" EVALUATION OF EFFECT OF METFORMIN ON CLOZAPINE INDUCED METABOLIC DERANGEMENT IN RATS"

By

DR.SYED SHOIB MD HUSSAINI

Dissertation submitted to the

BLDE University, Vijayapur, Karnataka

In partial fulfillment of the requirements for the award of the degree of

DOCTOR OF MEDICINE

IN

PHARMACOLOGY

Under the Guidance of

DR.A.A.NAIKAWDI$_{M.D}$

PROFESSOR & HEAD

DEPARTMENT OF PHARMACOLOGY

BLDE UNIVERSITY'S, SHRI B.M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPUR,

KARNATAKA.

2018

B.L.D.E UNIVERSITY'S

SHRI B.M.PATIL MEDICAL COLLEGE, HOSPITAL

& RESEARCH CENTRE, VIJAYAPUR

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled**"EVALUATION OF EFFECT OF METFORMIN ON CLOZAPINE INDUCED METABOLIC DERANGEMENT IN RATS"**is a bonafide and genuine research work carried out by me under theguidance of**DR.A.A.NAIKAWDI$_{M.D}$**Professor& Head, Department of Pharmacology. BLDEU ShriB.M.Patil Medical College, Hospital & RC, Vijayapur, Karnataka.

Date: **DR.SYED SHOIB MD HUSSAINI**

Place: Vijayapur

B.L.D.E UNIVERSITY'S

SHRI B.M.PATIL MEDICAL COLLEGE, HOSPITAL

& RESEARCH CENTRE, VIJAYAPUR

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **"EVALUATION OF EFFECT OF METFORMIN ON CLOZAPINE INDUCED METABOLIC DERANGEMENT IN RATS"**is a bonafide research work done by **DR. SYED SHOIB MD HUSSAINI** in partial fulfillment of the requirements for the degree of **Doctor of Medicine (Pharmacology)**

Date:

Place: Vijayapur

DR.A.A.NAIKAWDI **M.D**

Professor & Head,

Department of Pharmacology,

BLDEU ShriB.M.Patil Medical

College, Hospital & RC, Vijayapur,

Karnataka

B.L.D.E UNIVERSITY'S

SHRI B.M.PATIL MEDICAL COLLEGE, HOSPITAL

& RESEARCH CENTRE, VIJAYAPUR

ENDORSEMENT BY HEAD OF DEPARTMENT

This is to certify that the dissertation entitled **"EVALUATION OF EFFECT OF METFORMIN ON CLOZAPINE INDUCED METABOLIC DERANGEMENT IN RATS"**is a bonafide research work done by**DR. SYED SHOIB MD HUSSAINI** in partialfulfillment of the requirements for the degree of **Doctor of Medicine (Pharmacology).**

Date:

Place: Vijayapur

DR.A.A.NAIKAWDI$_{M.D}$

Professor and H.O.D,

Department of Pharmacology,

BLDEU ShriB.M.Patil Medical

College, Hospital &RC,

Vijayapur, Karnataka.

B.L.D.E UNIVERSITY'S

SHRI B.M.PATIL MEDICAL COLLEGE, HOSPITAL

& RESEARCH CENTRE, VIJAYAPUR

ENDORSEMENT BY PRINCIPAL / HEAD OF THE INSTITUTION

This is to certify that the dissertation **"EVALUATION OF THE EFFECT OF METFORMIN ON CLOZAPINE INDUCED METABOLIC DERANGEMENT IN RATS"**is abonafide research work done by **DR.SYED SHOIB MD HUSSAINI** in partialfulfillment of the requirements for the degree of **Doctor of Medicine (Pharmacology).**

Date:

Place: Vijayapur

Dr.S.P.GUGGARIGOUDAR

Principal,

BLDEU ShriB.M.Patil

Medical College, Hospital & RC,

Vijayapur, Karnataka.

,

B.L.D.E UNIVERSITY'S

SHRI B.M.PATIL MEDICAL COLLEGE, HOSPITAL

& RESEARCH CENTRE, VIJAYAPUR

COPYRIGHT

DECLARATION BY THE CANDIDATE

I hereby declare that the BLDE University, Karnataka shall have the rights to preserve, use and disseminate this dissertation / thesis in print or electronic format for academic / research purpose.

Date: **DR.SYED SHOIB MD HUSSAINI**

Place: Vijayapur

ACKNOWLEDGMENT

First of all, I am grateful to **The Almighty God** for establishing me to complete my dissertation .

I am grateful to **my parents, my wife and my family members** for their constant encouragement, support and prayers.

I wish to express my deep sense of gratitude to **Dr. Akram A Naikawdi,** Professor and head, Department of pharmacology for his constant guidance, supervision and encouragement throughout the course of my dissertation work.

I am very grateful to **Dr. V.U.Narsapur** Professor, Department of pharmacology for her constant support and valuable criticism in the preparation of this dissertation.

I express my sincere thanks to **Dr. AnantKhot**Asst prof,Department of pharmacology **Dr.LeelaHugar**Asst profDepartment of pharmacology, **Mr.GurudattaMoharir** lecturer Department of pharmacology and **Dr.AmbadasBharatha** former lecturer Department of pharmacology.

I also express my sincere thanks to **Dr.B.S.Patil** Lecturer Department of Anatomy for helping me in the histo-pathological work.

My sincere thanks to my seniors **Dr.Jyotipatil** and **Dr.Vijaylaxmiuppin** and to my co-pg**Dr.SupreetKamni.**

I am deeply endowed to the **Vice-chancellor** of BLDE University and **Principal** of BLDE University's, Shri B.M Patil Medical College for giving me an opportunity to carrying out this dissertation.

I finally acknowledge with gratitude to all the **non-teaching staff** of Department of pharmacology.

Date: **Dr. Syed ShoibMdHussaini**

Place: Vijayapur

LIST OF ABBREVATIONS USED

AMPK	AMP-activated protein kinase
ANOVA	One way analysis of variance
DA	Dopamine
DM	Diabetes Mellitus
FGAs	First generation antipsychotics
HDL	High density lipoprotein
5-HT	5- Hydroxytryptamine
LSD	Lysergic acid diethylamide
LDL	Low density lipoprotein
NMDA	N-methyl-D-aspartate
PET	Positron emission tomography
PCOS	Polycystic ovary syndrome
SGAs	Second generation antipsychotics
SEM	Standard Error of Mean
UKPDS	The UK prospective diabetes study
WHO	World health organization
YLD	Years lived with disability

TABLE OF CONTENTS

Sl. No.	Contents	Page No
1.	INTRODUCTION	1-4
2.	AIMS &OBJECTIVES	5-6
3.	REVIEW OF LITERATURE	7-23
4.	MATERIALS AND METHODS	24-28
5.	RESULTS	29-44
6.	DISCUSSION	45-49
7.	SUMMARY	50-51
8.	CONCLUSION	52-53
9.	LIMITATIONS OF THE STUDY	54-55
10.	REFERENCES	56-68
11.	ANNEXURE	69

LIST OF TABLES

SL. NO.	TABLES	PAGE NO
1.	CHANGE IN MEAN BLOOD GLUCOSE AMONG STUDY GROUPS ACCORDING TO TIME	30
2.	CHANGE IN MEAN LIPID PROFILE AMONG STUDY GROUPS ACCORDING TO TIME	32
3.	CHANGE IN MEAN FOOD INTAKE AMONG STUDY GROUPS ACCORDING TO TIME	34
4.	CHANGE IN MEAN BODY WEIGHT AMONG STUDY GROUPS ACCORDING TO TIME	36

LIST OF FIGURES

Sl. No.	Figure	Page No.
1.	STRUCTURE OF CLOZAPINE	16
2.	STRUCTURE OF METFORMIN	20
3.	CHANGE IN MEAN BLOOD GLUCOSE AMONG STUDY GROUPS ACCORDING TO TIME	31
4.	CHANGE IN MEAN LIPID PROFILE AMONG STUDY GROUPS ACCORDING TO TIME	33
5.	CHANGE IN MEAN FOOD INTAKE AMONG STUDY GROUPS ACCORDING TO TIME	35
6.	CHANGE IN MEAN BODY WEIGHT AMONG STUDY GROUPS ACCORDING TO TIME	37
7	HISTOPATHOLOGY OF LIVER (CONTROL)	39
8	HISTOPATHOLOGY OF LIVER (CLOZAPINE)	40
9	HISTOPATHOLOGY OF LIVER (CLOZAPINE + METFORMIN)	41
10	HISTOPATHOLOGY OF PANCREAS (CONTROL)	42
11	HISTOPATHOLOGY OF PANCREAS (CLOZAPINE)	43
12	HISTOPATHOLOGY OF PANCREAS (CLOZAPINE + METFORMIN)	44

INTRODUCTION

1.INTRODUCTION

In recent years Second generation antipsychotics (SGAs) are effective pharmacotherapeutic agents for various neuropsychiatric diseases , especially Schizophrenia, but also for bipolar disorder, autism, as a add on therapy in many major depressive disorder and are used by millions of patients in the world[1]. The major advantage of these drugs is that they are less likely to cause neurologic side effects like extrapyramidal symptoms, where as first generation antipsychotics(FGAs) produces movement disorders like acute dystonias, akinesia, akathesia and tardive dyskinesia. The neurologic side effects of first generation antipsychotics(FGAs) are due to potent dopamine D2 receptor blockade and are important limiting factors for long term use of these agents[2]. The second generation antipsychotics(SGAs) are currently more prescribed due to lack of these neurological side effects. SGAs act both 5HT2 and are weak D2 blocker. In addition they have alpha adrenergic blocking, anticholinergic and H1 antihistaminic activity. However, the clinical research in past decade has reported that most SGAs drugs can cause serious metabolic adverse effects, resulting in a metabolic syndrome that substantially increases the risk for cardio-metabolic disorders, such as type II diabetes mellitus and cardiovascular diseases[3-5].The incidence of diabetes mellitus has been shown to be increased in patients treated with SGAs in comparison with general population[6].Several retrospective studies shown increased in serum triglyceride in patients treated SGAs like Clozapine[7]. SGAs drugs induced metabolic syndrome is characterized by weight gain, hyperprolactemia, hyperlipidaemia, hyperglycaemia, glucose intolerance, hypertension cardiovascular disease and insulin resistance[8]. For the patients who are taking Clozapine , the risk of developing type 2 diabetes during

the first 6 years is greatest. It has been reported that among the patients who took Second generation antipsychotic like Clozapine more than 50% become overweight[9].

Treatment with Clozapine was associated with an average weight gain of 9.8lb over 10weeks which is as high as any other antipsychotics[10]. These metabolic changes are seen regardless of age , sex or duration of antipsychotic therapy[11-12]. In one of the study the prevalence of metabolic syndrome has been found to be as high as 24.6% [13]. Metabolic syndrome is also predictive of both cardiovascular diseases and type 2 diabetes mellitus. This may lead to early death[14]. several studies have reported that there are increased loss of life in patients with Schizophrenia. Besides , they have higher risks for cerebrovascular illnesses, respiratory disorders and mortality from suicide. However , the most common reason for death suggested to be cardiovascular disorders[15-16]. With widespread use of these drugs, the metabolic adverse effects of antipsychotics is a major public health problem and there is a great need for a better understanding for their management. Consistent with the literature on type II diabetes mellitus, some success has been obtained through lifestyle changes, including exercise and dietary modifications[17]. However, these changes may be more challenging in the psychiatric population[18], therefore the mainstay of treatment remains the use of antidiabetic drug like Metformin to minimise or to ameliorate metabolic derangement. Metformin is being currently used to treat metabolic syndrome and type II diabetes mellitus. Metformin acts through diverse pharmacological mechanisms. Metformin has been demonstrated to reduce the incidence of type 2 diabetes mellitus by 31% compared to control subjects who received placebo[19]. The present study proposes that reduced progression from pre-diabetes to diabetes should be a treatment goal for patients with Schizophrenia treated with SGAs. Regular use of Metformin would be expected to lower the progression

rate to a considerable extent. It is therefore important to determine whether Metformin is efficacious in treating SGAs drugs-induced metabolic syndrome. Therefore the present study is undertaken to evaluate the effects of Metformin on metabolic derangement caused by Clozapine, a SGA drug in a rat model[20].

AIMS & OBJECTIVES

AIMS AND OBJECTIVES

1. To evaluate metabolic derangement induced by second generation SGAs drug Clozapine.
2. To evaluate the mechanism of Clozapine induced metabolic derangement.
3. To evaluate the effect of Metformin in minimizing Clozapine induced metabolic derangement.

REVIEW OF LITERATURE

SCHIZOPHRENIA

HISTORICAL ASPECTS

Schizophrenia still remains mysterious or difficult to understand though its thought to be among the most common psychiatric disorders. The word is almost more than 100 years older however it most likely accompany human beings throughout its whole history. It had been first known as a distinct mental disease by Emil Kraepelin in 1887, who had used the word dementia preacox for individuals who had symptoms that we now associate with Schizophrenia. In 1911 swiss psychiatrist , Eugen Bleuler coined the word Schizophrenia for first time. "positive" or "negative" symptoms of Schizophrenia were also first described by him. Eugen Bleuler changed the name dementia preacox to Schizophrenia as it was obvious that Emil Kraeplin name was misleading as the disease was not a dementia (it did not always lead to mental deterioration) and could sometimes occur late as well as early in life. The word "Schizophrenia" comes from the Greek word schizo (split) and phrene (mind) to describe the fragmented thinking of people with the disorder.The information of historic growth of Schizophrenia allows us to grasp the various ideas within the comprehension of the pathological process of the disease and the way the definition, the concepts concerning its aetiology and its treatment have emerged[21-22].

CLINICAL MANIFESTATIONS

Patients suffering from Schizophrenia may present with positive symptoms which include hallucination (auditory), delusions, conceptual disorganization or negative symptoms which include lack of pleasure, poverty of speech, decreased emotional expression, social withdrawal. cognitive symptoms include defect in attention and working memory. The patients who are suffering from Schizophrenia feels cheerful or sad; they often are depressed.

To meet formal diagnostic criteria at least two of the above symptoms for a period of one month and continuous signs for a period of six months should present. There are no specific features. It is usually begins in late adolescence, has an insidious onset and very poor outcome. Negative symptoms occur in about one-third of the patients of Schizophrenia and it has very poor outcome and poor response to the drug therapy. prognosis does not depend on symptom severity but depends on response to the antipsychotic medications. Around 10% of schizophrenic population commits suicide[23]. In statistics generated by the WHO, Schizophrenia is listed as the 5th and 6th most significant cause of years lived with disability (YLD) in men and women, respectively [24].

EPIDEMIOLOGICAL ASPECTS INCIDENCE AND PREVALENCE

The life risk of Schizophrenia has historically been given at ~1% worldwide with life time prevalence ~1-1.5% . Around three lakhs acute Schizophrenia occur annually in US. Schizophrenia occurs in ~6.6% in first degree relatives of an affected proband. Risk of offspring is 40% if both the parents are affected with Schizophrenic disorder, 50% for monozygotic twins and 10% for dizygotic twins [23.]. precise diagnostic criteria yielded incidence rates starting from 6/100,000 to 14/100,000 in an exceedingly giant multinational WHO study (the alleged 10-country study) [25]. overall median incidence of Schizophrenia 15.2 per 100,000, with an calculable seven out of one thousand people diagnosed with the disorder at some purpose in their life[26]. Male: Female risk of developing Schizophrenia could also be ~ 1.4:1[27].

RISK FACTORS

The heritability of Schizophrenia is very high possibly as high as 80%[28]. Intrinsic possibility of being attacked is thought to coincide with external risk factors to trigger onset of Schizophrenia in an individual. External risk factors include increasing parental age[29]. Denovo mutations arising in paternal germ cells may be suggested as underlying mechanism[30].winter birth, cannabis use, cannabis is a known risk factor for Schizophrenia, roughly for around 50% cases for Schizophrenia[31-32],Obstetric complications and prenatal viral infections. The disorder is often described as multifactorial in order to trigger the symptoms.

PATHOGENESIS OF SCHIZOPHRENIA

Inspite of eager research the pathophysiology of schizophrenic disorder remains unclear. The most important mechanisms during the last fourty years has undoubtedly been the so-called dopamine hypothesis. It was suggested that excessive and dysregulated dopaminergic activity in mesolimbic pathway that leads to disturb function of the limbic system and that translate into positive symptoms. Decrease activity in mesocortical pathway leads to negative symptoms. In normal healthy person there is balance between mesolimbic pathway and mesocortical pathway. DA(Dopamine)hypothesis is based on the fact that drugs which bind to and blocks dopamine receptors particularly D2 receptors relieves symptoms of Schizophrenia[33-34]. Apart from that increased dopamine receptor density is seen in PET(positron emission tomography) and postmortem brain studies of the schizophrenic patients. Drugs that stimulate central dopaminergic activity like amphetamine also aggrevate Schizophrenia[35]. Demyelination of the primary component of the white matter, affects the neuronal property and is believed to be of significance within the pathophysiology of schizophrenic disorder. Signs of impaired myelination have been demonstrated in patients with schizophrenic disorder[36-37]. Defect in other neurotransmitter system have also been suggested. 5-HT hypothesis; It is based on the fact that LSD , 5-HT2 agonist produces hallucination but this hypothesis has fallen because psychotic symptoms associated with LSD is visual hallucination while in Schizophrenia it is auditory hallucinations predominate[35].Glutamate hypothesis; This is based on the findings that NMDA receptors antagonist like Ketamine when administered in normal individual produces psychotic symptoms[35].

FIRST GENERATION ANTIPSYCHOTICS

The First generation antipsychotics are also known as typical antipsychotics, classic antipsychotics or dopamine antagonist. Chlorpromazine was the first antipsychotic drug belonging to chemical class phenothiazines and was discovered in 1950. commercially launched for the treatment of psychiatric illness in 1953[38-39]. Its introduction has been labelled as greatest achievement in the history of psychiatry. Psychiatrists first observed regression of treatment induced positive symptoms in the patients who are suffering from Schizophrenia[40]. Haloperodol belongs to different chemical class butyrophenones was discovered by Paul Janssen in 1958 and in 1959 commercially launched in europe[41]. Pimozide was introduced at Janssen pharmaceutica in 1963. Flupenthixol introduced by Lundbeck in 1965.Several other antipsychotics were also introduced during the 1950s and 1960s. The antipsychotic action of typical antipsychotics is now believed to be produced by their competetive blockade of D2 receptors[42].Haloperidol is a potent D_2 antagonist and Chlorpromazine is a relatively weak D_2 antagonist compared to other early antipsychotics. Thus competetive blockade of D2 receptors produces unwanted dose dependent extrapyramidal side effects like acute dystonias occurs within few hours usually by the parenteral drugs because basal ganglia is responsible for posture regulation, Akinesia(parkinson like syndrome) occurs within few days, Akathesia occurs within weeks to months of course akathesia developed in those patient who are not having akinesia. Tardive dyskinesia occurs within weeks to months, tardive dyskinesia develops due to chronic use of antipsychotics. The serious extra-pyramidal side effects and less effective against negative symptoms encouraged the search for newer antipsychotic agents.

SECOND GENERATION ANTIPSYCHOTICS

The second generation antipsychotics are also known as atypical antipsychotics. Clozapine was the first atypical antipsychotic drug which was introduced in the year 1958. They generally have a lower propensity to cause extrapyramidal side effects like acute dystonias, akinesia, akathesia and tardive dyskinesia than first generation antipsychotics, SGAs helps to improve negative symptoms, they are less likely to cause hyperprolactinemia except for Risperidone. SGAs have greater affinity for 5-HT, alpha adrenergic, muscarinic, histaminergic and weak dopaminergic antagonist activity[43-44].Its affinity for serotonergic receptors ($5\text{-}HT_{2A}$, $5\text{-}HT_{2C}$) is as much as 20 times higher than its D_2 affinity. Imaging studies have demonstrated that Olanzapine's D_2 affinity is higher than that of Clozapine.[45] Risperidone was commercially introduced in 1994, Risperidone is more powerful D2 blocker than Clozapine. During Risperidone therapy prolactin levels rise disproportionately[46].Olanzapine was commercially introduced in 1996 and is chemically related to Clozapine[47].Quetiapine commercially introduced in 1998 It is a short acting with plasma half life of 6hrs, Quitiapine is quite sedating[46]. Ziprasidone commercially introduced in 2001 has D2 affinity, high affinity for 5-HT combined with serotonin and norepinephrine reuptake inhibition. Aripiprazole is first third generation antipsychotic commercially introduced in 2002, It has partial D2 agonist, partial agonism at $5\text{-}HT_{1A}$ receptors, as well as $5\text{-}HT_2$ antagonism[49]. Some of the adverse effects of SGAs are weight gain, lipid derangements and development of type 2 diabetes mellitus.

TREATMENT OF SCHIZOPHRENIA

Antipsychotic drugs are the cornerstone of acute and maintenance therapy of Schizophrenia and are effective in delusion, hallucination and thought disorder regardless of etiology. In the patients who are presenting with first episode, antipsychotic drugs are effective in about 70% cases[23]. The introduction of first generation antipsychotics(FGAs) like chlorpromazine offered a good choice for management of psychotic symptoms. FGAs are still effective therapeutic pharmacologic agents and additionally presently accessible to treat psychotic symptoms[51], their use usually led to extrapyramidal symptoms. Second generation antipsychotics(SGAs) have principally replaced First generation antipsychotics(FGAs) since 1990s. SGAs have very low risk of extrapyramidal symptoms compared to FGAs .SGAs are also effective in treating negative symptoms and improving cognitive functions.

CLOZAPINE IN TREATMENT OF RESISTANT SCHIZOPHRENIA

From the introduction of the primary neuroleptic drug, its become obvious that a large set of patients with Schizophrenia are treatment resistant. Between 20% and 60% of the patients with Schizophrenia don't respond sufficiently to normal treatment[52-53]. Although Clozapine is considered the standard pharmacotherapy as a last resort in the management of treatment-resistant Schizophrenia. Clozapine-resistant Schizophrenia characteristics include persistent active psychotic features despite daily doses of 300 to 900 mg/d for eight weeks to six months, with plasma drug levels of 1.0 nmol/l of the parent drug or higher[54]. Despite of wide range of adverse effects Clozapine remains the drug of choice for treatment resistant Schizophrenia, few of them like thromboembolism, agranulocytosis, cardiomyopathy and myocarditis are serious or potentially life-threatening adverse effects. However Clozapine is more effective compared to any other first generation or second generation antipsychotic. one or two thirds of these patients who are not responding to any other first or second generation drugs will adequately respond to Clozapine. Clozapine relieve cognitive deficits, decrease the suicidal tendency and it has been associated with reduce total mortality than any other antipsychotic drugs[55]. Despite these risks it may also be associated with the lowest mortality of all antipsychotics[56]. Around 30% of the patients who do not respond from older first generation antipsychotics will have a good response with Clozapine[23].

CLOZAPINE

Clozapine is a dibenzodiazepine derivative[57]. It is a prototype of SGAs. Clozapine has weak dopamine receptor activity at D1,D2,D3 and D5 and it has high affinity for D2 receptors, Additionally it has antimuscarinic, alpha-adrenergic blocking, antiserotonergic and sedative properties[57]. It is known to be more efficacious than any other first generation and second generation antipsychotics. It is more effective in treatment resistant positive symptoms and suicidality[55].Unfortunately due to more side effects it is usually considered as third line antipsychotic[58]**.** Clozapine produces few or no extra pyramidal side effects. No rise in prolactin level and tardive dyskinesia is rare. Both the positive as well as negative symptoms of Schizophrenia will get improve. Clozapine is structurally similar with SGAs like Olanzapine and Quetiapine [59].The structure of Clozapine is given below

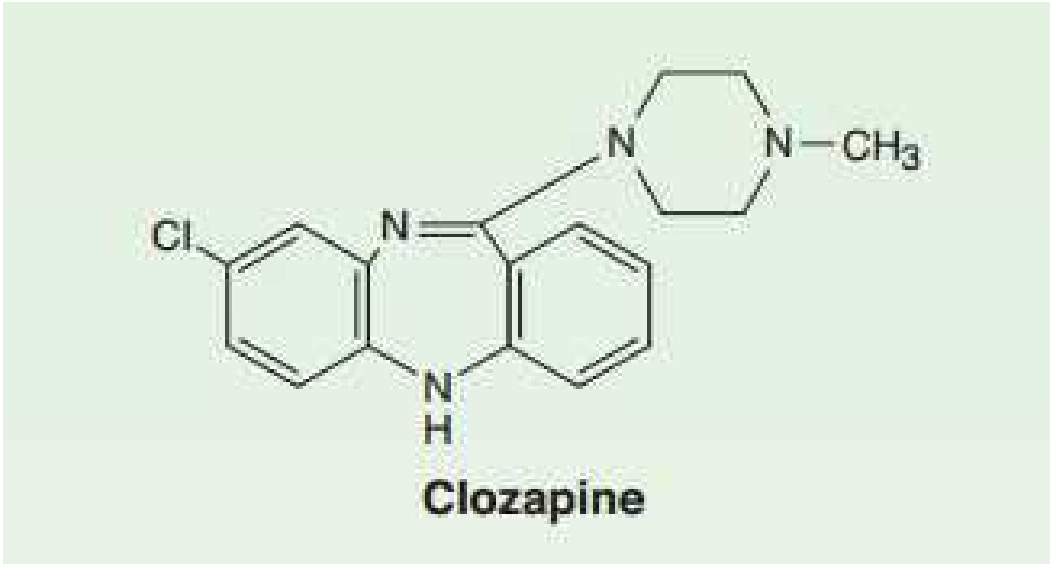


Figure1: structure of Clozapine

PHARMACOKINETICS

At therapeutic dose Clozapine has linear or First order kinetics this means the rate of absorption and elimination proportional to the drug concentration[60].The average t1/2 of Clozapine is 12hrs, The peak concentration is achieved at 1-3 hrs and it is matabolized into active and inactive metabolites by CYP2C19 , CYP1A2 and CYP3A4

ADVERSE EFFECTS

The major life threatening adverse effect of Clozapine is fatal agranulocytosis wich occur in approximately in 01-2% patients. Weekly monitoring of blood count is required for first six months and every month thereafter. It is usually reversible after discontinuance of the Clozapine. usually occurs at 6th to 18th week of treatment[61-62]. Other side effects include weight gain, sedation, tachycardia, unstable BP, urinary incontinence and myocarditis[63]. Most adverse effects are due to its pharmacological properties Orthostatic hypotension and sexual dysfunction are due to its alpha blockade, muscarinic antagonism causes anticholinergic side effects tachycardia, constipation, blurred vision and urinary retention, sedation is due to histamine blockage[64]. Clozapine has high affinity for H1 receptor and 5HT2C which is associated with weight gain[65].

CLOZAPINE AND GLUCOSE

It has mentioned in several reports that Clozapine is associated with hyperglycemia and in some patients occasionally debuting as diabetic ketoacidosis[66-67].The majority of such cases occur within three months of beginning of Clozapine[68].The retrospective studies have associated with occurence of relative risk of diabetes in the patients treated with Clozapine compared to typical antipsychotics[69]. Impaired glucose tolerance is twice as high as patients taking Clozapine compared to any other antipsychotics. In the previous study patients chronic treated with Clozapine upto five years about one third of the patient developed diabetes mellitus[70].Clozapine and Olanzapine have been demonstrated to increase the risk of insulin resistance and type 2 diabetes[71].

CLOZAPINE AND LIPID PROFILE

In 1994 first increased in triglyceride level in the patients who were treated with Clozapine was published as a case report[72].Several years later, elevated serum triglyceride levels were reported in the patients who are treated with Clozapine[73].later it was demonstrated to occur due to increased adipose tissue mass[74]. Increase in serum cholesterol levels has also been reported during Clozapine treatment[75].

CLOZAPINE AND WEIGHT

Weight gain in a patients who are treated with Clozapine is a common clinical finding. Average weight gain with Clozapine treatment is 1.72 kg/month which occurs during sixth to twealth month of treatment[76]. Clozapine had significant weight gain followed by Olanzapine and Quetiapine[77]. When treated with Clozapine weight gain continuous beyond the first year whereas with other antipsychotics weight may stabilize in short to medium term[78].little is known about the changes in appetite of these patients. It had been found that 20% gain in weight compared to the baseline weight in two years[79].The exact mechanism about the Clozapine induced weight gain is unknown. 5HT2C receptor blockade, Histamine H1 receptor affinity and dopamine D2 receptor blockade have been implicated[80].It has been implicated that leptin genotype may be involved[81].

METFORMIN

Metformin is a biguanide and was introduced in 1950s, Metformin does not stimulate β-cells of pancrease therefore it remarkably differ from sulfonylureas. It causes very little or no hypoglycemia in both non- diabetics as well as in diabetics. Metformin is reported to improve lipid profile. This agent helps to reduce LDL cholesterol and triglyceride levels, and is not associated with weight gain, and prevents the cardiovascular complications of diabetes. The structure of Metformin is shown below[82].

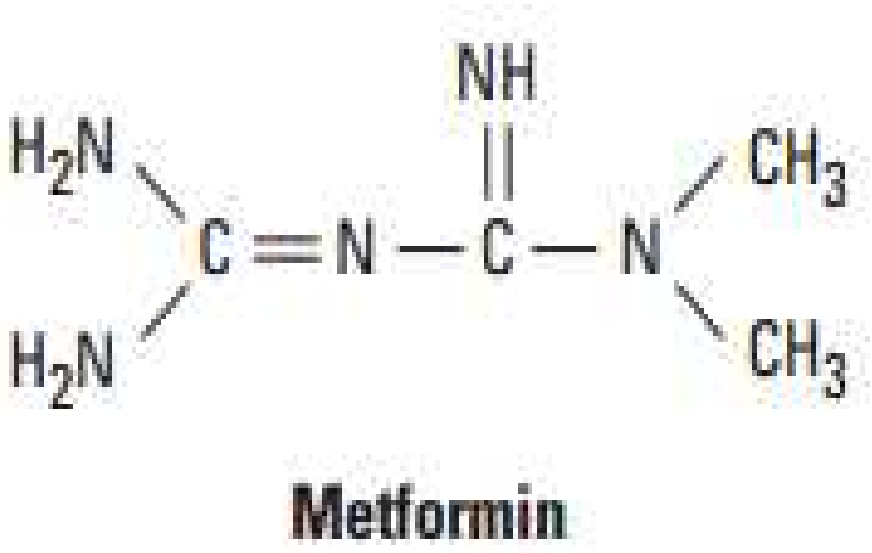


Figure 2: Structure of Metformin

MECHANISM OF ACTION

The full explanations about the mechanism of action of Metformin still remains elusive. Metformin leads to activation of AMP dependent protein kinase (AMPK) and leads to decrease hepatic glucose production. Other possible minor mechanism might be decrease glucose output from liver, Enhances insulin mediated glucose uptake in skeletal muscles, Metformin also reduces the intestinal absorption of amino acids, glucose, Vit B12 and other hexoses and Impairment of renal gluconeogenesis. Metformin does not stimulate insulin release from the B-cells but presence of insulin is essential for its action. it is not effective in Type-1 DM and pancreatectomized animals[82-84].

METABOLISM AND EXCRETION

The half life of Metformin is 1.5 to 3 hours. Metformin is not bound to plasma protein, is not metabolized and is excreted unchanged in the urine as the active compound. Active tubular secretion in the kidney is the principal route of metformin elimination. The drug is widely distributed into body tissues including the intestine, liver, and kidney by organic cation transporters. As a result of hepatic blokade of gluconeogenesis, it will impair the hepatic metabolism of lactic acid. In renal failure patients it will accumulate and causes lactic acidosis and which resembles to be dose related complications of metformin[82-83].

CLINICAL USES.

Metformin is recommended as first line therapy in the patients who are suffering from type 2 diabetes mellitus except when it is contraindicated or when it is not tolerated. Because it is a insulin sparing agent (it does not stimulate β-cells of pancrease to release insulin) and it doesnot cause hypoglycemia or weight gain, Metformin has a potential to prevent microvascular and macrovascular complications of diabetes mellitus, no β-cells exhaustion in type 2 DM patients, can combine with other oral or injectable anti-diabetic drug[82-86]. Patients who are treated with Metformin in Polycystic ovary syndrome (PCOS) produces considerable improvement in ovulation and decrease rate of miscarriage. In addition to improvements in insulin resistance, hyperglycemia and body weight which are mostly associated with the disease condition[87-88].Recently Metformin has also been associated with positive symptoms in the patients who are suffering from cancer. Metformin in vitro has shown to decrease the number of cancer cells including endometrial, colon, prostate, breast and gliomas[89]. Metformin has demonstrated to control weight both in patients who are suffering from diabetes as well as non-diabetic patients[90]. Chronic Metformin treatment increases the sensitivity to the leptins as the leptin resistance is more common in obese patients[91].Metformin has many beneficial effects on the cardiovascular system. In the UKPDS study, the risk of cardiovascular events decreased in the patients who are treated with Metformin[92].

ADVERSE EFFECTS

The common side effects with Metformin therapy is gastrointestinal disturbances like nausea, vomiting, abdominal discomfort, anorexia, metallic taste, tiredness and diarrhoea which occur in upto 20% of patients[82]. These gastrointestinal side effects are transient and are usually dose related and are liable to occur during the initiation of therapy. Due to persistent diarrhoea in about 3-5% patients Metformin possibly be discontinued[82]. Chronic treatment with Metformin therapy reduces the intestinal absorption of Vit. B12 therefore yearly screening of serum Vit. B12 should be advised for the patient. Lactic acidosis with Metformin is rare but little increase in blood lactate can occur. Alcohol can accelerate Lactic acidosis[83].

CONTRAINDICATIONS

- Alcoholism
- Renal failure
- Hepatic disease
- Tissue anoxia (chronic cardiopulmonary resuscitation)
- Hypotensive states and
- Heart failure.

MATERIALS AND METHODS

MATERIALS AND METHODS

STUDY DESIGN : An Experimental animal based study.

LOCUS OF STUDY : BLDEU's Shri B.M. Patil Medical College Hospital & Research Centre, Vijayapura.

SAMPLE SIZE : 18 Wistar rats

SEX : Either

DRUGS USED :

1)**Clozapine:** It was obtained from Rajesh Chemicals co. Mumbai . The dose used was 25mg / kg/ day orally. It was dissolved in dilute acetic acid.

2) **Metformin:** It was obtained from Rajesh Chemicals co. Mumbai . The dose used was 100mg / kg/ day orally. It was dissolved in distilled water.

Wistar rats weighing 180-240g either sex bred from a stock obtained from the Central Animal House, BLDEU's Shri BM Patil Medical College Hospital & Research Centre, Vijayapura, India, were used in the study. Animals were housed in separate room 3 each in polypropylene cages for one week acclimatization before the start of the study.

The cages were lined with paddy husk which was replaced every day and animals were kept under standard condition of illumination with a 12 - h light-dark cycle at room temperature of 25 ± 1° C and 45-70% relative humidity. The animals were fed with commercial pellet rat chow (manufactured by VRK Nutritional solutions, Sangli, Maharashtra) and water ad libitum.

ETHICAL CLEARANCE

The study was reviewed and approved by the **Institutional Animal Ethics Committee (IAEC)** vide letter reference number; 665/15, dated 07.12.2015 (letter enclosed). Study was carried out as per guidelines of Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA).

DOSE CALCULATION

Rat dose per day is calculated using average human dose/day and converted into rat dose using following formula[93]: Rat dose/200g = human dose x 0.18.

GROUPING OF ANIMALS, DOSE OF DRUG AND ROUTE OF ADMINISTRATION:

Animals will be divided into 3 groups of six rats each, each group having equal number of male and female rats.

Group 1: Control (n=6) was given distilled water p.o

Group 2: Received Clozapine (n=6)25mg/kg p.o[21]

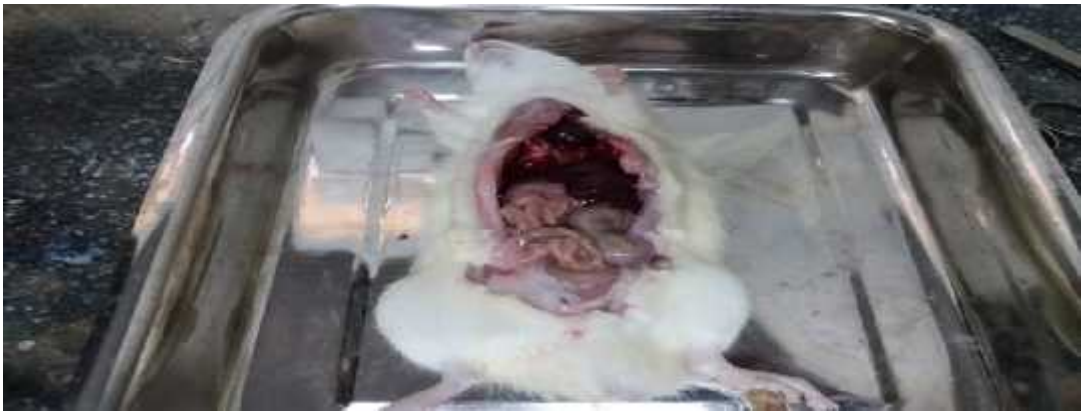
Group 3: Received Clozapine25mg/kg + Metformin (n=6)100mg/kg p.o[22]

All animals had access to food and water *ad libitum*.

STATISTICAL ANALYSIS

STATISTICAL ANALYSIS:

- All the values have been expressed as the mean ± SEM and analyzed by one-way analysis of variance (ANOVA) in order to test differences between groups.
- The level of statistical significance has been set at $p<0.05$.



RESULTS AND OBSERVATIONS

RESULTS AND OBSERVATIONS:

TABLE 1 : CHANGE IN MEAN BLOOD GLUCOSE AMONG STUDY GROUPS ACCORDING TO TIME

Blood Glucose	CONTROL		CLOZAPINE		CLOZAPINE + METFORMIN		ANOVA p value
	Mean	SEM	Mean	SEM	Mean	SEM	
0 DAY	82.7	1.3	83.5	2.1	83.2	2.0	0.948
14DAY	82.8	1.4	87.0	1.9	83.5	1.7	0.201
28 DAY	82.8	1.2	91.3	2.0	83.8	1.9	0.007*

Note: *means significant at 5% level of significance ($p<0.05$)

FIGURE 3 : CHANGE IN MEAN BLOOD GLUCOSE AMONG STUDY GROUPS ACCORDING TO TIME

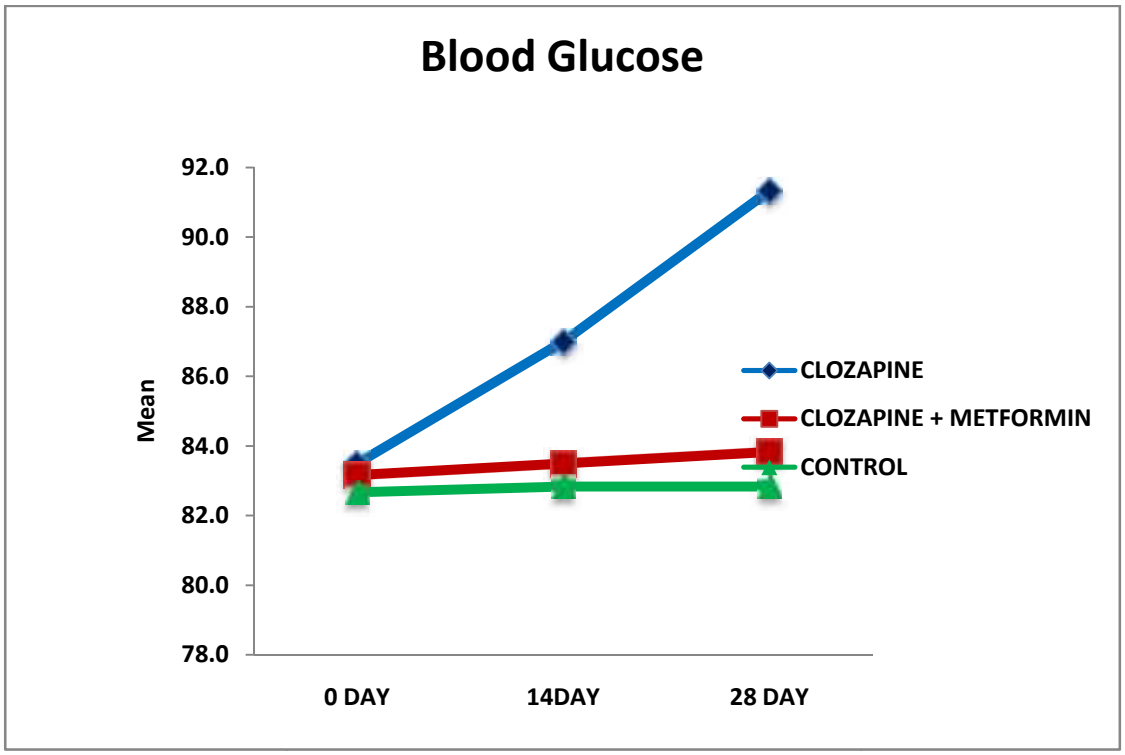


Table No.1 and Figure No.3 shows that Blood glucose level was measured initially,on day 14th and at the end of the study, by glucometer. There was significant increase in Blood glucose level in Group 2(Clozapine treated rats) than to Group 1(Control). Administration of Clozapine along with Metformin in Group 3(Clozapine+Metformin treated rats) there was no increase and was comparable to those rats in untreated control group.

TABLE 2 : CHANGE IN MEAN LIPID PROFILE AMONG STUDY GROUPS ACCORDING TO TIME

Lipid profile	Days	CONTROL		CLOZAPINE		CLOZAPINE + METFORMIN		ANOVA p value
		Mean	SEM	Mean	SEM	Mean	SEM	
Triglyceride	0 DAY	91.5	2.6	92.8	1.9	92.0	1.9	0.906
	28 DAY	93.0	2.6	103.3	1.7	94.7	1.7	0.006*
Total cholesterol	0 DAY	101.0	1.4	101.5	0.6	101.7	0.6	0.878
	28 DAY	103.7	1.5	113.7	1.6	102.8	0.8	<0.001*
HDL	0 DAY	29.8	0.7	29.3	0.3	28.8	0.5	0.427
	28 DAY	31.7	1.1	31.0	0.4	30.3	0.6	0.444
LDL	0 DAY	52.9	1.7	53.6	0.6	54.4	1.0	0.648
	28 DAY	53.4	1.8	62.0	1.7	53.6	0.6	0.001*
VLDL	0 DAY	18.3	0.5	18.6	0.4	18.4	0.4	0.906
	28 DAY	18.6	0.5	20.7	0.3	18.9	0.3	0.006*

Note: *means significant at 5% level of significance ($p<0.05$)

FIGURE 4 : CHANGE IN MEAN LIPID PROFILE AMONG STUDY GROUPS ACCORDING TO TIME.

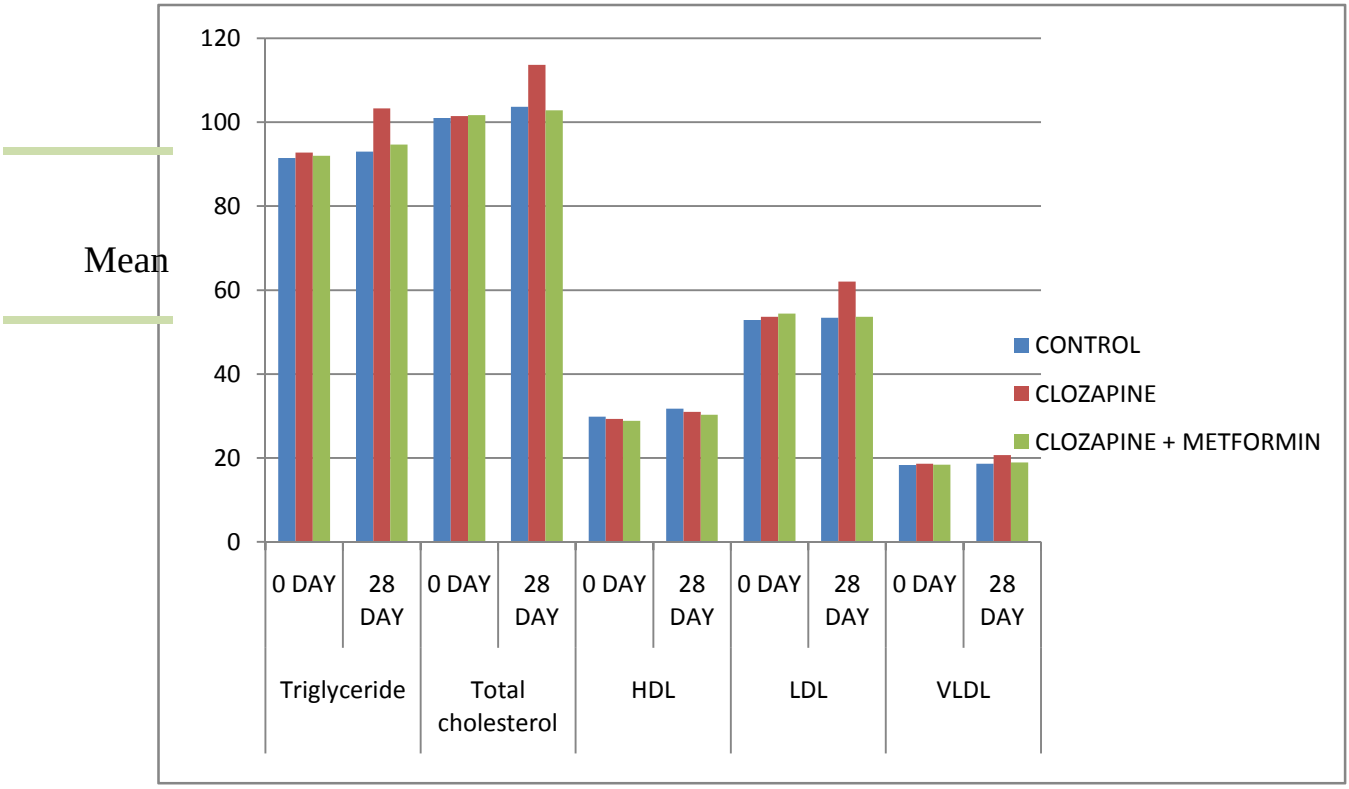


Table No.2 and Figure No.4 shows that Group 2 (Clozapine treated rats) showed alterations in Serum Triglycerides, Total cholesterol, LDL and VLDL levels compared to those in normal control group over study period. These alterations in Lipid Profile were reduced by Group 3(Clozapine + Metformin treated rats) compared to untreated control group.

TABLE 3 : CHANGE IN MEAN FOOD INTAKE AMONG STUDY GROUPS ACCORDING TO TIME

Food intake	CONTROL		CLOZAPINE		CLOZAPINE + METFORMIN		ANOVA p value
	Mean	SEM	Mean	SEM	Mean	SEM	
D1	11.2	0.3	11.3	0.3	11.0	0.4	0.785
D2	11.3	0.3	10.3	0.3	10.8	0.3	0.472
D3	11.7	0.2	11.8	0.3	11.2	0.2	0.149
D4	11.2	0.2	11.8	0.3	11.3	0.3	0.245
D5	11.3	0.2	11.8	0.2	11.2	0.3	0.149
D6	11.5	0.3	12.2	0.3	11.3	0.2	0.136
D7	11.3	0.3	12.3	0.3	11.3	0.3	0.08
D8	11.2	0.2	12.5	0.4	11.3	0.2	0.011*
D9	11.3	0.2	12.7	0.2	11.5	0.2	0.001*
D10	11.3	0.2	13.0	0.3	11.5	0.2	<0.001*
D11	11.7	0.3	13.3	0.2	12.0	0.0	<0.001*
D12	11.5	0.2	13.0	0.4	11.7	0.2	0.008*
D13	11.0	0.0	13.8	0.3	11.8	0.2	<0.001*
D14	11.5	0.4	13.7	0.3	11.7	0.3	0.001*
D15	11.8	0.2	13.8	0.3	12.0	0.3	<0.001*
D16	11.5	0.2	13.8	0.3	11.5	0.2	<0.001*
D17	11.5	0.2	14.2	0.5	11.7	0.3	<0.001*
D18	11.8	0.2	14.5	0.2	11.8	0.3	<0.001*
D19	11.3	0.2	14.0	0.3	12.0	0.3	<0.001*
D20	12.2	0.2	14.5	0.4	12.0	0.4	<0.001*
D21	12.2	0.3	14.8	0.4	11.8	0.2	<0.001*
D22	12.0	0.3	15.2	0.4	11.8	0.3	<0.001*
D23	12.0	0.3	15.0	0.5	11.8	0.3	<0.001*
D24	12.5	0.2	15.0	0.5	12.0	0.3	<0.001*
D25	12.0	0.4	15.7	0.4	12.2	0.3	<0.001*
D26	11.8	0.2	16.2	0.4	12.0	0.3	<0.001*
D27	11.8	0.2	16.3	0.3	12.0	0.3	<0.001*
D28	12.0	0.4	16.8	0.3	12.5	0.2	<0.001*

Note: *means significant at 5% level of significance ($p<0.05$)

FIGURE 5 : CHANGE IN MEAN FOOD INTAKE AMONG STUDY GROUPS ACCORDING TO TIME

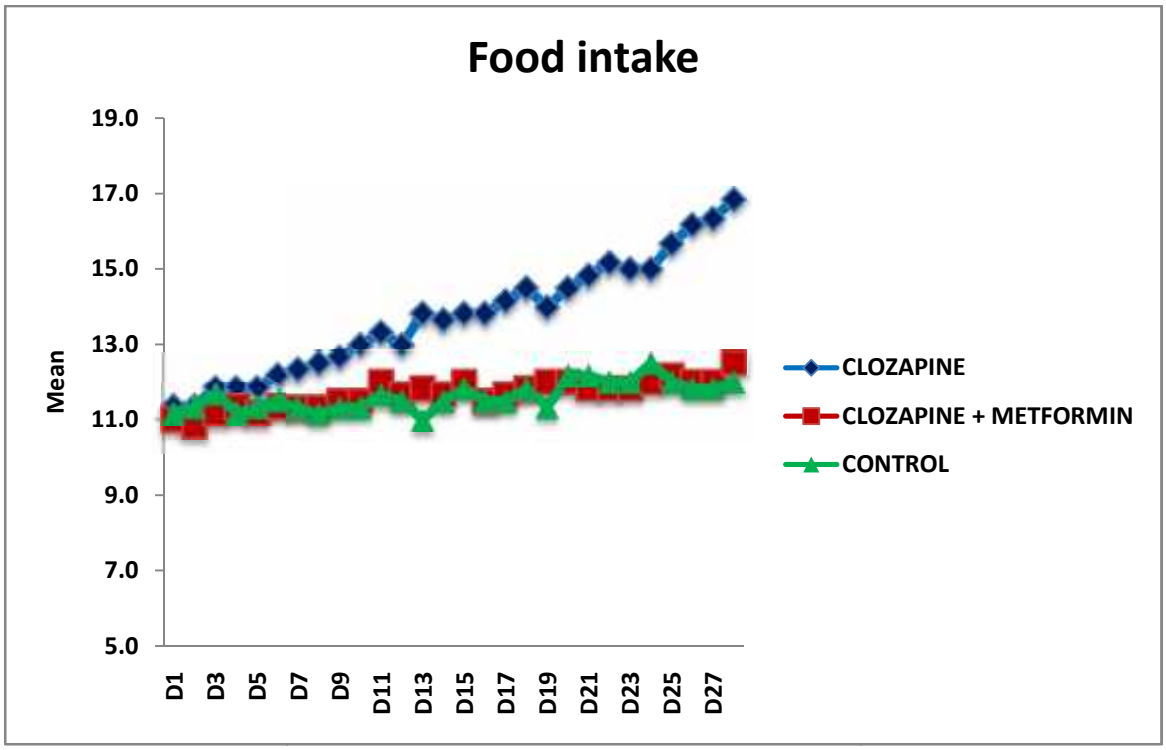


Table No. 3 and Figure No. 5 shows that food intake was monitored in all animals daily from day 1 to 28 days. There was significant increase in food intake in group2 (Clozapine treated rats) than to the Group 1 (control) rats. Administration of Clozapine along with Metformin prevented this increase and was comparable to those rats in untreated control group.

TABLE 4 : CHANGE IN MEAN BODY WEIGHT AMONG STUDY GROUPS ACCORDING TO TIME

Body weight	CONTROL		CLOZAPINE		CLOZAPINE + METFORMIN		ANOVA p value
	Mean	SEM	Mean	SEM	Mean	SEM	
0 DAY	196.8	5.3	196.7	6.8	196.3	3.6	0.998
1 DAY	197.0	5.2	197.5	6.9	196.2	3.6	0.985
2 DAY	198.3	5.5	198.7	6.9	197.5	3.5	0.988
7 DAY	203.7	5.5	211.7	6.8	204.0	3.4	0.511
14 DAY	208.8	5.2	225.8	6.5	210.3	3.7	0.07
21 DAY	215.7	4.4	240.3	6.1	218.8	3.5	0.005*
28 DAY	222.0	4.4	252.3	6.4	226.3	3.2	0.001*

Note: *means significant at 5% level of significance ($p<0.05$)

FIGURE 6 : CHANGE IN MEAN BODY WEIGHT AMONG STUDY GROUPS ACCORDING TO TIME

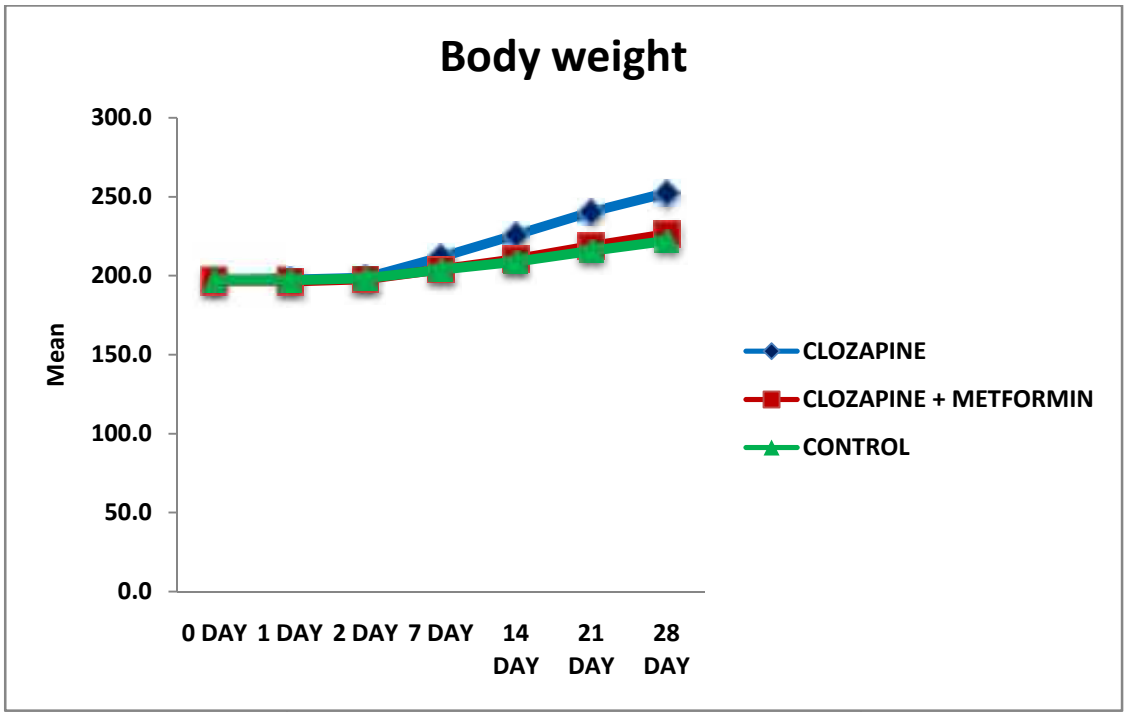
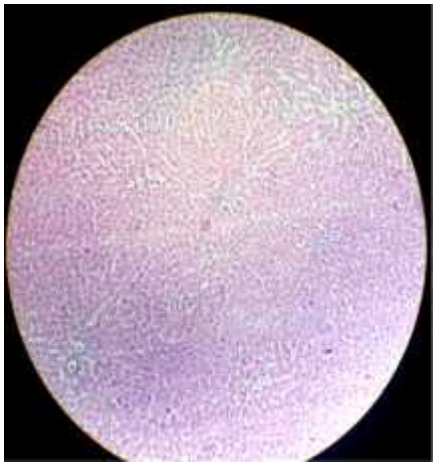


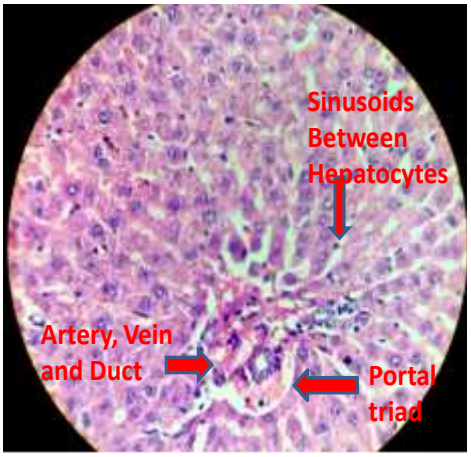
Table No.4 and Figure No.6 shows that animals in all the groups gained body weight. However Group2(Clozapine treated rats) showed significant increase in body weight after 4 weeks compared to those in the untreated control group. Rats in group 3(Clozapine + Meformin treated rats) arrested this increase in body weight significantly and were comparable to untreated conrol group.

HISTOPATHOLOGY

Histopathology of Liver (Control)



H&E Stained 10X of Liver Control group

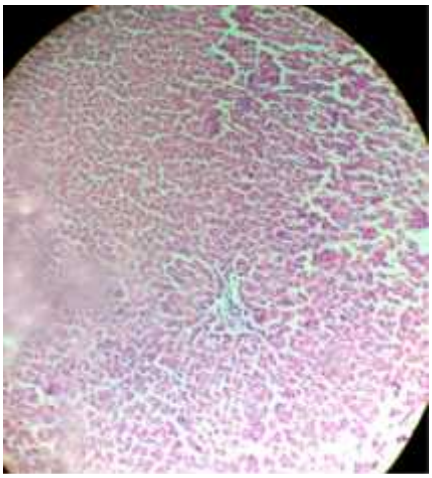


H&E Stained 40X of Liver Control group

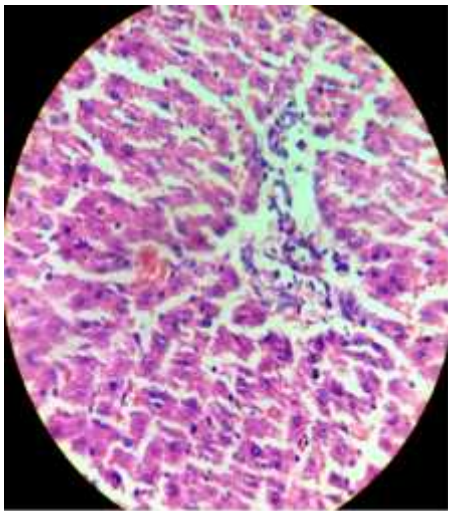
FIGURE 7

10X and 40X section of liver showing normal hepatic architecture compressed of hepatic lobules formed by central vein and cords of hepatocytes with indistinct sinusoidal dilatation . In the middle of 40X a prominent structure is called portal triad and it contains Hepatic Artery, portal Vein and bile duct seen from right to left in Group 1.

Histopathology of Liver (Clozapine Group)



H&E Stained 10X of Liver Clozapine group

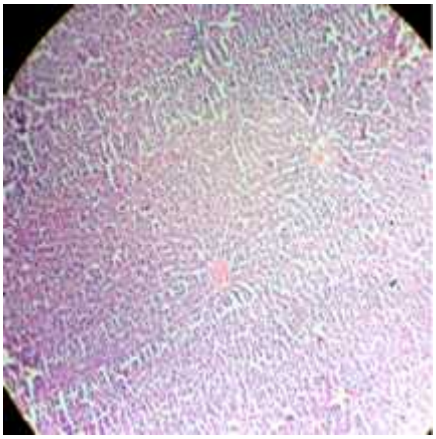


H&E Stained 40X of Liver Clozapine group

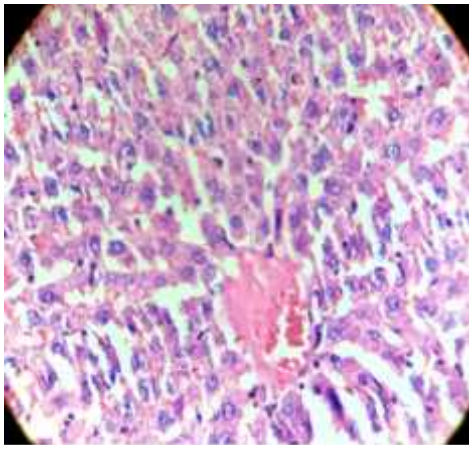
FIGURE 8

Histopathology of liver in 10X and 40X **showing deranged** architecture, consist of irregular hepatocytes with less prominent nucleus, sinusoids are prominently dilated and running irregular hepatic plate towards central vein. With pathological changes of portal triad.

Histopathology of Liver
(Clozapine+ Metformin)



H&E Stained 10X of Liver Clozapine + Metformin group

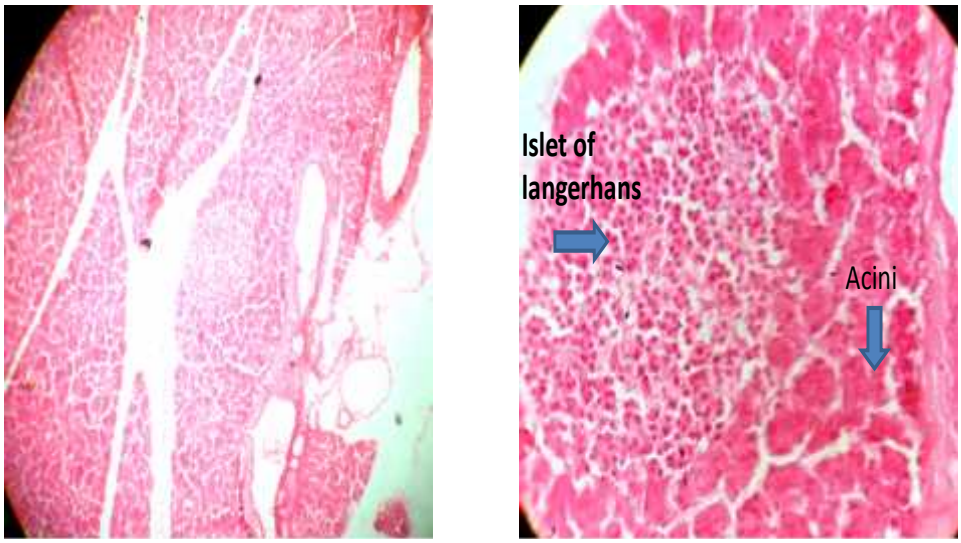


H&E Stained 40X of Liver Clozapine + Metformin group

FIGURE 9

Histopathology of liver in 10X and 40X showing normal hepatic architecture with compressed hepatic lobules formed by central vein and cords of hepatocytes with indistinct sinusoidal dilatation . In 40X a prominent structure is called central vein showing normal and the portal triad containing Hepatic Artery, portal Vein and bile duct are normal.

Histopathology of Pancreas (Control Group)

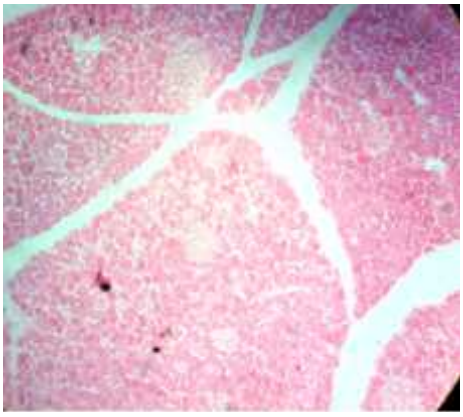


H&E Stained 10X of Pancreas Control group H&E Stained 40X of Pancreas Control group

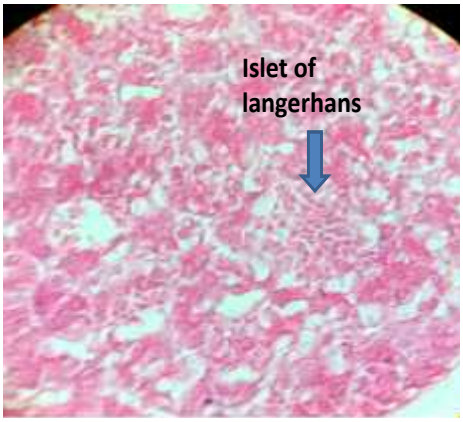
FIGURE 10

The section of the pancreas 10X and 40X control group stained with H & E showing normal acini and islet of langerhans.

Histopathology of Pancreas (Clozapine Group)



H&E Stained 10X of Pancreas Clozapine group

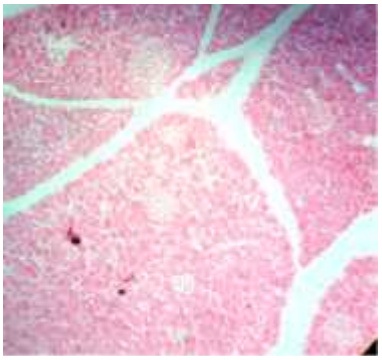


H&E Stained 40X of Pancreas Clozapine group

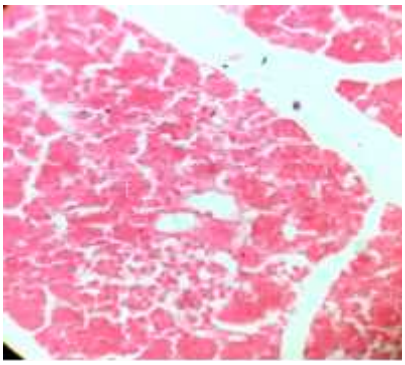
FIGURE 11

The section of the pancreas 10X and 40X control group stained with H & E showing dissociation of most of the islet cells of the Langerhans with normal histopathology of acini.

Histopathology of Pancreas (Clozapine+ Metformin)



H&E Stained 10X of Pancreas
Clozapine+ Metformin group



H&E Stained 40X of Pancreas
Clozapine+ Metformin group

FIGURE 12

The section of the pancreas 10X and 40X control group III stained with H & E showing improvement in the islet of langerhans with normal histopathology of acini.

DISCUSSION

DISCUSSION

- Major psychosis, Schizophrenia is characterised by its chronicity, disordered behavioral function and disturbed emotions and thinking. Availability of effective antipsychotic drugs has considerably improved the outcome. Older or first generation antipsychotic drugs have been used for years. But undesirable effects like disorders of movements have been major limiting factor. This resulted in development of newer more effective and safe drugs like Clozapine. However, they are also not completely free from adverse effects like metabolic derangement on long term use. This metabolic derangement can be reversed by simultaneous administration of drug like Metformin.
- In this study, we found a significant increase in Blood Glucose levels in Group 2(Clozapine treated rats) during 28 days of continuous intake of Clozapine and these results are similar with Smith G et al (2008), He investigated the effects of therapeutically relevant 3 different antipsychotic drugs (Haloperidol, Queitapine and Clozapine) on glucose tolerance, measures of insulin resistance and hepatic glucose production as well as on insulin and glucagon secretions in rats and found that impaired glucose tolerance in rats was greater with Clozapine. Their findings indicate that SGAs can cause acute derangements in glucose homeostasis not by direct induction of insulin resistance but by increasing glucagon secretion which stimulates hepatic glucose production[96].Similar study done by Hedenmalm k et al(2002), He utilized WHO database for adverse reactions. All reports suggestive of glucose intolerance for Clozapine, Olanzapine and Risperidone were identified. Clozapine, Olanzapine and Risperidone were significantly associated with glucose intolerance(GI). From 1968 to 2000, total no. of 868

reports of glucose intolerance were received, out of which 480 had received Clozapine, 253 had received Olanzapine and 138 had received risperidone[97].Glucose intolerance and hyperglycemia has been shown to be increasingly associated with use of atypical antipsychotic drug like Clozapine[98].

- A study by Boyda H et al(2010), a pre-clinical evaluation of SGAs induced metabolic side effects with focus on glucose dysregulation and insulin resistance provided insight into the metabolic influences of SGAP drugs on peripheral tissues such as liver, pancreas and skeletal muscle.[99]
- In our study administration of Clozapine along with Metformin prevented this rise in blood glucose levels and these are also similar with studies conducted by Boyda H et al (2012), He studied the effects of 3 classes of antidiabetic drugs on SGAs closely similar to Clozapine induced glucose dysregulation and insulin resistance in female rats. The treatment with Metformin and Rosiglitazone produced significant amelioration in glucose dysregulation and insulin resistance whereas Glyburide was found be ineffective. This study provides insight into the metabolic influences of SGAs on peripheral tissues such as liver, pancreas and skeletal muscles and its amelioration predominantly by Metformin. Fasting glucose levels in the rats before Olanzapine administration did not differ between groups, however fasting glucose levels measured after 60 min treatment with Olanzapine, but before the administration of Metformin showed significant increase in glucose level by antipsychotic drugs($p<0.001$).[100]
- In our study there was a statistically significant increase in serum levels of TG,TC,LDL and VLDL in Group 2 compared to group 1. whereas in Group

3(treated with Clozapine+ Metformin) there was no significant increase in TG, TC, LDL and VLDL Similar results have been reported by Chen C et al (2013), He studied effects of adjunctive Metformin on metabolic derangement in non-diabetic schizophrenic patients treated with Clozapine and found that Metformin significantly reversed the metabolic derangement particularly due to its effect on triglycerides caused by Clozapine. Metformin also reduced body weight significantly. However, beneficial effect of Metformin on body weight in Clozapine treated patients disappeared on discontinuation of Metformin. Metformin is well tolerated by these patients. Thus, it supports the strategy for long term Metformin supplementation in Clozapine treated patients with Schizophrenia and pre-existing metabolic abnormalities.[101]

- In our study there was no significant change in serum HDL levels and these are also similar with study conducted by Ozenoglu et al [102] and he also did not report any significant changes in serum levels of HDL.
- Rats treated with Clozapine have significant increase in body weight due to altered Clozapine induced glucose metabolism. The body weight of rats treated with Clozapine and Metformin(Group 3) did not increase significantly as compared to Group 1 . No increase in body weight in Group 3 rats might be due to treatment with Metformin. Hence in Known cases of Schizophrenia treated with Clozapine addition of Metformin can prevent increase in the bodyweight.
- Housekeneche k et al(2007), studied acute effects of atypical antipsychotics on whole body insulin resistance in rats and provided the first evidence that profound whole body insulin resistance following a single dose of Olanzapine and Clozapine is primarily due to hepatic insulin resistance and is sustained

with sub- chronic dosing. Their data and work of others (Ader et al 2005), Pre-clinical models for insulin resistance suggests that hepatic insulin resistance is an early response to Olanzapine or Clozapine treatment and that the hepatic effects are maintained with the development of obesity and hyperlipidemia. A better understanding of underlying molecular mechanisms will provide appropriate guide lines for preventing or minimizing SGAs induced metabolic derangement. Single dose Olanzapine and Clozapine 10mg/kg significantly impaired the ability of insulin to inhibit hepatic glucose production, 18.5 and 22.7 fold increase in hepatic glucose production vs insulin only respectively, consistent with severe hepatic insulin resistance ($p<0.001$)[103]

- In the present study treatment with Clozapine produced histopathological early changes in pancreas characterised by dissociation (hyperplasia)of islets. Previous studies reported Clozapine induced pancreatic islets hyperplasia responsible for hyperglycemia [104].Pancreatic islets hyperplasia reversed by Metformin is suggestive of functional defect rather than destruction of pancreatic beta cells [105].
- Abdelrahim Eman A et al(2013), studied histopathological change of endocrine pancreas in male rat treated with atypical antipsychotic Clozapine and found that chronic treatment with Clozapine caused changes in pancreas in the form of islets hyperplasia overlapped by atrophic changes as fibrosis and thick engorgement of islets capillaries.[104] These changes were similar to those in type 2 diabetes mellitus.[105]

SUMMARY

SUMMARY

- Our study showed hyperglycemia, increased levels of Total cholesterol, Triglycerides & Body weight produced by Clozapine.
- It also showed histological changes in Liver & Pancreas Similar to those seen in Type II Diabetes Mellitus.
- Our study results show improved glycemic control, Lipid profile, Weight gain and histopathological alterations caused by Clozapine thereby reducing risk factors for coronary heart disease
- Metformin appears to be involved in the regulation of insulin action and it's effects on carbohydrate and lipid metabolism by enhancing insulin sensitivity.
- Insulin resistance with or without the presence of metabolic syndrome significantly increases cardiovascular risk.
- By enhancing the insulin sensitivity Metformin lowers the risk of cardiovascular adverse events.
- This study increases the possibility of using Metformin supplementation to improve carbohydrate metabolism, lipid metabolism, weight gain and histopathological alterations in liver & pancreas in a patient treated with Clozapine

CONCLUSION

CONCLUSION

Our present pre clinical study is concluded with the following important observations and outcome

- The result of the study suggests that Clozapine produces increase in Blood glucose ,Triglycerides, Total cholesterol & body weight.
- It also produces histo-pathological changes in a liver & pancreas similar to those seen in Type II Diabetes Mellitus.
- The results of this study suggest that Metformin reverses the metabolic derangement caused by Clozapine in albino rats
- This raises the possibility that Metformin supplementation can be considered to improve metabolic derangement in the patients who are treated with Clozapine
- Careful monitoring of risk patients may help in the prevention of metabolic derangements as well as the management of any possible symptoms which they occur.
- Further studies are required to confirm the more beneficial effects of Metformin in the patients who are treated with Clozapine.

LIMITATIONS OF THE STUDY

LIMITATIONS OF THE STUDY

- Metformin has diverse pharmacological action which cannot be attributed to single mechanism. This requires further analysis.
- Effect on insulin levels have not been studied, which is necessary to assess the insulin resistance.
- Comparison with other antidiabetic drugs needs to be considered.
- Mass utilization of this drug requires further studies

REFERENCES

REFERENCES

1. Honer W, Thornton A, Sherwood M, MacEwan G, Ehmann T, Williams R et al. Conceptual and methodological issues in the design of clinical trials of antipsychotics for the treatment of Schizophrenia. CNS Drugs. 2007;21(9):699-714.
2. Rummel-Kluge C, Komossa K, Schwarz S, Hunger H, Schmid F, Kissling W et al.Second-generation antipsychotic drugs and extrapyramidal side effects: a systematic review and meta-analysis of head-to-head comparisons. Schizophrenia Bulletin. 2010;38(1):167-177.
3. Kessing L, Thomsen A, Mogensen U, Andersen P. Treatment with antipsychotics and the risk of diabetes in clinical practice. The British Journal of Psychiatry. 2010;197(4):266-271.
4. Newcomer J. Antipsychotic medications: metabolic and cardiovascular risk. *J Clin Psychiatry* 2007;68 (4):8-13.
5. Procyshyn Ric M, Wasan Kishor M, Thornton Allen E, Barr Alasdair M, Chen Eric Y, Pomarol-Clotet E et al. Changes in serum lipids, independent of weight, are associated with changes in symptoms during long-term Clozapine treatment. J Psychiatry Neurosci2007;32(5):331-8.
6. Buse JB, Cavazzoni P, Hornbuckle K, Hutchins D, Breier A, Jovanovic L.A retrospective cohort study of diabetes mellitus and antipsychotic treatment in the United States. J Clin Epidemiol 2003;56: 164–70.
7. Henderson DC, Cagliero E, Gray C, et al. Clozapine, diabetes mellitus, weight gain, and lipid abnormalities: a five-year naturalistic study. Am J Psychiatry2000;157:975-81.
8. Hasnain M, Fredrickson S, Vieweg W, Pandurangi A. Metabolic Syndrome

Associated with Schizophrenia and Atypical Antipsychotics. Curr Diab Rep. 2010;10(3):209-216.

9. Umbricht DS, P.S., Kane JM., Clozapine and weight gain.J Clin Psychiatry., 1994. 55 Suppl B: p. 157-60.

10. Allison DB, Mentore JM, Heo M, Chandler LP, Cappelleri JC, Infante MC, Weiden PJ: Antipsychotic-induced weight gain: a comprehensive research synthesis. Am J Psychiatry 1999; 156: 1686–1696.

11. Buse JB, C.P., Hornbuckle K, Hutchins D, Breier A, Jovanovic L. , A retrospective cohort study of diabetes mellitus and antipsychotic treatment in the United States.J Clin Epidemiol. , 2003. 56(2): p. 164-70.

12. Citrome LL, H.R., Zachry WM, Clewell JD, Orth PA, Karagianis JL,Hoffmann VP., Risk of treatment-emergent diabetes mellitus in patients receiving antipsychotics.Ann Pharmacother., 2007. 41(10): p. 1593-603.

13. Bobes J, Arango C, Aranda P. Cardiovascular and metabolic risk with outpatient Schizophrenia treated with antipsychotics. Results of the CLAMORS study. Schizophr Res 2007;90:162-73.

14. Grundy SM, Brewer HB, Cleeman JL, et al. Definition of the metabolic syndrome. Circulation. 2004;109:433–438.

15. Colton CW, Manderscheid RW. Congruencies in increased mortality rates, years of potential life lost, and causes of death among public mental health clients in eight states. Prev Chronic Dis. 2006;3:A42.

16. Brown S, Inskip H, Barraclough B. Causes of the excess mortality of Schizophrenia. Br J Psychiatry. 2000;177:212–217.

17. Park T, Usher K, Foster K. Description of a healthy lifestyle intervention for people with serious mental illness taking second-generation antipsychotics.

International Journal of Mental Health Nursing. 2011;20(6):428-437.

18. Von Hausswolff-Juhlin Y, Bjartveit M, Lindstrom E, Jones P. Schizophrenia and physical health problems. Acta Psychiatrica Scandinavica. 2009;119:15-21.

19. Knowler W. C, Barrett-Connor E , Fowler S. E , Hamman, R. F , Lachin, J. M., Walker E. A. et al.. Diabetes Prevention Program Research, Group. (2002). Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med, 346(6), 393-403.

20. Hafidh S, Senkottaiyan N, Villarreal D, et al. Management of the metabolic syndrome. *Am J Med Sci.* 2005;330(6):343-51.

21. Kyziridis T. Notes on the History of Schizophrenia. German Journal of Psychiatry. 2005;8:42-48.

22. Jablensky A. The diagnostic concept of Schizophrenia: its history, evolution, and future prospects. *Dialogues in Clinical Neuroscience*. 2010;12(3):271-287.

23. Kasper D, Harrison T. Harrison's Principles of internal medicine. 19th ed. New York: McGraw-Hill Education; 2015;2720-2722.

24. World Health Organization. The Global Burden of Disease 2004 Update. 2004.

25. Jablensky A, Sartorius N, Ernberg G, et al. Schizophrenia: manifestations, incidence and course in different cultures. A World Health Organization ten-country study. Psychol Med Monogr Suppl 1992;20:1-97.

26. McGrath J, Saha S, Chant D, Welham J. Schizophrenia: a concise overview of incidence, prevalence, and mortality. Epidemiol Rev 2008;30:67-76.

27. Aleman A, Kahn RS, Selten JP. Sex differences in the risk of Schizophrenia:

evidence from meta-analysis. Arch Gen Psychiatry 2003;60:565-71.

28. Sullivan PF, Kendler KS, Neale MC. Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. Arch Gen Psychiatry 2003;60:1187-92.

29. Tsuchiya K, Takagai S, Kawai M, Matsumoto H, Nakamura K, Minabe Y et al. Advanced paternal age associated with an elevated risk for Schizophrenia in offspring in a japanese population. 2007

30. Petersen L, Mortensen P, Pedersen C. Paternal Age at Birth of First Child and Risk of Schizophrenia. American Journal of Psychiatry. 2011;168(1):82-88.

31. Moore TH, Zammit S, Lingford-Hughes A, Barnes TR, Jones PB, Burke M, *et al*. annabis use and risk of psychotic or affective mental health outcomes: A systematic review. Lancet 2007;370:319-28.

32. Baldacchino A, Blair H, Scherbaum N, Grosse-Vehne E, Riglietta M, Tidone L, *et al*. Drugs and Psychosis Project: A multi-centre European study on comorbidity. Drug Alcohol Rev 2009;28:379-89.

33. Lang UE, Puls I, Muller DJ, Strutz-Seebohm N, Gallinat J. Molecular mechanisms of Schizophrenia. Cell Physiol Biochem 2007;20:687-702.

34. Davis KL, Kahn RS, Ko G, Davidson M. Dopamine in Schizophrenia: a review and reconceptualization. Am J Psychiatry 1991;148:1474-86.

35. Sharma H, sharma k. Principles of pharmacology. 2nd ed. hyderabad: paras medical; 2013;451-452.

36. Hof PR, Haroutunian V, Copland C, Davis KL, Buxbaum JD. Molecular and cellular evidence for an oligodendrocyte abnormality in Schizophrenia. Neurochem Res 2002;27:1193-200.

37. Davis KL, Stewart DG, Friedman JI, et al. White matter changes in Schizophrenia: evidence for myelin-related dysfunction. Arch Gen Psychiatry 2003; 60:443-56.

38. Ban T. Fifty years chlorpromazine: a historical perspective. Neuropsychiatric Disease and Treatment. 2017;3 (4):495-500.

39. Curzon G. How reserpine and chlorpromazine act: the impact of key discoveries on the history of psychopharmacology. Trends Pharmacol Sci 1990;11:61-3.

40. Cancro R. The introduction of neuroleptics: a psychiatric revolution. Psychiatr Serv 2000;51:333-5.

41. Lopez-Munoz F, Alamo C. The consolidation of neuroleptic therapy: Janssen, the discovery of haloperidol and its introduction into clinical practice. Brain Res Bull 2009;79:130-41.

42. Sharma H, sharma k. Principles of pharmacology. 2nd ed. hyderabad: paras medical; 2013;453-454.

43. Satoskar R, Rege N, Bhandarkar S. Pharmacology and pharmacotherapeutics. 23rd ed. Mumbai: Popular prakashan private Limited ; 2015;201-203.

44. Stahl SM. Describing an atypical antipsychotic: receptor binding and its role in pathophysiology. Primary Care Companion J Clin Psychiatry 2003;5:9-13.

45. Kapur S, Zipursky RB, Remington G. Clinical and theoretical implications of 5-HT2 and D2 receptor occupancy of Clozapine, risperidone, and Olanzapine in Schizophrenia. Am J Psychiatry 1999;156:286-93.

46. Tripathi K. Essentials of medical pharmacology. 7th ed. New Delhi: Jaypee Brothers; 2013;439-442.

47. Mullen J, Jibson MD, Sweitzer D. A comparison of the relative safety, efficacy, and tolerability of Quetiapine and risperidone in outpatients with Schizophrenia and other psychotic disorders: the Quetiapine experience with safety and tolerability (QUEST) study. Clin Ther 2001;23:1839-54.

48. Goodnick PJ. Ziprasidone: profile on safety. Expert Opin Pharmacother 2001;2:1655-62.

49. Mailman RB, Murthy V. Third generation antipsychotic drugs: partial agonism or receptor functional selectivity? Curr Pharm Des 2010;16:488-501.

50. Miyamoto, S., Duncan, G.E., Marx, C.E.Lieberman, J.A. 2005. Treatments for Schizophrenia: a critical review of pharmacology and mechanisms of action of antipsychotic drugs. Mol Psychiatry 10, 79-104.

51. Tandon, R., Belmaker, R.H., Gattaz, W.F., Lopez-Ibor, J.J., Jr., Okasha, A., Singh, B., Stein, D.J., Olie, J.P., Fleischhacker, W.W.Moeller, H.J. 2008. World Psychiatric Association Pharmacopsychiatry Section statement on comparative effectiveness of antipsychotics in the treatment of Schizophrenia. Schizophr Res 100, 20-38.

52. Miller, A., McEvoy, J.Jeste, D. 2006. Treatment of chronic Schizophrenia. J. A. Lieberman, T. S. Stroup and D. O. Perkins. Washing-ton DC, American Psychiatric Publishing**:** 365-381.

53. Meltzer H, Kostacoglu A. Treatment-resistant Schizophrenia. In: Lieberman J, Murray R, eds. Comprehensive Care of Schizophrenia: A Textbook of Clinical Management. London: Martin Dunitz; 2001:181-203.

54. Umbricht, D.S.,Wirshing, W.C., Wirshing, D.A., McMeniman, M., Schooler,

N.R., Marder, S.R.Kane, J.M. 2002.Clinical predictors of response to Clozapine treatment in ambulatory patients with Schizophrenia. J Clin Psychiatry 63, 420-424.

55. Essali, A., Al-Haj Haasan, N., Li, C.Rathbone, J. 2009. Clozapine versus typical neurolep-tic medication for Schizophrenia. Cochrane Database Syst Rev, CD000059

56. Tiihonen, J., Lonnqvist, J., Wahlbeck, K., Klaukka, T., Niskanen, L., Tanskanen, A.Hauk-ka, J. 2009. 11-year follow-up of mortality in patients with Schizophrenia: a popu-lation-based cohort study (FIN11 study). Lancet 374, 620-627.

57. Brayfield A, Martindale W. Martindale. 38th ed. London: Pharmaceutical Press; 2014;1057-1062

58. Wang, P.S., Ganz, D.A., Benner, J.S., Glynn, R.J.Avorn, J. 2004. Should Clozapine continue to be restricted to third-line status for Schizophrenia?: a decision-analytic model. J Ment Health Policy Econ 7, 77-85.

59. Markowitz, J.S., Brown, C.S.Moore, T.R. 1999. Atypical antipsychotics. Part I: Pharmacology, pharmacokinetics, and efficacy. Ann Pharmacother 33, 73-85.

60. Guitton, C., Abbar, M., Kinowski, J.M., Chabrand, P.Bressolle, F. 1998. Multiple-dose pharmacokinetics of Clozapine in patients with chronic Schizophrenia. J Clin Psychopharmacol 18, 470-476.

61. Sharma H, sharma k. Principles of pharmacology. 2nd ed. hyderabad: paras medical; 2013;460.

62. Iqbal MM, Rahman A, and Husain Z. et al. Clozapine: a clinical review of adverse effects and management. Ann Clin Psychiatry. 2003 15:33–48.

63. Nielsen, J., Correll, C.U., Manu, P.Kane, J.M. 2013. Termination of Clozapine treatment due to medical reasons: when is it warranted and how can it be avoided? J Clin Psychiatry 74, 603-613; quiz 613.

64. Markowitz, J.S., Brown, C.S.Moore, T.R. 1999. Atypical antipsychotics. Part I: Pharmacology, pharmacokinetics, and efficacy. Ann Pharmacother 33, 73-85.

65. Wagstaff, A.J.Bryson, H.M. 1995. Clozapine: A Review of its Pharmacological Properties and Therapeutic Use in Patients with Schizophrenia Who Are Unresponsive to or Intolerant of Classical Antipsychotic Agents. CNS Drugs 4, 370-400.

66. Yazici K, Erbas T, Yazici A. The effect of Clozapine on glucose metabolism. Exp Clin Endocrinol Diabetes. 1998;106(6):475-477.

67. Haupt DW, Newcomer JW. Hyperglycemia and antipsychotic medications. J Clin Psychiatry 2001;62 Suppl 27:15-26; discussion 40-1.

68. Koller E, Schneider B, Bennett K, et al. Clozapine-associated diabetes. Am J Med. 2001;111:716–723.

69. Sernyak MJ, Leslie DL, Alarcon RD, et al. Association of diabetes mellitus with use of atypical neuroleptics in the treatment of Schizophrenia. Am J Psychiatry. 2002;159:561–566.

70. Henderson DC, Cagliero E, Gray C. Clozapine, diabetes mellitus, weight gain, and lipid abnormalities: a five year naturalistic study. American Journal of Psychiatry. 2000;157:975–981.

71. Lindenmayer JP, Czobor P, Volavka J, et al. Changes in glucose and cholesterol levels in patients with Schizophrenia treated with typical or atypical antipsychotics. Am J Psychiatry 2003;160:290-6.

72. Vampini C, Steinmayr M, Bilone F. The increase of plasma levels of triglyceride during Clozapine treatment: a casereport[abstract]. *Neuropsychopharmacology* 1994;10:249s.

73. Ghaeli P, Dufresne RL. Serum triglyceride levels in patients treated with Clozapine. Am J Health Syst Pharm 1996;53:2079-81.

74. Graham KA, Perkins DO, Edwards LJ, Barrier RC, Jr., Lieberman JA, Harp JB. Effect of Olanzapine on body composition and energy expenditure in adults with first-episode psychosis. Am J Psychiatry 2005;162:118-23.

75. Lindenmayer JP, Czobor P, Volavka J, et al. Changes in glucose and cholesterol levels in patients with Schizophrenia treated with typical or atypical antipsychotics. Am J Psychiatry 2003;160:290-6.

76. Wetterling T. Bodyweight gain with atypical antipsychotics: A comparative review. Drug Safety. 2001;24:59–73.

77. Taylor DM, McAskill R. Atypical antipsychotics and weight gain – A systematic review. Acta Psychiatr Scand. 2000;101:416–32.

78. Haddad P. Weight change with atypical antipsychotics in the treatment of Schizophrenia. *J Psychopharmacol*. 2005;19(6 Suppl):p16–27.

79. Covell, N.H., Weissman, E.M.Essock, S.M. 2004. Weight gain with Clozapine compared to first generation antipsychotic medications. Schizophr Bull 30, 229-240.

80. Reynolds, G.P.Kirk, S.L. 2010. Metabolic side effects of antipsychotic drug treatment--pharmacological mechanisms. Pharmacol Ther 125, 169-179.

81. Zhang, X.Y., Tan, Y.L., Zhou, D.F., Haile, C.N., Cao, L.Y., Xu, Q., Shen, Y., Kosten, T.A.Kosten, T.R. 2007. Association of Clozapine-induced weight gain with a polymorphism in the leptin promoter region in patients

with chronic Schizophrenia in a Chinese population. J Clin Psychopharmacol 27, 246-251.

82. Katzung B. Basic and Clinical Pharmacology. 13th ed. India: Mc Graw Hill Education Private Limited; 2015.

83. Tripathi K. Essentials of medical pharmacology. 7th ed. New Delhi: Jaypee Brothers; 2013;275-276.

84. Correia, S., Carvalho, C., Santos, M. S., Seiça, R., Oliveira, C. R., & Moreira, P. I. (2008).Mechanisms of action of metformin in type 2 diabetes and associated complications: an overview. Mini Reviews in Medicinal Chemistry, 8(13), 1343–54.

85. Shlomo Melmed, Kenneth S. Polonsky, P. Reed Larsen, Henry M. Kronenberg. Williams Textbook of Endocrinology. 12th ed. Philadelphia: Elsevier; 2011.

86. Metformin. Available at: https://www.drugs.com/metformin.html

87. amanti-Kandarakis E, Economou F, Palimeri S, Christakou C 2010 Metformin in polycystic ovary syndrome. Ann N Y Acad Sci 1205:192-198

88. Pugeat M, Ducluzeau PH 1999 Insulin resistance, polycystic ovary syndrome and metformin. Drugs 58 Suppl 1:41-46.

89. Dowling RJ, Goodwin PJ, Stambolic V 2011 Understanding the benefit of metformin use in cancer treatment. BMC Med 9:33

90. Desilets AR, Dhakal-Karki S, Dunican KC 2008 Role of metformin for weight management in patients without type 2 diabetes. Ann Pharmacother 42:817-826

91. Kim YW, Kim JY, Park YH, Park SY, Won KC, Choi KH, Huh JY, Moon KH 2006 Metformin restores leptin sensitivity in high-fat-fed obese rats

with leptin resistance. Diabetes 55:716-724

92. Anfossi G, Russo I, Bonomo K, Trovati M 2010 The cardiovascular effects of metformin: further reasons to consider an old drug as a cornerstone in the therapy of type 2 diabetes mellitus. Curr Vasc Pharmacol 8:327-337.

93. MN Ghosh, Pagets and Barnes 1964. Table 25.2. in: Fundamentals of Experimental Pharmacology. 5th ed. India: Hilton & Company: 2011.

94. Jassim G, Skrede S, Vázquez MJ, et al. Acute effects of orexigenic antipsychotic drugs on lipid and carbohydrate metabolism in rat. *Psychopharmacology*. 2012;219(3):783-794.

95. Cheng J, Huang C, Liu I, Tzeng T, Chang C. Novel Mechanism for Plasma Glucose-Lowering Action of Metformin in Streptozotocin-Induced Diabetic Rats. Diabetes. 2006;55(3):819-825.

96. Smith G, Chaussade C, Vickers M, Jensen J, Shepherd P. Atypical antipsychotic drugs induce derangements in glucose homeostasis by acutely increasing glucagon secretion and hepatic glucose output in the rat. Diabetologia.2008;51(12):2309-2317.

97. Hedenmalm k, Hagg S,Stahl M,OlavS. Glucose Intolerance with atypical Antipsychotics. Drug safety – 2002;25(15):1107-1116.

98. Hägg S, Joelsson L, Mjörndal T, et al. Prevalence of diabetes and impaired glucose tolerance in patients treated with Clozapine compared with patients treated with conventional depot neuroleptic medications. J Clin Psychiatry 1998; 59:294-9.

99. Boyda H, Tse L, Procyshyn R, Honer W, Barr A. Preclinical models of antipsychotic drug-induced metabolic side effects. Trends in Pharmacological Sciences. 2010;31(10):484-497.

100. Boyda H, Procyshyn R, Tse L, Hawkes E, Jin C, Pang C et al. Differential effects of 3 classes of antidiabetic drugs on Olanzapine-induced glucose dysregulation and insulin resistance in female rats. Journal of Psychiatry & Neuroscience. 2012;37(6):407-415.

101. Chen C, Huang M, Kao C, Lin S, Kuo P, Chiu C et al. Effects of Adjunctive Metformin on Metabolic Traits in Nondiabetic Clozapine-Treated Patients With Schizophrenia and the Effect of Metformin Discontinuation on Body Weight. J Clin Psychiatry. 2013;74(05):424-430.

102. Ozenoglu A, Ugurlu S, Balci H, Eker E. Nutritional approach to metabolic changes arising out of Schizophrenia therapy: Case report. Intern Med. 2007;46:1213–8.

103. Houseknecht K, Robertson A, Zavadoski W, Gibbs E, Johnson D, Rollema H. Acute Effects of Atypical Antipsychotics on Whole-Body Insulin Resistance in Rats: Implications for Adverse Metabolic Effects. Neuropsychopharmacology. 2006;32(2):289-297.

104. Abdelrahim Eman A. Histopathological change of the endocrine pancreas, in male Albino rat treated Atypical Antipsychotic Clozapine. Rom J Morphol Embryol 2013;54(2):385-394.

105. Scheen AJ, De Hert MA, Abnormal glucose metabolism in patients treated with antipsychotics, Diabetes Metab, 2007, 33(3):169–175.

ANNEXURE

ETHICAL CLERANCE CERTIFICATE