TO STUDY THE EFFECT OF RANITIDINE ON OLANZAPINE INDUCED WEIGHT GAIN IN RATS

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BY

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DECLARATION

*I declare that the thesis entitled "**To Study the Effect of Ranitidine on Olanzapine Induced Weight Gain in Rats**" has been prepared by me under the guidance of Dr. Jagruti A. Patel, Associate Professor, Department of Pharmacology, Institute of Pharmacy, Nirma University of Science and Technology. No part of this thesis has formed the basis for award of any degree of fellowship previously.*

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CERTIFICATE

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Chapter 1:- Abstract

Objective

Olanzapine is an atypical antipsychotic drug exhibiting a low incidence of extrapyramidal side effects. It is not only effective in treating positive symptoms of schizophrenia, but also more efficacious against negative and depressive symptoms than classical antipsychotics. Olanzapine has been recommended as the first-line drug for the treatment of schizophrenia. Unfortunately, a common side effect of olanzapine, namely weight gain, has also been observed. A comprehensive literature analysis revealed that olanzapine induced higher weight gain than most other antipsychotics, only second to clozapine. The incidence of olanzapine-induced weight gain and related diseases, such as diabetes and hypertension, is higher than that of the general population. These unwanted side effects have decreased the adherence to treatment. Various studies showed that H_2 receptor antagonist class of drugs may be useful to treat the weight gain induced by olanzapine treatment. So, present study was conducted to investigate the effect of ranitidine as an adjuvant therapy to minimize the unwanted effect of olanzapine.

Material and method

In this study, olanzapine suspension (3 mg/kg) was given orally to induce weight gain in female wistar rats for 3 weeks followed by test drug, ranitidine at three dose level of 10, 20, & 30 mg/kg orally for 2 weeks. Sibutramine was taken as standard drug at dose level of 6 mg/kg. At the end of treatment, various parameters like food intake, body weight, body mass index (BMI), serum lipid profile, blood sugar level and blood pressure were measured in the treatment, normal control as well as olanzapine treated group and were compared.

Results

Results show that, Olanzapine treated group showed marked increased in food intake, body weight, BMI, serum total cholesterol, triglyceride, LDL and VLDL level and significant decreased in serum HDL level compared to normal control group. Olanzapine treatment also increased blood sugar level compared to normal control group. There was marked decreased in food intake, body weight and BMI after the treatment with ranitidine and sibutramine. Also, there was significant decrease in serum total cholesterol, triglyceride, LDL and VLDL level and raised in serum HDL level compared

to olanzapine treated group. There was also significant decrease in blood sugar level. Significant decrease in mean blood pressure was observed compared to olanzapine treated group. Histological examination showed that,.

Conclusion

Our interest with this study was to demonstrate that ranitidine can be a potentially simple and useful response for the treatment of weight gain and other risk factors associated with Olanzapine use.

Chapter:- 2 Introduction

People with schizophrenia are at increased risk of morbidity and mortality, mainly from cardiovascular disease, compared with the general population[1]. Additionally patients with mental illness tend to have a higher body mass index (BMI) than the general population [2]. It is, therefore very important to treat antipsychotics induced weight gain.

Although conventional antipsychotic agents significantly reduced symptoms in chronically psychotic patients, a substantial number of patients remain impaired by residual symptoms. In a recently published meta-analysis of the efficacy of second-generation antipsychotics, several second-generation antipsychotics (clozapine, amisulpride, risperidone, and olanzapine) were found to be significantly more efficacious than first-generation antipsychotics [3]. The introduction of atypical antipsychotics in psychopharmacology represented a major advance in the treatment of schizophrenia, providing an effective therapy for both positive and negative symptoms of psychosis while minimizing the extrapyramidal effects characteristic of earlier therapeutic options. Indeed, these medications are widely prescribed (about 3% of the U.S. population) for treatment of schizophrenia, as well as bipolar disorder, depression, and dementia. In the face of their widespread use, concern has arisen regarding treatment-associated weight gain and apparent increased diabetes risk [4]. In recent years, particular effort has been paid in the investigation of the health-threatening association between atypical antipsychotics medication and increased risk of developing obesity, diabetes mellitus and lipid abnormalities [5]. Olanzapine and risperidone, which collectively account for >80% of all drugs prescribed of their class of atypical antipsychotics, have also been associated with metabolic abnormalities, though Olanzapine is generally linked to greater relative risk for diabetes [6, 7] and more marked obesity [6, 8, 9]. Patients who gain more than 10% of their total body weight are at risk for developing hypertension and type 2 diabetes mellitus. The risk for diabetes mellitus is increased approximately twofold in the mildly obese, fivefold in the moderately obese, and tenfold in severely obese persons [10]. In clinical trials, olanzapine treatment was associated with significant weight increases (greater than 7% of baseline weight) in 32% of patients who were underweight, 18% of normal-weight patients, and 11% of overweight patients [11]. In other studies, the mean incidence of weight gain of 7% or greater, was 41% in patients treated with

olanzapine, compared to 12% of those receiving haloperidol and 3% of those receiving placebo [11]. A study of 25 inpatients demonstrated a mean weight gain of 12 lb after 12 weeks of treatment with olanzapine at a mean dose of 13.8 mg/day [12]. In another study, 94% of day-treatment patients treated with olanzapine (mean dose, 14.1 mg/day) experienced weight gains of greater than 7%. The mean weight gain was 22.1 lb over a 7-month period and correlated with clinical response [13]. As a result, Pharmacological and nonpharmacological strategies for antipsychotic-associated weight gain and associated metabolic disturbances have been tried, such as: medication switching, medication addition to influence weight loss or prevent weight gain, or even to increase insulin sensitivity.

Among these pharmacological strategies, it has been found that H_2 blocker compounds such as cimetidine were reported to reduce weight gain in patients with type-2 diabetes [14]. Nizatidine, which is similar to cimetidine, is also a histamine H_2 receptor antagonist and has been proposed to have weight-reducing effects in patients taking olanzapine [15]. But cimetidine shows severe side effects like breast enlargement in males, hair loss etc [16] and also, ranitidine is quite easily available in India than nizatidine at cheaper cost. Clinical study showed positive results for ranitidine for treatment of olanzapine induced weight gain [17]. The mechanism by which H_2 antagonists might induce weight loss is not known but based on the study in rats [18] does not appear to be due to inhibition of gastric acid secretion. A possible explanation involves an increased plasma concentration of cholecystokinin, the peptide associated with reducing appetite [19]. So, present study was conducted on rats to evaluate to what extend ranitidine is effective to reverse the weight gain induced by antipsychotic drug olanzapine.

Chapter:- 3 Literature Review

3.1 Introduction

The term "psychosis" denotes a variety of mental disorders. Schizophrenia is a particular kind of psychosis characterized mainly by a clear sensorium but a marked thinking disturbance [20]. By any kind of reckoning, schizophrenia is the most severe mental disorder. It is found worldwide in almost all cultures and countries. Schizophrenia is a debilitating disorder of the central nervous system. Its symptoms have been divided into two classes: positive symptoms, including hallucinations, delusions and conceptual disorganization; and negative symptoms, including social withdrawal, blunted affect, and poverty of speech [21]. Whether schizophrenia is a single disorder with multiple degrees of severity and manifestations, or whether it is a syndrome whose clinical manifestations represent a number of disorders of differing etiologies, is still a matter of some debate [22].

3.2 Symptoms

Psychosis is primarily a disorder of thought, but emotions and behavior can also be affected.

Hallucinations Hallucinations are false perceptions. Auditory hallucinations (“hearing voices”) are the most common, but visual, olfactory (smell), tactile (touch), and gustatory (taste) hallucinations occur as well.

Delusions A delusion is a false belief. Persecutory, religious, somatic (a false belief about your body), and grandiose delusions are most common. Delusions can appear to have some semblance of truth, such as the belief that one is the target of a conspiracy. Other delusions can be bizarre, such as the belief that one’s internal organs have been removed and replaced by worms.

Disorganized Thinking Disorganized thinking occurs when the usual flow of thoughts is disrupted. It is manifested primarily through incoherent speech. When marked, speech becomes incomprehensible and is sometimes called “word salad.”

Loose Associations This form of disorganized thinking occurs when your thoughts follow such a rapid flow of associations that the focus becomes lost.

Thought Broadcasting The belief that your thoughts can be perceived by others.

Thought Insertion The belief that other people are putting thoughts into your head.

Ideas of Reference The belief that events in the environment are specifically directed at you. A common idea of reference is the belief that the radio or television is sending special messages only to you.

Alogia Alogia, derived from Greek and meaning "without words," is a pronounced decrease in the amount of thoughts and speech.

Avolition Avolition is the lack of motivation and willingness to pursue goal directed activities.

Paranoia Intense fearfulness that is greater than warranted.

Impaired Insight You may have difficulty accurately perceiving and understanding yourself and the world if your thinking is affected by delusional or disorganized thinking. For example, you may believe that your voices are part of a religious experience and not a symptom of a mental illness.

Impaired Judgment Your decisions may be so affected by psychotic thinking that you inaccurately assess what is happening in your life and how you should act. For example, if you are paranoid and fear attack from any direction, you may think medication is a poison and refuse it.

Flat Affect *Affect* refers to the facial expression of emotion. Flat affect is the description given to a face that is largely immobile and unresponsive.

Inappropriate Affect Inappropriate affect is the facial expression of an emotion that doesn't fit the content of thought. Examples include smiling calmly when talking about painful experiences, giggling continuously no matter what the subject, or crying for hours when there is nothing to be sad about.

Catatonic Behavior Catatonic behavior occurs if you are so preoccupied with your thoughts that you move in odd ways. You may assume rigid postures and remain still, in spite of efforts by others [23].

3.3 Epidemiology

One percent of the world's population suffers from schizophrenia and symptoms usually first present in late adolescence or early adulthood. While equally prevalent between genders, symptoms generally appear earlier in males, and males have a younger age at first hospitalization (15 to 24 years) compared to females (25 to 34 years) [24].

Epidemiological studies report prevalence rates for psychiatric disorders from 9.5 to 370/1000 populations in India [25].

3.4 Etiology

The etiology of schizophrenia remains largely unknown, though the evidence strongly supports a genetic basis for the disorder [26]. The disease shows a strong, but incomplete, hereditary tendency. In first-degree relatives, the risk is about 10%, but even in monozygotic twins, one of whom has schizophrenia, the probability of the other being affected is only about 50%, pointing towards the likely importance of environmental factors. Genetic linkage studies have identified a number of susceptibility genes [27], but it is clear that no single gene is responsible. There are significant associations between polymorphisms in individual genes and the likelihood of an individual developing schizophrenia, but many are quite weak, and there appears to be no single gene that has an overriding influence. The first, and most robust, association found was with the gene for *neuregulin-1*, a gene involved with synaptic development and plasticity, with effects on NMDA receptor expression. Transgenic mice that under express neuregulin-1 show a phenotype resembling human schizophrenia in certain respects. This discovery was followed by the identification of about eight other susceptibility genes, several of which were involved in one way or another with glutamate-mediated transmission. They include the gene for d-amino acid oxidase (DAAO), the enzyme responsible for making D-serine, an allosteric modulator of NMDA receptors and G72, an activator of DAAO. Among the other genes involved, some are thought to affect monoamine transmission. Apart from focusing attention on glutamate [28] and confirming the likely involvement of amines such as dopamine, genetic studies have not so far pointed to any specific neurochemical abnormality underlying the schizophrenic phenotype. Environmental stimuli or triggers along with genetic liability may contribute to the expression of the illness.

Some environmental influences early in development have been identified as possible predisposing factors, particularly maternal virus infections. This and other evidence suggests that schizophrenia is associated with a neurodevelopmental disorder affecting mainly the cerebral cortex and occurring in the first few months of prenatal development [29]. This view is supported by brain-imaging studies showing cortical atrophy, with enlargement of the cerebral ventricles. These structural changes are present

in schizophrenic patients presenting for the first time, and are probably not progressive, suggesting that they represent an early irreversible aberration in brain development rather than a gradual neurodegeneration. Studies of post-mortem schizophrenic brains show evidence of misplaced cortical neurons with abnormal morphology. It appears to be through a combination of such genetic and developmental factors with social and environmental factors that schizophrenia becomes manifest in particular individuals. One of the environmental factors now thought to play a significant role is consumption of cannabis [30].

3.5 Pathophysiology

The oldest theory associated with the pathophysiology of schizophrenia is the dopamine hypothesis, which proposes that psychosis is due to excessive dopamine in the brain. There is evidence from many pharmacologic challenge studies that drugs that lead to an increase in dopamine (e.g., cocaine and amphetamines) increase psychotic symptoms, while drugs that decrease dopamine (as do all current antipsychotic medications) decrease psychotic symptoms. A wide array of scientific work over the last several decades, including functional magnetic resonance imaging (MRI), positron emission tomography (PET), computed tomography (CT), and studies of dopamine metabolites have revealed a more complicated picture with both hyperdopaminergic, as well as hypodopaminergic brain regions. Hypodopaminergic activity observed in the prefrontal lobe is thought to relate to the core negative symptoms associated with schizophrenia. Thus, a more modern reworking of the dopamine hypothesis is the "dysregulation hypothesis" which takes these findings into account [31]. It is possible; however, that the dopamine abnormalities hypothesized to underlie the etiology of schizophrenia may represent compensatory changes that occurs secondary to other pathophysiologic abnormalities intrinsic to the illness. Despite the accumulation of knowledge surrounding dopamine's etiologic relationship to schizophrenia, other neurotransmitter systems have been implicated. Some investigators have suggested that a combined dysfunction of the dopamine and glutamate transmitter systems may better explain the disorder [32]. There has also been a great deal of speculation regarding a role for serotonin receptor antagonism in antipsychotic efficacy [33], as many second-generation antipsychotics (SGAs) are active at serotonin receptors. Serotonin receptor binding may be important to drug action, possibly by modulating dopamine activity in

mesocortical pathways. However, a compelling pathophysiologic theory relating to dopamine and serotonin receptor affinities does not yet exist. It is important to note that to date, antipsychotics without any primary or secondary dopamine modulating properties have been ineffective for the treatment of positive symptoms of schizophrenia [24]. Many antipsychotic drugs have been developed based on pathophysiology of schizophrenia.

3.5.1 Mechanisms of action of antipsychotic drugs

Although their mechanisms of action are not fully understood, the ability of antipsychotic drugs to block dopamine D_2 and other neurotransmitter receptors is considered pivotal for the major clinical effects.

D_2-receptor antagonism:-

All established antipsychotic drugs share the property of moderate to high dopamine D_2- receptor affinity [34]. The typical antipsychotic drugs exhibit strong dopamine D_2-receptor antagonism, with D_2-receptor affinity correlated to their ability to reduce psychotic (positive) symptoms [35-37]. Clinical effects of dopamine receptor blockade can be numerous, and the brain region to which binding occurs seems to be relevant for clinical outcome. Binding to dopamine D_2-receptors in mesolimbic circuits probably contributes to the antipsychotic effect, whereas D_2-receptor blockade and reduced dopamine firing in nigrostriatal projections may lead to extrapyramidal side effects [38]. In order to balance the therapeutic and adverse effects, it is important to treat patients with optimal drug doses. A D_2-receptor blockade of 70-80% is correlated with therapeutic effect with tolerable side effects, whereas occupancy above 80% generally leads to EPS [38, 39]. However, clozapine and quetiapine have therapeutic effect at D_2-receptor occupancy as low as in the range 40-60% [38, 40], suggesting that mechanisms other than D_2-blockade are important for therapeutic effect.

The role of serotonin (5-hydroxytryptamine; 5-HT):-

The diverse receptor binding profiles of atypical drugs suggest that antipsychotic effect can be mediated via receptors other than dopamine [41, 42]. Many antipsychotics demonstrate 5-HTreceptor antagonism, and the combination of strong 5-HT-receptor

binding and low dopamine D_2-receptor affinity has been suggested as a key mechanism for the improved therapeutic profile observed for several atypical drugs [43]. 5-HT antagonism can lead to increased dopamine signalling in mesocortical projections, which is a proposed mechanism for the more beneficial effects of atypical drugs on the negative symptoms [44, 45]. The 5-HT_1-receptor, to which clozapine binds, has been suggested to be involved in reduced anxiety and depression and improvement of cognitive and negative symptoms [46, 47].

N-methyl-D-aspartic acid (NMDA)-receptor antagonism:-

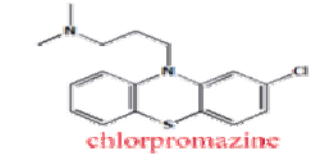
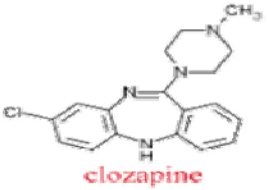
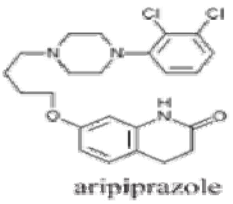
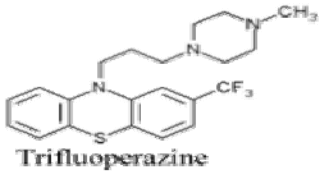
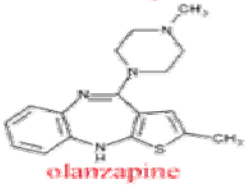
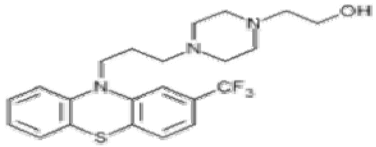
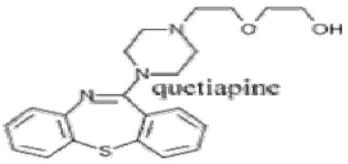
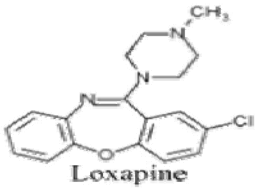
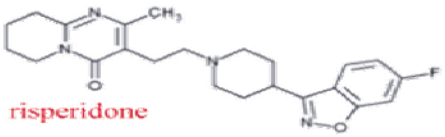
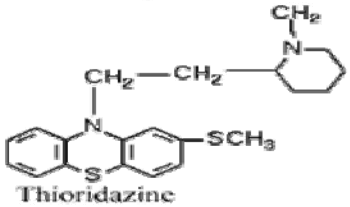
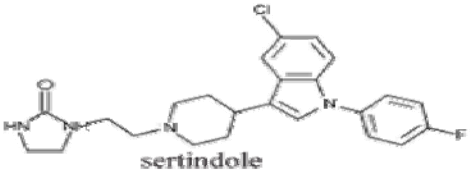
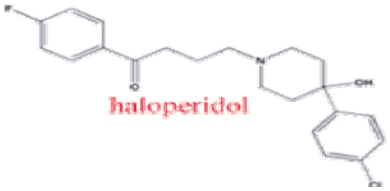
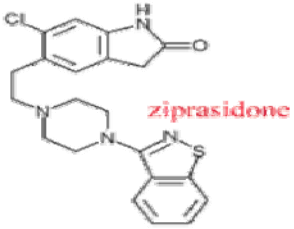
Ketamine and Phencyclidine (PCP) are NMDA-receptor antagonists that reduce glutamate signaling and that can induce a psychotic state both in patients and in animal models, including the full range of positive and negative symptoms [48]. These findings suggest that glutamatergic neurotransmission should be taken into account when designing new antipsychotic drugs. Specific NMDA-agonists such as the amino acids glycine and D-serine have been demonstrated to promote learning and memory in rats and monkeys [49, 50] and may improve on negative symptoms in schizophrenia [51, 52].

3.6 Drug therapy for schizophrenia

Antipsychotic drug therapy is considered a cornerstone in the treatment of schizophrenia. Usually, they reduce or ameliorate the positive symptoms of the disorder. The effect of the presently used drugs on negative and cognitive symptoms is limited, and future drugs should have increased focus on these symptoms. Antipsychotic drugs are categorized based on their clinical efficacy and their side effect profiles.

3.6.1 Classification of antipsychotic drugs

Table 1 Chemical structure of a selection of first-, second-, and third generation antipsychotic drugs defined by Roth et al [53]

1st generation (typical)	2nd generation (atypical)	3rd generation
 chlorpromazine	 clozapine	 aripiprazole
 Trifluoperazine	 olanzapine	
 Fluphenazine	 quetiapine	
 Loxapine	 risperidone	
 Thioridazine	 sertindole	
 haloperidol	 ziprasidone	

3.6.1.1 First generation drugs

The first antipsychotic drug was chlorpromazine. Originally applied to treat preoperative anxiety, its antipsychotic properties were discovered by chance in the early 1950's. The effectiveness of chlorpromazine stimulated the synthesis of other antipsychotic agents, some of which are still in use today. These first generation (typical) antipsychotic drugs are effective against the *positive* symptoms of schizophrenia, but unfortunately they often induce highly unpleasant side effects, such as extrapyramidal side effects (EPS) and hyperprolactinemia [54].

3.6.1.2 Second generation drugs

Clozapine is the prototype of the second generation (atypical) antipsyhotic drugs and was ntroduced in the early 1970's. Due to the serious and sometimes fatal side effect of agranulocytosis [55], clozapine was withdrawn from the market, but was re-introduced and approved by the Food and Drug Administration (FDA) in 1989, since clozapine was found effective in otherwise treatment-resistant patients [56].

In addition to being equally effective as the first generation drugs against the positive symptoms, the second generation drugs seem to have better effect on negative and cognitive symptoms [46, 57, 58]. A meta-analysis demonstrated that some atypicals (clozapine, olanzapine, risperidone) apparently proved better on overall clinical efficacy than other atypicals (ziprasidone, sertindole, quetiapine, remoxipride) and typical drugs (haloperidol), with effect sizes calculated from the Positive and Negative Syndrome Scale (PANSS) [3, 59]. Unfortunately, clozapine and several other atypical drugs are associated with various metabolic disturbances, such as weight gain, hyperglycemia and hypertriglyceridemia [60-63]. These metabolic adverse effects are of great concern since they increase the risk for obesity-related complications and death [64]. They also reduce patient compliance [65, 66] . It is therefore noteworthy that olanzapine, which is associated with considerable weight gain, recently was ranked as the most effective antipsychotic drug in terms of discontinuation rates [67].

3.6.1.3 Third generation drugs

Aripiprazole and other benzamides function as partial dopamine agonists. Due to their separate mechanism of action, these drugs have been described as the third generation antipsychotic drugs [68]. Aripiprazole is claimed to be effective against

positive, negative and cognitive symptoms of schizophrenia, whereas EPS and metabolic adverse effects appear to be quite infrequent [69].

3.6.2 Side-effects

3.6.2.1 First generation drugs:-

Acute neurological side-effects occur secondary to D_2 receptor blockade in the extrapyramidal system (and are also called **acute EPS**). They can appear on the first day of treatment and can take various forms of involuntary muscle spasm, particularly involving of the jaw, tongue, neck and eyes. A dramatic form is oculogyric crisis – in which the neck arches back and the eyes roll upward. A potentially dangerous form is laryngospasm – an early warning sign may be the patient's voice becoming higher pitched.

The extrapyramidal system is composed of two pathways, in one the neurotransmitter is dopamine and in the other, acetylcholine. When the dopamine pathway is blocked by the antipsychotic the balance in the system is disrupted, resulting in spasm. Acute treatment is oral or intramuscular injection of an anticholinergic – such as benztropine (2 mg). The response is immediate and pleasing.

Medium-term neurological side-effects are also due to D_2 blockade. **Akathisia** usually occurs within the first few day of treatment and involves either a mental and/or motor restlessness. Mental restlessness presents as increasing distress and agitation. Motor restlessness usually affects the lower limbs, with shifting from one foot to the other while sitting and constant crossing and uncrossing of the legs while sitting. This is a difficult condition to manage. Useful steps include lowering the dose of the antipsychotic (if possible), adding diazepam or propranolol, or adding an anticholinergic (this latter option is not dramatically effective). **Parkinsonism** usually occurs some days or weeks after the commencement of treatment. There is a masklike face, rigidity of limbs, bradykinesia, and loss of upper limb-swing while walking. Tremor and festinating gait are less common. The best management is reduction in dose of the antipsychotic (if possible) and the addition of an anticholinergic agent.

Table 2 Shared side effects and dosages of commonly used antipsychotic agents. [70]

	Recommended Dose Range (mg/day)	Extrapyramidial Side Effects / Tardive Dyskinesia	Weight Gain	Other Notable Side Effects[c]
First-generation Agents				
Haloperidol	5-20	+++	+	Prolactin elevation[c]
Perphenazine	16-64	++	+	
Thioridazine	300-800	+	+	QTc prolongation[c]
Second-generation Agents				
Aripiprazole	10-30	0[b]	0	Nausea and headaches[d]
Clozapine	150-600	0[b]	+++	Glucose and lipid abnormalities,[c] sedation,[c] hypotension,[c] anticholinergic side effects,[c] agranulocytosis,[d] seizures,[d] and myocarditis[d]
Olanzapine	10-30	0[b]	+++	Glucose and lipid abnormalities[c]
Quetiapine	300-800	0[b]	++	Warning about development of cataracts[d]
Risperidone	2-8	+	++	Prolactin elevation[c]
Ziprasidone	120-200	0[b]	0	QTc prolongation[c]

0 = No risk or rarely causes side effects at therapeutic dose.

+ = Mild or occasionally causes side effects at therapeutic dose.

++ = Sometimes causes side effects at therapeutic dose.

+++ = Frequently causes side effects at therapeutic dose.

[b]Possible exception of akathisia

[c]Side effects frequently caused at therapeutic dose (+++)

[d]Side effects attributable to a specific antipsychotic

Chronic neurological side-effects (also known as chronic or **late EPS**) usually occur after months or years of continuous D_2 blockade. **Tardive dyskinesia (TD)** manifests as continuous choreoathetoid movements of the mouth and tongue, frequently with lip-smacking, and may also involve the head, neck and trunk. Late EPS may continue after cessation of the typical antipsychotic.

Neuroendocrine effects result from blockade of dopamine transmission in the infundibular tract. Prolactin levels rise, producing galactorrhea, amenorrhoea and infertility.

Neuroleptic malignant syndrome (NMS) is probably due to disruption of dopaminergic function, but the mechanism is not understood. Untreated the mortality rate is 20%, so immediate medical attention is mandatory. The symptoms include muscle

rigidity, hyperthermia, autonomic instability and fluctuating consciousness. Renal failure secondary to rhabdomyolysis is a major complication and the cause of mortality.

Anticholinergic side-effects include dry mouth, difficulty with micturition, constipation, blurred vision and ejaculatory failure. Anticholinergic effects can contribute to a toxic confusional state.

Histamine blockade may produce severe sedation.

Alpha adrenergic blockade may produce postural hypotension, cardiac arrhythmias and impotence.

Dermatological side-effects include skin rash and photosensitivity.

Weight gain is common with most typical antipsychotics. [71]

3.6.2.2 Second generation drugs:-

Weight gain

Weight gain is problem in schizophrenia and other mental disorders, in part because of poor eating habits and lack of exercise. However, the atypical antipsychotics exacerbate this problem. A meta-analysis estimated that over a 10 week period the mean increase was as follows:

1) clozapine 4.45 kg
2) olanzapine 4.15 kg
3) risperidone 2.1 kg (quetiapine probably similar)
4) ziprasidone 0.04 kg (aripiprazole probably similar). [71]

Clozapine and olanzapine are the antipsychotics that cause more weight gain. A metaanalysis carried out by Allison et al. has estimated mean change in weight secondary to antipsychotics after 10 weeks. In decreasing order: clozapine (+3.99 kg), olanzapine (+3.51 kg), thioridazine (+3.49 kg), chlorpromazine (+2.10 kg), risperidone (+2.0 kg) and haloperidol (+0.48). Fluphenazine (+0.43 kg) and ziprasidone (+0.04 kg) are not associated with statistically significant weight gain. Molindone was associated with slight weight loss (-0.81 kg). Pimozide apparently does not cause weight gain, but data found did not allow adequate analysis. It is worth stressing that weight gain may continue for a much longer period than what was demonstrated in another study, reaching up to 46 weeks in patients taking clozapine [72].

Mechanisms of Weight Gain:-

Obesity and weight gain are the result of a complex confluence of environmental, behavioral, genetic, and neurochemical factors. AAs exhibit pleiotropic receptor affinity. Many, but not all, patients experiencing weight gain with antipsychotic drugs re port in creased appetite, binge eating, carbohydrate craving, food preference changes, and decreased satiety.

Dopamine or noradrenaline antagonism at the lateral hypothalamus may affect satiety. Increasing the availability of serotonin or activating serotonin receptors reduces food consumption, while blocking serotonin receptors increases food intake. The serotonin receptor sub type(s) responsible for stimulating food in take remain a matter of speculation. Currently, the 5-HT_{2C} receptor is under active investigation.

Antihistamines and low potency antipsychotics have well documented effects on weight gain. Histamine antagonism stimulates appetite. Clozapine exhibits histamine blockade affinity more than 20- fold greater than risperidone.

It has been hypothesized that prolactin elevation may stimulate feeding centres in the brain by changing the estrogen–testosterone ratio, which in turn modifies the functioning of satiety- related neurons in the ventral medial and paraventricular hypothalamus. Further, in vitro experiments have demonstrated adipocyte insulin insensitivity in hypeprolactinemic conditions. How ever, evidence militating against prolactin elevation as a sufficient mechanistic variable is the absence of sustained prolactin elevation with clozapine and olanzapine. Several pep tides have been implicated in the control of appetite, including leptin. Leptin is a product of the obese gene found in several tissues. Leptin, released by adipocytes, is believed to act at the level of the hypothalamus, modulating appetite, energy expenditure, and the neuroendocrine axis. It has been reported that serum leptin concentrations correlate positively with BMI and percentage of body fat [73]**.**

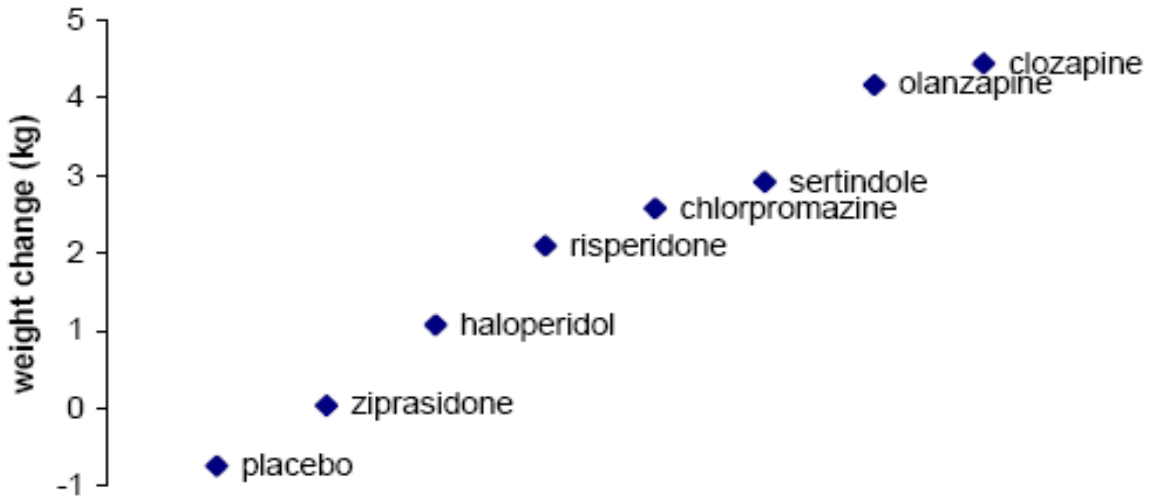


Fig. 1 Weight change induced by antipsychotics after 10 weeks on standard drug doses, estimated from a random effect model [74].

Type 2 diabetes

The prevalence of type 2 diabetes is twice as high in people with schizophrenia compared to the general population. Over recent years there has been concern this may be the result of atypical antipsychotic treatment [71]. The WHO Collaborating Centre for International Drug Monitoring receives summary clinical reports of individual adverse drug reactions from the national centres in 63 countries around the world. This information is stored in a large international database containing more than 2.5 million adverse drug reaction reports. Reports were identified for clozapine, olanzapine and risperidone in the WHO database, which included the following diagnoses: glucose tolerance abnormal, hyperglycaemia, diabetes mellitus, diabetes mellitus aggravated, ketosis, diabetic coma and glycosuria [75]**.** Hyperglycemia, exacerbation of existing diabetes, new-onset type 2 diabetes, and diabetic ketoacidosis have all been associated with newer antipsychotic medications, with multiple reports for clozapine and olanzapine, and more limited reports of significant hyperglycemia for quetiapine and risperidone [76, 77]. Henderson et al. reported that >30% of schizophrenic patients receiving clozapine developed diabetes within a 5-year follow-up, and those with preexisting diabetes required increased insulin dosing. Federal Drug Administration reports, based in part on the Medwatch Surveillance Program, provide further evidence

of the excessive occurrence of new-onset diabetes and exacerbation of preexisting disease with clozapine compared with disease incidence in untreated individuals. More recently, olanzapine and risperidone, which collectively account for >80% of all drugs prescribed of their class of atypical antipsychotics, have also been associated with metabolic abnormalities, though olanzapine is generally linked to greater relative risk for diabetes and more marked obesity compared with RIS**.** [78]

These side effects have involved such agents as clozapine, olanzapine, quetiapine, and a combination of clozapine and quetiapine. The development of diabetes has been reported to occur anywhere from 10 days to 18 months after starting therapy. One theory is that diabetes might result from the weight gain caused by these agents. Other studies suggest that these agents affect glucose transport metabolism peripherally in patients, possibly increasing the potential for hyperinsulinemia and peripheral insulin resistance. Further hypotheses point to the activity of atypical antipsychotic drugs at the serotonin receptors of the beta cells in the pancreas, more specifically $5HT_{1A}$ and $5HT_2$ receptors. This activity might lead to derangement of beta cell function, with resulting increases in glucose levels in patients Pre-clinical studies have indicated differences between antipsychotic in their response to insulin release. Best et al. (2005) studied the effects of clozapine and haloperidol on rat pancreatic β-cells in-vitro [79]. The authors demonstrated the contrasting effects of clozapine and haloperidol on pancreatic β- cell function. Clozapine had no effect on β-cell membrane potential at fasting glucose levels but hyperpolarized the membrane potential, when glucose concentrations were high. In contrast haloperidol depolarized the membrane at both fasting and stimulatory levels of glucose. The effects of these two drugs on electrical activity only partially explained their effect on insulin release. Clozapine inhibited secretion of insulin in response to glucose, which could explain the hyperglycaemia and diabetes associated with it, but did not affect 'basal insulin release'. Interestingly, haloperidol had no effect on insulin release [80]**.**

Hyperlipidemia

Hyperlipidemia (raised cholesterol and triglycerides) appears to be associated with the atypical antipsychotics (clozapine, olanzapine and quetiapine). Among the atypical antipsychotics, clozapine and olanzapine, which produce the greatestweight gain, are associated with the greatest increases in total cholesterol, LDL cholesterol, and

triglycerides and with decreased HDL cholesterol. Aripiprazole and ziprasidone, which are associated with the least amount of weight gain, do not seem to be associated with a worsening of serum lipids. Risperidone and quetiapine appear to have intermediate effects on lipid**.** In one study Serum cholesterol concentrations were also measured in the 14-week prospective trial. Clozapine and olanzapine were found to significantly increase serum cholesterol at week 8, but only remained marginally significant at week 14. The mean cholesterol concentrations for each of the four groups was <200 mg% at each study point, One study also shows that High-potency conventional antipsychotics (e.g., haloperidol) and the atypical antipsychotics, ziprasidone, risperidone and aripiprazole, appear to be associated with lower risk of hyperlipidemia. Low-potency conventional antipsychotics (e.g., chlorpormazine, thioridazine) and the atypical antipsychotics, quetiapine, olanzapine and clozapine, are associated with higher risk of hyperlipidemia. Possible hypotheses for lipid dysregulation include weight gain, dietary changes and the development of glucose intolerance [81].

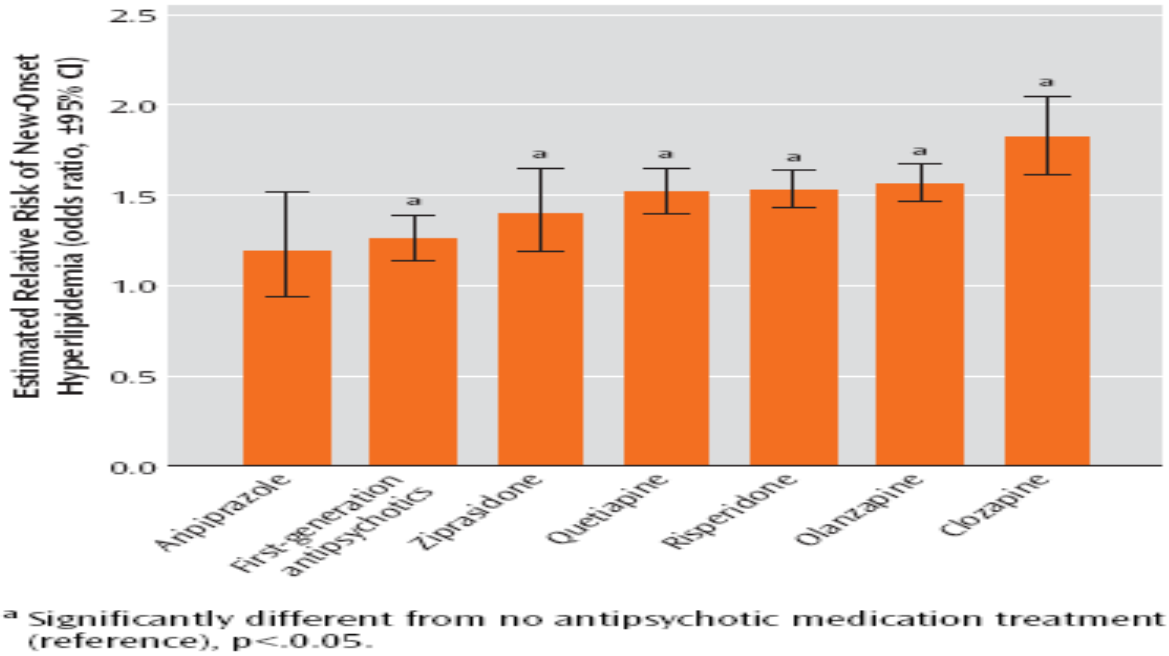


Fig. 2 Association of Antipsychotic Medication Treatment with New-Onset Hyperlipidemia in Adults with Psychotic Disorders [82]

The antipsychotic drugs activate SREBP [83] controlled lipogenic gene expression in cell cultures and in rats, and preliminary results indicate that such effects also occur in humans. In the cell culture experiments, SREBP activation was associated with elevated levels of cholesterol and triglycerides.(Fig.3)

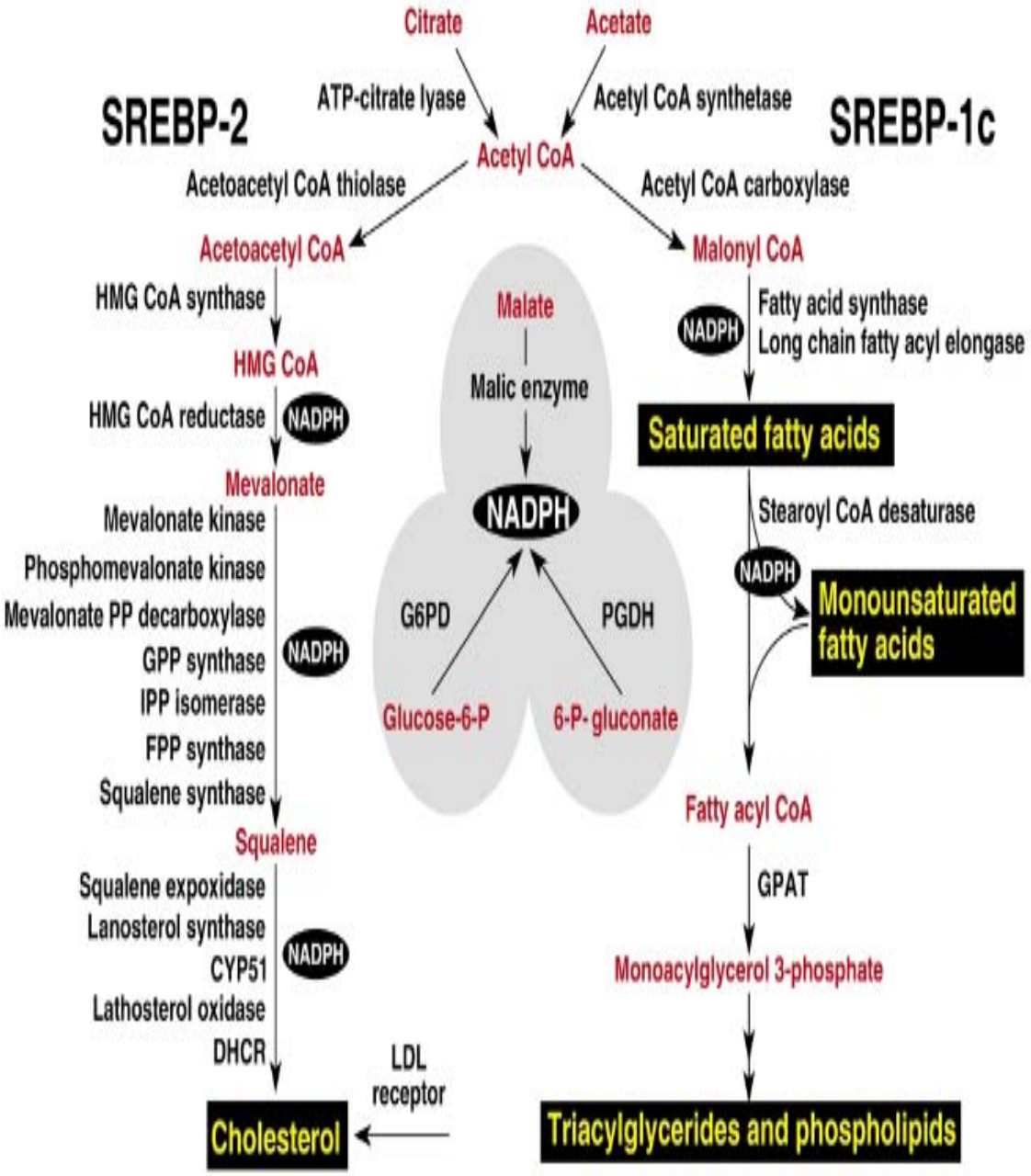


Fig.3 Genes regulated by SREBPs. The diagram shows the major metabolic intermediates in the pathways for synthesis of cholesterol, fatty acids, andtriglycerides. In vivo, SREBP-2 preferentially activates genes of cholesterol metabolism, whereas SREBP-1c preferentially activates genes of fattyacid and triglyceride metabolism. DHCR, 7-dehydrocholesterol reductase; FPP, farnesyl diphosphate; GPP, geranylgeranyl pyrophosphate synthase;CYP51, lanosterol 14α-demethylase; G6PD, glucose-6-phosphate dehydrogenase; PGDH, 6-phosphogluconate dehydrogenase; GPAT,glycerol-3-phosphate acyltransferase.

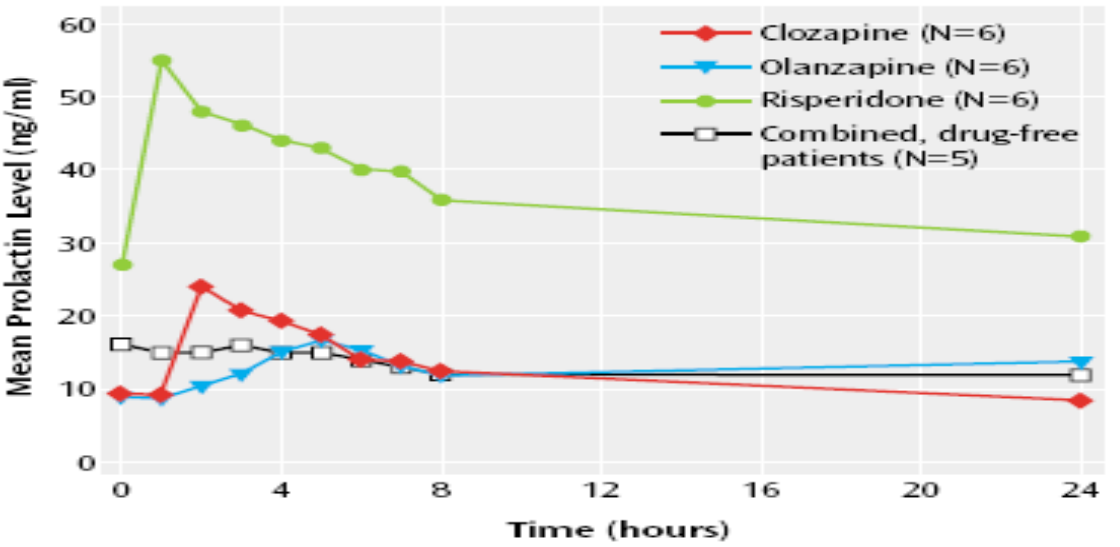
Table 3 Metabolic complications of atypical antipsychotics [84]

Reference value compared to baseline	Olanzapine	Quetiapine	Risperidone	Ziprasidone	Clozapine	Aripiprazole
Weight Change (lbs/month)	2	0.5	0.4	-0.3	0.5	–
HbA1c (%)	0.4	0.04	0.07	0.1	0.1	–
Blood Glucose (mg/dl)	13.7	7.5	6.6	2.9	13.2	0.90
Total Cholesterol (mg/dl)	9.4	6.6	-1.3	-8.2	7.3	-0.7
Triglycerides (mg/dl)	40.5	21.2	-2.4	-16.5	52.6	0.6

Hyperprolactemia

In the case of prolactin, the primary hypothalamic regulation is a tonic inhibition of its secretion which is mediated by dopamine, a neurotransmitter produced in the hypothalamus and secreted into the hypothalamic–pituitary portal capillaries which then carry it to the anterior pituitary gland. Dopamine acts on D_2–dopamine receptors on the pituitary lactotroph cells to inhibit both synthesis and secretion of prolactin. Although the hypothalamus also produces prolactin–stimulating factors such as thyrotropin–releasing hormone (TRH) and vasoactive intestinal polypeptide (VIP), these appear to play minor roles in regulating prolactin secretion in humans. All conventional (first generation) antipsychotic agents act as antagonists on the D_2–dopamine receptor. Atypical (second generation) antipsychotics are more variable in their affinity for the D_2 receptor and thus vary in their effects on prolactin secretion. The relative potency of antipsychotic agents in elevating serum prolactin is:

Risperidone = Paliperidone > Haloperidol > Olanzapine > Ziprasidone > Quetiapine > Clozapine > Aripiprazole [85].



[a] One of the patients taking clozapine, two taking olanzapine, and two taking risperidone had their prolactin levels measured twice: first after taking their usual dose of the medication and second, at least 1 month later, after not taking their usual dose.

Fig. 4 Mean Plasma Prolactin Level Changes Over 24 Hours in 18 Patients After Taking Clozapine, Olanzapine, or Risperidone and in Five of the Same Patients After Not Taking the Drugs [86]

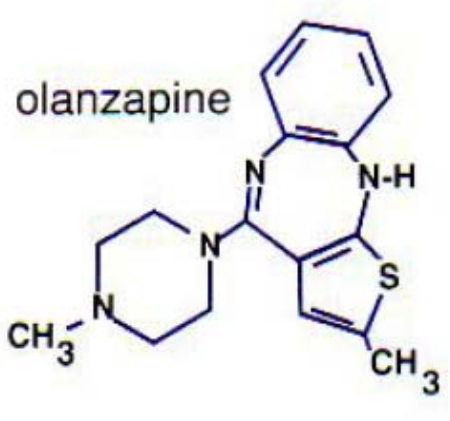
Hypertension

Long term treatment of antipsychotic drug may rise in systolic and diastolic blood pressure [87]. Treatment with olanzapine in rats also raised the systolic blood pressure which could be due to weight gain effect of olanzapine [88].

Arrhythmia

Antipsychotic medications can cause a wide range of adverse effects from significant arrhythmia to unexplained sudden deaths in schizophrenic patients. Among the antipsychotic-induced adverse effects, both direct and indirect effects on various central or peripheral neurotransmitters and neuro-hormonal systems have been noted. Dysfunction of cardiac autonomic nervous system (ANS) may play a role in the arrhythmic adverse effect in schizophrenic patients receiving antipsychotics. A link between ANS dysfunction and sudden death caused by arrhythmia has been suspected since decreased parasympathetic tones may lower the threshold for ventricular tachycardia, which is likely to cause sudden deaths [89].

3.7. Olanzapine



The chemical structure of olanzapine

The FDA approved Olanzapine, an antipsychotic drug manufactured by the Eli, Lily and company, in October 1996, for the treatment of psychotic disorders. It is a thieno-benzodiazepine analog with the chemical name of 2-methyl-4-(4-methyl-1-piperazinyl)-10 thieno [2, 3-b][1,5] benzodiazepine. Olanzapine is a yellow crystalline solid and practically insoluble in water.

MECHANISM OF ACTION OF OLANZAPINE:

Experiments *in vitro* have shown that olanzapine affects key receptors, which are believed to be related to schizophrenia. The binding profile indicates that olanzapine has high affinity for 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C} and 5-HT_{6}, and histamine (H_1) receptors, as well as a moderate affinity for D_2 and acetylcholine muscarinic receptors, and a low affinity for β-adrenergic receptors [90, 91]. Olanzapine also interacts non-selectively with other dopamine receptors and 5-HT_1, 5-HT_3, 5-HT_4 and 5-HT_7 receptors. The limbic selectivity of olanzapine reflects its atypical antipsychotic properties. *c-fos* is an early oncogene and its expression was used as a marker for neuronal activity. Olanzapine induces *c-fos* expression in the nucleus accumbens, which also can be increased by other atypical antipsychotics, and usually correlates, with efficacy in treating positive symptoms. Olanzapine also induces *c-fos* in the prefrontal cortex, which might be related to its efficacy in treating negative symptoms. These effects are similar to what has been observed with clozapine [90].

DOSE:

The antipsychotic efficacy of olanzapine was demonstrated in the dose range of 5-20 mg/day.

PHARMACOLOGY:

Behavioural and pharmacological studies of olanzapine confirm that it interacts with dopamine, serotonin and muscarinic receptors, and exhibits clozapine-like profiles, including mesolimbic selectivity, blocking 5-HT receptors at a lower doses than that for dopamine receptors, and inhibiting the conditioned avoidance response (indication of antipsychotic efficacy) at lower dose than that required to induce catalepsy (indication of EPS) [92]. Double blind clinical studies show that olanzapine is highly efficacious in treating psychotic symptoms, while it has low liability to induce tardive dyskinesia or Parkinsonism [91]. In contrast to clozapine, olanzapine does not cause agranulocytosis in patients. Although clozapine has clear advantages over traditional antipsychotics, and causes less extrapyramidal side effects in patients, the agranulocytosis can be very serious and could be life threatening. Therefore, olanzapine has been considered as the first line drug and widely used in treatment of psychosis[93], [90].

PHARMACOKINETICS:

There is complete absorption of olanzapine after oral administration. The maximum concentration (Cmax) and the time required to reach (tmax) after single dose of 12 mg in six healthy male subjects were 11 ± 1 ng/mL and 4.9 ± 1.8 hrs, respectively.

METABOLISM AND EXCRETION:

It is metabolized extensively in humans via glucuronidation, allylic hydroxylation, N-oxidation, N-dealkylation and a combination thereof. This is the most important pathway both in terms of contribution to drug related circulating species and as an excretory product in the species [94]. The major metabolites found in humans are 10-N-glucuronide and 4-desmethylolanzapine [95]. *In vitro* evaluations of the human cytochrome P450 isoenzymes involved in the formation of the three major metabolites of olanzapine have found that CYP 1A2, CYP 2D6, and the flavin containing mono-oxygenase system are involved in the oxidation of olanzapine [96]. The major route of elimination seems to be urine (first pass metabolism) in humans [94]. It displays linear

kinetics over the clinical dosing range. The systemic clearance of olanzapine takes about 26.1 ± 12.1 hrs. The plasma elimination half-life ($t_{1/2b}$) is 33.1 ± 10.3. Compared with young men, young women demonstrated decreased clearance. Similarly, elderly subjects showed a decreased clearance compared to younger patients [97].

UNWANTED EFFECTS:

Unfortunately, olanzapine also exhibits a common unwanted side effect, namely, increase in weight. Several clinical reports have shown that olanzapine-induced weight gain is accompanied with increased body fat, serum levels of glucose, triglyceride, insulin and leptin [12, 98, 99]. Allison's report show that among different atypical antipsychotics, olanzapine cause the second highest degree of weight gain at 10 weeks using a random effects model, only lower than the weight gain caused by clozapine [74]. Based on the definition from Federal Drug and Food Administration (U.S.A) that weight gain induced by any given drug is an increase in body weight of more than 7% before treatment, a study showed that 29% of patients with olanzapine obtained 7% or more increase in body weight, while only 25% of patients treated with quetiapine, 18% with risperidone, and 9.8% with ziprasidone did [100]. Investigations also show that olanzapine significantly increases the risk of developing diabetes over conventional antipsychotics. McIntyre and colleagues reported that Zolar retrospectively assessed 396 antipsychotic-treated patients and the result indicated that olanzapine tends to cause a higher incidence of type II diabetes than other antipsychotics. In this report, the prevalence of type II diabetes in the general population is 5%-7%. However, it was estimated that the incidence of type II diabetes caused by olanzapine was 11%, while 6.6% by haloperdol, 6% by risperidone, and 4.5% by fluphenazine [101]. Excess weight gain and obesity in these patients would increase the risk of hypertension [88], chronic cardiovascular disease and cancer. This side effect also causes distress in patients, affects their self-esteem and decreases treatment adherence.

3.7.1 Olanzapine induced weight gain models

Various rat models are established to check the weight gain effect of olanzapine. In one model, Female Sprague-Dawley rats received olanzapine or diluent (1.2mg/kg per day) via gavage for 10 days. Rats receiving olanzapine exhibited significant increases in body weight when compared with control rats. Body weight returned to control levels once olanzapine treatment was discontinued. Food consumption among the olanzapine

treated group was significantly greater than among control rats between 6 and 10 days of treatment. Between 4 and 10 days of treatment, feed efficiency (grams of weight gained/grams of food consumed) was also significantly greater among animals receiving olanzapine. In contrast, chronic administration of haloperidol (0.04mg/kg; q.d.; gavage) did not influence body weight or food consumption of treated rats. Data show that an animal model of olanzapine-induced weight gain is readily generated, and suggest that the weight gain results at least in part from increased food intake, reduced gross motor activity, and enhanced feed efficiency [102]. In another animal study, the effects of olanzapine on weight gain, food and water intake, intra-abdominal fat, the oestrous cycle and uterine weight were assessed in group-housed adult female hooded-Lister rats. Olanzapine (0.5-4.0 mg/kg i.p.) or vehicle was administered once daily for 21 days and body weight, food and water intake measured, with histological examination of vaginal lavage to determine the stage of the oestrous cycle. On day 22, animals were sacrificed and intra-abdominal fat, wet and dry uterine weights measured. Olanzapine induced significant weight gain with concomitant increases in food and water intake and intra-abdominal fat without an effect on the oestrous cycle, wet and dry uterine weights or plasma prolactin levels. The results confirm the ability of olanzapine to induce weight gain in female rats on unrestricted normal diet with a concomitant increase in food and water intake and increased intra-abdominal fat [103]**.**

3.7.2 Treatment of olanzapine induced weight gain

Nearly all antipsychotics can induce weight gain, which in turn lowers self-esteem and causes increased morbidity and mortality. There are differing degrees of weight gain among patients treated with different antipsychotics. Of the atypical antipsychotics those associated with the greatest weight gain are clozapine and olanzapine. Chlorpromazine and thioridazine are the typical antipsychotics reported to cause the most weight gain. Generally pharmacotherapy for obesity should only be considered when non-pharmacological measures have failed. It is usually only used in patients with a BMI > 30, or a BMI > 27 with other risk factors.

3.7.2.1 Available treatments:-

SIBUTRAMINE:-

Sibutramine was originally developed as an antidepressant, but the serendipitous observation during early depression trials that weight loss also occurred, particularly in obese depressed patients, led to its redevelopment as an anti-obesity agent and its subsequent introduction in the UK in 2001 [104]. Administered orally, sibutramine acts centrally to increase satiety and stimulate energy expenditure or thermogenesis [105]. Sibutramine is recommended only for individuals with a BMI of greater than 30 kg/m2 or greater than 27 kg/m2 with co-morbidities, who have failed to lose weight by non-pharmacological [106].

Sibutramine is a b-phenylethylamine that acts by inhibiting the reuptake of the neurotransmitters noradrenaline, serotonin and, to a lesser extent, dopamine. The pharmacological effect of sibutramine is largely attributable to its active metabolites, M1 and M2.

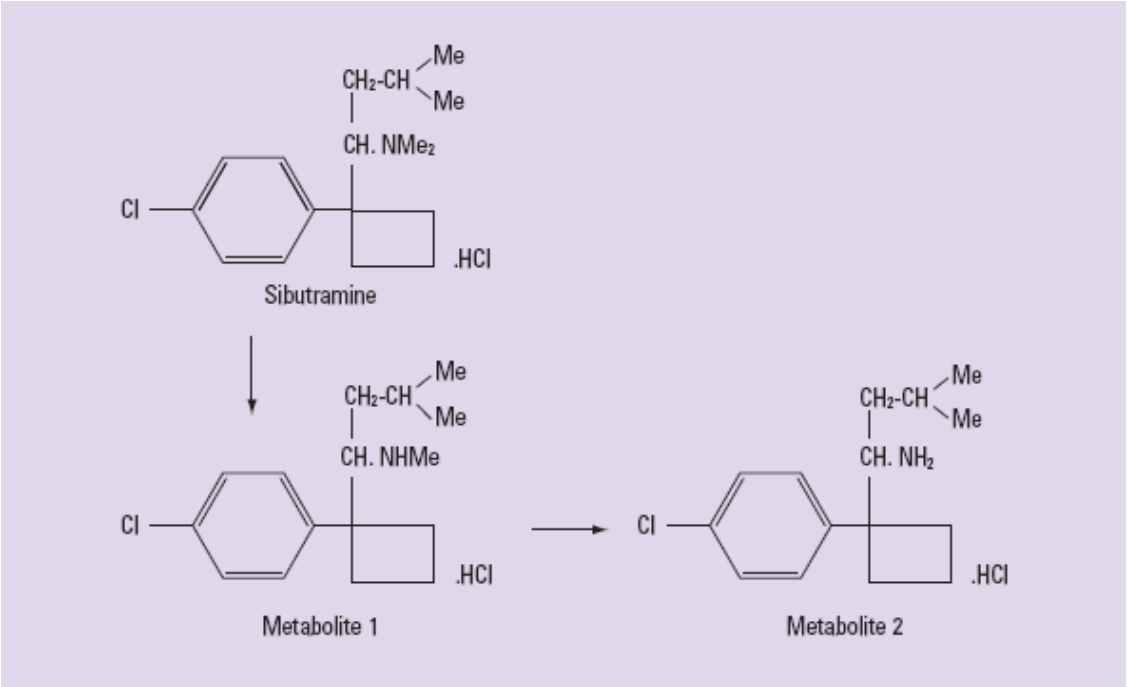


Fig. 5 The chemical structure of sibutramine hydrochloride monohydrate; chemical formula $C_{17}H_{29}C_{l2}NO$, molecular weight 334.33, and its metabolites [107].

PHARMACOLOGY:-

The pharmacological profile of sibutramine differs from that of other anti-obesity compounds. *In vitro* studies have shown that sibutramine acts through the central inhibition of the reuptake of noradrenaline and serotonin (5-HT), and to a lesser extent,

dopamine (Table 3) [107]. This activity contrasts with that of D-fenfluramine and D-amphetamine which enhance the release of either serotonin alone, or noradrenaline and dopamine [104]. The secondary and primary metabolites of sibutramine desmethylsibutramine (M1) and didesmethylsibutramine (M2) – evoke more potent inhibition of noradrenaline and serotonin *in vitro* than the parent compound [108] and it is likely that they are primarily responsible for the hypophagic and weight-loss effects. In contrast, the dopaminergic action of sibutramine is not thought to contribute to its antiobesity effects but may impact on its side-effect profile [109]. By increasing serotonin and noradrenaline activity, sibutramine also activates adrenergic and serotonergic receptors through an indirect mechanism, yet exhibits negligible affinity for serotonergic, adrenergic, dopaminergic and histaminergic receptors themselves and does not affect monoamine oxidase activity [110].

Table 4 Potencies as determined by the inhibition constants (Ki) of sibutramine and its metabolites, M1 and M2, as monoamine reuptake inhibitors in human and rat brain [107].

	Potency to inhibit monoamine reuptake (K_i[nM])		
	Noradrenaline	Serotonin	Dopamine
Human brain tissue			
Sibutramine	5451	298	943
M_1	20	15	49
M_2	15	20	45
Rat brain tissue			
Sibutramine	283	3131	2309
M_1	2.7	18	24
M_2	4.9	26	31

Effect on food intake and body weight:-

Sibutramine decreases the size and duration of meals consumed by enhancing satiety signals and hence causes a reduction in food intake and body weight. Extensive

studies have established that these effects are due largely to the synergistic interaction of central serotonergic and noradrenergic mechanisms. In an experiment conducted in free-feeding Sprague-Dawley rats, sibutramine induced hypophagia which was reversed (to varying degrees) by selective noradrenergic and serotonergic receptor antagonists, specifically implicating a1 adrenoceptors, B_1- adrenoceptors and 5-$HT_{2B/2C}$ receptors in appetite control [111, 112]. Furthermore, fluoxetine, a selective serotonin reuptake inhibitor, and nisoxetine, a noradrenaline reuptake inhibitor, had no effect on food intake when given alone, yet when administered together, elicited significant hypophagia, equivalent to that observed following sibutramine [111].

Effect on thermogenesis:-

Obesity results from a combination of low energy expenditure and increased energy intake. Patients with a low metabolic rate, perhaps as a result of low sympathetic nervous system activity, may be more at risk of weight gain [105]. By stimulating energy expenditure, or thermogenesis, through a central mechanism, [108] sibutramine restores the energy balance that is disrupted during periods of weight gain and thus encourages efficient weight loss [105].

PHARMACOKINETICS:-

Sibutramine is rapidly absorbed from the gastrointestinal tract after oral administration and is rapidly demethylated, primarily by cytochrome P450 (CYP) 3A4 to form the pharmacologically active metabolites, M1 and M2. These metabolites then undergo hydroxylation and conjugation to form the inactive metabolites, M5 and M6.29 The elimination of sibutramine and its active metabolites is predominantly hepatic, with 85% of inactive metabolites excreted in the urine and faeces and, for this reason, sibutramine is not recommended for use in patients with severe hepatic impairment [113].

Table-5 The pharmacokinetic properties of sibutramine in patients with obesity [113]

Pharmacokinetic parameter	
Fasting bioavailability (%)	77
t_{max} (hours)	sibutramine 1.2
	M_1 3.1–4.1
	M_2 3.2–3.8
C_{max} (ng/L)	M_1 3.2–4.8
	M_2 5.6–7.2
Plasma protein binding (%)	sibutramine 97%
	M_1 94%
	M_2 94%
$t_{1/2}$ (hours)	sibutramine 1.1
	M_1 14
	M_2 16
Plasma clearance (L/hour)	1750
AUC (ng/L.hour)	M_1 18.1–32.9
	M_2 81.2–103

AUC, area under the concentration-time curve; tmax, time to reach maximum drug plasma concentration; $t_{1/2}$, elimination half-life; M1, secondary metabolite; M2, primary metabolite.

SAFETY AND TOLERABILITY:-

Experience drawn from clinical trials shows that sibutramine is generally well tolerated and adverse events do not represent a significant factor governing treatment discontinuation. The most common side-effects associated with sibutramine include headache, constipation, dry mouth, insomnia, nausea, tachycardia, hypertension, light-headedness and depression (Table 7).

Table 6 Incidence of side-effects associated with the therapeutic use of sibutramine [113]

Adverse event	Sibutramine (n=2068)	Placebo (n=884)
Headache	30.3	18.6
Back pain	8.2	5.5
Asthenia	5.9	5.3
Abdominal pain	4.5	3.6
Tachycardia	2.6	0.6
Hypertension	2.1	0.9
Constipation	11.5	6.0
Nausea	5.9	2.8
Dry mouth	17.2	4.2
Insomnia	10.7	4.5
Dizziness	7.0	3.4
Depression	4.3	2.5
Generalised oedema	1.2	0.8

Table 7 Changes in various parameters when treatment of olanzapine induced weight gain patient with Sibutramine [114]

	Baseline				Week 4				Week 12			
	Placebo		Sibutramine		Placebo		Sibutramine		Placebo		Sibutramine	
Measurement	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Lipids												
Total (mg/dl)	223.6	33.4	213.4	32.6	228.9	39.9	186.6	44.4	232.8	41.6	202.7	52.5
HDL (mg/dl)	40.9	12.6	38.4	14.5	39.4	9.9	36.4	11.2	36.8	10.1	38.1	13.1
LDL (mg/dl)	134.4	31.7	129.1	24.7	136.3	50.0	105.2	30.6	146.4	43.4	112.6	38.9
Triglycerides (mg/dl)	247.8	116.8	251.6	216.8	233.3	95.7	215.9	124.4	274.7	181.0	286.6	312.2
Glucose (mg/dl)	89.5	21.5	100.2	29.0	103.0	39.4	89.4	28.0	84.8	28.8	88.6	27.3
Uric acid (mg/dl)	5.7	2.0	5.7	1.2	5.9	1.7	5.6	1.2	6.1	1.5	5.6	1.7
Cortisol (μg/dl)	9.5	4.8	8.4	3.9	8.9	4.0	8.6	4.2	9.6	4.6	10.4	4.9
Hemoglobin A_{1c} (%)	5.7	0.9	5.6	1.0	5.7	0.8	5.3	0.7	6.0	1.0	5.3[b]	0.7
Liver function												
Alkaline phosphatase (U/liter)	111.3	45.5	112.6	65.9	112.7	64.2	91.1	27.2	107.8	59.9	93.2	34.6
SGOT (U/liter)	30.9	16.0	26.6	14.2	42.1	34.3	21.3	5.1	30.2	14.2	21.3	5.8
SGPT (U/liter)	38.7	27.4	27.7	12.4	44.1	34.6	24.4	10.2	35.9	21.3	24.5	6.0
Lactate dehydrogenase (U/liter)	187.3	42.4	173.2	53.7	202.9	46.7	161.9	28.8	199.4	62.0	171.2	40.6
BUN (mg/dl)	14.3	3.7	13.2	4.1	13.9	4.8	14.7	3.0	14.3	5.5	12.5	2.9
Creatinine (mg/dl)	1.0	0.1	1.1	0.2	1.0	0.1	1.1	0.1	1.1	0.2	1.1	0.2
T_3 uptake (%)	29.2	2.7	30.8	2.4	29.9	2.6	32.1	2.7	30.2	2.4	30.5	1.3
T_4 thyroxine (μg/dl)	7.5	1.3	7.0	1.2	7.7	1.2	7.4	1.3	7.2	1.3	7.3	1.4
Free thyroxine index ($T_3 \times T_4$)	2.2	0.4	2.1	0.4	2.3	0.4	2.4	0.4	2.2	0.5	2.3	0.4
Olanzapine (ng/ml)	43.3	39.7	27.2	17.6	45.3	54.2	27.8	17.8	—	—	—	—

[a] Numbers of subjects vary because of different patterns of missing data.
[b] Significant difference between groups in change from baseline, controlled for baseline levels ($p<0.05$, two-tailed t test).

ORLISTAT:-

Orlistat reacts with serine residues at the active sites of gastric and pancreatic lipases, irreversibly inhibiting the enzymes and thereby preventing the breakdown of dietary fat to fatty acids and glycerols. It therefore causes a dose-related decrease in fat absorption and a corresponding increase in faecal fat excretion that plateaus at some 30% of dietary fat. Given with a low-calorie diet in obese individuals, it produces a modest but consistent loss of weight compared with in placebo-treated control subjects. In a recent meta-analysis of 11 long-term placebo-controlled trials encompassing over 6000 patients, orlistat was found to produce a 2.9% greater reduction in body weight than in the control group, and 12% more patients lost 10% or more of their body weight compared with the controls.

Orlistat is also reported to be effective in patients suffering from type 2 diabetes and other complications of obesity, to reduce leptin levels and blood pressure, to protect against weight loss-induced changes in biliary secretion, to delay gastric emptying and gastric secretion, to improve several important metabolic parameters, and not to interfere with the release or action of thyroid and other important hormones. It does not induce changes in energy expenditure.

PHARMACOKINETIC ASPECTS:-

Virtually all (97%) of orlistat is excreted in the faeces (83% unchanged), with only negligible amounts of the drug or its metabolites being absorbed.

UNWANTED EFFECTS:-

Abdominal cramps, flatus with discharge and faecal incontinence can occur, as can intestinal borborygmi (rumbling) and oily spotting. Surprisingly, in view of the possibility of these antisocial effects occurring, the drug is well tolerated. Supplementary therapy with fat-soluble vitamins may be needed, and there has been a report of decreased absorption of contraceptive pills [30].

ORLISTAT IN TREATMENT OF OLANZAPINE INDUCED WEIGHT GAIN:-

Study shows that, without a hypocaloric diet, the effect of orlistat in overweight/obese clozapine-or olanzapine-treated patients is modest and may only be seen in men [115].

3.7.2.2 Other possible treatments for olanzapine induced weight gain.

METFORMIN:-

ACTIONS AND MECHANISM:-

Biguanides lower blood glucose by mechanisms that are complex and incompletely understood. They increase glucose uptake and utilisation in skeletal muscle (thereby reducing insulin resistance) and reduce hepatic glucose production (gluconeogenesis). Metformin, while preventing hyperglycaemia, does not cause hypoglycaemia [30]. In diabetic patients, it suppresses endogenous glucose production and may also act as an insulin sensitizer. It also helps diabetic patients lose weight or at least keep their weight stable. In addition to its use in treatment of diabetes, metformin has also become commonly prescribed for patients with polycystic ovary syndrome (PCOS), and its use has resulted in weight reduction in those patients as well [116].

ABSORPTION, EXCRETION, AND DOSING:-

Metformin is absorbed mainly from the small intestine. The drug is stable, does not bind to plasma proteins, and is excreted unchanged in the urine. It has a t1/2 of ~2

hours. The maximum recommended daily dose of metformin is 2.5 g divided into three doses with meals [117].

PRECAUTIONS AND ADVERSE EFFECTS:-

Patients with renal impairment should not receive metformin. Other contraindications include hepatic disease, a history of lactic acidosis, cardiac failure requiring drug therapy, or chronic hypoxic lung disease. These conditions all predispose to the potentially fatal complication of lactic acidosis. Metformin should be discontinued temporarily prior to the administration of intravenous *contrast media* and prior to any surgical procedure. The drug should not be readministered any sooner than 48 hours after such procedures and should be withheld until renal function is determined to be normal.

METFORMIN IN TREATMENT OF OLANZAPINE INDUCED WEIGHT GAIN:-

Table 8 Change in Weight and Body Mass Index in Patients with First-Episode Schizophrenia Randomly Assigned to 12 Weeks of Double-Blind Treatment with Olanzapine plus Metformin or Olanzapine plus Placebo [118]

	Change From Baseline					
	Olanzapine Plus Metformin (N=18)		Olanzapine Plus Placebo (N=19)		Between-Group Difference	
Variable and Week	Mean	SD	Mean	SD	t	p
Weight (kg)						
Week 2	1.37*	2.68	1.49*	2.72	−0.122	0.862
Week 4	1.86*	2.79	2.13*	3.01	−0.216	0.742
Week 8	1.87*	2.81	5.14*	3.67	−2.573	<0.02
Week 12	1.90*	2.72	6.87*	4.23	−2.861	<0.02
Body mass index (kg/m^2)						
Week 2	0.41*	0.89	0.45*	0.72	−0.17	0.791
Week 4	0.53*	0.82	0.59*	0.86	−0.223	0.724
Week 8	0.53*	0.56	1.92*	1.03	−2.882	<0.01
Week 12	0.54*	0.92	2.26*	1.12	−3.063	<0.01

*p<0.05.

AMANTADINE:-

MECHANISM:-

Amantadine is a dopamine agonist that is approved for the treatment of extrapyramidal side effects of medications, idiopathic Parkinsonism, and influenza A virus. The mechanism by which it stabilizes weight is unknown, but it is postulated to be related to its ability to decrease prolactin and thereby influence gonadal and adrenal steroids or to decrease appetite through its dopaminergic anorexic effects [119].

AMANTADINE IN TREATMENT OF OLANZAPINE INDUCED WEIGHT GAIN:-

Many studies were carried out to check the effect of amantadine in patients of schizophrenia with olanzapine induced weight gain. Amantadine appears to stabilize

weight gain related to psychotropic medications. Decreased weight and body mass index may occur with continued amantadine usage [120, 121]. Weight gain is commonly associated with lipid and carbohydrate disturbances. It was found that despite weight stabilization, 12 weeks of amantadine did not improve fasting insulin or lipid levels. It is possible that longer treatment may show a metabolic effect [119].

REBOXETINE:-

Reboxetine is a selective norepinephrine reuptake inhibitor, and its addition to conventional antipsychotics has been found to be safe and well tolerated in patients with schizophrenia. In one study, it is hypothesized that stimulation of adrenergic activity by the selective norepinephrine reuptake inhibitor reboxetine may diminish olanzapine-induced weight gain [122].

TOPIRAMATE:-

Topiramate normalized hippocampal NPY-LI in flinders sensitive line 'depressed' rats and upregulated NPY, galanin, and CRH-LI in the hypothalamus, which may account for the drug's weight loss effects [123]. Although the mechanism of topiramate-induced weight loss likely involves potentiation of GABAergic transmission and antagonism of AMPA glutamate receptors, its precise mechanism remains under investigation [124].

One study shows that topiramate was well tolerated and seems to be effective and safe in the long-term treatment of olanzapine-related adiposity in women [125].

H_2 RECEPTOR ANTAGONIST:-

Various studies show that, this class of drug may be useful to treat the weight gain induced by olanzapine treatment [15, 17, 126]. It has bee proposed that this class of drug decreases the food intake by suppression of gastric acid secretion. Gastrointestinal regulatory peptides, such as cholecystokinin, have been proposed to mediate the satiety signal from gut to brain. In normal subjects, cimetidine increased the basal cholecystokinin and may be one mechanism by which it reduces the appetite [127].

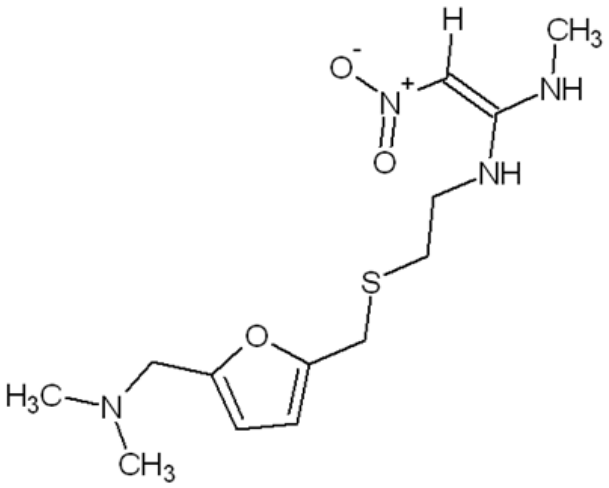
Cholecystokinin:-

Cholecystokinin (CCK) is the major hormone responsible for gallbladder contraction and pancreatic enzyme secretion. CCK, like other gastrointestinal hormones, is produced in discrete endocrine cells that line the mucosa of the small intestine [126].

The application of modern molecular biological techniques has identified two CCK receptors, CCK-A receptor (CCK-AR) and CCK-B receptor (CCK-BR), that mediate the actions of CCK and gastrin; gastrin receptors have been found to be identical to CCK-BR. CCK-AR, found predominantly in the GI system and select areas of the CNS, have high affinity for CCK and the nonpeptide antagonist L-364,718, whereas CCK-BR, found predominantly in the CNS and select areas of the GI system, have high affinity for CCK and gastrin and the nonpeptide antagonist L-365,260 [128].

After food is processed for a bit by the physical and chemical processes of the stomach it becomes a semi-liquid material called chyme. The contents of chyme include dietary fat, protein, and carbohydrate. Chyme is periodically squirted into the small intestine from the stomach in a process called gastric emptying. Epithelial cells in the upper third of the small intestine (duodenum) release CCK into the bloodstream when chyme high in fat and protein reaches them. CCK in the bloodstream quickly travels to the CNS, stomach, liver, gallbladder, and pancreas. The hormone instructs the stomach to stop emptying by contracting the pyloric sphincter. It then acts as a neurotransmitter in the CNS, causing the sensation of satiety. CCK signals the liver to create more bile and for the pancreas to release digestive enzymes. It also signals the gallbladder to spasm and secretes stored bile into the small intestine. The combination of bile and pancreatic enzymes then breaks down the chyme and facilitates the absorption of nutrients [129].

RANITIDINE



DRUG CLASS AND MECHANISM**:-**

Histamine is a natural chemical that stimulates the stomach cells to produce acid. Ranitidine belongs to a class of medications, called H_2-blockers that block the action of histamine on stomach cells, thus reducing stomach acid production.

PREPARATIONS:-

Tablets (150 mg, 300 mg), Capsules (150 mg, 300 mg); Syrup (15 mg/ml)

INDICATION:-

Duodenal ulcers (ulcers in the first part of the intestine after the stomach) -- Ranitidine can be used to treat a duodenal ulcer and also to prevent ulcers from coming back. Gastric ulcers (stomach ulcers) -- Ranitidine helps to heal a stomach ulcer and to keep ulcers from returning. Gastroesophageal reflux disease (GERD). Erosive esophagitis (damage to the esophagus, usually due to stomach acid) -- Ranitidine can be used to heal erosive esophagitis and to prevent it from returning. Pathological hypersecretory conditions (in which too much stomach acid is produced), such as Zollinger-Ellison syndrome or systemic mastocytosis. The over-the-counter form is approved for the following conditions:

Heartburn**,** Acid indigestion, Sour stomach.

Over-the-counter ranitidine can be used to treat these problems once they have started. Also, it can be used to prevent these problems if taken before eating or drinking foods or beverages that may cause them.

DOSING:-

May be taken with or without food. Since ranitidine is excreted by the kidney and metabolized by the liver, dosages of ranitidine need to be lowered in patients with significantly abnormal liver or kidney function.

DRUG INTERACTIONS:-

Antacids may decrease the absorption of ranitidine. Safety of ranitidine in children has not been established. Ranitidine is not habit forming. Ranitidine can interfere with the metabolism of alcohol. Patients taking ranitidine who drink alcohol may have elevated blood alcohol levels.

SIDE EFFECTS:-

Stop using ranitidine and get emergency medical help if you have any of these signs of an allergic reaction: hives; difficulty breathing; swelling of your face, lips, tongue, or throat. Call your doctor at once if you have any of these serious side effects:

- chest pain, fever, feeling short of breath, coughing up green or yellow mucus;
- easy bruising or bleeding, unusual weakness;
- fast or slow heart rate;
- problems with your vision;
- fever, sore throat, and headache with a severe blistering, peeling, and red skin rash; or
- nausea, stomach pain, low fever, loss of appetite, dark urine, clay-colored stools, jaundice (yellowing of the skin or eyes).

Less serious side effects may include:

- headache (may be severe);
- drowsiness, dizziness;
- decreased sex drive, impotence, or difficulty having an orgasm; or
- swollen or tender breasts (in men);
- nausea, vomiting, stomach pain; or

- diarrhea or constipation.

CONTRAINDICATION:-

H_2-blockers may be contraindicated in: Renal disease, hepatic disease: the H_2-blocker may build up in the bloodstream, which increases the risk of side effects. Immunodeficiency: Decrease in stomach acidity caused by H_2-blockers may increase the risk [130].

RANITIDINE IN TREATMENT OF OLANZAPINE INDUCED WEIGHT GAIN:-

In one study, concomitant administration of Ranitidine prevented or corrected weight gain in 59.6% of cases. Patients followed by 16 weeks had shown the following results: Olanzapine without Ranitidine exhibited an average weight gain of 3.4 kilograms, ranging between -2.5 and +16 kg. This implies an average increase of 1.19 in BMI for this group. Patients treated additionally with Ranitidine at doses of 300 mg, a 0.9 kilogram weight gain ranging between -4 and +10.6 kg was observed, implying an average BMI change of 0.34. In patients treated with Ranitidine at doses of 600 mg, the weight gain curve trended toward normalization with a 1.6 kilogram decrease, ranging between -15 and +7 kilograms, accounting for a decrease of 0.6 points in BMI [17].

Chapter:-4 Materials & Methods

4.1.Materials:

4.1.1 Drug: Drugs were purchased from local retailer.

a)

Ranitidine Hydrochloride Tablets I.P. **RANITIN-150**	
Each film coated tablet contains Ranitidine Hydrochloride I.P. equivalent to Ranitidine 150 mg	
Manufactured by .	TORRENT PHARMACEUTICAL LTD. VIII. Bhud & Makhnu Majra, Baddi-179205 Dist. Solan (H.P.), INDIA.
Mfg. Lic. No.	MNB/05/183
Batch No.	C 4148062
Mfg. Date	OCT. 2008
Exp.Date	JUN. 2012

Formulations used: oral suspension

Concentrations: 10 mg/kg, 20 mg/kg, 30 mg/kg of ranitidine hydrochloride

b)

Sibutramine Hydrochloride Monohydrate Capsules **OBEGO-10**	
Each hard gelatin capsule contains Sibutramine Hydrochloride Monohydrate 10 mg	
Manufactured by .	INTAS PHARMACEUTICAL Selaqui, Dehradun-248197. INDIA.
Mfg. Lic. No.	15/UA/2006
Batch No.	DJ1938
Mfg. Date	AUG. 2008
Exp.Date	JUL. 2010

Formulations used: oral suspension

Concentration: 6 mg/kg

c)

Olanzapine Tablets **OLEANZ 10**	
Each film coated tablet contains Olanzapine 10 mg	
Manufactured by. .	SUN PHARMACEUTICAL INDUTRIES 6-9 epip, Kartholi, Bari brahmana, Jammu-181133 (J&K). INDIA
Mfg. Lic. No.	JK/01/58
Batch No.	AO81902
Mfg. Date	10/2008
Exp.Date	09/2010

Formulations used: oral suspension

Concentration: 3 mg/kg

4.1.2 Animal treatment:

Female wistar rats weighing in range of 150 – 250 g were obtained from Animal house of Nirma University, Institute of Pharmacy (Ahmedabad, India) and maintained at temperature (23±2 °C) and humidity (55-60%) controlled room with a 12h light-dark cycle. They were kept in group of 6 animals in polypropylene cages with paddy husk as bedding, cleaned weekly, and fed with Pranav Agrochemicals lab chow without antibiotic and water *ad libitum*. Ethical approval for the study was obtained by the CPCSEA and the Institutional Animal Ethics Committee; protocol no: IPS/PCOL/MPH09/002. Rats were divided in six groups. No drug treatment was given to first group of animals. Second group of animals were give 3 mg/kg of olanzapine suspension by oral administration for 5 weeks. Third group was give olanzapine 3 mg/kg p.o. for 3 weeks and then for two weeks, sibutramine 6 mg/kg suspension p.o. was given along with olanzapine suspension. Next three groups were treated with olanzapine suspension (3 mg/kg p.o.) for three weeks and then ranitidine suspension by oral administration at dose level of 10 mg/kg, 20 mg/kg, and 30 mg/kg respectively for two weeks along with olanzapine suspension.

4.2. Method:

4.2.1 Preparation of formulation

The all tablets were triturated and the drug was prepared as an aqueous suspension using 0.5% Carboxy Methyl Cellulose (CMC).

4.2.2 Food intake and body weight measurement

Six rats were housed per cage for feeding measurement. Individual food consumption per cage was assessed and recorded at the end of one week of treatment. The body weight of individual animals was assessed and recorded at the end of each week.

4.2.3 Body mass index (BMI)

The body mass index (BMI) is a statistical measure of the weight of a person scaled according to height. BMI is frequently used to assess how much an individual's body weight departs from what is normal or desirable for a person of his or her height. Weight of animal in kilogram and length of animal from nose to opening of anal route in meter was measured and BMI was calculated by formula- **kg/m^2.**

4.2.4 Biochemical Estimations:-

4.2.4.1 Collection of serum:

The blood samples were withdrawn from retro-orbital plexus under light ether anesthesia without any anticoagulant and allowed to clot for 10 minutes at room temperature. It was centrifuged at 2500 rpm for 20 minutes. The serum obtained was stored at 4ºC until used.

4.2.4.2 Determination of Serum lipid profile:-

Estimation of total cholesterol

In vitro quantitative determination of the activity of cholesterol in serum was done using enzymatic kit **(Lab Care Diagnostics, India).**

Principle

Cholesterol esters are hydrolyzed by Cholesterol esterase to produce cholesterol.
Hydrogen Peroxide is then produced from oxidation of cholesterol by cholesterol

oxidase. The indicator quinoneimine is formed from hydrogen peroxide and 4 - aminoantipyrine in the presence of phenol and peroxide. The absorption of the red quinoneimine dye is proportional to the concentration of cholesterol in the sample.

$$\text{Cholesterol} - \text{ester} + H_2O \xrightarrow[\text{esterase}]{\text{Cholesterol}} \text{Cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow[\text{Oxidase}]{\text{Cholesterol}} \text{Cholestene–3– one} + H_2O_2$$

$$2H_2O_2 + \text{phenol} + \text{4-Aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4\ H_2O$$

Procedure:

Pipette into 3 test tubes labeled Blank (B), Standard (S) and Sample (Total Cholesterol) as shown below:

	Blank	Standard	Sample
Sample	-	-	10 µl
Standard	-	10 µl	-
Reagent	1000 µl	1000 µl	1000 µl

Mix, Incubate 5 mins at 37°C (or 10 mins at 20 - 25°C) Measure absorbance of the sample (AT) and standard (AS) against reagent blank at 505 nm. The colour is stable for 90 mins at 20 - 25°C

Calculations:

Total Cholesterol (mg/dl) = AT / AS. X conc. Standard (200 mg/dl)

Estimation of triglycerides:

In vitro quantitative measurement of triglyceride (neutral fat) concentration in serum was done by using kit **(Lab Care Diagnostics, India)**.

Principle:

Triglycerides are determined after enzymatic hydrolysis with lipases. The quinonemine indicator is formed from hydrogen peroxide, 4-aminophenazone, and 4-chlorophenol under the catalytic influence of peroxidase.

$$\text{Triglycerides} + H_2O \xrightarrow{\text{lipases}} \text{glycerol} + \text{fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPO}} \text{dihydroxyacetone} + \text{phosphate} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{4 chlorophenol} \xrightarrow{\text{POD}} \text{quinoneimine} + HCl + 4H_2O$$

Procedure:

Pipette into 3 test tubes labeled Blank (B), Standard (S) and Sample (Triglycerides) as shown below;

	Blank	Standard	Sample
Sample	-	-	10 µl
Standard	-	10 µl	-
Reagent	1000 µl	1000 µl	1000 µl

Mix, Incubate 5 mins at 37°C (or 10 mins at 20 - 25°C) Measure absorbance of the sample (AT) and standard (AS) against reagent blank at 505 nm. The colour is stable for 30 mins at 20 - 25°C

Calculations:

Triglycerides (mg/dl) = AT / AS. X conc. Standard (200 mg/dl)

Estimation of LDL Cholesterol:

In vitro quantitative measurement of LDL-C concentration in serum was done by using kit **(Lab Care Diagnostics, India)**.

Principle:

Direct determination of serum LDL-C (low density lipoprotein cholesterol) levels without the need for any pre-treatment of centrifugation steps. The assay takes place in two steps.

-1^{o} elimination of lipoprotein non-LDL

$$\text{Cholesterol esters} \xrightarrow{\text{CHE}} \text{Cholesterol + Fatty acids}$$

$$\text{Cholesterol + } O_2 \xrightarrow{\text{CHOD}} \text{4-Cholestenone + } H_2O_2$$

$$2\ H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

-2^{o} measurement of LDL-C

$$\text{Cholesterol esters} \xrightarrow{\text{CHE}} \text{Cholesterol + Fatty acids}$$

$$\text{Cholesterol + } O_2 \xrightarrow{\text{CHOD}} \text{4-Cholestenone + } H_2O_2$$

$$2\ H_2O_2 + \text{TOOS + 4-AA} \xrightarrow{\text{POD}} 2H_2O + O_2$$

The intensity of color formed is proportional to the LDL-C concentration in the sample.

Procedure:

Pipette into 3 test tubes labeled Blank (B), Standard (S) and Sample (LDL-C) as shown below:

	Blank	Standard	Sample
R1 (µl)	300	300	300
Standard (µl)	-	4	-
Sample (µl)	-	-	4
Mix and incubate for 5 mins at 37°C			
R2 (µl)	100	100	100

Mix and incubate for 5 min at 37^{o}C and Measure absorbance of the sample (AT) and standard (AS) against reagent blank at 546 nm.

Calculations:

LDL-C (mg/dl) = AT / AS. X conc. Standard (114 mg/dl)

Estimation of HDL Cholesterol:

In vitro quantitative measurement of HDL-C concentration in serum was done by using kit **(Lab Care Diagnostics, India**).

Principle:

Direct determination of serum HDL-C (High Density Lipoprotein Cholesterol) levels without the need for any pre-treatment or centrifugation of the sample. The method depends on the properties of a detergent which solubilizes only the HDL so that HDL-C is released to react with the cholesterol esterase, cholesterol oxidase and chromogens to give colour. The non HDL lipoprotein LDL, VLDL and chylomicrons are inhibited from reacting with the enzymes due to abruption of the detergents on their surfaces. The intensity of the color formed is proportional to the HDL-C concentration in the sample.

Procedure:

Pipette into 3 test tubes labeled Blank (B), Standard (S) and Sample (HDL-C) as shown below:

	Blank	Standard	Sample
R1 (µl)	300	300	300
Standard (µl)	-	3	-
Sample (µl)	-	-	3
Mix and incubate for 5 mins at 37°C.			
R2 (µl)	100	100	100

Mix and incubate for 5 min at 37^{o}C and measure absorbance of the sample (AT) and standard (AS) against reagent blank at 6oo nm against blank.

Calculations:

HDL-C (mg/dl) = AT / AS. X conc. Standard (56 mg/dl)

Estimation of VLDL Cholesterol

Estimation of VLDL-cholesterol was done using the Friedwald's formula.

VLDL cholesterol = triglycerides / 5.

4.2.4.3 Determination of Blood glucose level

In vitro quantitative measurement of glucose concentration in serum was done by using kit **(Lab Care Diagnostics, India)**.

Principle:

Glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed reacts, under catalysis of peroxidase, with sodium phenolate and 4- aminophenazone to form a red-violet quinoeimine dye as indicator.

$$\text{D-Glucose} + O_2 \xrightarrow{\text{GOD}} \text{D-Gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{4-AAP} + \text{sodium phenolate} + H_3O + \xrightarrow{\text{POD}} \text{Quinoneimine} + 5H_2O$$

Procedure:

Pipette in to test tube

	Blank	Standard	Sample
Sample	-	-	10 µl
Standard	-	10 µl	-
Reagent	1000 µl	1000 µl	1000 µl

Mix & Incubate for 15 min at 37°C or 30 min at RT. Measure absorbance of Sample (AT) and Standard (AS) at wavelength of 505 nm against Reagent Blank. The colour is stable for 30 min at R.T.

Calculations:

Total Glucose (mg/dl) = AT/AS x conc. Standard (100 mg/dl)

4.2.5 Determination of Blood Pressure

Direct or invasive method of blood pressure measurement was used in rats. The procedure was followed as below:-

1. Rat was anesthetized using ketamine (70 mg/kg i.p.)
2. An anaesthetized rat was placed on a small-animal operating board and secured by tying the limbs.
3. A midline incision was made in the neck; the trachea was exposed and cannulated to ensure a free air way.
4. The common carotid artery on one side was then exposed and ligated at the superior end and clamped at the inferior end.
5. A polythene cannula attached to 23-gauge needle was inserted in to the artery through a small incision and connected via three ways stopcock to a recording device filled with 0.9% NaCl solution containing heparin 1000 units per ml.
6. The system was flushed briefly with heparin saline solution and blood pressure is recorded by releasing the clamp.

Blood pressure was recorded by software LabScribe2 version: 2.05000 using iWorx 114 recorder, iWorx Systems, Inc. - One Washington Street, Suite 404 - Dover NH – 03820

4.2.6 Histochemical Examination

Adipose tissue

Rats were sacrificed by decapitation. The hind limb's skin was widely incised. The subcutaneous adipose pad was collected, weighed and frozen on dry ice quickly. All the samples were stored at –70 ^{o}C until further analysis.

Hematoxylin stain

The slides were coated with 1% poly-L-lysine. The adipose tissue was cut into sections of 12-15 μm with a rotary microtome and dried at 37 ^{o}C. After being fixed in 4% paraformaldehyde for 1 minute, the section was stained using Mayer's hematoxylin for 5 minutes, and rinsed in the tap water following dehydration in a series of alcohols from 70%, 80%, 90%, and 95% to 100%. The slide was immersed in xylene for 3 minutes. The section was then mounted in xylene-based medium and observed under a light microscope. The biopsy study was carried out by HISTOPATHOLOGY DEPARTMENT, NHL-MUNICIPAL COLLEGE, AHMEDABAD.

4.3 Statistical Analysis:

One way ANOVA followed by Post Hoc Dunnet t-Comparisons test was employed for determining the statistical significance of difference among all groups.

Chapter:-5 Results

5.1 Food intake:-

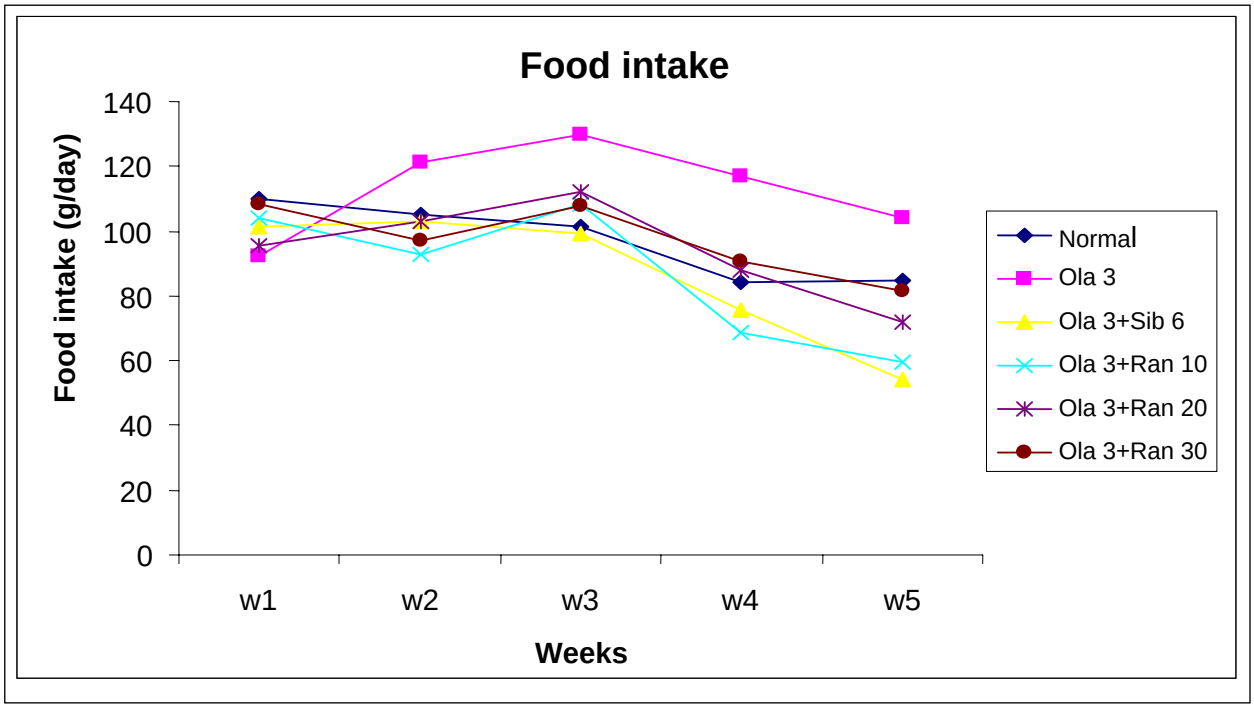
Olanzapine suspension (3 mg/kg p.o.) treated animals showed marked increase in food intake compared to normal group at the end of 5 week of treatment. After treatment with olanzapine for 3 weeks in test groups, when it was given along with ranitidine (10, 20, 30 mg/kg p.o.) for two weeks, there was significant decrease in food intake at the end of 5th week of treatment. Decrease in food intake was not dose dependant (Table 9). Sibutramine (6 mg/kg p.o.) was given as standard drug showed significant decrease in food intake compared to all treatment groups (Fig 6).

Table 9: Effect of ranitidine on food intake to Olanzapine treated rats

week/groups		**Normal**	**Ola 3**	**Ola 3+Sib 6**	**Ola 3+Ran 10**	**Ola 3+Ran 20**	**Ola 3+Ran 30**
Average food intake (g)	**w1**	110.00	92.14	101.43	104.28	95.71	108.57
	w2	105.00	121.43	102.86	92.85	102.86	97.14
	w3	101.43	130.00	99.28	108.57	112.14	107.86
	w4	84.29	117.14	75.71	68.57	87.85	90.71
	w5	85.00	104.28	54.28	59.28	72.14	81.43

Normal-Normal group
Ola 3-Olanzapine 3 mg/kg
Ola 3 + Sib 6- Olanzapine 3 mg/kg + Sibutramine 6 mg/kg
Ola 3 + Ran 10- Olanzapine 3 mg/kg + Ranitidine 10 mg/kg
Ola 3 + Ran 20- Olanzapine 3 mg/kg + Ranitidine 20 mg/kg
Ola 3 + Ran 30- Olanzapine 3 mg/kg + Ranitidine 30 mg/kg

Fig 6: Effect of ranitidine on food intake to Olanzapine treated rats



Normal-Normal group
Ola 3-Olanzapine 3 mg/kg
Ola 3 + Sib 6- Olanzapine 3 mg/kg + Sibutramine 6 mg/kg
Ola 3 + Ran 10- Olanzapine 3 mg/kg + Ranitidine 10 mg/kg
Ola 3 + Ran 20- Olanzapine 3 mg/kg + Ranitidine 20 mg/kg
Ola 3 + Ran 30- Olanzapine 3 mg/kg + Ranitidine 30 mg/kg

5.2 Body weight:

Table 10 shows that olanzapine (3 mg/kg p.o.) treated group increased body weight by 18.95% compared to normal group which shows only 6.64% increase in body weight at the end of 5 weeks. Ranitidine given p.o. at 10 mg/kg, 20 mg/kg, and 30 mg/kg of dose level shows rose in body weight by 5.98%, 4.86% and 6.93% respectively. While treatment with Sibutramine reduced the body weight to its basal body weight (Fig 7).

Table 10: Effect of ranitidine on body weight to Olanzapine treated rats

week/groups		Normal	Ola 3	Ola 3+Sib 6	Ola 3+Ran 10	Ola 3+Ran 20	Ola 3+Ran 30
% change in body weight	w1	-7.52 ± 2.10	-0.81 ± 4.53	22.33 ± 1.62	10.68 ± 1.22	8.91 ± 1.85	12.55 ± 1.24
	w2	6.64 ± 2.35	8.47 ± 3.29	17.48 ± 1.30	17.95 ± 3.03	14.98 ± 3.10	12.12 ± 1.97
	w3	9.29 ± 2.81	19.76 ± 3.45	25.73 ± 2.95	20.51 ± 4.91	23.89 ± 3.22	21.65 ± 2.19
	w4	8.41 ± 2.32	21.77 ± 4.00	15.53 ± 1.44	12.82 ± 3.38	17.81 ± 2.32	12.12 ± 2.09
	w5	6.64 ± 3.20	18.95 ± 3.15 *	-1.94 ± 0.97 #	5.98 ± 2.36 #	4.86 ± 3.01 #	6.93 ± 1.10 #

Each group contain 6 numbers of animals

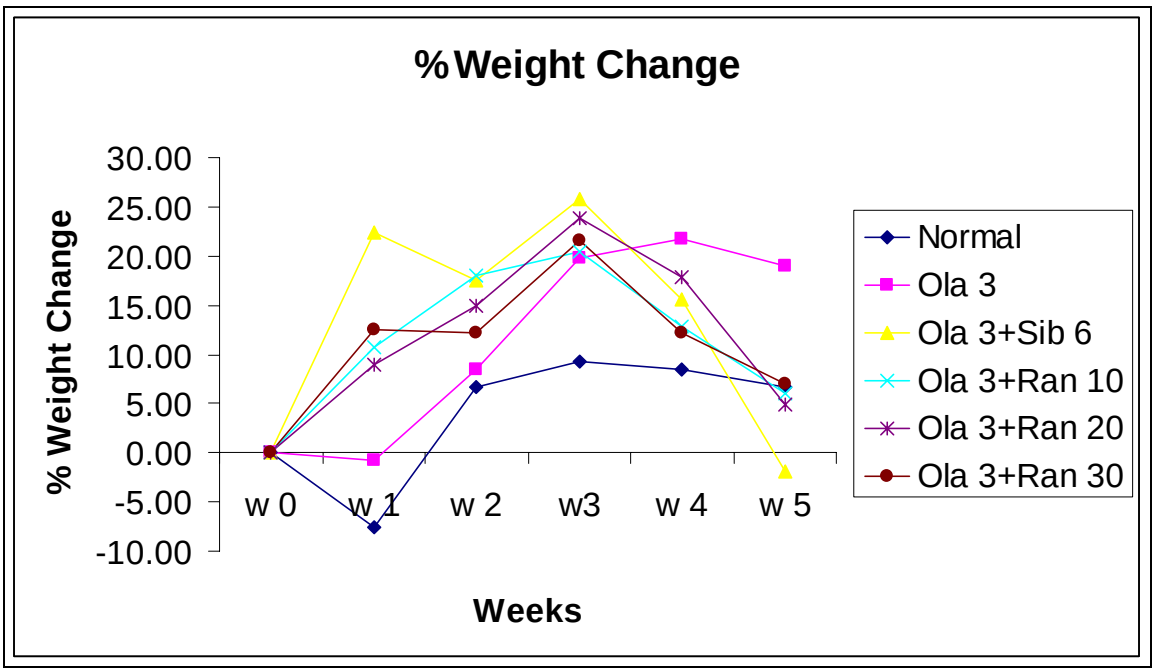
Values are expressed as Mean ± SEM

* indicates significantly different from normal group (P<0.05, One way ANOVA followed by Dunnett's test)

# indicates significantly different from Olanzapine treated group (P<0.05, One way ANOVA followed by Dunnett's test)

Normal-Normal group
Ola 3-Olanzapine 3 mg/kg
Ola 3 + Sib 6- Olanzapine 3 mg/kg + Sibutramine 6 mg/kg
Ola 3 + Ran 10- Olanzapine 3 mg/kg + Ranitidine 10 mg/kg
Ola 3 + Ran 20- Olanzapine 3 mg/kg + Ranitidine 20 mg/kg
Ola 3 + Ran 30- Olanzapine 3 mg/kg + Ranitidine 30 mg/kg

Fig 7: Effect of ranitidine on body weight to Olanzapine treated rats



Each group contain 6 numbers of animals

Values are expressed as Mean ± SEM

* indicates significantly different from normal group (P<0.05, One way ANOVA followed by Dunnett's test)

# indicates significantly different from Olanzapine treated group (P<0.05, One way ANOVA followed by Dunnett's test)

Normal-Normal group
Ola 3-Olanzapine 3 mg/kg
Ola 3 + Sib 6- Olanzapine 3 mg/kg + Sibutramine 6 mg/kg
Ola 3 + Ran 10- Olanzapine 3 mg/kg + Ranitidine 10 mg/kg
Ola 3 + Ran 20- Olanzapine 3 mg/kg + Ranitidine 20 mg/kg
Ola 3 + Ran 30- Olanzapine 3 mg/kg + Ranitidine 30 mg/kg

5.3 Body Mass Index (BMI):

Table 11 shows that olanzapine (3 mg/kg p.o.) treated group increased BMI by 19.58% compared to normal group which shows only 5.37% increased in BMI at the end of 5 weeks. Ranitidine given p.o. at 10 mg/kg, 20 mg/kg, and 30 mg/kg of dose level shows raised in BMI by 6.19%, 5.68% and 7.27% respectively. While treatment with Sibutramine reduced the BMI to its basal value (Fig 8).

Table 11: Effect of ranitidine on BMI to Olanzapine treated rats

week/groups		Normal	Ola 3	Ola 3+Sib 6	Ola 3+Ran 10	Ola 3+Ran 20	Ola 3+Ran 30
% change in BMI	w1	-7.30 ± 2.04	-0.14 ± 5.22	24.99 ± 2.56	10.57 ± 1.42	8.82 ± 1.78	12.59 ± 1.25
	w2	6.49 ± 2.24	9.38 ± 4.06	20.08 ± 2.67	17.73 ± 3.09	15.00 ± 2.96	12.33 ± 2.21
	w3	9.53 ± 2.92	20.62 ± 4.42	28.60 ± 4.36	21.13 ± 4.48	24.69 ± 3.45	21.88 ± 2.29
	w4	7.06 ± 2.31	22.33 ± 4.45	17.18 ± 3.44	12.25 ± 3.74	18.08 ± 2.54	12.19 ± 2.11
	w5	5.37 ± 3.28	19.58 ± 3.80 *	-1.94 ± 0.98 #	6.19 ± 2.52 #	5.68 ± 3.43 #	7.27 ± 1.31 #

Each group contain 6 numbers of animals

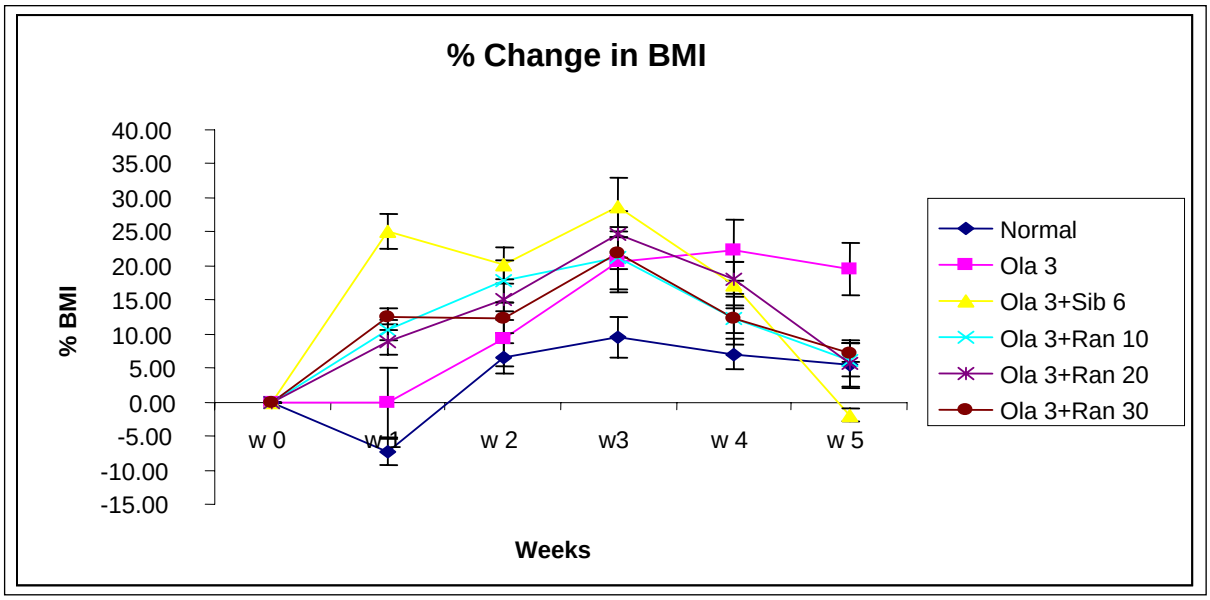
Values are expressed as Mean ± SEM

* indicates significantly different from normal group ($P<0.05$, One way ANOVA followed by Dunnett's test)

# indicates significantly different from Olanzapine treated group ($P<0.05$, One way ANOVA followed by Dunnett's test)

Normal-Normal group
Ola 3-Olanzapine 3 mg/kg
Ola 3 + Sib 6- Olanzapine 3 mg/kg + Sibutramine 6 mg/kg
Ola 3 + Ran 10- Olanzapine 3 mg/kg + Ranitidine 10 mg/kg
Ola 3 + Ran 20- Olanzapine 3 mg/kg + Ranitidine 20 mg/kg
Ola 3 + Ran 30- Olanzapine 3 mg/kg + Ranitidine 30 mg/kg

Fig 8: Effect of ranitidine on BMI to Olanzapine treated rats



Each group contain 6 numbers of animals

Values are expressed as Mean ± SEM

* indicates significantly different from normal group (P<0.05, One way ANOVA followed by Dunnett's test)

# indicates significantly different from Olanzapine treated group (P<0.05, One way ANOVA followed by Dunnett's test)

Normal-Normal group
Ola 3-Olanzapine 3 mg/kg
Ola 3 + Sib 6- Olanzapine 3 mg/kg + Sibutramine 6 mg/kg
Ola 3 + Ran 10- Olanzapine 3 mg/kg + Ranitidine 10 mg/kg
Ola 3 + Ran 20- Olanzapine 3 mg/kg + Ranitidine 20 mg/kg
Ola 3 + Ran 30- Olanzapine 3 mg/kg + Ranitidine 30 mg/kg

5.4 Serum lipid profile:

Normal values of Total Cholesterol (TC), Triglyceride (TG), Low Density Lipoprotein Cholesterol (LDL-C), Very Low Density Lipoprotein Cholesterol (VLDL-C), and High Density Lipoprotein Cholesterol (HDL-C) were found to be 77.05 ± 6.22 mg/dl, 56.29 ± 2.48 mg/dl, 51.53 ± 2.91 mg/dl, 11.25 ± 0.49 mg/dl, 46.49 ± 1.36 mg/dl respectively in normal control animals. Treatment with olanzapine (3 mg/kg) for 5 week showed significant increase in serum TC levels (110.02 ± 13.19), TG levels (99.99 ± 5.90 mg/dl), LDL-C levels (78.53 ± 4.53 mg/dl) and VLDL-C (19.99 ± 1.18 mg/dl) when compared to normal control animals. Also, a significant decrease in HDL-C (27.77 ± 1.69 mg/dl`) levels was seen in high cholesterol diet fed animals (Table - 12).

Administration of different doses (10 mg/kg, 20 mg/kg and 30 mg/kg p.o.) of ranitidine showed a significant reduction in levels of TC, TG, LDL-C and VLDL-C as compared to olanzapine treated group and results were comparable with the standard drug Sibutramine (Fib 9a-e). Also, a remarkable increase in HDL-C levels was seen with ranitidine and Sibutramine treated animal.

Table 12: Effect of ranitidine on serum lipid profile to Olanzapine treated rats at the end of 5 weeks

Groups	TC (mg/dl)	TG (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)	HDL-C (mg/dl)
Normal	77.05 ± 6.22	56.29 ± 2.48	51.53 ± 2.91	11.25 ± 0.49	46.49 ± 1.36
Ola 3	110.02 ± 13.19 *	99.99 ± 5.90 *	78.53 ± 4.53 *	19.99 ± 1.18 *	27.77 ± 1.69 *
Ola 3 + Sib 6	54.18 ± 3.92 #	57.44± 8.34 #	53.06 ± 2.97 #	11.48 ± 1.67 #	39.79 ± 1.37 #
Ola 3 + Ran 10	81.96 ± 4.85 #	58.84 ± 7.30 #	53.77 ± 4.24 #	11.76 ± 1.46 #	36.99 ± 1.23 #
Ola 3 + Ran 20	64.20 ± 10.47 #	52.50 ± 4.92 #	65.84 ± 2.79 #	10.50 ± 0.98 #	37.63 ± 1.25 #
Ola 3 + Ran 30	104.82 ± 8.98 #	64.28 ± 6.85 #	61.64 ± 3.75 #	12.85 ± 1.38 #	39.66 ± 1.12 #

Each group contain 6 numbers of animals

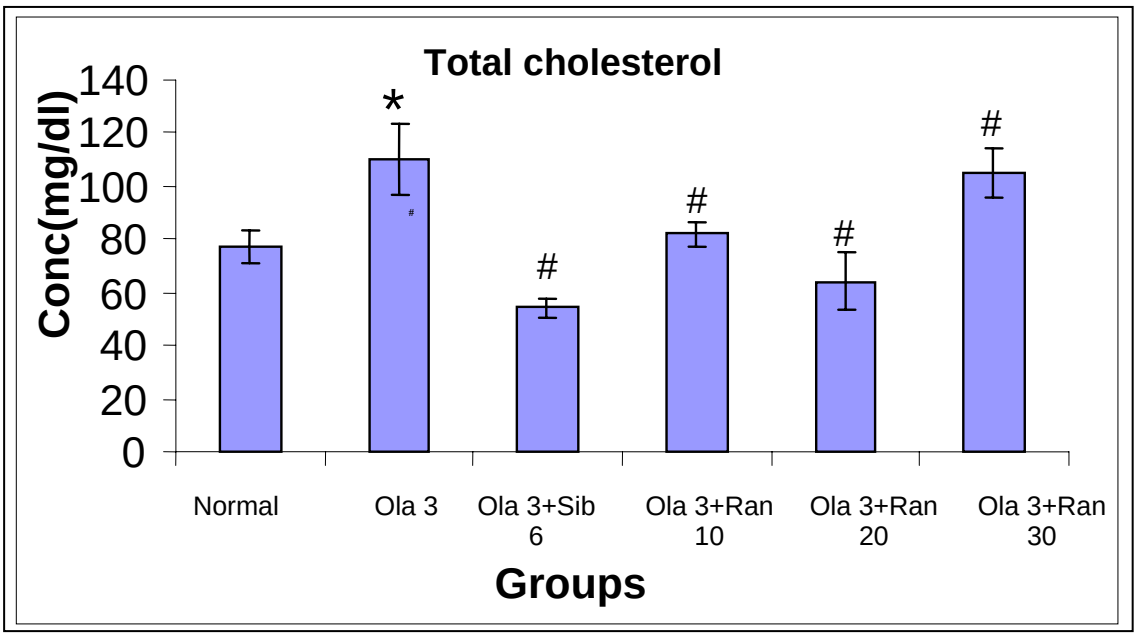
Values are expressed as Mean ± SEM

* indicates significantly different from normal group (P<0.05, One way ANOVA followed by Dunnett's test)

# indicates significantly different from Olanzapine treated group (P<0.05, One way ANOVA followed by Dunnett's test)

Normal-Normal group
Ola 3-Olanzapine 3 mg/kg
Ola 3 + Sib 6- Olanzapine 3 mg/kg + Sibutramine 6 mg/kg
Ola 3 + Ran 10- Olanzapine 3 mg/kg + Ranitidine 10 mg/kg
Ola 3 + Ran 20- Olanzapine 3 mg/kg + Ranitidine 20 mg/kg
Ola 3 + Ran 30- Olanzapine 3 mg/kg + Ranitidine 30 mg/kg

Fig 9(a): Effect of ranitidine on serum total cholesterol level to Olanzapine treated rats at the end of 5 weeks



Each group contain 6 numbers of animals

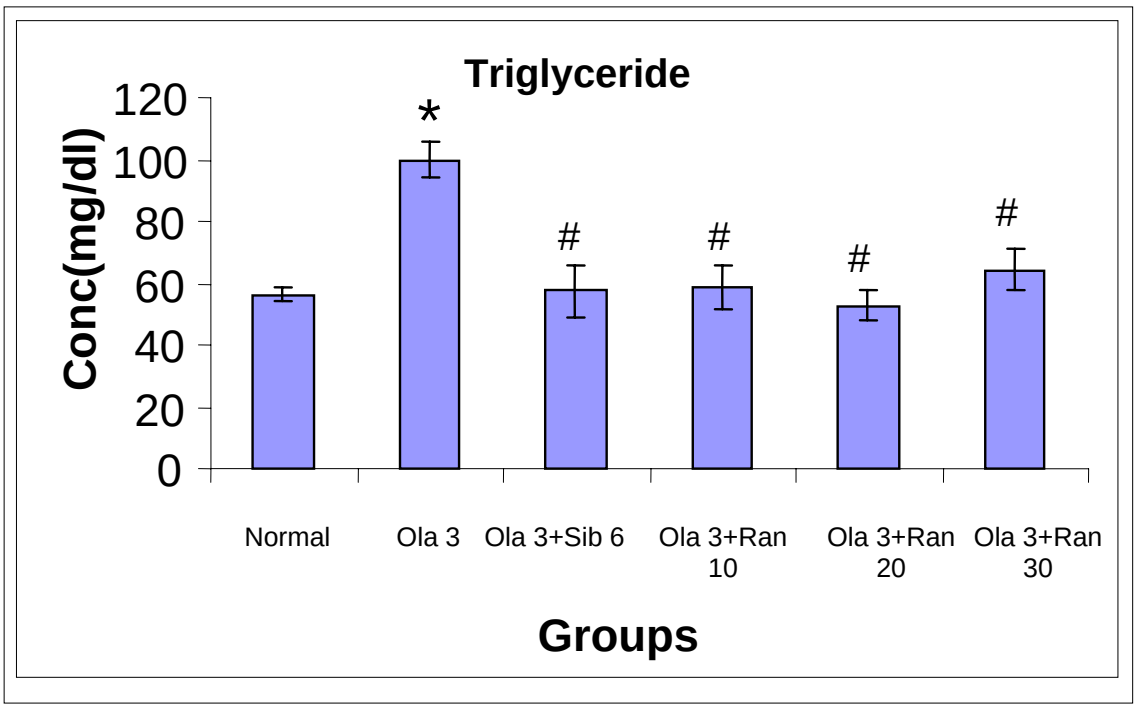
Values are expressed as Mean ± SEM

* indicates significantly different from normal group (P<0.05, One way ANOVA followed by Dunnett's test)

# indicates significantly different from Olanzapine treated group (P<0.05, One way ANOVA followed by Dunnett's test)

Normal-Normal group
Ola 3-Olanzapine 3 mg/kg
Ola 3 + Sib 6- Olanzapine 3 mg/kg + Sibutramine 6 mg/kg
Ola 3 + Ran 10- Olanzapine 3 mg/kg + Ranitidine 10 mg/kg
Ola 3 + Ran 20- Olanzapine 3 mg/kg + Ranitidine 20 mg/kg
Ola 3 + Ran 30- Olanzapine 3 mg/kg + Ranitidine 30 mg/kg

Fig 9(b): Effect of ranitidine on serum triglyceride level to Olanzapine treated rats at the end of 5 weeks



Each group contain 6 numbers of animals

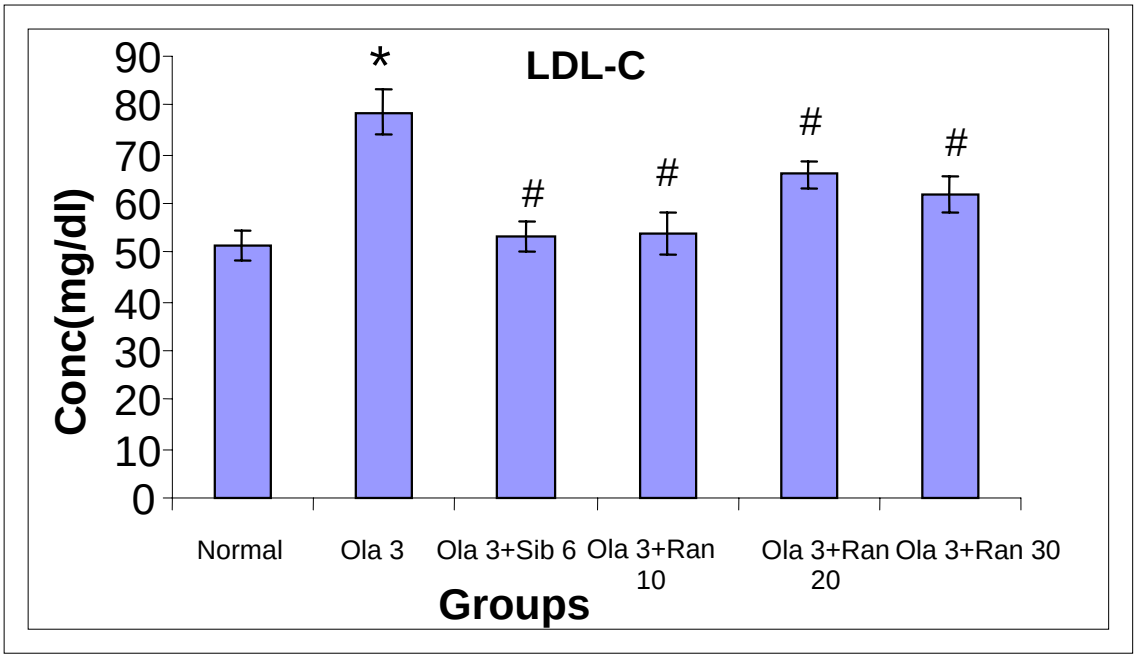
Values are expressed as Mean ± SEM

* indicates significantly different from normal group (P<0.05, One way ANOVA followed by Dunnett's test)

# indicates significantly different from Olanzapine treated group (P<0.05, One way ANOVA followed by Dunnett's test)

Normal-Normal group
Ola 3-Olanzapine 3 mg/kg
Ola 3 + Sib 6- Olanzapine 3 mg/kg + Sibutramine 6 mg/kg
Ola 3 + Ran 10- Olanzapine 3 mg/kg + Ranitidine 10 mg/kg
Ola 3 + Ran 20- Olanzapine 3 mg/kg + Ranitidine 20 mg/kg
Ola 3 + Ran 30- Olanzapine 3 mg/kg + Ranitidine 30 mg/kg

Fig 9(c): Effect of ranitidine on serum LDL-C level to Olanzapine treated rats at the end of 5 weeks



Each group contain 6 numbers of animals

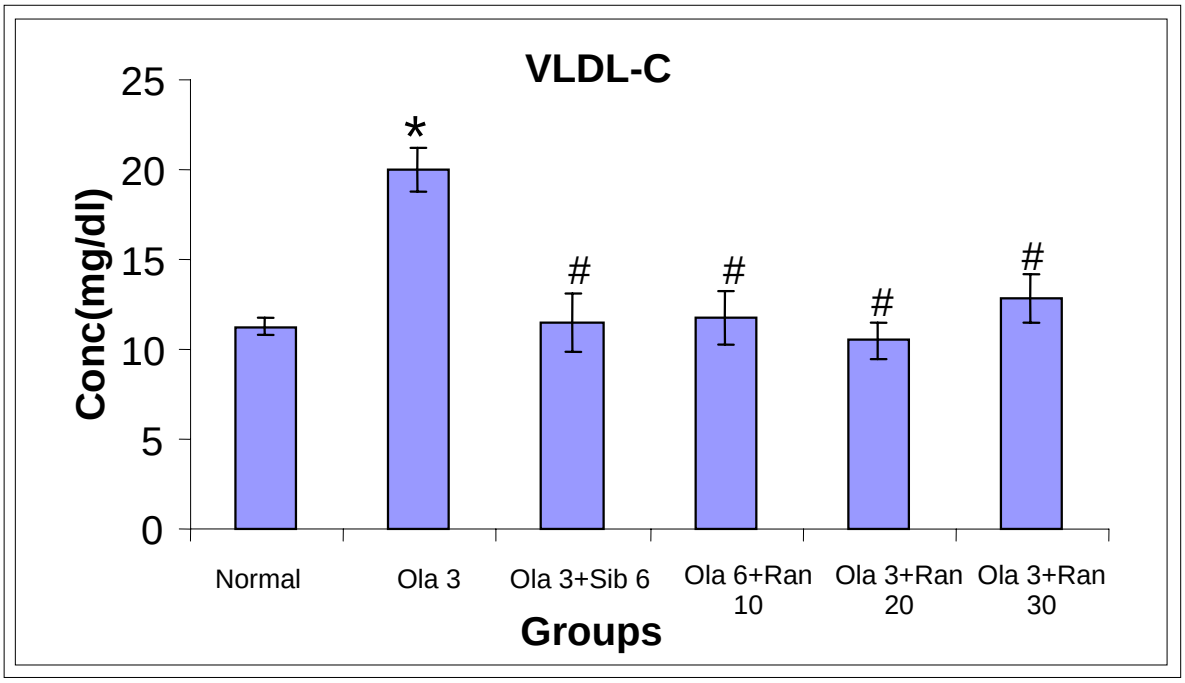
Values are expressed as Mean ± SEM

* indicates significantly different from normal group (P<0.05, One way ANOVA followed by Dunnett's test)

# indicates significantly different from Olanzapine treated group (P<0.05, One way ANOVA followed by Dunnett's test)

Normal-Normal group
Ola 3-Olanzapine 3 mg/kg
Ola 3 + Sib 6- Olanzapine 3 mg/kg + Sibutramine 6 mg/kg
Ola 3 + Ran 10- Olanzapine 3 mg/kg + Ranitidine 10 mg/kg
Ola 3 + Ran 20- Olanzapine 3 mg/kg + Ranitidine 20 mg/kg
Ola 3 + Ran 30- Olanzapine 3 mg/kg + Ranitidine 30 mg/kg

Fig 9(d): Effect of ranitidine on serum VLDL-C level to Olanzapine treated rats at the end of 5 weeks



Each group contain 6 numbers of animals

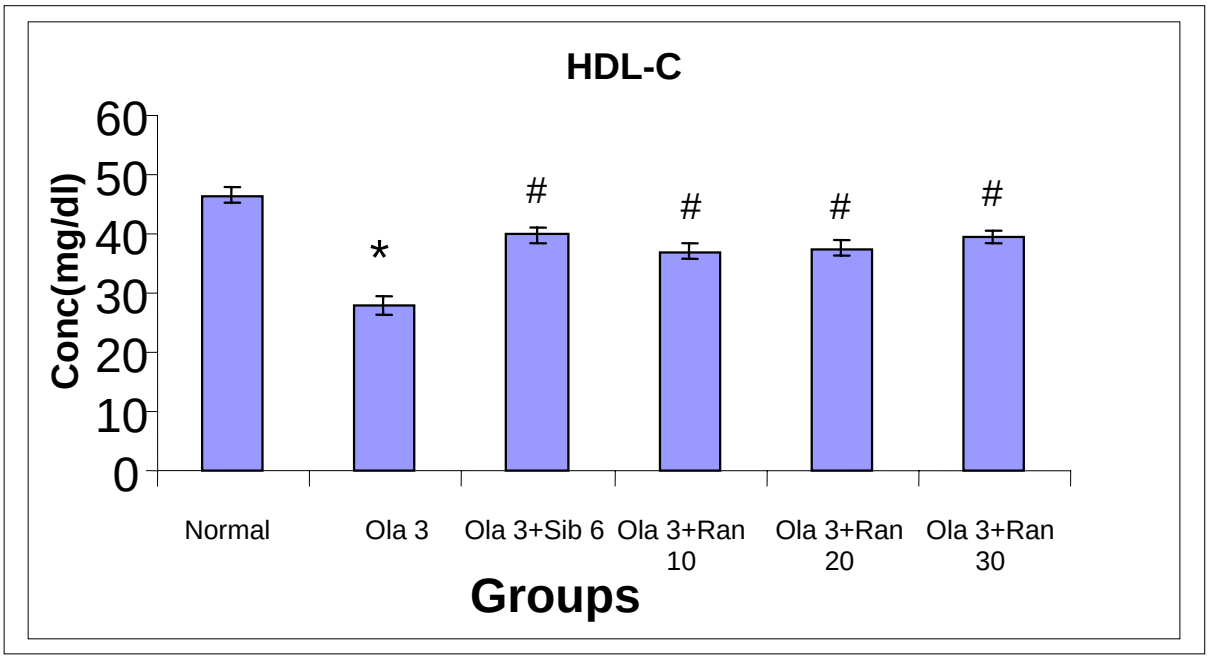
Values are expressed as Mean ± SEM

* indicates significantly different from normal group (P<0.05, One way ANOVA followed by Dunnett's test)

# indicates significantly different from Olanzapine treated group (P<0.05, One way ANOVA followed by Dunnett's test)

Normal-Normal group
Ola 3-Olanzapine 3 mg/kg
Ola 3 + Sib 6- Olanzapine 3 mg/kg + Sibutramine 6 mg/kg
Ola 3 + Ran 10- Olanzapine 3 mg/kg + Ranitidine 10 mg/kg
Ola 3 + Ran 20- Olanzapine 3 mg/kg + Ranitidine 20 mg/kg
Ola 3 + Ran 30- Olanzapine 3 mg/kg + Ranitidine 30 mg/kg

Fig 9(e): Effect of ranitidine on serum HDL-C level to Olanzapine treated rats at the end of 5 weeks



Each group contain 6 numbers of animals

Values are expressed as Mean ± SEM

* indicates significantly different from normal group (P<0.05, One way ANOVA followed by Dunnett's test)

# indicates significantly different from Olanzapine treated group (P<0.05, One way ANOVA followed by Dunnett's test)

Normal-Normal group
Ola 3-Olanzapine 3 mg/kg
Ola 3 + Sib 6- Olanzapine 3 mg/kg + Sibutramine 6 mg/kg
Ola 3 + Ran 10- Olanzapine 3 mg/kg + Ranitidine 10 mg/kg
Ola 3 + Ran 20- Olanzapine 3 mg/kg + Ranitidine 20 mg/kg
Ola 3 + Ran 30- Olanzapine 3 mg/kg + Ranitidine 30 mg/kg

5.5 Serum glucose level:

Normal value of serum sugar level was found to be 88.63 ± 13.47 mg/dl. Olanzapine (3 mg/kg) treated group showed significant increased in serum glucose level (137.94 ± 5.34 mg/dl) compared to normal group at the end of five week of treatment. There was significant decrease in sugar level with treatment of ranitidine compared to olanzapine treated group. Fig 10 shows dose dependent decrease in sugar level with treatment of ranitidine (10, 20, 30 mg/kg p.o.). Standard drug sibutramine (6 mg/kg p.o.) also significantly decreased in sugar level compared to olanzapine group (Table 13).

Table 13: Effect of ranitidine on serum glucose level to Olanzapine treated rats at the end of 5 weeks

Groups	Serum glucose level (mg/dl)
Normal	88.63 ± 13.47
Ola 3	137.94 ± 5.34 *
Ola 3 + Sib 6	105.55 ± 4.58 #
Ola 3 + Ran 10	105.55 ± 6.60 #
Ola 3 + Ran 20	91.79 ± 12.46 #
Ola 3 + Ran 30	87.68 ± 13.07 #

Each group contain 6 numbers of animals

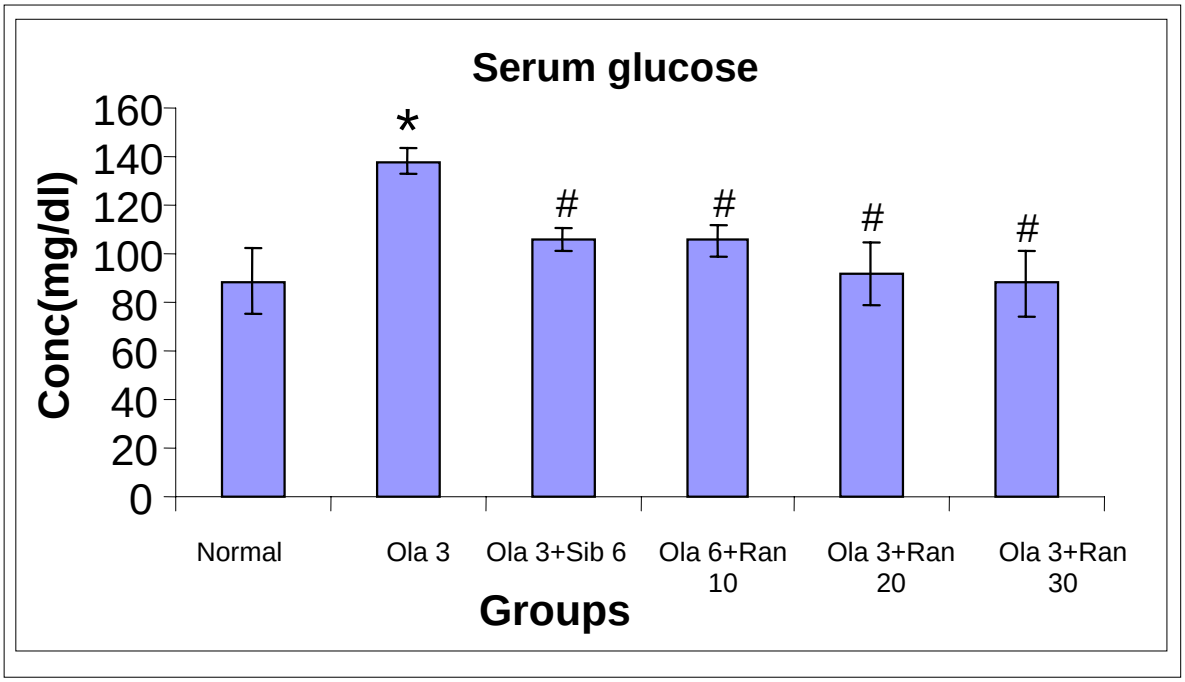
Values are expressed as Mean ± SEM

* indicates significantly different from normal group (P<0.05, One way ANOVA followed by Dunnett's test)

# indicates significantly different from Olanzapine treated group (P<0.05, One way ANOVA followed by Dunnett's test)

Normal-Normal group
Ola 3-Olanzapine 3 mg/kg
Ola 3 + Sib 6- Olanzapine 3 mg/kg + Sibutramine 6 mg/kg
Ola 3 + Ran 10- Olanzapine 3 mg/kg + Ranitidine 10 mg/kg
Ola 3 + Ran 20- Olanzapine 3 mg/kg + Ranitidine 20 mg/kg
Ola 3 + Ran 30- Olanzapine 3 mg/kg + Ranitidine 30 mg/kg

Fig 10: Effect of ranitidine on serum glucose level to Olanzapine treated rats at the end of 5 weeks



Each group contain 6 numbers of animals

Values are expressed as Mean ± SEM

* indicates significantly different from normal group (P<0.05, One way ANOVA followed by Dunnett's test)

# indicates significantly different from Olanzapine treated group (P<0.05, One way ANOVA followed by Dunnett's test)

Normal-Normal group
Ola 3-Olanzapine 3 mg/kg
Ola 3 + Sib 6- Olanzapine 3 mg/kg + Sibutramine 6 mg/kg
Ola 3 + Ran 10- Olanzapine 3 mg/kg + Ranitidine 10 mg/kg
Ola 3 + Ran 20- Olanzapine 3 mg/kg + Ranitidine 20 mg/kg
Ola 3 + Ran 30- Olanzapine 3 mg/kg + Ranitidine 30 mg/kg

5.6 Blood pressure:

Normal mean blood pressure in rats observed was 128.71 mmhg. Results show that, olanzapine (3mg/kg p.o.) at the end of five week of treatment raised the mean blood pressure significantly compared to normal group. Ranitidine reduced the blood pressure in dose dependent manner, but significant decrease in blood pressure was observed only at dose of 30 mg/kg. Standard drug Sibutramine increased the blood pressure higher than olanzapine treated group (Table 14).

Table 14: Effect of ranitidine on Blood pressure to Olanzapine treated rats at the end of 5 weeks

Groups	Mean Blood Pressure (mmhg)
Normal	128.71 ± 4.01
Ola 3	146.64 ± 3.49 *
Ola 3 + Sib 6	166.35 ± 7.68
Ola 3 + Ran 10	130.29 ± 2.70
Ola 3 + Ran 20	112.06 ± 11.24
Ola 3 + Ran 30	109.82 ± 15.74 #

Each group contain 6 numbers of animals

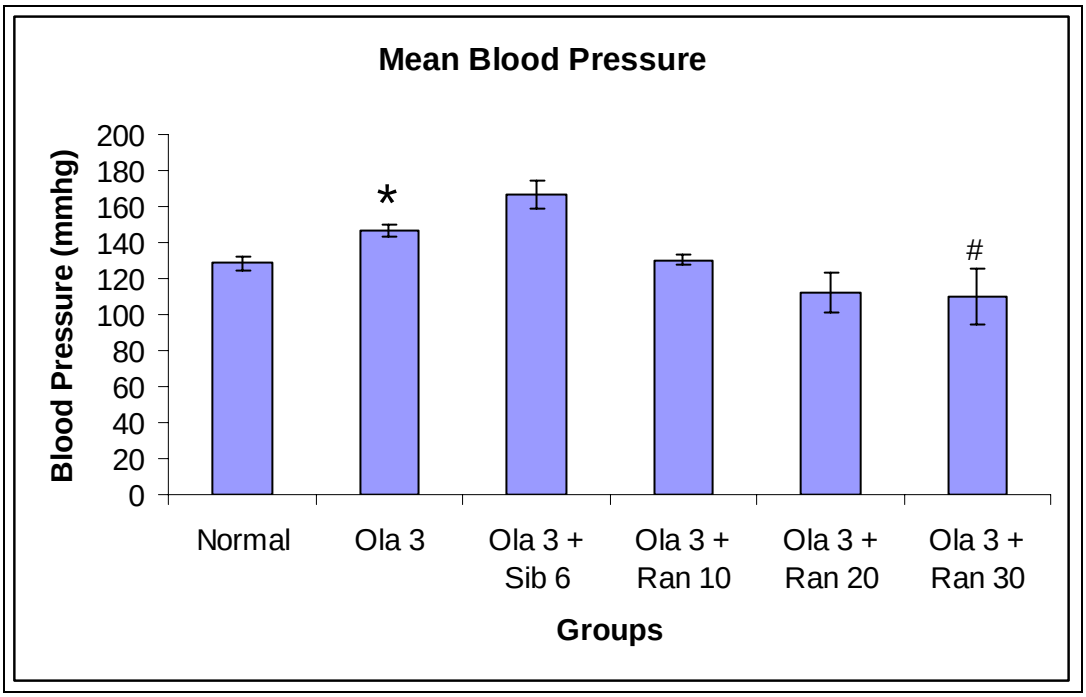
Values are expressed as Mean ± SEM

* indicates significantly different from normal group (P<0.05, One way ANOVA followed by Dunnett's test)

# indicates significantly different from Olanzapine treated group (P<0.05, One way ANOVA followed by Dunnett's test)

Normal-Normal group
Ola 3-Olanzapine 3 mg/kg
Ola 3 + Sib 6- Olanzapine 3 mg/kg + Sibutramine 6 mg/kg
Ola 3 + Ran 10- Olanzapine 3 mg/kg + Ranitidine 10 mg/kg
Ola 3 + Ran 20- Olanzapine 3 mg/kg + Ranitidine 20 mg/kg
Ola 3 + Ran 30- Olanzapine 3 mg/kg + Ranitidine 30 mg/kg

Fig 11: Effect of ranitidine on Blood pressure to Olanzapine treated rats at the end of 5 weeks



Each group contain 6 numbers of animals

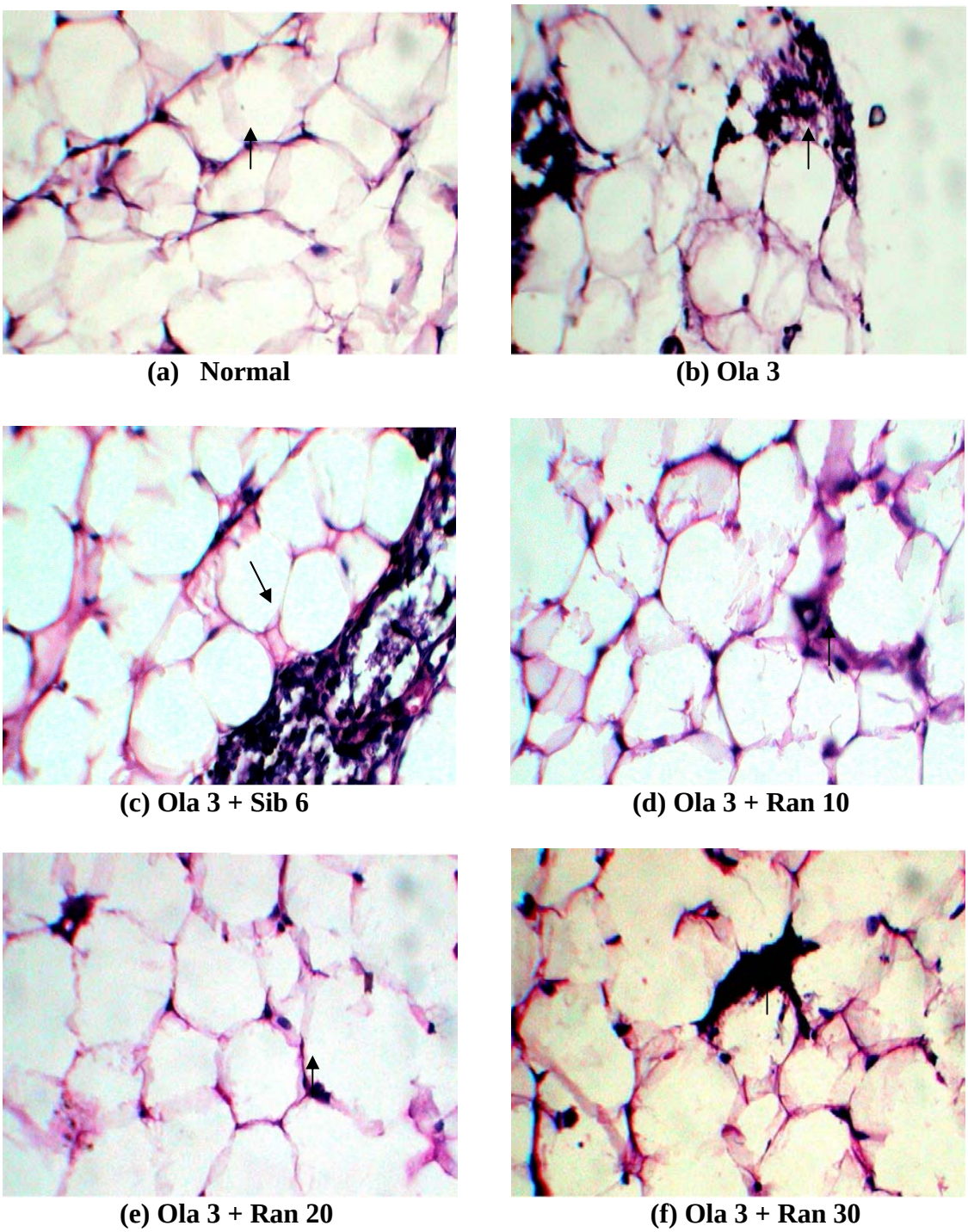
Values are expressed as Mean ± SEM

* indicates significantly different from normal group (P<0.05, One way ANOVA followed by Dunnett's test)

# indicates significantly different from Olanzapine treated group (P<0.05, One way ANOVA followed by Dunnett's test)

Normal-Normal group
Ola 3-Olanzapine 3 mg/kg
Ola 3 + Sib 6- Olanzapine 3 mg/kg + Sibutramine 6 mg/kg
Ola 3 + Ran 10- Olanzapine 3 mg/kg + Ranitidine 10 mg/kg
Ola 3 + Ran 20- Olanzapine 3 mg/kg + Ranitidine 20 mg/kg
Ola 3 + Ran 30- Olanzapine 3 mg/kg + Ranitidine 30 mg/kg

5.7 Histochemical Examination



(a) Normal **(b) Ola 3**

(c) Ola 3 + Sib 6 **(d) Ola 3 + Ran 10**

(e) Ola 3 + Ran 20 **(f) Ola 3 + Ran 30**

Fig. 12 Histochemical examination of subcutaneous adipose tissue.
↑ = nucleus and extracellular matrix

The Histochemical examination revealed the difference between adipose tissue obtained from normal and olanzapine treated groups (3 mg/kg p.o.). More nuclei and extracellular matrix were observed surrounding the adipocytes in olanzapine treated groups compared to normal group (Fig 12a, b). Treatment with ranitidine (10, 20, 30 mg/kg p.o) lower nuclei and extracellular matrix surrounding the adipocytes compared to olanzapine treated group (Fig 12d, e, f). While sibutramine treatment (6 mg/kg p.o.) showed more nuclei and extracellular matrix (Fig 12c)

Chapter:-6 Discussion

The results of the present study indicate that weight gain can be induced in female wistar rats by giving olanzapine at dose of 3 mg/kg p.o. for 3 weeks. Significant increase in weight gain and BMI was observed during the period of 3rd to 5th week of treatment (Fig 7, 8). Body mass index (BMI) is a measure of body fat based on height and weight that applies to both adult men and women. BMI is frequently used to assess how much an individual's body weight departs from what is normal or desirable for a person of his or her height. The excess weight or deficiency may, in part, be accounted for by body fat. So, increase in BMI indicates fat deposition in the body during treatment with olanzapine. Clinical data also shows that treatment with olanzapine in schizophrenic patient raised body weight and BMI [98, 131]. Also, food intake was raised after second week of treatment with olanzapine. However, no consistent increase in food intake was observed during the whole period of treatment. Nevertheless, the finding of this study is consistent with a number of clinical observations and animal studies that olanzapine induces short-term hyperphagia in both patients and rodents. Two clinical studies indicated that although inconsistent with time course of treatment, increase in caloric intake was observed in treated patients, and there was no change in physical activity of these patients [98, 131].

Increased triglyceride (TG) level, small LDL particle diameter, and decreased HDL-C levels appear to reflect underlying metabolic perturbations with adverse consequences for risk of myocardial infarction [132]. The present study shows that olanzapine causes significant increase in serum total cholesterol (TC), triglyceride (TG), LDL-C and VLDL-C and decrease the level of HDL-C compared to normal group (Table 12). Clinical study supports this effect of olanzapine [82].

Hyperglycemia is a common side effect with most atypical antipsychotics [75]. Hyperglycemia, exacerbation of existing diabetes, new-onset type 2 diabetes, and diabetic ketoacidosis have all been associated with newer antipsychotic medications, with multiple reports for clozapine and olanzapine, and more limited reports of significant hyperglycemia for quetiapine and risperidone [76, 77]. The present study shows significant increase in blood sugar level following compared to normal group of animals (Fig 10). Also, present study shows that there is increase in mean blood pressure. Increase in blood pressure was significant when compared to normal group (Table 14).

The deposition of white adipose tissues is known to be dependent on gender. In humans, abdominal fat mass is predominant in the male, while subcutaneous fat mass is more prominent in the female [133]. Increased abdominal adipose tissue, especially visceral abdominal adiposity can increase insulin resistance and contribute to hyperglycemia and diabetes [134]. It present study, olanzapine treated group show marked nuclei and extracellular matrix surrounding the adipocytes (Fig 12a, b) compare to normal group. This is due to that, adipocytes differentiation might be influenced by olanzapine

At present, there is no standardized pharmacological treatment for antipsychotic-related body weight gain. Some studies have assessed the effects of agents such as amantadine, orlistat, metformin, nizatidine, and topiramate as pharmacological alternatives to manage this adverse event [15, 116, 121, 124, 135]. Treating weight gain with pharmacological agents in psychiatric patients must be done with caution as some drugs used to this purpose may exacerbate the psychiatric condition, as their primary site of action is the central nervous system. Thus, the use of a medication to manage weight gain in psychiatric patients without central activity would be of particular interest. Previous studies have shown that H_2 antagonists might be related to weight loss in humans [14, 18]. Causing weight loss by a mechanism that is yet to be defined; H_2 antagonists are more likely to have their primary site of action outside the central nervous system, as H_2 antagonists as a class are very hydrophilic and cross the blood brain barrier to a limited extent [136]. Due to their peripheral site of action, H_2 antagonists were used in some studies to manage weight gain in schizophrenic patients on olanzapine [127]. In present study, rats were treated with olanzapine by oral route for three weeks; then ranitidine was given at three different dose levels (10, 20, 30 mg/kg) in rats along with olanzapine for two weeks. Results show that, at the end of 5 weeks of study, ranitidine treatment in rats with olanzapine significantly reduced the body weight and BMI. In only olanzapine treated animal % raise in body weight was 18.95, while in ranitidine treated animal % raise in body weight was 5.98, 4.86, and 6.93. Which was significantly lower than only olanzapine treated group. Same results were obtained for BMI (Table 10, 11). Clinical study also supports this result [17]. Results also show a marked decrease in food intake compared to only olanzapine treated group

Results show that, co-administration with ranitidine lowers the hyperlipidemia due to olanzapine treatment. Co-administration with ranitidine showed significant

decrease in TC, TG, LDL-C, and VLDL-C, which are major risk factors for congestive heart failure, and it also; increases the level of HDL-C.

Diabetes is the major risk factor for schizophrenic patients medicated with olanzapine [75]. The present study shows that, treatment with ranitidine significantly lowers the blood sugar level from hyperglycemia to normal level (Fig 10). Mechanism is not understood, but probably this can be attributed to the weight reducing effect of ranitidine. Ranitidine treatment also lowered the blood pressure to normal level (Fig 11).

Ranitidine treatment showed less nuclei and extracellular matrix surrounding the adipocytes compared to olanzapine treated group (Fig 12d, e, f). So, it might be possible that, ranitidine treatment can decrease the adipocytes differentiation and fat deposition in subcutaneous adipose tissue and ultimately insulin resistance and hyperglycemia.

In present study, Sibutramine was used as a standard drug. Treatment with Sibutramine in olanzapine induced weight gain significantly lowered all the parameters measured in present study except blood pressure. Supportive clinical data have been obtained for Sibutramine [114]. In present study, Sibutramine markedly increased the blood pressure when compared to olanzapine treated hypertensive rats. This can be a major drawback of Sibutramine when used as an adjuvant treatment in schizophrenia along with olanzapine. The results of the present preclinical study favor ranitidine as a better adjuvant therapy in schizophrenic patients with least side effects and better tolerability. But detailed clinical studies are further warranted.

Chapter:-7 Conclusion

Present study showed that, ranitidine an adjuvant therapy for the treatment of schizophrenia along with olanzapine may be useful to reverse the weight gain and hyperlipidemia, which are the major risk factors for congestive heart failure. Co-administration of ranitidine also lowered the blood sugar level. So, it may be help to reverse the hyperglycemia which are the major side effects of olanzapine. Ranitidine may also be useful to lower the blood pressure which is elevated in the olanzapine treatment. So, add-on therapy of ranitidine could be an effective option for the control of weight gain and other metabolic side effects in olanzapine treated patients.

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