kees *et al*., 1990) and is not dose-dependent. The serum half-life of Cefixime is prolonged in patients with impaired renal function (Nakashima *et al*., 1987). About 65% of Cefixime is bound to plasma proteins and it crosses the placenta. Relatively high concentrations may be achieved in bile and urine.

There is no evidence that Cefixime is metabolized *in vivo*, no microbiologically active metabolites have been detected in serum or urine following oral administration of the drug (Nakashima *et al.,* 1987; Brodgen, 1989). Cefixime is eliminated by renal and non renal mechanisms. About 20% of an oral dose (or 50% of an absorbed dose) is excreted unchanged in the urine within 24 hours. The drug is excreted principally by glomerular filtration and to a lesser extent by tubular secretion; the remainder of the dose (up to 60%) is eliminated by non renal mechanisms.

2.6.3 Indications

Cefixime is effective against various gram-positive and gram-negative organisms. Its broad-spectrum antibacterial activity includes many Enterobacteriaceae, *Streptococcus spp*, and *Haemophilus spp in vitro*. It also has some activity against *Staphylococcus aureus* but not against *Bordetella bronchiseptica* or *Pseudomonas aeruginosa*. In humans, Cefixime is often used to treat urinary tract infections (UTIs), otitis media, pharyngitis, tonsillitis, and bronchitis. Cefixime has excellent *in vitro* activity against pathogenic bacteria such as *Escherichia coli, Proteus* and other Gram-ve organisms. These organisms are most commonly associated with UTIs in diabetic patients (Joshi *et al.,* 1999).

2.6.4 Adverse Reactions

Mild to moderate reactions are seen and this include

Gastrointestinal Tract (GIT) effects: These occur in 2-10% of patients and include diarrhea/abdominal pain, nausea, dyspepsia, flatulence, loose stools, vomiting and hepatitis.

Reactions that are seen in < 2% of patients are haematological reactions such as leukopenia, neutropenia, prolonged Prothrombin Time (PT), thrombocytopenia and eosinophilia. Others include pseudomembranous colitis, rash, pruritus, urticaria, serum sickness-like reaction, erythema multiforme, toxic epidermal necrolysis anaphylactic/anaphylactoid reactions and Stevens-Johnson syndrome. Also seen are acute renal failure, increase in creatinine levels, angioedema, dizziness, drug fever, facial oedema, headache, hyperbilirubinemia, jaundice, seizure, increased liver enzyme

(transaminases) levels, increased candidiasis and vaginitis. Adverse reactions reported with other cephalosporins not associated with Cefixime include interstitial nephritis, aplastic anemia, hemolytic anemia, hemorrhage, pancytopenia, agranulocytosis, colitis and superinfection.

2.6.5 Drug Interactions

Niwa *et al*, (2004) demonstrated the effects of two kinds of oral cephalosporins, Cefixime and cefdinir on cytochrome P450 (CYP) activities in human hepatic microsomes and the results suggested that both Cefixime and cefdinir at concentrations of 2millimoles would not cause clinically significant interactions with other drugs which are metabolized by CYPs via the inhibition of metabolism.

The simultaneous administration of nifedipine and Cefixime has been shown to increase the oral absorption of the antibiotic. Nifedipine could increase Cefixime absorption via several mechanisms, these are:

(1) Slowing down of fluid movement through the gut, thus allowing more time for absorption to occur.

(2) Increase in intestinal blood flow, thus favoring passive absorption (provided that transmembrane passage is not a limiting factor);

(3) Increase in (directly or indirectly,) the rate of uptake by epithelial carriers, (Harcouet *et al.*, 1997).

Cefixime also forms complexes with many essential and trace elements present in the body. Antibacterial studies of metal complexes of Cefixime, with copper, zinc and

cadmium showed a decrease in antimicrobial activity of Cefixime and increase in MIC values after complexation (Arayne *et al*., 2002). Increased prothrombin time (with or without bleeding) has been reported following concomitant use of Cefixime with an anticoagulant (e.g., warfarin). Cephalosporins may be associated with a fall in prothrombin activity especially those that possess the N- Methylthiotetrazole side chain le.g. Cefixime, cefminox and cefmetazole. (Stockley, 2002).Those at risk include patients with renal or hepatic impairment, or poor nutritional state, as well as patients receiving a protracted course of antimicrobial therapy, and patients previously stabilized on anticoagulant therapy.

Concomitant administration of Cefixime and carbamazepine has resulted in increased plasma carbamazepine concentrations. Carbamazepine concentrations should be monitored if the drug is used concomitantly with cefixime. The concomitant administration of probenecid reportedly increases peak serum concentrations and the Area under the curve (AUC) of Cefixime and decreases renal clearance and volume of distribution of the drug. The increase in cephalosporin serum concentration is due to inhibition of tubular secretion by probenecid. In one *in vitro* study in pooled serum, salicylic acid apparently displaced Cefixime from protein binding sites, resulting in more than a 50% increase in concentrations of free cefixime; this effect appeared to be concentration-dependent (Bialer *et al.*, 1998).

Concomitant administration of a 650 mg oral dose of aspirin and a 400 mg oral dose of cefixime in healthy adult men did not appear to affect protein binding, serum half-life, or

renal clearance of Cefixime but did result in a 20–25% decrease in peak serum concentrations and AUCs of the anti-infective agent. Although the manufacturer states that this effect was not considered clinically important since serum concentrations of Cefixime remained higher than the MIC values reported for most susceptible organisms (Suprax® (Cefixime) product monograph. 1989).

Chapter Three

MATERIALS AND METHODS

3.1 Animals and Animal Care

Adult wistar rats, (150 -200g) of both sexes obtained from the animal house facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University (ABU), Zaria were used in this study. The rats were maintained on feed formulated by the department and water was supplied to the animals from the public water supply of the A.B.U. Dam. The rats were housed in cages and transferred to the main pharmacology laboratory where they were allowed to acclimatize for 72 hrs prior to the study.

3.2 Drugs, Chemicals and Reagents

The following Drugs were used in the experiment

i. Metformin 500mg tabs (Glucophage) manufactured by Merke Sante s.a.s France. Batch No 102682, Manufacturing date: 10/2008, Expiry date: 10/ 2013 purchased from Osbuk K pharmacy, Kaduna.

ii. Metformin powder from (Sigma Aldrich) collected from Department of Pharmacology and Therapeutics, A.B.U. Zaria

iii. Cefixime 200mg tabs manufactured by Fredun pharmaceuticals LTD. India. Batch No T-8039, Manufacturing date: 06/2008 Expiry date: 05/2010 purchased from Reals pharmaceuticals, Lagos.

iv. Cefixime powder for reconstitution into suspension manufactured by Vapi Care Pharma PVT. Ltd. Gujarat India. Manufacturing Date February 2009, Expiry Date January 2011. For: Swiss Pharma Nigeria Ltd.

v. Alloxan monohydrate (Sigma Aldrich, St. Louis USA) from Department of Pharmacology and Therapeutics, A.B.U. Zaria.

vi. Mueller- Hinton Agar (Oxoid, England) from Department of Pharmaceutics and Pharmaceutical microbiology, A.B.U. Zaria.

Sundry consumables and other materials

i. Animal cages and animal feeds

ii. Test tubes, conical flasks and measuring cylinders

iii. Stop clock

iv. Scissors, cotton wool, gloves and antiseptics.

v. Normal saline and distilled water

vi. Disposable syringes and needles (1ml, 2mls, 5mls and 10mls).

vii. Digital glucometer and glucose strips (Accucheck Active)

viii. Microrganisms: Clinical isolates of *Escherichia coli, Klebsiella spp, Proteus mirabilis and Haemophylus Influenza* were obtained from the Department of Microbiology A.B.U.T.H. Zaria.

3.3 Induction of Experimental Diabetes

Twenty four wistar rats of both sexes were weighed, fasted overnight and their fasting blood glucose levels taken on day 0. Alloxan (freshly prepared in 0.9% Normal saline solution) at a dose of 140mg/kg body weight was administered through the tail vein. After 72 hours, the blood glucose levels in the animals were again determined following an overnight fast, using the glucose oxidase method with the aid of a digital glucometer.

The glucose oxidase enzyme (GOx) is an oxido-reductase that catalyses the oxidation of glucose to hydrogen peroxide and D-glucono-δ-lactone. Hydrogen peroxide produced as a result of enzyme-catalysed oxidation of glucose is detected, quantified and read from the digital glucometer (Wilson, 1992). Rats having blood glucose level ≥ 250mg/dl were considered diabetic and grouped for the experiment (Ayoola *et al.*, 2009).

3.4 Effect of Metformin -Cefixime Co-administration on Blood Glucose Levels

The diabetic rats were weighed and randomly divided into 2 groups of 6 rats per group (groups 3 and 4), based on their blood glucose level on day 1. Additional 12 normoglycaemic rats were also weighed and grouped into 2 groups of 6 rats per group (groups 1 and 2). Group 1 served as the control and was given normal saline (10 mls/kg), group 2 and 4 received Cefixime (400 mg/kg) from day14 to 28 while groups 3and 4 were given Metformin (200 mg/kg) throughout the 28 days.

The dose of Metformin used (200 mg/kg) is based on the oral LD_{50} as reported by Sanofi adventis Inc which is 1000 mg/kg body weight in rats (Sanofi adventis Inc, 2006), while Cefixime (400 mg/kg) is also based on the oral LD_{50} of Cefixime (>2000 mg/kg body weight in rats) documented in the safety data sheet of (Oxoid, 2005).

The Fasting Blood Glucose (FBG) and Random Blood Glucose (RBG) of all rats were taken weekly using the glucose oxidase method with the aid of a digital glucometer. The weights of the rats were also taken weekly.

3.5 Evaluation of Haematological and Biochemical Indices

At the end of the fourth week blood was obtained from the tail of the rats using careful aseptic technique and haematological parameters (PCV, RBC and WBC) were determined following the procedure described by Ibu and Adeniyi (1989) at Physiology department, Faculty of medicine. The rats were lightly anaesthesized with chloroform, sacrificed whole blood collected allowed to clot and centrifuged. The sera were analysed for urea, electrolyte and creatinine at the Chemical Pathology department, Faculty of Medicine, Ahmadu Bello University Teaching Hospital (ABUTH).

3.5.1 Determination of Packed Cell Volume (PCV)

For each rat, its tail was prepped with methylated spirit with the aid of cotton wool. Thereafter the tail was snipped at about the terminal 3-5mm after which blood was obtained by gentle forward pull along the length of the tail. Blood was collected into heparinized micro capillary tubes by capillary action, allowing about one-third of the tube unfilled. The unfilled end of the tube was then sealed with flame. After all samples were collected, arranged and labeled sequentially, the blood was centrifuged using a micro capillary tube centrifuge over a ten minute duration. For the purpose of avoidance of loss of samples that may be consequence on accidental breakage, each rats blood was collected in duplicate. The PCV was then read off using a PCV reader and values obtained were recorded.

3.5.2 Determination of Red Blood Cell Count (RBC)

The RBC pipette with its corresponding rubber mouth piece was used to suction blood up to the 0.5 mark. The blood so collected was diluted to the 101 mark with the RBC diluting fluid (solution containing mercuric chloride) while ensuring that there was no backflow of the blood into the fluid in the watch glass. After that, the diluted blood in the pipette was left lying horizontally on the bench while the counting chamber was prepared. The pipette was then shaken thoroughly and the pure diluting fluid in the stem was discarded along with a few drops of blood. A drop of blood was then introduced to the counting chamber after the cover slip had been placed appropriately on the counting chamber. This blood runs into the chamber, after which the chamber is mounted on the microscope and left for a few minutes before the systematic counting of RBCs in 5 major grided squares. Appropriate calculations using the dilution factor were then carried out to determine the RBC count.

5 large squares = (16 x 5) = 80

Each small square has a volume of (1/20 x 1/1/20 x 1/10 – 1/4000) cu mm

Hence 80/4000 cu mm of (undiluted) blood contains 4000/30 corpuscles and 1 cu mm of the undiluted blood contains

$\frac{4000n\,x\,200}{80}$ x 10,000 copuscules

3.5.3 Determination of White Blood Cell Count (WBC)

The procedure for determination of the WBC count was similar to that for the RBC count, except that the diluting fluid used (2% glacial acetic acid with 1% gentian violet) and the pipette are specific for WBC and four major grided squares were counted. The

calculation for the WBC was done using the dilution factor and the data obtained recorded.

3.5.4 Determination of Serum Urea, Electrolytes and Creatinine levels.

Serum Urea: Determination of serum urea level is based on the Diacetyl Monoxime method using Thiosemicarbazide (Natelson 1951, Marsh *et al.,* 1965). The principle is based on the fact that when urea is heated in strongly acidic conditions with substances such as diacetyl $CH_3COCOCH_3$ containing two adjacent carbonyl groups or monoxime CH_3COC NOH, yellow condensation compounds are formed. This reaction is intensified by the presence of polyvalent ions such as ferric ions, such that a red coloured complex is formed which is more linear with concentration of the urea present than the initial yellow complex. The intensity of the red complex is measured colorimetricaly and its intensity is proportional to the concentration of urea in the sample.

Method: 0.1ml of serum from each individual rat was mixed thoroughly with 10mls of distilled water and 0.1ml of the standard (Urea10mmol /L) was also mixed with 10mls of distilled water. 1ml each of the test sample and standard was pipetted into separate test tubes and 1ml of distilled water added to get 2mls, a blank sample is also prepared using 2mls of the distilled water. 2mls of the working colour reagent (containing a mixture of Diacetyl Monoxime and Thio-semicarbaxide) was added into the test, standard and blank sample tubes respectively and mixed thoroughly. 2mls of the prepared mixed acid reagent (Ferric Chloride $FeCl_3$, Concentrated sulphuric acid and phosphoric acid) was added to the mixture in each tube and mixed thoroughly. The tubes were then placed in boiling water at 100°C for 20mins, cooled and the Optical Density read at 520nm

Calculations:

$$\frac{T-B}{S-B} x10$$

Where

T= Test sample, B= Blank sample and S= Standard.

Serum Sodium and Pottasium: The Flame Photometry method was used. The principle is based on the fact that potassium or sodium under carefully controlled condition when finely sprayed (aspirated) into a burner, is dissolved by the flame leaving solids (salts) which dissociates to give neutral ground states atoms. Some of these atoms are excited in the flame thus moving into a higher energy state. When these excited atoms fall back to the ground state, light of characteristic wavelength is emitted (590nm for sodium and 770 nm for potassium). This light passes through a suitable filter unto a photo-sensitive element. The amount of current thus produced is measured and is proportional to the amount of sodium and potassium present in the sample.

Method: The working standard was prepared by mixing 7.0mls of potassium stock standard (0 .746 g KCl/Litre) solution with 8.0mls of sodium stock standard (11.69 g NaCl/ Litre) solution and the volume was made up to 1000mls with distilled water. 0.1ml of serum from each rat was added into 9.9mls of deionized distilled water in universal test bottles as the test sample. 2mls of the working standard and 2mls of deionized distilled water was introduced into test bottle to act as standard sample and blank sample respectively. The test bottles were capped with paraffin and mixed by inversion.

For Sodium: Sodium light filter was inserted into the galvanometer, switched on and the flame ignited. The gas supply was adjusted to obtain discreet cones of flames. The galvanometer scale was set to zero with water and the galvanometer reading to 80 with the working standard and reset to zero. Each test sample was then introduced and the reading was taken

Sodium (Mmol/L) = galvanometer reading x 2.

For Potassium: Potassium light filters were used, the galvanometer was set to 70 with the working standard and procedure as for sodium above.

Potassium (Mmol/L) = galvanometer reading x 0.1.

Serum Chloride: Determination of serum chloride was carried out using Mecuric nitrate titrimetric method of Schales and Schales, (1941). The principle is such that when mercuric nitrate solution is added to a solution containing chloride, unionized but soluble mercuric chloride is formed. At the end point, the first excess mercuric ions combine with the indicator (diphenylcarbazone) to give a violet-blue coloured complex. The titre value against that of a standard is used to quantify the amount of chloride present.

Method: The standard chloride solution was prepared by dissolving 585mg of sodium chloride in water and the volume made up to 1000mls. 0.2mls of serum was added to 1.8mls of de-ionized water followed by 3 drops of the incicator (diphenylcarbazone) and mixed well. This mixture was titrated against mecuric nitrate solution until a sharp violet colour is formed. The titration was also repeated using 2.0mls of the standard chloride solution.

Serum chloride (mg%) = Vol (ml) mercuric Nitrate required for the unknown x 585.

Vol(ml) mercuric nitrate required for the Std

Serum Creatinine: Determination of serum creatinine is based on Rehberry method. Creatinine gives a red colour with alkaline solutions of picric acid (Jaffe,s reaction). The red colour is measured in the presence of a back ground (yellow colour) and the solutions appear orange at low creatinine concentrations.

Method: The working creatinine standard used was 1mg%. Two separate test tubes containing 3mls and 4mls of distilled water representing the test (T) and blank (B) sample tubes were used for each animal. The serum (1ml) was added into the test sample tube, followed by 1ml each of 10% Sodium tungstate and concentrated sulphuric acid. The reagents were also added to the blank sample tubes respectively. The tubes were then centrifuged and 3mls of the supernatant withdrawn. To the supernatant, 1ml each of 0.75N NaOH and Picric acid was added and also to 3mls of the working creatinine standard(S). The solution was thoroughly mixed, allowed to stand for 15mins and the absorbance read in the UV spectrophotometer at the wavelength of 520 nm.

Creatininine (Umol/L) = $\frac{T-B}{S-B}$ X 530

Where T= Absorbance of Test sample, B=Absorbance of Blank sample and S= absorbance of Standard.

3.6 Histopathological Studies

3.6.1 Processing of tissues

At the end of the 4th week, the rats were weighed, sacrificed and their hearts, liver, kidneys and pancreas were removed, weighed and relative organ weight (ROW) determined using the formula:

$$ROW = \frac{\text{Absolute Organ Weight (g)}}{\text{Body weight of rat on day of sacrifice (g)}}$$

The organs were then fixed in 10% formalin. The protocol used for the study was as described by Tulpule and Ghaji, (1987). Processing of tissues involves dehydration, clearing, impregnation and embedding. The tissues that had been previously fixed in 10% formalin solution were dehydrated with successive ascending concentrations of ethanol starting from a 50% to 70%, 90% and finally absolute (100%) ethanol. Besides the 50% ethanol in which dehydration was left to continue overnight, the tissues were subjected to dehydration twice at duration of 30 minutes in each of 70%, 90%, and 100% ethanol respectively. After the dehydration of the tissues was completed, the tissues were cleared in three changes of xylene lasting for one hour each. The tissues were then impregnated through two changes of molten paraffin wax for a period of one hour each. This was followed by embedding in molten paraffin wax and the tissues were then allowed to solidify. The embedded tissue was mounted on a wooden block and trimmed. Sections were then cut to a thickness of about 6 microns using a rotary microtome. The resulting serial sections were allowed to float in a warm water bath and then selected sections were placed on clean albuminised microscope slides. The tissues were then dewaxed in two

changes of xylene lasting for a period of three minutes each. Rehydration was then carried out through descending concentrations of ethanol (100%-50%).

3.6.2 Staining of Tissues: The tissues were dewaxed in xylene for two changes and passed through running water for a few minutes, stained in Harris haematoxylin solution for ten minutes and further rinsing under tap water. The tissues were then differentiated using 1% ethanol and washed again in running tap water. They were stained with 1% Eosin solution for one minute and subsequently dehydrated in graded concentrations of ethanol. The tissues were thereafter cleared in xylene and mounted with cover slips using tricresylphosphate and xylene (DPX). Slides were allowed to dry overnight, preserved in microscope slide box and photomicrographs of representative lesions taken.

3.7 Effect of Interaction between Metformin and Cefixime on the Antimicrobial properties of Cefixime

To investigate the possible effect of Cefixime-Metformin drug-drug interaction on the antimicrobial properties of Cefixime, the Cork and Bore method was used. An initial test run was carried out using two large plates 24cm by 24cm to investigate the effects of varying concentrations of Metformin, Cefixime, and their combinations on clinical isolates of *Klebsiella spp* and *Proteus mirabilis,* based on the peak plasma concentrations achieved at Steady State in plasma. It is 1 – 2mcg/ml for Metformin, 2 – 4.6mcg/ml between 2 – 6 hours, for Cefixime.

The concentrations used were as shown

Cefixime		Metformin	
10 mcg/ml	C1	4mcg/ml	M1
5 mcg/ml	C2	2mcg/ml	M2
2.5mcg/ml	C3	1mcg/ml	M3

3.7.1 Sterilization Procedures

Sterilization of all the materials, utensils, equipments, agar and distilled water was carried out using the steam autoclave for 1hr at 15psi pressure at 121°C and the experiment was carried out in an aseptic environment.

3.7.2 Sample Preparation

Metformin powder (40mg) was weighed and dissolved in 10mls of water in a sample bottle to get a stock solution which was further diluted serially to obtain the various concentrations used. Cefixime powder (100mg) was also dissolved in 5mls of sterilized water in a volumetric flask and made up to the 100ml mark with the same solvent. The stock solution was further diluted serially to obtain the various concentrations used.

3.7.3 Culture and Media

The stock culture agar was used for the maintenance and sub culturing of the different organisms. It is a soft almost semi solid medium. The advantage of the medium is that it contains a small amount of dextrose which serves as a readily available source of energy.

The medium supports luxuriant growth of pathogenic bacteria and preserve their viability over a long period of time.

Standardization of the cultures: 0.9% normal Saline was used for the standardization of the already prepared overnight culture. Since all organisms to be used are gram-negative organisms, a serial dilution of 1 : 5000 of the stock culture was carried by transferring 1ml or the subculture into 9mls of 0.9% normal saline and diluting further in test tubes to obtain the cell population of the test cultures used. A volume of 2ml of the suspension was used as innoculum.

Method: The agar plate diffusion method as described by (Reeves and Bywater, 1976) was used. The already prepared nutrient agar (200mls) was melted and poured into the sterilized large plates 24cm by 24cm. This was allowed to set, after which another 200mls of molten nutrient agar was seeded with 2mls of the test micro organism and poured into the already set agar in the large plates. The large plates where then covered and allowed to set finally. Sterile cork borer having a diameter of 8mm was used to bore 36 holes into the seeded plates containing 400mls of solidified nutrient agar each. Sterile loop was used to remove the cut agar to expose the holes and 0.1ml of agar was introduced into each of the holes and allowed to set prior to drug administration, this was to prevent the drug sample to be used from interacting directly with the surface of the plates.

The holes in the large plates were as labelled

C_1	C1M1	C1M2	C1M3	C2	C2M1
C2M2	C2M3	C3	C3M1	C3M2	C3M3
M1	M1C1	M1C2	M1C3	M2	M2C1
M2C2	M2C3	M3	M3C1	M3C2	M3C3
C1	C1M1	C1M2	C1M3	C2	C2M1
C2M2	C2M3	C3	C3M1	C3M2	C3M3

0.1ml each of the varying concentrations of Cefixime and Metformin were introduced into labeled wells in the plates as shown above using needles and syringes. The plates were then incubated at 37°C for 24 hours and the inhibition zone diameters (IZD) was measured with a ruler.

Based on the result of the initial test run using large plates to evaluate the effects of varying concentrations of Metformin and Cefixime on *Proteus mirabilis* and *Klebsiella spp*, the second concentration of Cefixime i.e. 5 µg/ml was selected and the effect of varying concentrations of Metformin on the antimicrobial effect of Cefixime using small plates on the four test microorganisms was investigated.

Method: Previously sterilized small dishes were used, 20mls of already prepared agar was melted and poured into eight petri dishes and allowed to set, two plates for each microorganism. 2mls of the standardized culture was poured into the plates (Fluotation method) and left for 5mins after which five wells were made using sterile cork borer on each plate.0.1ml of molten agar was poured into each well and allowed to set.

The labeling of the holes in the small plates is as shown below

C2 C2M1

C0

C2M2 C2M3

0.1 ml each of the varying concentrations of Metformin and Cefixime and 0.1ml of Sterile Water were introduced into the appropriately labeled wells in the plates as shown above using sterile needles and syringes under an aseptic environment. The plates were then incubated at 37°C for 24hrs and the inhibition zone diameters (IZD) was measured with a ruler.

3.8 Analysis and Presentation of Data

Data from the various experiments were expressed as mean ± standard error of mean (SEM). Data obtained was analysed using Analysis of variance tool (ANOVA) followed by Turkey post Hoc test. A p-value <0.05 was taken to be statistically significant. Data are presented as tables, bar chart and plates as appropriate.

Chapter four

RESULTS

4.1 Effect of Metformin-Cefixime Co-Administration on Fasting Blood Glucose (FBG) in Wistar Rats

Statistically significant decrease (P< 0.001) in FBG on Day 21 between diabetic rats treated with Metformin alone (231.3±26.64) and rats treated with Metformin and Cefixime (90.75±8.450) and in FBG of Met-Cef group between Day 1 and Days 14, 21 and 28. Statistically significant decrease (P<0.01) seen in FBG of Metformin alone group between Day 1 and Days 14 and 28. There is also an appreciable numerical difference on Day 28 in the FBG of the two diabetic groups Metformin and Met-Cef. (Table 4.1)

Table 4.1: Effect of Metformin-Cefixime Co-Administration on Fasting Blood Glucose (FBG) in Wistar Rats

	Fasting Blood Glucose (mg/dl)			
Day/Group	**Day 1**	**Day 14**	**Day 21**	**Day 28**
Control	77.20±1.390	73.83±5.624	65.17±2.535	60.17±3.664
Cefixime (400mg/kg)	81.80±1.820	83.71±9.631	61,67±1.308	65.00±4.183
Metformin (200mg/kg)	412.3±41.81**	128.3±11.84	231.3±26.64*	137.3±50.67
Met(200mg/kg)-Cef(400mg/kg)	390.8±57.29*	89.00±13.01	90.75±8.450	66.75±10.71

Significant difference P<0.001* Two Way ANOVA, Metformin vs Met-Cef on Day21 and Met-Cef, Day1 vs Days 14, 21 and 28. P<0.01** Metformin, Day1 vs Day14 and Day28. Values are mean ± SEM, n = 5.
Vs = compared to.

4.2 Effect of Metformin-Cefixime Co-Administration on Random Blood Glucose (RBG) in Wistar Rats

There is a statistically significant decrease (P<0.01) in RBG values of animals in Metformin group (323.7±65.38) as against Met-Cef Group (166.5±29.27) on Day 28. There is also a statistically significant decrease (P<0.01) in RBG of Metformin group from Day1 (440±67.34) to Day 28 (166±29.27). The RBG of the control and Cefixime alone rats however did not change significantly throughout the experiment. (Table 4.2)

Table 4.2: Effect of Metformin-Cefixime Co-Administration on Random Blood Glucose (RBG) in Wistar Rats

	Random Blood Glucose (mg/dl)			
Day/Group	**Day 1**	**Day 8**	**Day 14**	**Day 28**
Control	112.3±3.116	108.8±2.344	100.5±2.643	75.33± 3.343
Cefixime (400mg/kg)	113.5±2.861	113.7±3.211	107.3±6.31	75.67±3.744
Metformin (200mg/kg)	412.3±41.81	378.3±29.17	345.3±4.09	323.7±65.38*
Met(200mg/kg)-Cef(400mg/kg)	440.0±67.34**	305.0±39.70	326.8±29.20	166.5±29.27

Significant difference P<0.01* Two Way ANOVA Metformin vs Met- Cef , P<0.01** Met-Cef Day 1 vs Day 28. Values are in mean ± SEM, n = 5 per group.

4.3 Effect of Metformin-Cefixime Co-Administration on Mean Body Weight in Wistar Rats

There was no statistically significant difference in the weights of the rats in each group comparing day 1 with day 28. However time dependent, non significant increase in mean body weights of rats in all the groups. (Table 4.3)

Table 4.3: Effect of Metformin-Cefixime Co-Administration on Mean Body weight in Wistar Rats

Day/Group	Day 1	Day 8	Day 14	Day 21	Day 28
Control	165.0±13.50	179.7±10.03	192.2±9.502	200.3±11.09	210.3±10.46
Cefixime (400mg/kg)	159.0±12.86	172.5±9.801	187.0±6.698	188.0±6.904	194.5±7.424
Metformin (200mg/kg)	189.8±12.40	188.8±12.57	193.5±13.43	194.5±16.64	197.5±14.36
Met(200mg/kg)-Cef(400mg/kg)	171.4±11.04	178.0±15.00	181.0±13.09	185.8±13.52	188.8±15.42

No statistically significant difference, ($P > 0.05$ ANOVA). Table shows Mean ± SEM of weight in (g), $n = 5$.

4.4. Effect of Metformin-Cefixime Co-Administration on Some Hematological Parameters

There was no statistically significant difference ($P>0.05$) in all the haematological parameters studied (PCV, RBC and WBC) compared to the control (Table 4.4).

4.5. Effect of Metformin-Cefixime Co-administration on Some Serum Electrolytes.

No significant difference was seen in serum Sodium, Potassium Chloride and Bicarbonate ion concentration of the rats in the control group as compared to the drug treated groups (Table 4.5).

Table 4.4: Effect of Metformin-Cefixime Co-Administration on Some Hematological Parameters

Group	PCV (%)	RBC ($x10^{12}$/L)	WBC ($x10^{9}$/L)
Control	44.83±0.7491	4.130±0.2709	1.608±0.04715
Cefixime (400mg/kg)	44.60±1.208	4.498±0.5040	1.536±0.05810
Metformin (200mg/kg)	46.25±1.031	4.448±0.5156	1.490±0.09460
Met(200mg/kg)-Cef(400mg/kg)	43.00±1.183	4.784±0.3789	1.836±0.1954

No statistically significant difference ($P > 0.05$, ANOVA). Table shows Mean ± SEM of PCV, RBC and WBC, n = 5.

Table 4.5: Effect of Metformin-Cefixime Co-Administration on Some Serum Electrolytes

Group	Sodium (mmoles/l)	Potassium (mmoles/l)	Chloride (mg%)	Bicarbonate (mg%)
Control	133.5±4.249	4.800±0.1155	96.17±2.810	24.00±2.556
Cefixime (400mg/kg)	127.2±8.470	4.900±0.4848	95.60±3.124	23.40±1.860
Metformin (200mg/kg)	137.8±11.05	4.525±0.3683	96.67±4.055	28.00±0.8165
Met(200mg/kg)-Cef(400mg/kg)	140.2±8.558	4.860±0.5870	96.80±3.929	25.20±2.223

Values are Mean ± SEM, n=5. ($P > 0.05$ ANOVA).

4.6 Effect of Metformin-Cefixime Co-administration on Urea and Creatinine in Wistar Rats

There was no significant difference in the value of Creatinine and Urea between the control group and the drug treated groups. Though, the Cefixime treated group showed a higher mean Creatinine value 113.2 as compared to 100, 94 and 98.8 of Control Metformin and Metformin-Cefixime groups respectively (Figure 4.1).

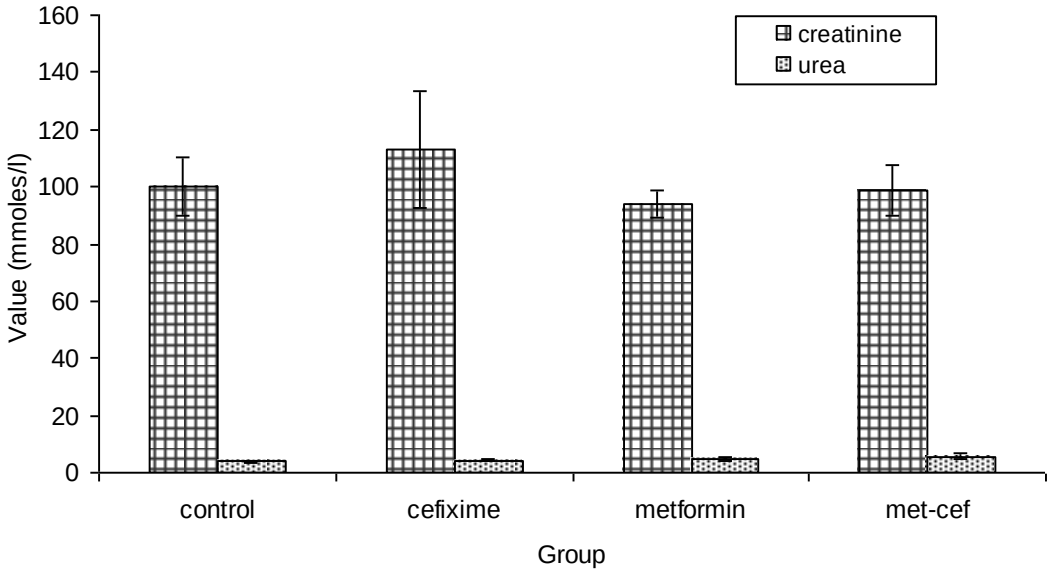


Figure 4.1: Effect of Metformin-Cefixime Co-administration on Urea and Creatinine in Wistar Rats

n =5 per group. (P > 0.05 ANOVA)

4.7 Effect of Metformin-Cefixime Co-Administration on Relative Organ Weights

The liver of the rats treated with vehicle (control) showed a mean of 7.680 which was not significantly different from 7.396, 6.623 and 6.852 obtained from Cefixime, Metformin, and Cefixime-Metformin pretreated groups respectively. The relative organ weights for the kidney, pancreas and heart for the various groups were also not significantly different from the control (Table 4.6).

Table 4.6: Effect of Metformin-Cefixime Co-Administration on Relative Organ Weights

	Relative Organ Weight (Percentage)			
Group/Organ	**Liver**	**Kidney**	**Pancreas**	**Heart**
Control	7.680±0.3881	0.6383±0.04045	0.6683±0.08171	0.7283±0.02455
Cefixime	7.396±0.2945	0.6060±0.03265	0.6740±0.1065	0.6460±0.01913
Metformin	6.623±0.6409	0.7533±0.09597	0.7733±0.1650	0.7333±0.08110
Metformin-Cefixime	6.852±0.4729	0.6360±0.04578	0.7900 ±0.1990	0.6440 ±0.0290

No statistically significant differences in the sizes of tissues ($P > 0.05$ ANOVA)
Values are mean ±SEM, n = 5.

4.8 Effect of Graded Concentrations of Metformin-Cefixime Combination on Antimicrobial Effect of Cefixime against *Klebsiella species* and *Proteus Mirabilis*

The IZD of graded concentrations of Cefixime and Metformin, showed no statistically significant difference compared to IZD of all Cefixime concentrations against the two test microorganisms. An increase in antimicrobial activity of Cefixime, though not statistically significant is seen with increase in concentration of the drug against the two organisms. This is evidenced by the gradual decrease in the IZD measured as the concentration reduced. (Table 4.7)

Table 4.7: Effect of Graded Concentrations of Metformin-Cefixime Combination on Antimicrobial Effect of Cefixime against *Klebsiella species* and *Proteus Mirabilis* *in vitro*

	C1	**C1M 1**	**C1M 2**	**C1M 3**	**C2**	**C2M 1**	**C2M 2**	**C2M 3**	**C3**	**C3M 1**	**C3M 2**	**C3M 3**
KS	32.50 ±0.50	30.33 ±0.33	29.33 ±1.20	28.00 ±1.15	29.00 ±1.00	26.33 ±1.45	28.33 ±0.8	29.00 ±1.00	24.50 ±1.50	20.67 ±1.45	21.33 ±2.02	19.00 ±1.52
PM	40.00 ±2.00	39.33 ±0.66	39.00 ±1.00	37.00 ±1.00	36.00 ±0.0	36.00 ±0.0	36.67 ±0.66	35.00 ±1.00	36.00 ±0.0	32.00 ±0.0	34.00 ±0.0	33.00 ±3.0

Key : KS - *Klebsiella specie*s, PM - *Proteus mirabilis*, n= 3.
CI=10µg/ml, C2= 5µg/ml and C3=2.5µg/ml of Cefixime
M1=4µg/ml, M2= 2µg/ml and M3= 1µg/ml of Metformin

4.9 Effect of Metformin on Anti-Microbial Activity of Cefixime on Selected Micro Organisms

No variations between the Inhibition Zone Diameter (IZD) of the test antimicrobial agent Cefixime (Control) and its combination with varying concentration of Metformin against *K. species, P. mirabilis* and *H. influenza.* The test antimicrobial agent was inactive against *E.coli* probably due to resistance by the strain of organism used (Table 4.8).

Table 4.8: Effect of Metformin on Anti-Microbial Activity of Cefixime on Selected Micro-Organisms

Micro-organisms	C2	C2M1	C2M2	C2M3
Klebsiella spp	27.50± 0.50	27.50±0.50	26.50±0.50	25.00±0.0
P. mirabilis	41.50±1.50	42.50±1.50	43.50±0.50	43.50±0.50
Haemophillus influenza	19.25±1.750	17.50±0.50	19.00±0.0	17.00±0.0
E.coli	NI	NI	NI	NI

Key: C2=5µg/ml of Cefixime. M1 = 4µg/ml, M2 = 2µg/ml and M3 = 1µg/ml of Metformin. NI = No inhibition.

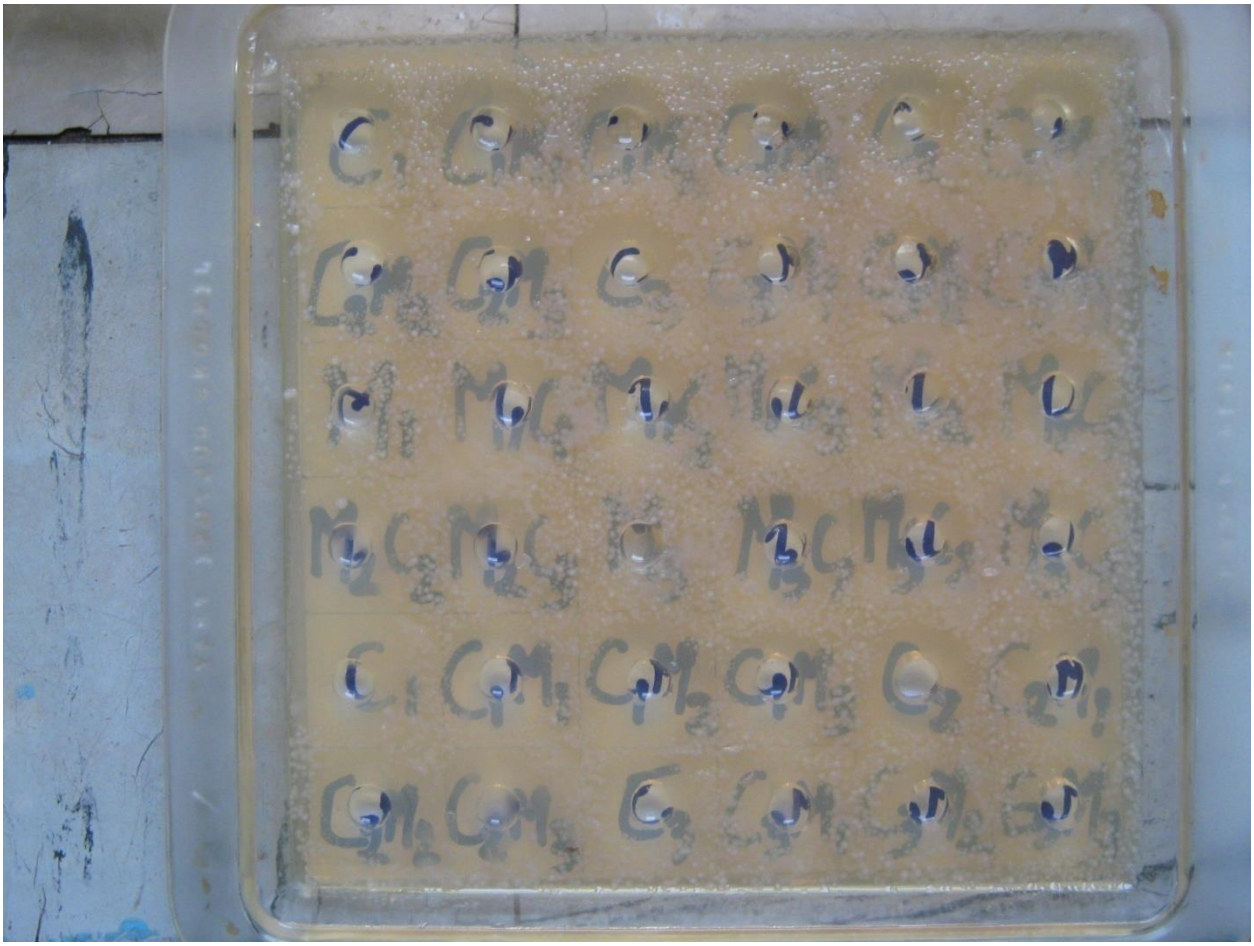


Plate 1 Sample Picture of Large Plate Showing the Inhibition Zone Diameter (IZD) of Graded Concentration of Cefixime-Metformin Against *Klebsiella Spp.*

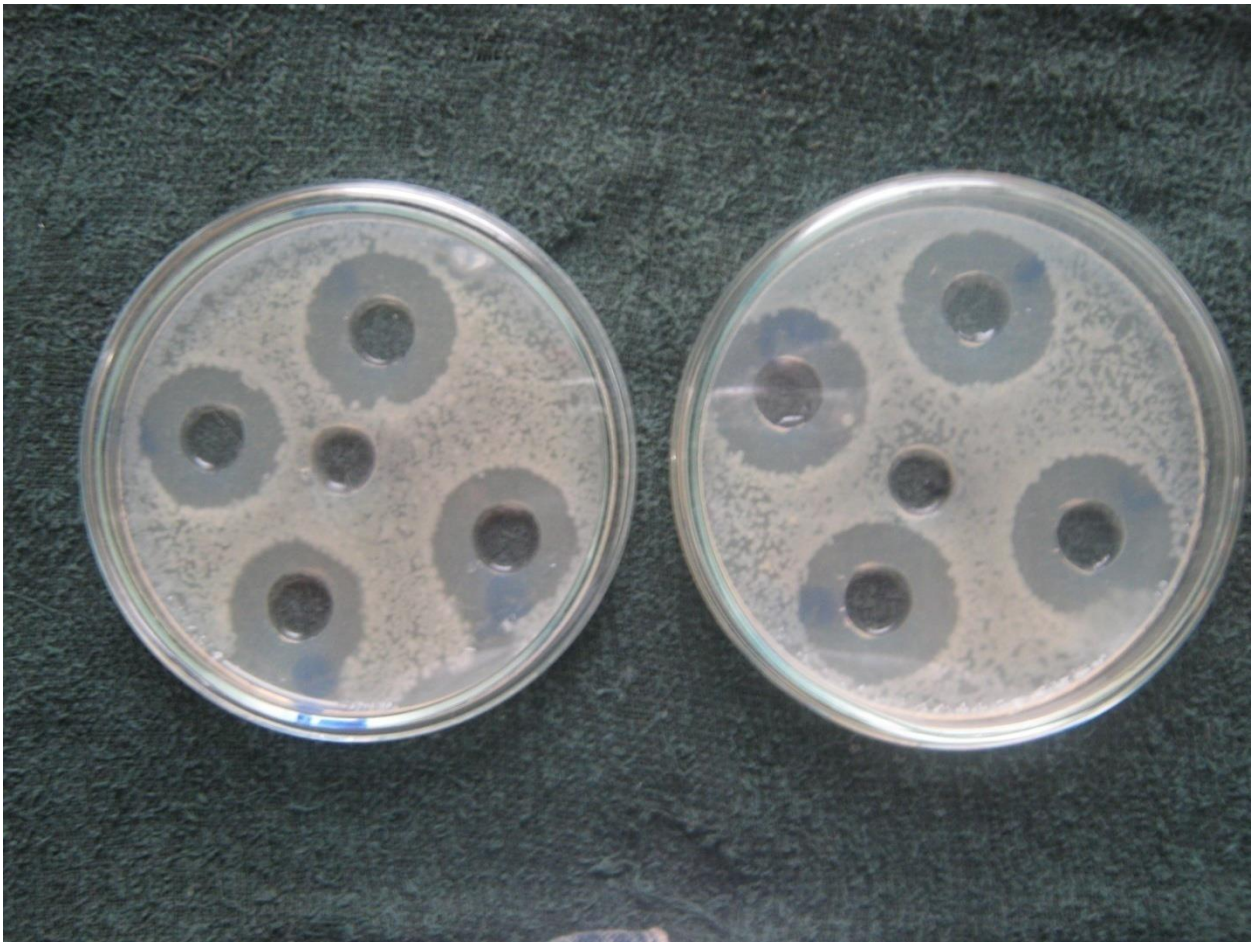


Plate II Sample picture of small plate showing the Inhibition Zone Diameter (IZD) of Cefixime (C2 =5µg/ml) interacted with Metformin M1 =4µg/ml, M2 = 2µg/ml and M3 = 1µg/ml. In the middle, showing no zone of inhibition is the vehicle used i.e. Sterile Water.

4.11. Effect of Metformin-Cefixime Co-Administration on Histology of Selected Organs Namely the Kidney, Pancreas, Liver and Heart

Following 28 days of drug administration, the effect of Co-administration of Metformin-Cefixime on the histological appearance of the selected organs was microscopically evaluated following Haematoxylin and Eosin stain.

Table 4.9: Effect of Metformin – Cefixime Co – Administration on Histology of Selected Organs

Group/Organ	Kidney	Pancreas	Liver	Heart
Control	NSHF	NSHF	NSHF	NSHF
Cefixime	NSHF	NSHF	Dilated sinusoids	NSHF
Metformin	Collapse and necrosis of Renal tubular cells	Necrosis of pancreatic acini	Dilated sinusoids with necrosis of hepatocytes	NSHF
Met – Cef	Collapse and necrosis of Renal tubular cells	NSHF	Dilated sinusoids with necrosis of hepatocytes	NSHF

Key: NSHF= No Significant Histopathological Findings Seen
Met – Cef = Metformin/Cefixime combination

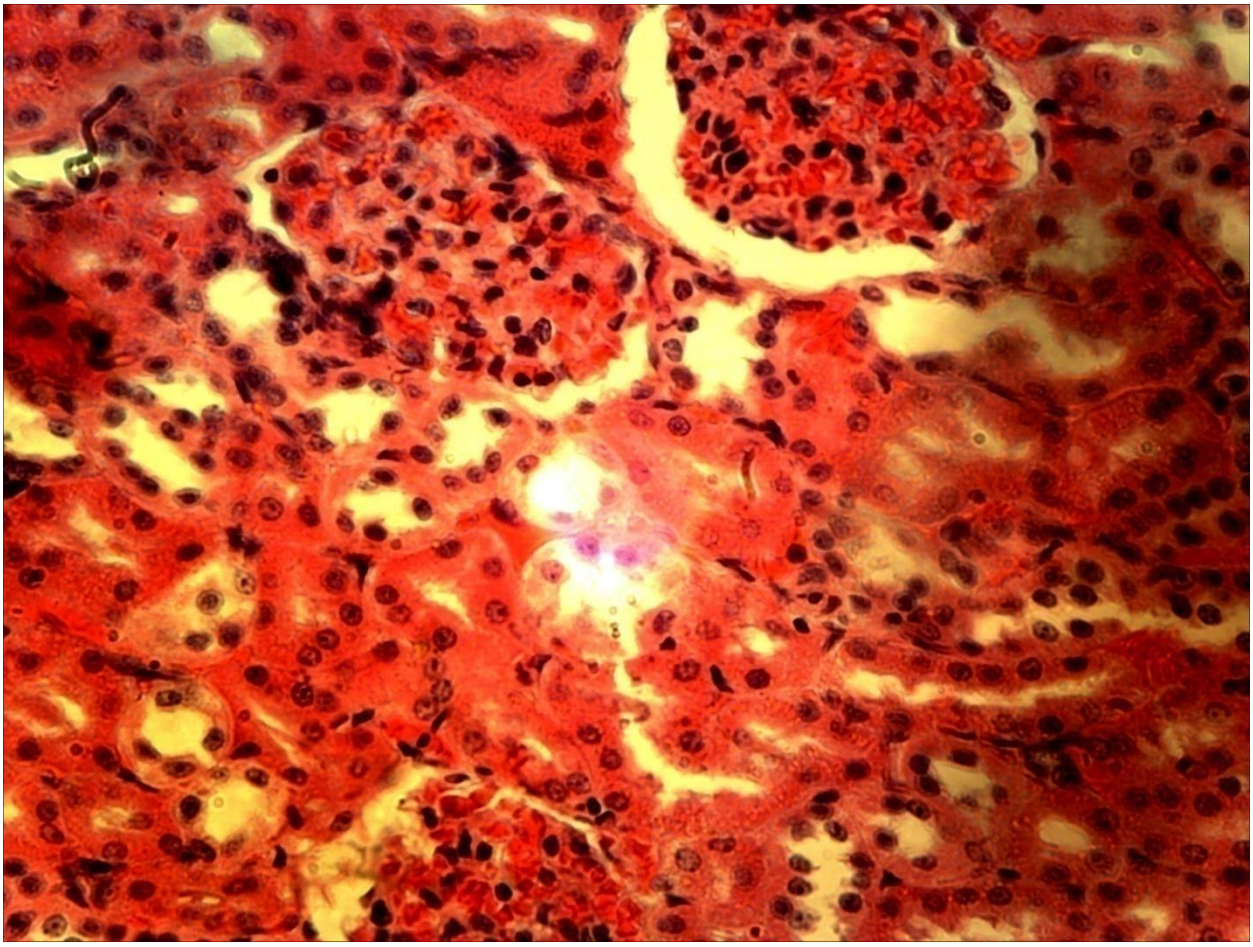


Plate III Photomicrograph of a section of the kidney of a control rat (H & E x400)

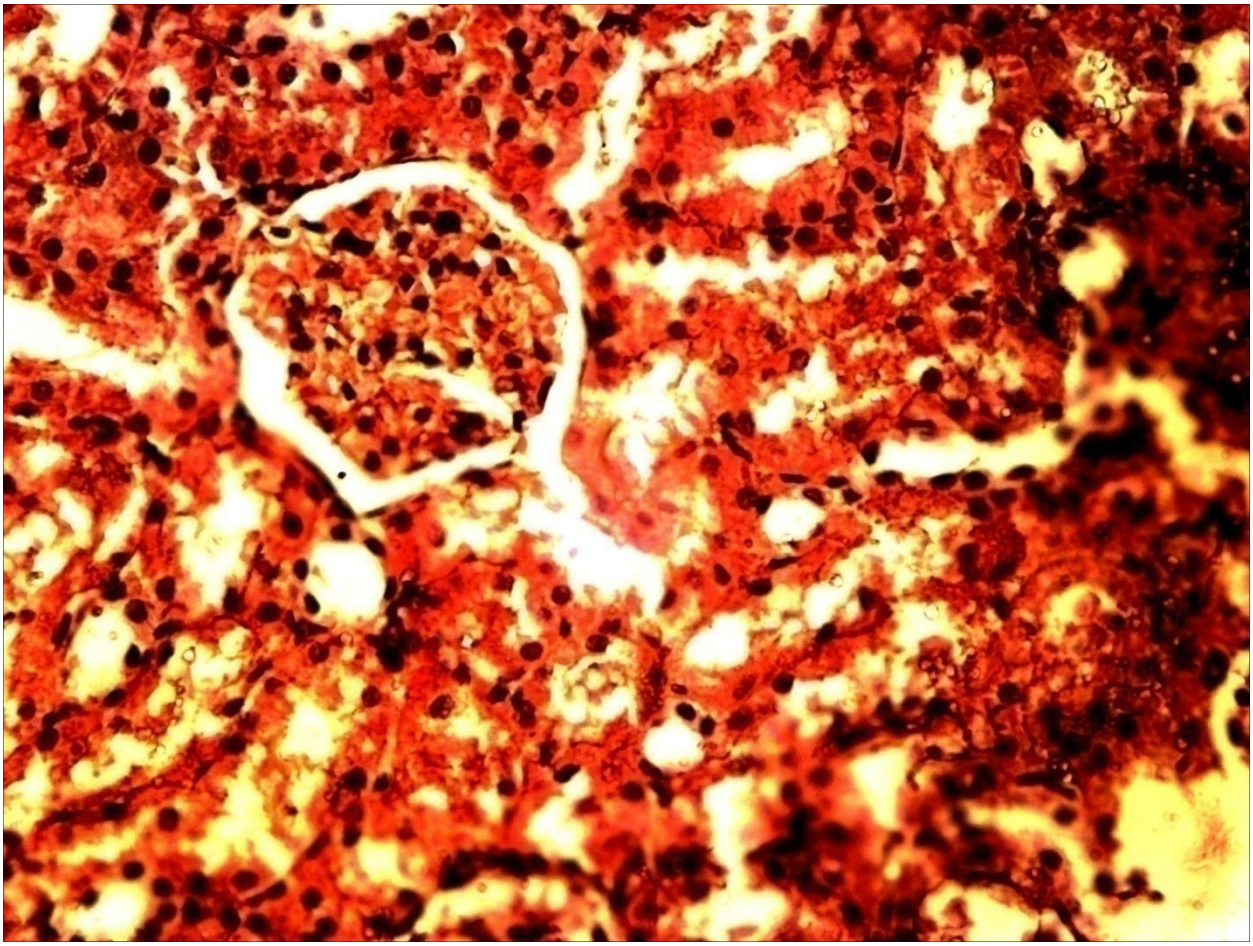


Plate IV Photomicrograph of a section of the kidney of a Cefixime treated rat (H & E X400)

There are no observable pathological findings.

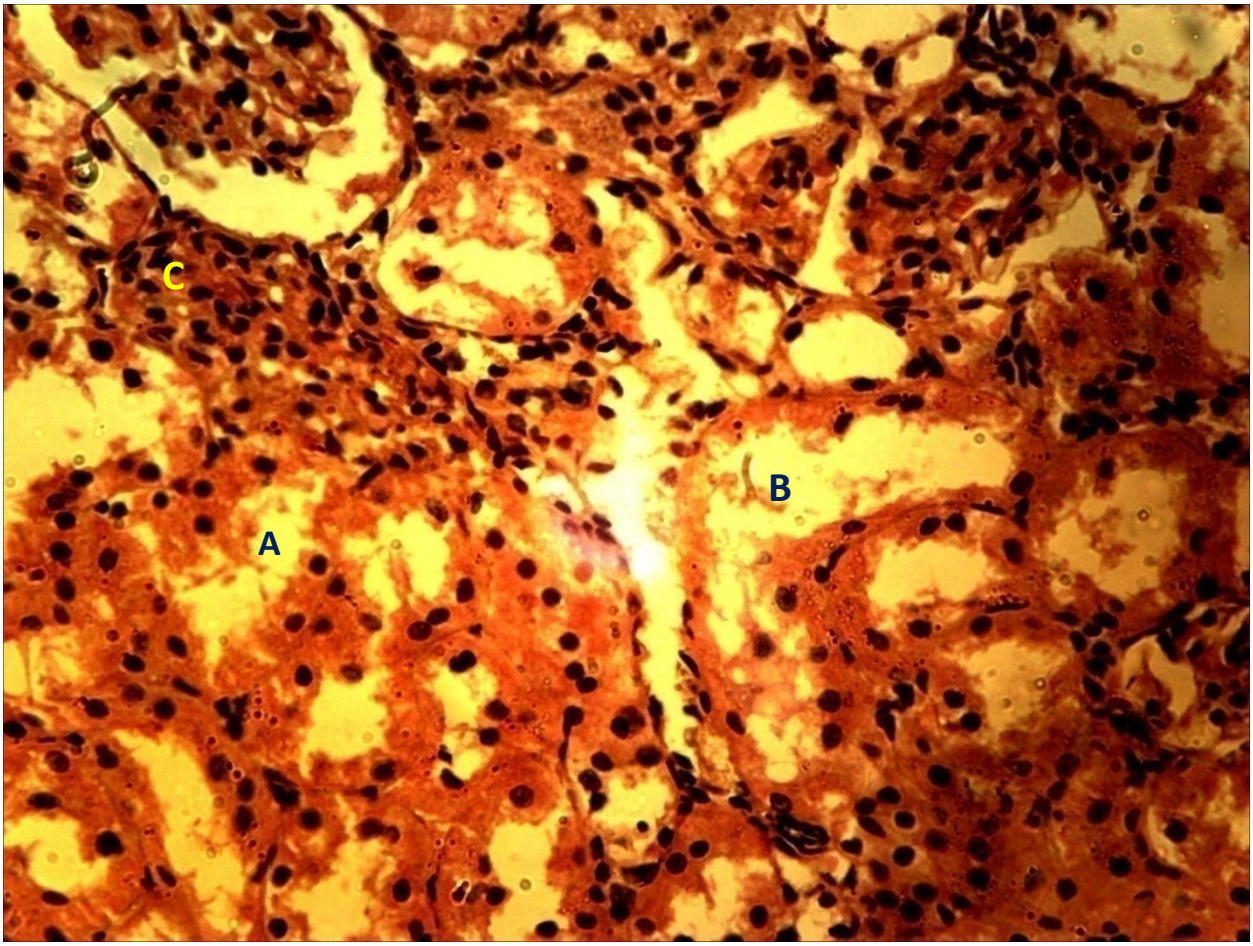


Plate V Photomicrograph of a section of the kidney of a Metformin treated alloxan induced hyperglycaemic rat (H & E X400)

Kidney of metformin only treated group showing some renal tubular collapse (A), focal area of necrosis of renal tubular epithelial cells (B) and mononuclear cellular infiltration (C).

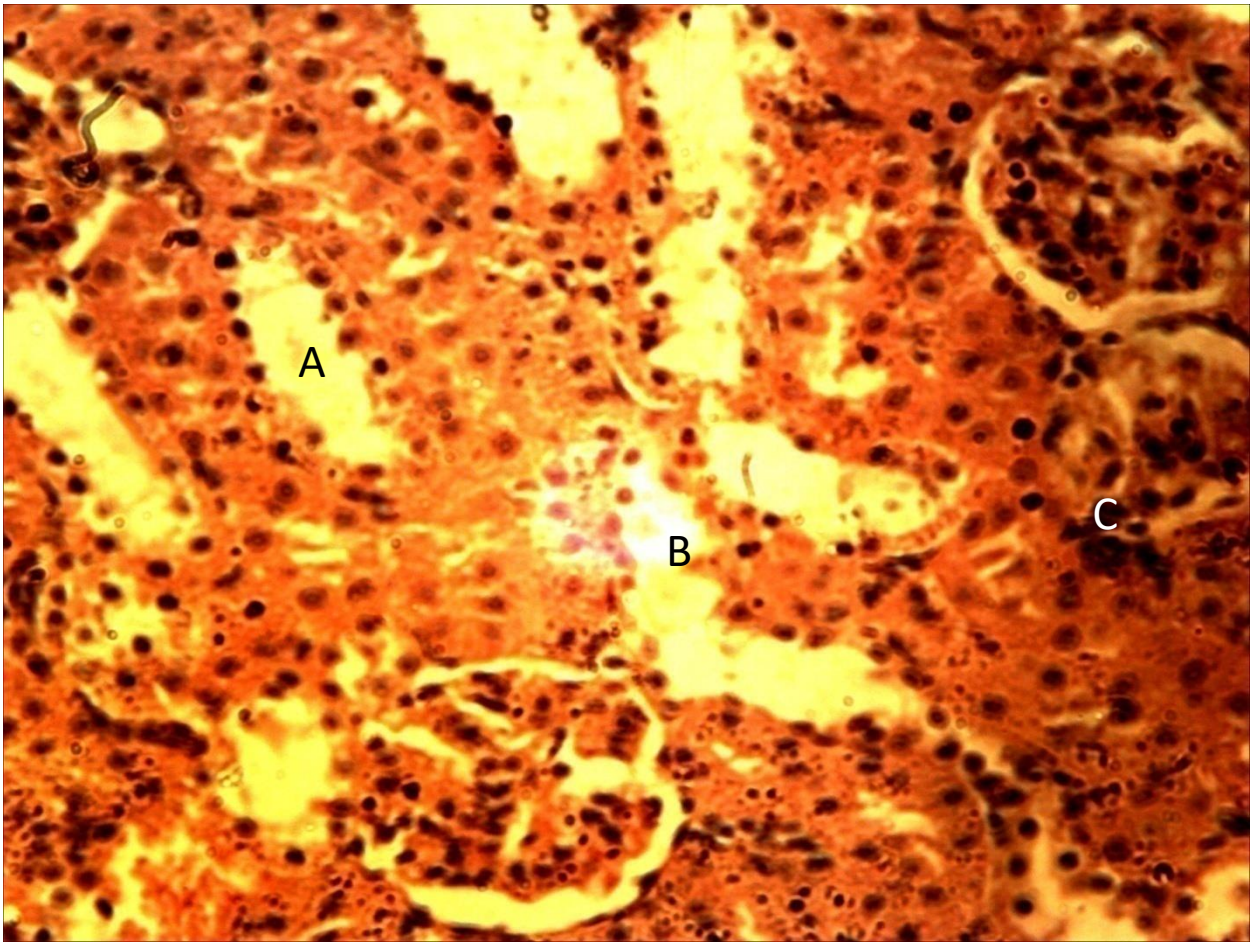


Plate VI Photomicrograph of a section of the kidney of a Metformin–Cefixime treated alloxan induced hyperglycaemic rat (H&E X400)

Kidney of Cefixime-metformin treated group also showing some renal tubular collapse (A), focal area of necrosis of renal tubular epithelial cells (B) and mononuclear cellular infiltration (C).

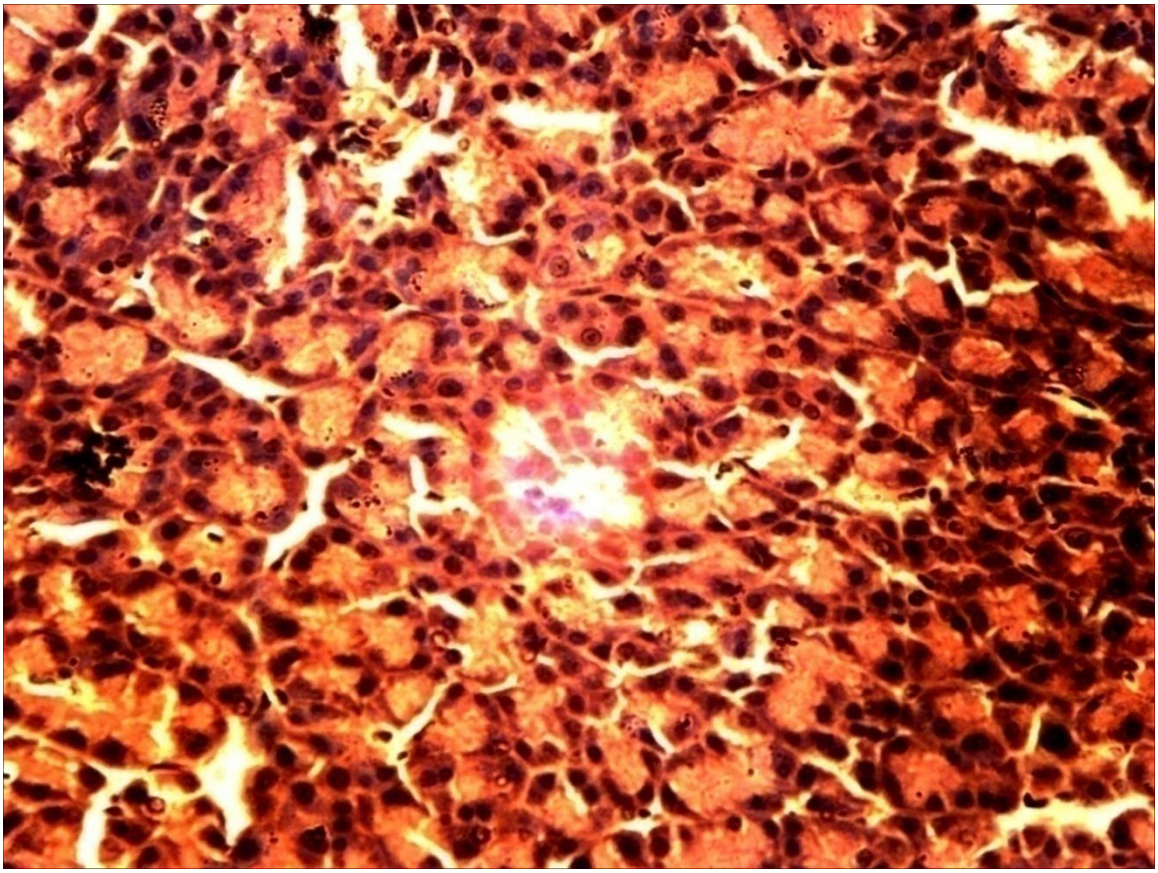


Plate VII Photomicrograph of a section of the pancreas of a control rat. (H&E X400).

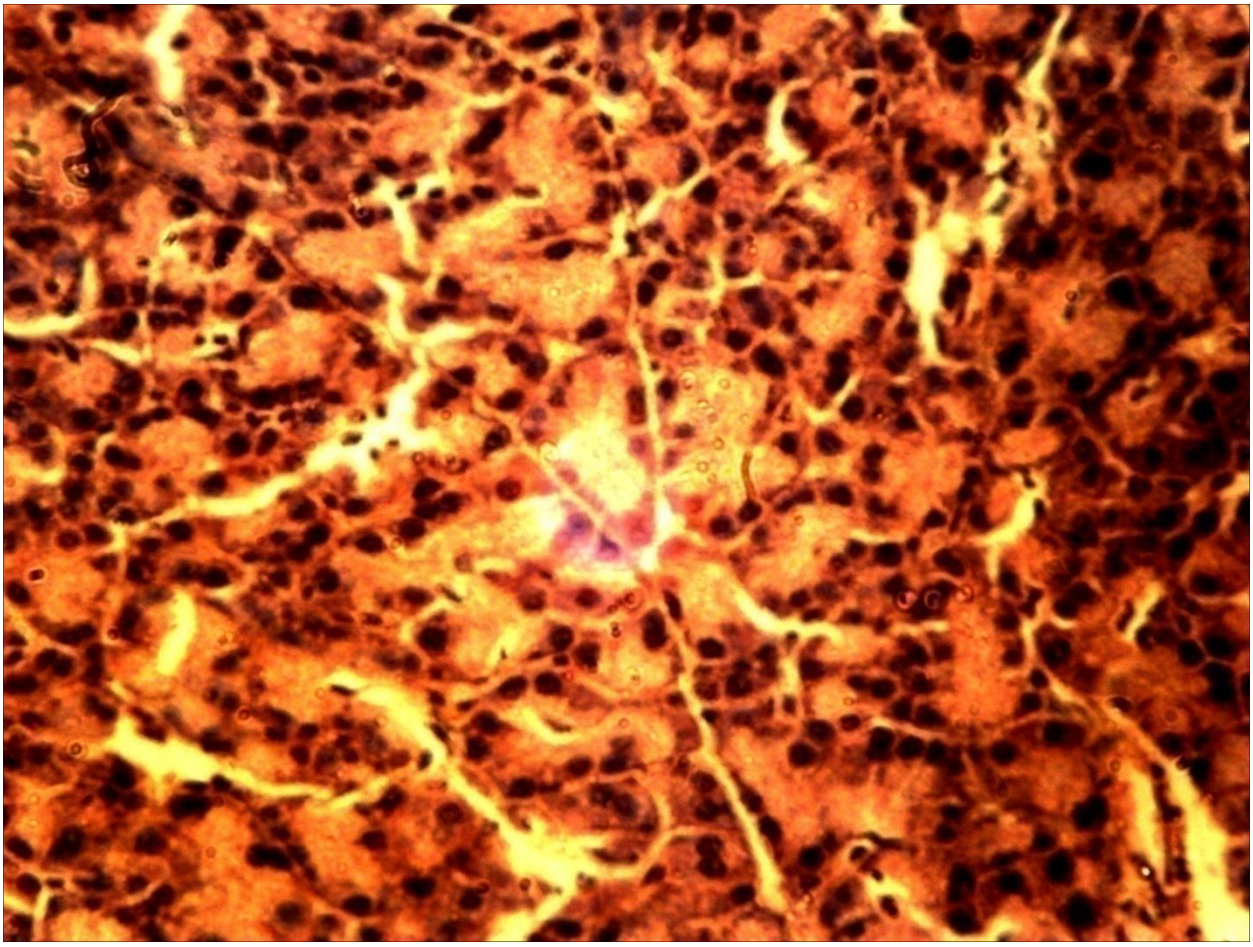


Plate VIII Photomicrograph of the section of the pancreas of a Cefixime treated rat (H&E X400)

No significant histopathological findings seen.

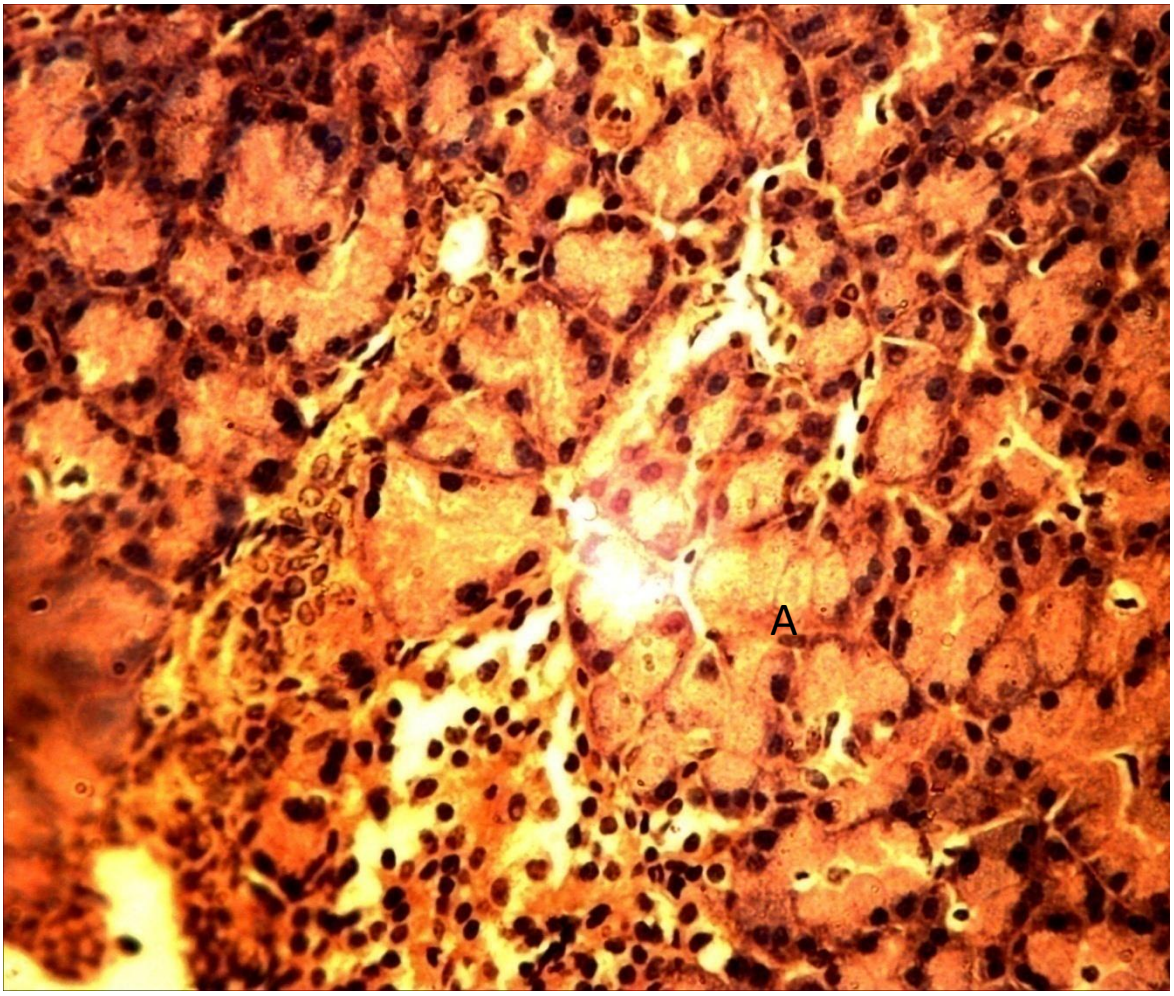


Plate IX Photomicrograph of the section of the pancreas of a Metformin (200mg/kg) treated alloxan induced hyperglycaemic rat (H & E X400)

The plate shows focal areas of necrosis of pancreatic acini with less cellular endocrine portion of pancreas (A).

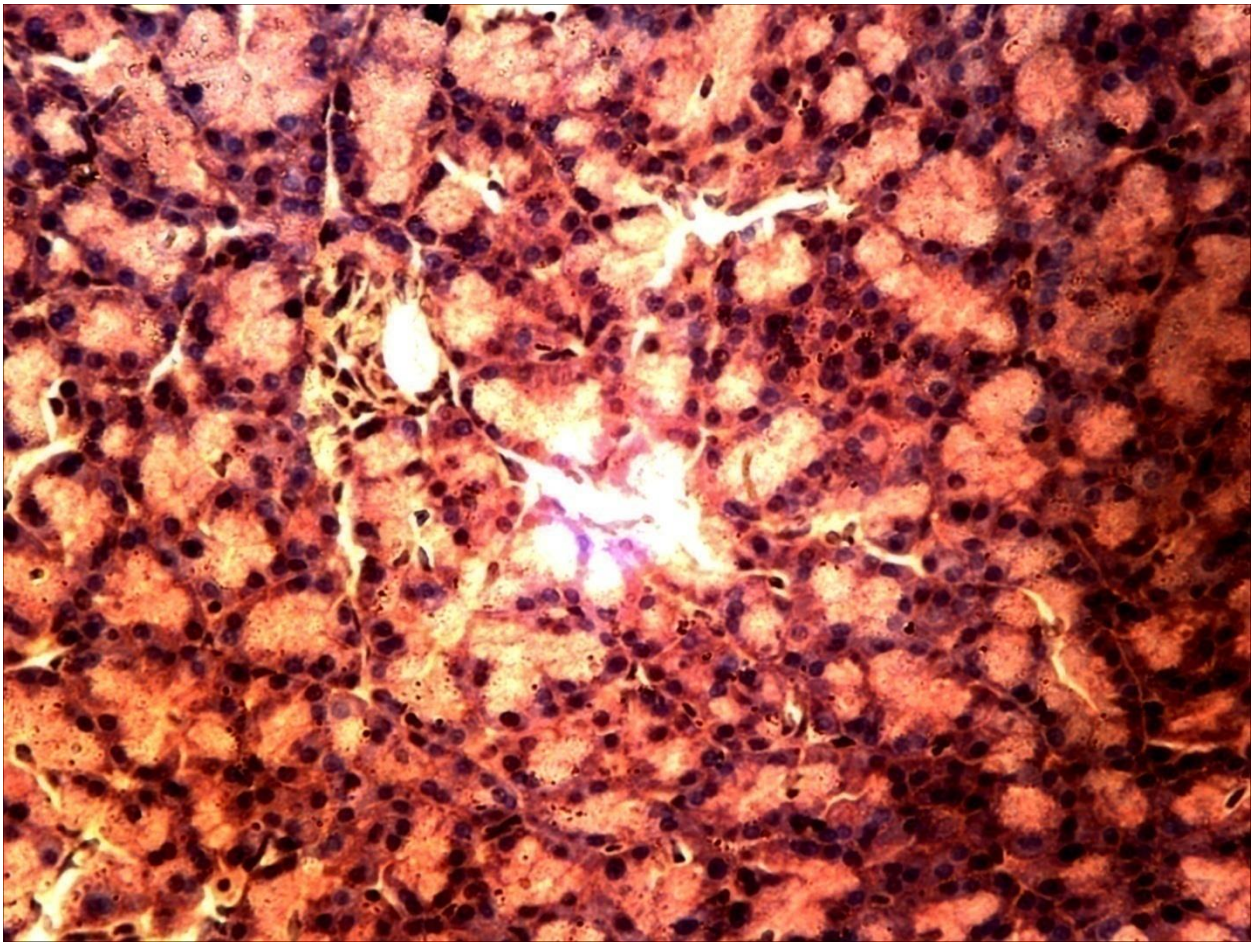


Plate X Photomicrograph of a section of the pancreas of a Metformin- Cefixime treated alloxan induced hyperglycaemic rat (H&E X400)

No significant histopathological findings seen.

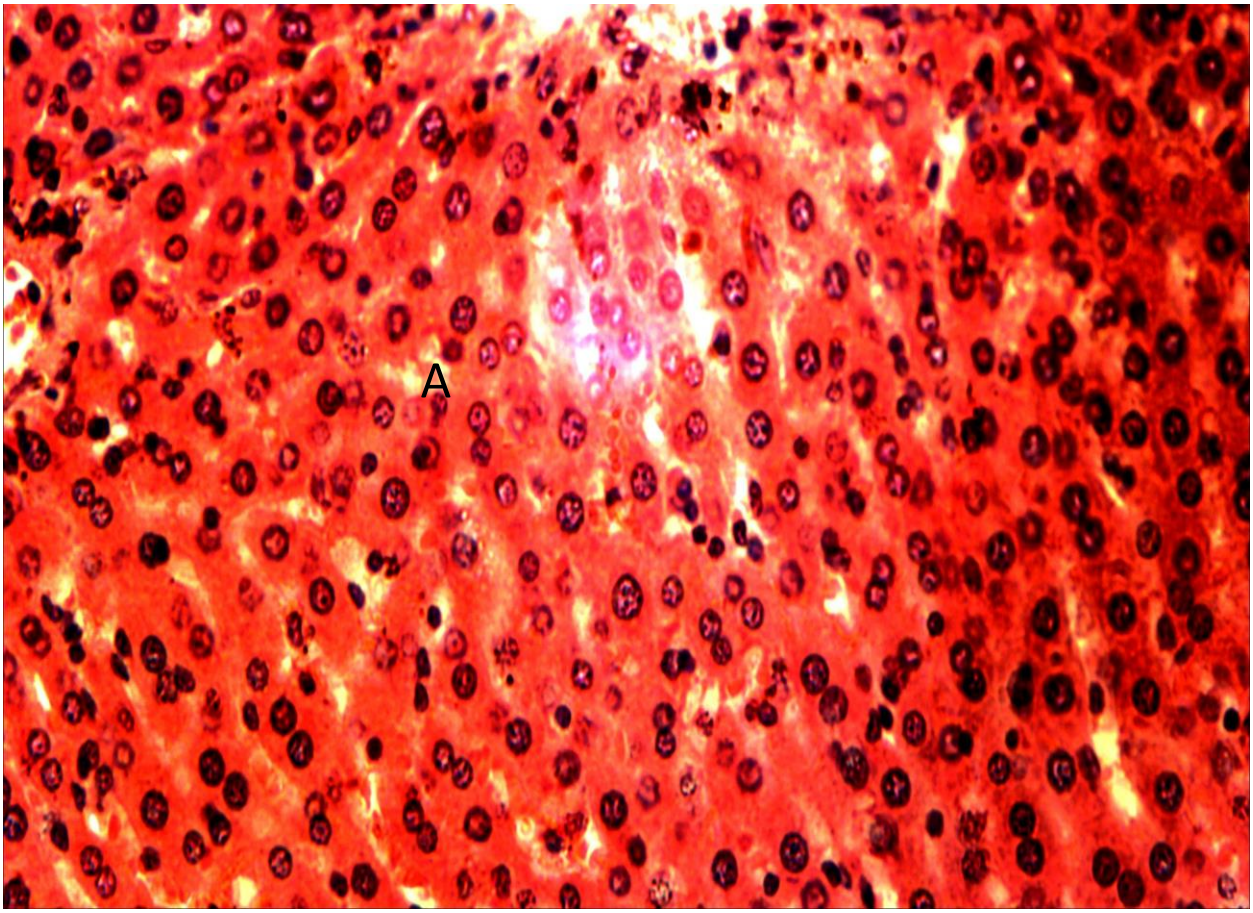


Plate XI Photomicrograph of a section of the liver of a Control rat (H&E X400)

The tissue section shows normal sinusoidal cards of hepatocytes (A).

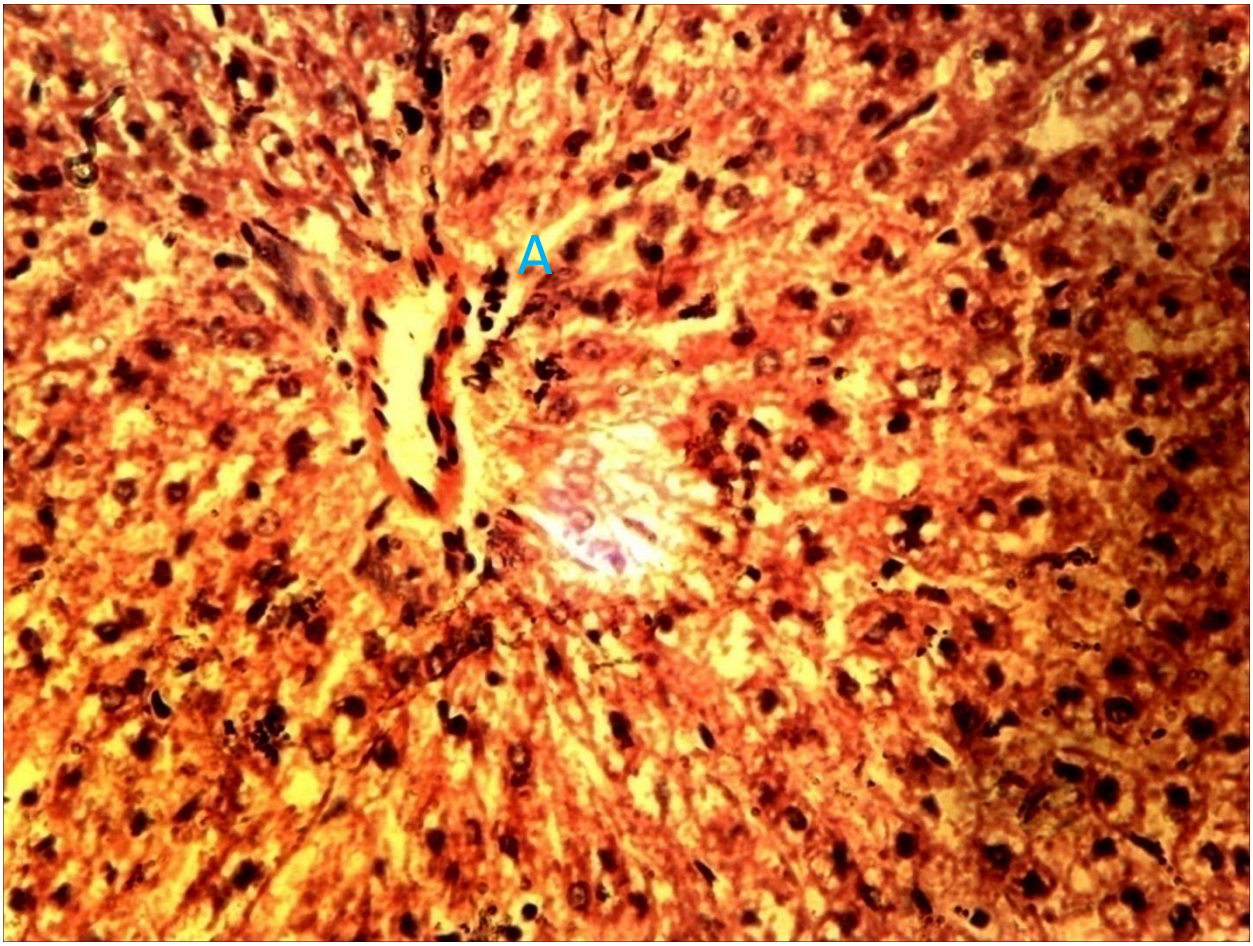


Plate XII Photomicrograph of a section of the liver of a Cefixime treated rat

(H&E X400)

The plate shows sections of dilated sinusoids of the liver of Cefixime treated rat (A).

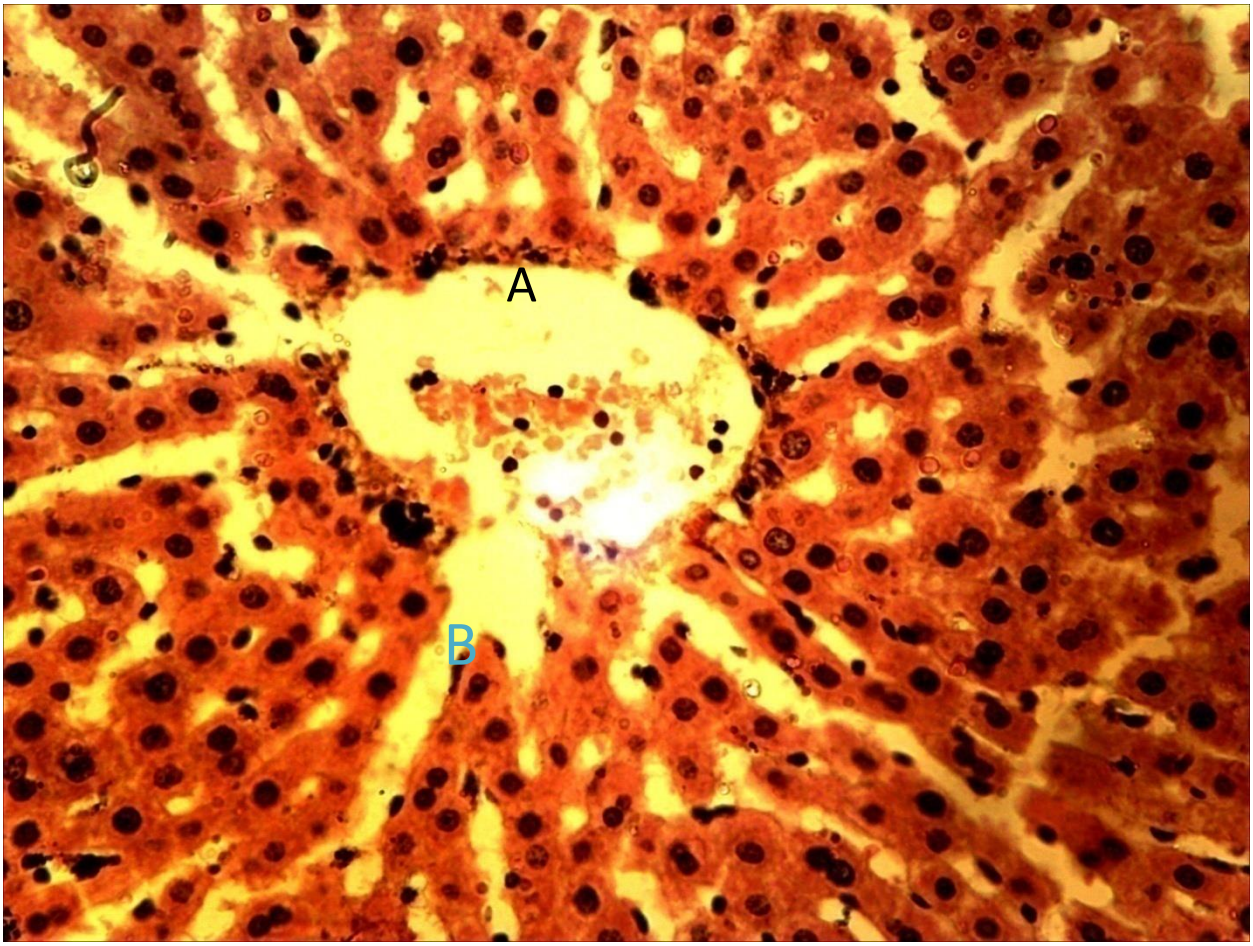


Plate XIII Photomicrograph of a section of the liver of a Metformin treated alloxan induced hyperglycaemic rat (H&E X400)

It shows distortion in the arrangement of cells around the central vein (A), periportal dilated sinusoids with focal area of necrosis of hepatocytes (B).

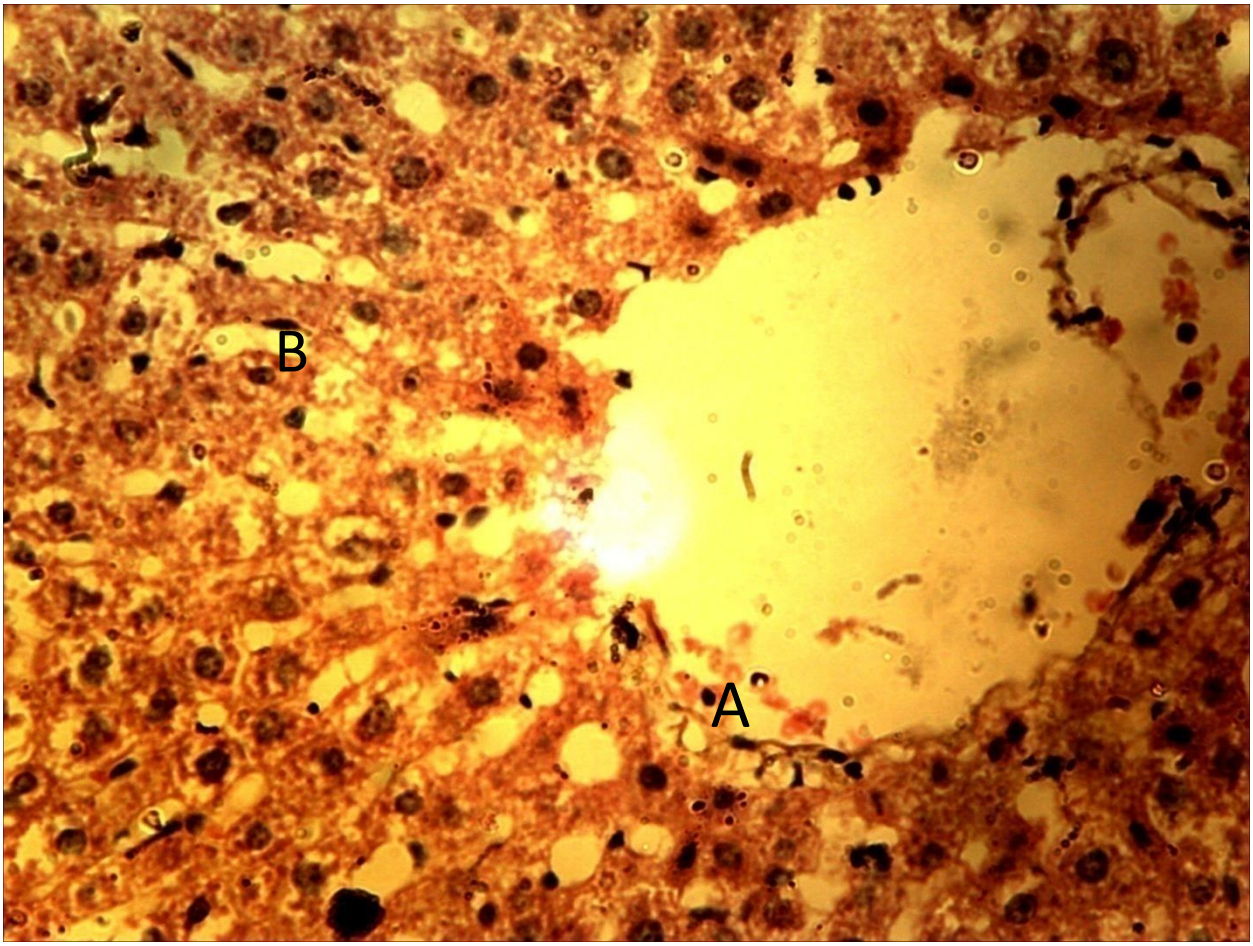


Plate XIV Photomicrograph of a section of the liver of a Metformin-Cefixime treated alloxan induced hyperglycaemic rat (H&E X400)

It shows distortion in the arrangement of cells around the central vein (A), periportal dilated sinusoids (B), with focal area of necrosis of hepatocytes.

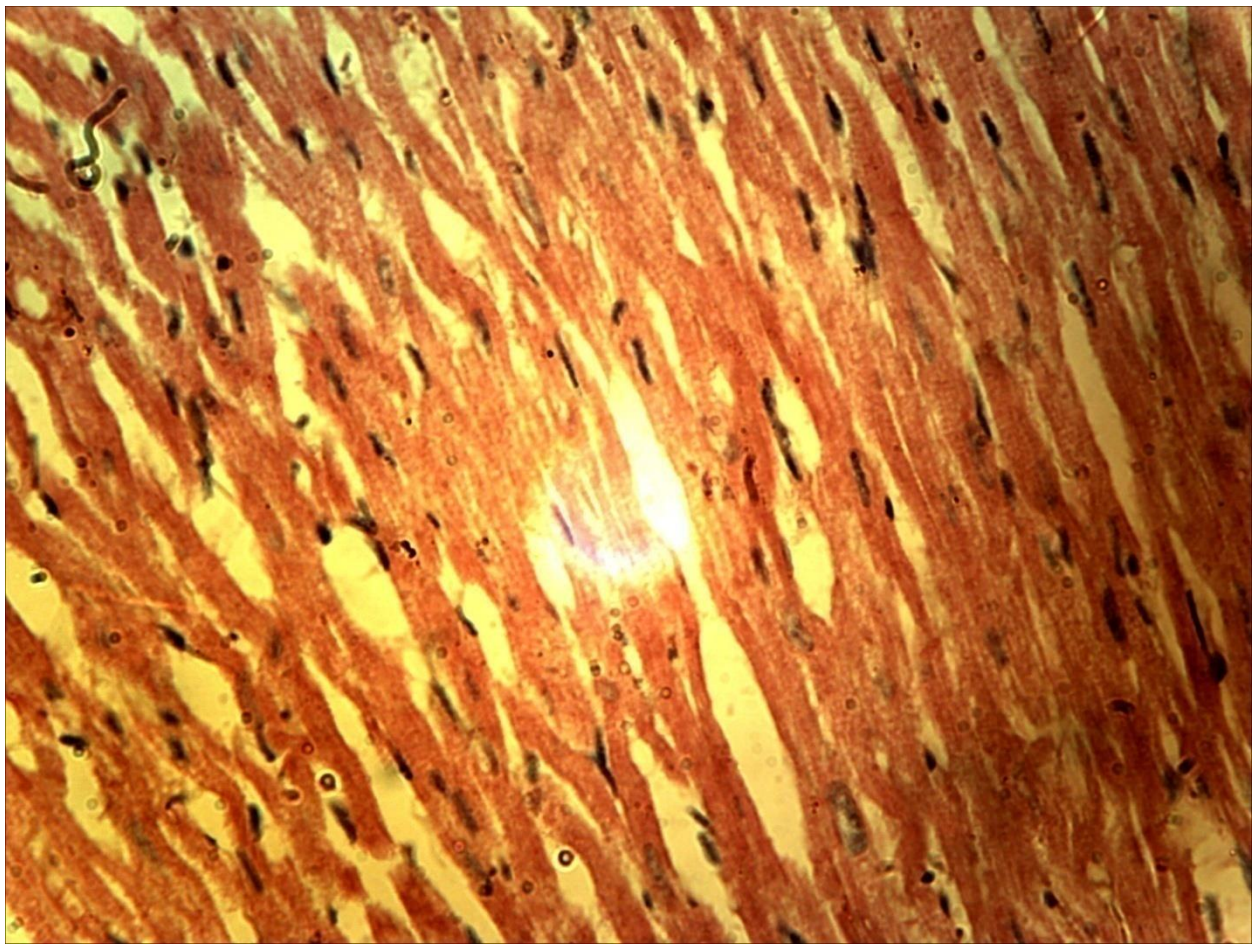


Plate XV Photomicrograph of a section of a normal heart (H&E X400)

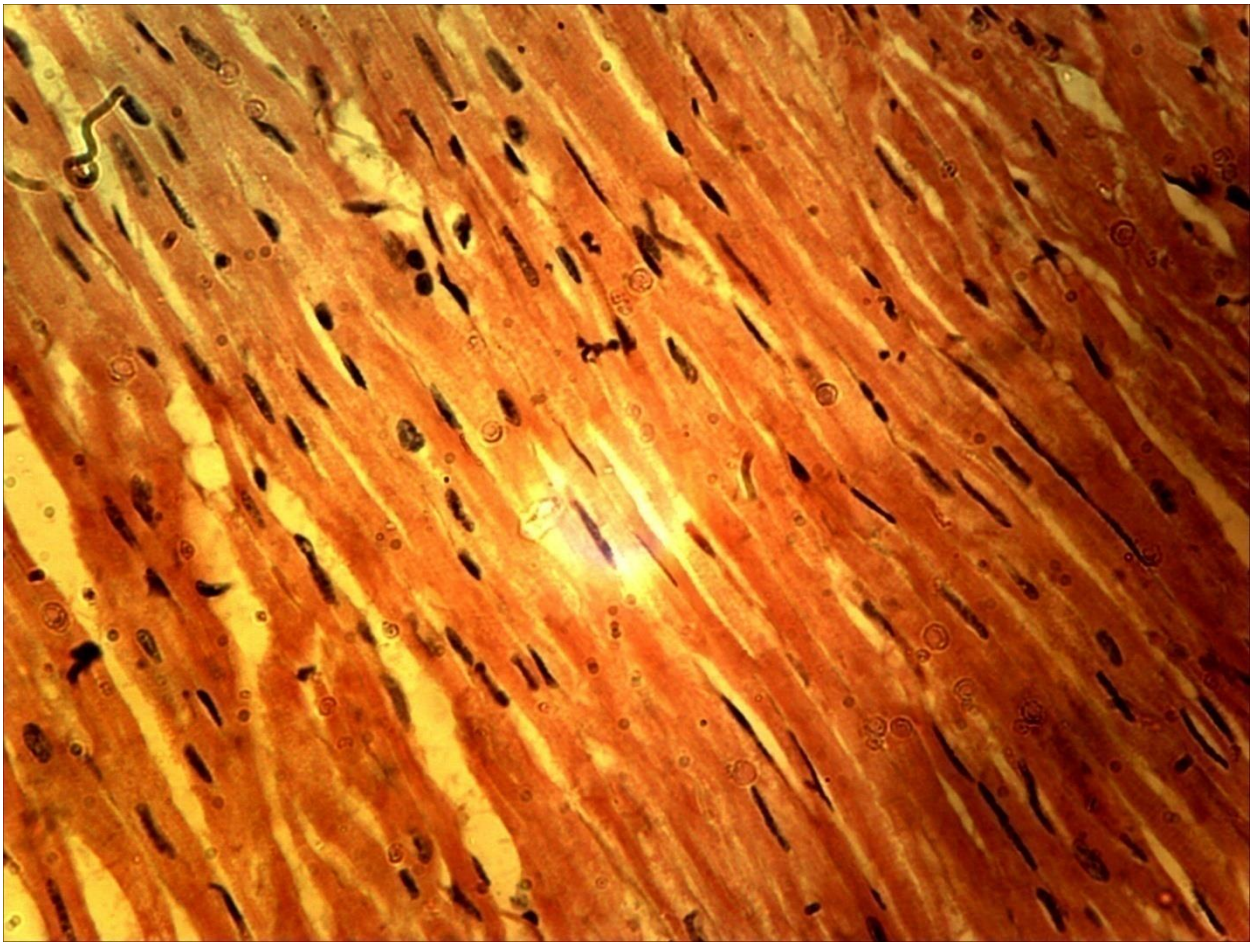


Plate XVI Photomicrograph of a section of the heart of a Cefixime treated rat

(H&EX400)

No significant histopathological findings seen.

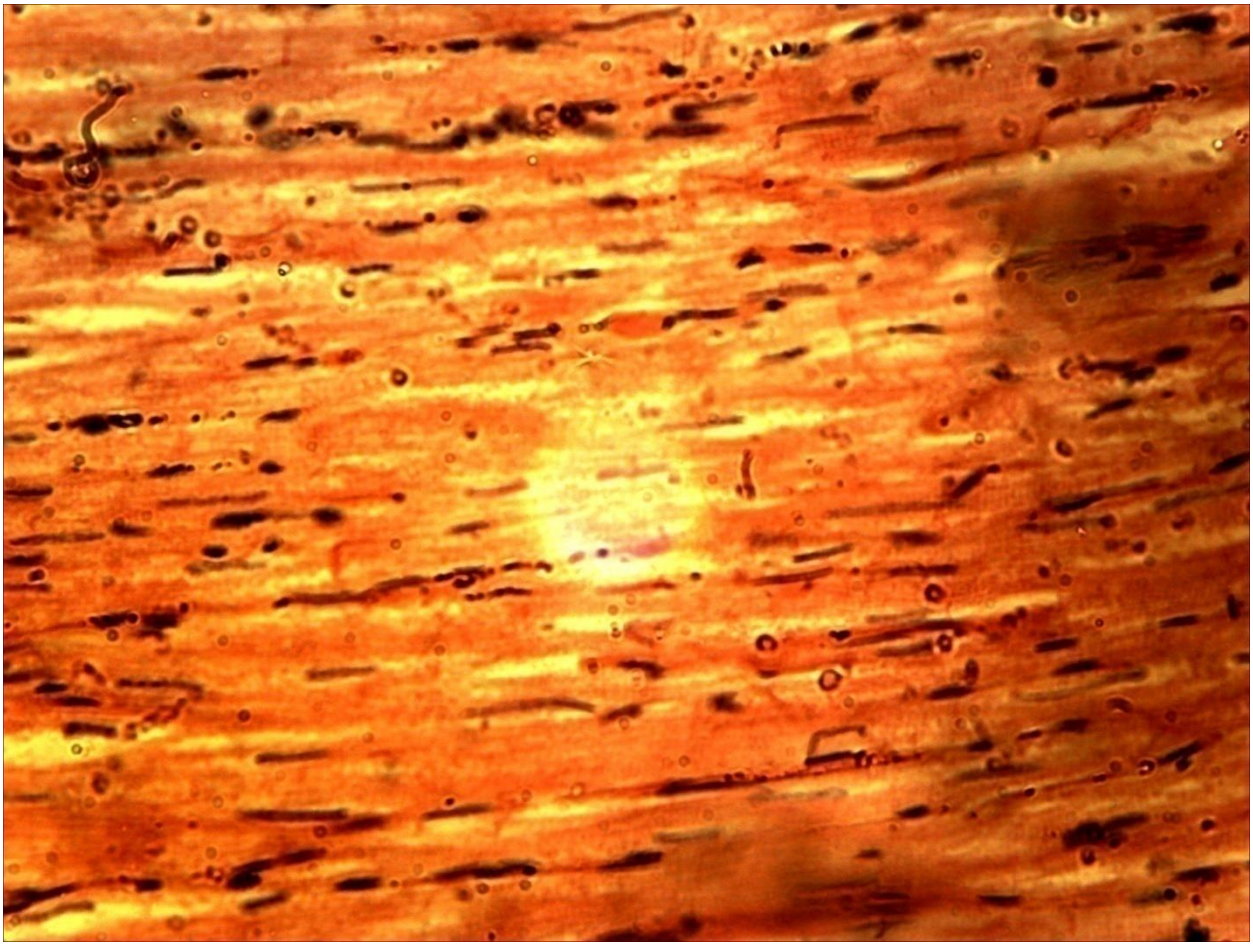


Plate XVII Photomicrograph of a section of the heart of a Metformin treated alloxan induced hyperglycaemic rat (H&E X400)

No significant histopathological findings seen.

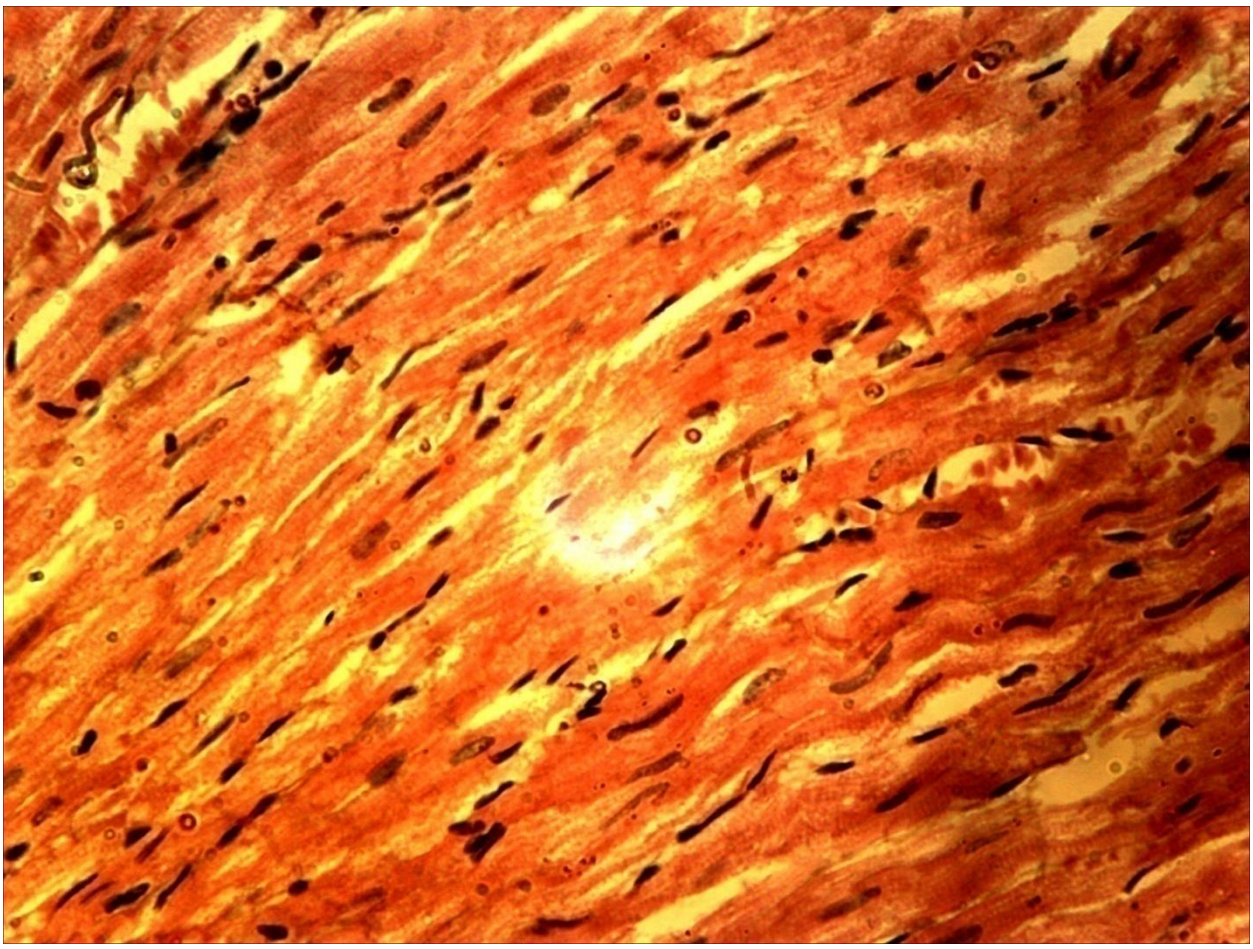


Plate XVIII Photomicrograph of a section of the heart of a Metformin-Cefixime treated alloxan induced hyperglycaemic rat (H&E X400)

No significant histopathological findings seen.

Chapter Five

DISCUSSION

Diabetes was induced in two groups of wistar rats by a single intraperitoneal injection of Alloxan (freshly prepared in 0.9% Normal saline solution) at a dose of 140 mg/kg body weight. Alloxan induces diabetes by producing dose dependent destruction of pancreatic β – cells (Rerup 1970; Jelodar *et al.,* 2007). There was a significant elevation in the blood glucose level, after administration of alloxan and the diabetic rats clearly showed a statistically significant difference in FBG and RBG values as compared to the non diabetic rats on Day 1. Administration of 200 mg/kg of Metformin caused significant reduction in the Fasting Blood Glucose levels of the hyperglycaemic rats seen on Day 8 i.e. groups 3 and 4. This is in agreement with Sheehan (2003) that the effect of Metformin on glucose control is seen fairly quickly, at an interval of one to two weeks.

The significant reduction seen in the FBG of the hyperglycaemic rats which was more evident than the reduction in RBG from day1 to day 14 might be as a result of peripheral utilization of the glucose in the system of the animals from the last meal without further replacement from another meal or gluconeogenesis from the liver. This is because glucose lowering by Metformin occurs primarily by decreasing hepatic glucose production (gluconeogenesis) and to lesser extent by decreasing peripheral insulin resistance, (Mahler and Adler 1999; Votey 2008). It could also possibly be due to food drug interaction decreasing the bioavailability of the drug. According to Bristol Myer Squib Company in an Official monograph of Glucophage® (Metformin hydrochloride tablet) distributed in 2008, “Food decreases the extent of and slightly delays the

absorption of Metformin, by approximately a 40% lower mean peak plasma concentration (Cmax), a 25% reduction in area under the plasma concentration versus time curve (AUC), and a 35-minute prolongation in time to attain peak plasma concentration (Tmax) following administration of Metformin with food".

By day 28th, the last day of the experiment, results of FBG of the Metformin-Cefixime combination group had returned to 66.75 and was comparable to the control group and Cefixime alone groups. Considering the fasting blood glucose levels of the diabetic animals in group 4 at the beginning of the experiment and at the end, it is clear that there had been a time dependent significant reduction in the FBG which is attributed to the hypoglycaemic action of Metformin. But the decrease in FBG of the diabetic rats given Metformin alone was still statistically significant compared to Metformin-Cefixime combination. Cefixme on its own has no effect on blood glucose levels, but from the result, when co--administered with Metformin may probably increase hypoglycaemic effect of Metformin. Cefixime and Metformin are both excreted renally via glomerular filtration and tubular secretion, and there is a possibility of competitive excretion as seen with concomitant administration of Cephalexin and Metformin. According to Jayasagar *et al.*, (2002), Cephalexin decreased renal tubular secretion of Metformin by 14% due to competitive inhibition leading to an increase in serum concentration of Metformin i.e. C_{max} by 34% .

The body weights of the rats in all groups also showed a steady increase, though no significant difference throughout the course of the experiment. The result of these

experiments is in conformity with a study to determine the effect of Metformin on leptin sensitivity in adult and aged rats by Kim *et al.,* (2006) were they established a very slight increase after an initial fall in the first week of the weight of adult wistar rats been given Metformin. Metformin is known to be advantageous in obese diabetic patients in that it is often weight neutral or induces slight weight loss through appetite suppressive action, (Sheeham, 2003).

The increase in the prevalence of type 2 diabetes is closely linked to the upsurge in obesity (Hossain *et al.,* 2007). The study did not reveal any significant difference in the weights of the rats in the four groups throughout the course of the study, suggesting that the use of Cefixime in diabetic patients been treated with Metformin is not likely to bring about an increase in weight. Metformin, is weight neutral and is the preferred drug of choice in diabetic obese patient. Increase in weight is not desirable in diabetic patients as obesity also increases the risk of complications of cardiovascular disease (Hossain *et al.,* 2007).

The haematological parameters that were evaluated in this study i.e. (PCV, RBC and WBC) did not differ significantly between the groups and within the groups. From the result, neither the individual drugs nor their combination affected the haematological parameters in the rats. Though Metformin is documented to be associated with megaloblastic anaemia due to malabsorption of Vitamin B_{12}, this effect is mostly seen after prolonged use of the drug and at such did not manifest in these study. Cefixime administration is also associated with a number of haematological anomalies e.g.

Leucopaenia, neutropaenia and eusinophilia, these effects are seen in less than 2% of the population. The wistar rats in the Cefixime alone group did not manifest any anomaly in the haematological parameters investigated, neither did its combination with Metformin increase nor decreases these values significantly. This result is of importance because drug/drug interactions involving two or more drugs with a same adverse effect could lead to increase in severity of such effect.

Body electrolytes e.g. sodium, potassium and bicarbonate ions are altered in the acute presentation of Diabetes Mellitus, as a result of dehydration from loss of body fluids. There is also an increase in Glomerular Filtration Rate (GFR) in Diabetes Mellitus leading to sodium retention and potassium depletion, (Shadid and Mahboob, 2008). This inverse relationship between sodium and potassium has been seen in diabetic coma, progression of diabetic nephropathy and other diabetic complications such as cardiovascular diseases (Shadid and Mahboob, 2008). It is therefore important to maintain a normal electrolyte homeostasis and acid-base balance in the diabetic patient.

The ions principally involved in fluid and electrolyte homoeostasis and acid-base balance are sodium, chloride, bicarbonate, and potassium ions. Only the mean potassium level of diabetic animals in Metformin alone group was slightly lower. This is in agreement with a study by Vallon *et al.*, (1999) to determine the role of tubular reabsorption in glomerular hyperfiltration in experimental diabetic rats where it was observed that plasma concentrations of sodium or chloride ion were not significantly altered whereas plasma potassium ion concentration was slightly lowered. Diabetic rats also exhibited an

increase in GFR (by approximately 27%). Elevated potassium ions are known to inhibit glucagon secretion while stimulating the secretion of insulin (Smith *et al.*, 1982). On the other hand, elevated sodium ion is associated with increased risk of cardiovascular diseases; this implies that it is important for potassium and sodium ions to be kept within normal limits in a T2DM patient. Drug combinations used in management of T2DM and other co-morbid diseases should therefore not bring about significant alterations in the levels of these ions.

Metformin-Cefixime co-administration did not affect urea and creatinine levels in hyperglycaemic rats as compared to control and the Cefixime and Metformin alone treated groups. The use of Cefixime has been associated with increase in serum creatinine levels (Tally *et al.*, 1987), and the result of this study also showed that rats in the Cefixime alone group had the highest mean serum creatinine level compared to control and the other drug treated groups. This is of importance because serum urea and creatinine levels are indices used in monitoring renal function. Metformin is substantially excreted by the kidneys and the risk of accumulation and life threatening Metformin Associated Lactic Acidosis (MALA) increases with the degree of impairment of renal function and abnormal creatinine clearance from any cause. Metformin as such is contraindicated in any condition predisposing to increase lactic acid production or decrease lactic acid metabolisim. This therefore suggests the need to monitor kidney function when this combination is used.

The relative organ weights of the various organs of the drug treated groups and control showed no statistical difference in sizes. This however, may not necessarily predict an absence of pathological conditions or functional alterations. The relative pancreas weight of the hyperglycaemic animals in Metformin alone group and Metformin-Cefixime group was higher than in the normoglycaemic animals i.e. Control and Cefixime group. There was also an increase in relative kidney weight of the hyperglycaemic rats treated with Metformin alone compared to Control and Metformin-Cefixime groups. Vallon *et al* (1999) reported an increase in the kidney weights of diabetic animals compared to non diabetic control.

Cefixime displayed antimicrobial activity against the selected microorganisms used in this study as seen from the Inhibition Zone Diameter (IZD) measured. Nevertheless there was no statistically significant difference seen in the *in vitro* Metformin-Cefixime combination on anti-microbial effect of Cefixime against the selected microorganisms. This result suggests that the use of Metformin and Cefixime concomitantly, is not likely to compromise the antimicrobial activities of Cefixime in Type 2 diabetic patient. The consequences of untreated or improperly treated infections in diabetes mellitus would result in decompensation of the disease. The result also showed that the three different concentrations of Metformin used, did not exhibit intrinsic antimicrobial activity against the test organisms as no zone of inhibition was seen in the plates.

Histopathological results of the diabetic rats treated with Metformin alone showed necrosis of pancreatic acini and less cellular endocrine portion of pancreas, which was

due to alloxan used in this study. This observation is in agreement with Ragavan and Krishnakumari (2006), where the ultra structure of alloxan induced diabetic rats showed considerable reduction in the islets. Jelodar *et al* (2007) and Edem (2009) also reported that alloxan caused severe necrotic changes of pancreatic islets, especially in the centre of islets of diabetic rats.

However the section of the pancreas of diabetic rats in the Metformin-Cefixime treated group did not show any significant histological findings. This probably suggests that there has been a pancreatic regenerative process since the final value of RBG and FBG of this group had reduced significantly as compared to the first day and also to Metformin only group. This finding reveals the probability of DDIs on co administration of Metformin and Cefixime.

Histologically, liver section of alloxan induced diabetic rats showed dilated sinusoids and necrosis of hepatocytes. The kidney histopathology data of alloxan induced diabetic rats in Metformin treated and Metformin-Cefixime treated groups showed marked tubular damage and necrosis of tubular epithelial cells. This effect of alloxan is associated with hyperglycaemia and was responsible for dilatation of proximal and distal tubules. The degenerative changes in the histology of liver and kidney brought about by alloxan administration are similar to earlier observations, (Ragavan and Krishnakumari, 2006). Where they reported marked tubular and glomerular damage in the kidney and necrosis of hepatocytes in the liver histopathology of alloxan induced diabetic rats.

Chapter Six

SUMMARY, CONCLUSION AND RECOMMENDATION

In this study on possible drug interaction between Metformin and Cefixime it was discovered that Cefixime may slightly increase the hypoglycaemic activity of Metformin when co administered. This effect may not appear to be of much clinical significance when both drugs are administered at therapeutic doses, but will call for caution when used for a longer period or in other situations that may predispose patient to possible accumulation of Metformin.

Metformin and Cefixime when co administered did not seem to affect body weight, haematological parameters, Urea, Creatinine and Electrolyte levels in the treated rats. Antibacterial study revealed that Metformin at therapeutic doses is not likely to affect the antimicrobial effect of Cefixime against the selected microorganisms used. This implies that Metformin and Cefixime may be used safely in real life clinical situations without the fear of compromising the antimicrobial effect of Cefixime.

In Conclusion, from the result of the study Metformin and Cefixime can be safely co administered when Diabetes Mellitus and infections exist as co morbid states in the absence of other pathological or disease conditions. It may also be necessary to monitor blood glucose levels and renal function if Cefixime is to be used for more than one week.

It is recommended that further studies be carried out on possible pharmacokinetic drug interactions between Metformin and Cefixime on both animals and human models. This will help to determine whether Cefixime increases the plasma concentrations of

Metformin at therapeutic doses and vice versa. Studies on possible interactions between Metformin and Cefixime can also be carried out in the presence of other pathological or disease conditions, to ascertain the possibility of Drug, drug / drug, disease state interactions.

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