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Study of effect of pioglitazone on pharmacokinetic and antidepressant activity of desvenalofaxine

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ABSTRACT

The interaction processes of Pioglitazone with Desvenalofaxine have been investigated practically. Some studies reported that depression and Diabetes disorders present in same patient. Therefore, both the disorders are managed clinically by administrating numbers of drugs for long duration. In such a scenario, there is a possibility that one drug may alter the effects of other drugs. The presence of depression resulted in a significant deterioration in quality of life in individuals with diabetes. Depression and diabetes are common conditions that often coexist and may clinically interact with each other. It is found that both the drugs are metabolized by common enzymes CYP3A4.The possibility of these two drugs to interact is by alteration in the absorption site, replacement at protein binding site (distribution) also at metabolism site and elimination site. The study on antidepressant activity will help to predict the extent of interaction whereas the study on pharmacokinetics parameters will help to predict the mechanism of action of drug interaction and help to support the pharmacodynamic interaction. So, it is very much significant and justified to conduct experiment on animals to verify the drug-drug interaction between Pioglitazone and Desvenlafaxine.

Keywords: Depression, Diabetes, Pioglitazone, Desvenlafaxine and CYP3A4.

1. INTRODUCTION

Depression is a condition with high prevalence worldwide. It is a major cause of morbidity, mortality and disability [1] and is associated with workplace absenteeism, diminished or lost work productivity and increased use of healthcare resources[1] . In 2000, it was estimated that depressive disorders were higher in women (4930 per 100,000) than men (3199 per 100,000) and that globally depressive disorders were the fourth leading cause of disease burden in women and seventh leading cause in men [2].

The presence of depression resulted in a significant deterioration in quality of life in individuals with diabetes. Diabetes and depression are debilitating conditions that are associated with significant morbidity, mortality, and healthcare costs. Co-existing depression in people with diabetes is associated with decreased adherence to treatment, poor metabolic control, elevated complication rates, decreased quality of life, increased healthcare use and cost, increased disability and lost productivity, and increased risk of death. In a systematic review designed to estimate the prevalence of clinically depressed patients with type-2 diabetes, research shown that the prevalence of depression was significantly higher among patients with type-2 diabetes (17.6%) than those without diabetes (9.8%) [3].

More than 300 years ago Dr. Thomas Willis, a British physician made the observation that there was a relationship between diabetes and depression when he suggested that diabetes was the result of ‘‘sadness or long sorrow’’.

Worldwide estimates of depression prevalence among individuals with diabetes appear to vary by diabetes type and among developed and developing nations. [4] Evidence

suggests a bi-directional relationship between depression and type-2 diabetes. Researchers suggests that in addition to depression being a consequence of diabetes, depression may also be a risk factor for the onset of diabetes [5] . A review of studies from 1950 to 2007 of diabetes and depression to examine the bi-directional relationship between diabetes and type-2 diabetes[6] concluded the pooled relative risk for incident depression associated with baseline diabetes was 1.15 (95% CI 1.02–1.30) while the relative risk for incident diabetes associated with baseline depression was 1.60 (95% CI 1.37–1.88).Depression was associated with a 60% increase of type-2 diabetes while type-2 diabetes was only associated with a moderate (15%) risk of depression[1].

This bidirectional relationship was confirmed among individuals without elevated depressive symptoms at baseline, patients treated for diabetes had higher odds of developing depressive symptoms during the follow-up period [7] . In contrast, individuals with impaired fasting glucose and those with untreated diabetes had reduced risk of incident depressive symptoms. The authors found that these findings were comparable across racial/ethnic groups.

Two major hypotheses currently exist to explain the causal pathway between diabetes and depression[8]. One hypothesis asserts that depression precedes type-2 diabetes (i.e. depression increases the risk of developing diabetes). Unfortunately, the mechanisms underlying the association between diabetes and depression are not clearly understood [1]. In theory, the increased risk of type-2 diabetes in individuals with depression is believed to result from increased counter regulatory hormone release and action, alterations in glucose transport function, and increased immunoinflammatory activation[8] .These physiologic alterations are thought to contribute to insulin resistance and beta islet cell dysfunction, which ultimately lead to the development of type-2 diabetes.

The second hypothesis is that depression in patients with both type-1 and type-2 diabetes results from chronic psychosocial stressors of having a chronic medical condition.

Diabetes complications are also greater among individuals with depression. In a meta-analysis of 27 studies including adults with type-1 and type-2 diabetes, there were significantly greater diabetes complications including: diabetic retinopathy, nephropathy, neuropathy, micro vascular complications and sexual dysfunction [9]. The onset and prevalence of coronary heart disease was affected in women with diabetes who were depressed [1]. Recent studies have shown that coexisting depression increases the risk of death among people with diabetes [11].

Drug–drug interactions may occur when more than one drug is administered in a patient to treat a single ailment or multiple ailments[12]. The concomitant use of several drugs is often desired to obtain a therapeutic objective or to treat co-existing diseases. Simultaneous use of several drugs may lead to drug–drug interactions. There are several diseases that require lifelong treatment such as diabetes and depression. Patients with such diseases will often need to be administered drugs for treatment of other co-existing diseases, either for a short period or lifelong.

Table - 1: Antidepressant drug use in patients with diabetes mellitus type 1 the effect of medication on mental problems and glycemic control

Antidepressant drugs	Important effects in diabetic patients
TCA (amitriptyline)	Increase glycemia, increase body weight
SSRI(citalopram, sertraline)	Reduce glycemia, increase body weight
IMAO (moclobemide)	Reduce glycemia, do not increase body weight
NDRI (bupropion)	Do not increase body weight
NaSSA (mirtazapine)	Increase body weight and glycosylated hemoglobin
SNRI(venlafaxine, duloxetine)	Do not increase body weight, duloxetine increases fasting glycemia

The major CYP enzymes involved in human drug metabolism are CYP3A4, CYP2D6, CYP2C8, CYP2C9, CYP2C19, CYP1A2 and CYP2B6. The liver is the main organ responsible for CYP-mediated drug metabolism. However, there are significant amounts of CYP enzymes in other tissues as well. For example, the enterocytes in the small intestine are an important site of metabolism for many drugs. CYP activities can vary markedly between individuals due to genetic and environmental factors and some diseases, which can lead to major differences in drug response and adverse effects.

Inhibition of CYP enzymes can lead to increased plasma concentrations of drugs metabolized by the same enzymes, thereby enhancing their pharmacological effects and increasing the likelihood of adverse effects. Induction of CYP enzymes can reduce the plasma

concentrations and effects of substrate drugs. Some prodrugs need to be metabolically activated by CYP enzymes, and inhibition of their metabolism can reduce their effects, while induction can either enhance or reduce their effects and toxicity, depending on the effects of induction on the further elimination of the active metabolite.

2. Depression

2.1. Definition

Depression is an illness that involves the body, mood, and thoughts, that affects the way a person eats and sleeps, the way one feels about oneself, and the way one thinks about things. The signs and symptoms of depression include loss of interest in activities that were once interesting or enjoyable. The principle types of depression are major depression, dysthymia, and bipolar disease (also called manic-depressive or manic depression disease). Depression is the most common of the affective disorders (defined as disorders of mood rather than disturbances of thought or cognition); it may range from a very mild condition, bordering on normality, to severe (psychotic) depression accompanied by hallucinations and delusions [13].

Mania in most aspects is exactly opposite, with excessive exuberance, enthusiasm, and self-confidence, accompanied by impulsive actions. These signs are often being combined with mania [14].

2.1.1. Classification of depression

Major affective disorders

Bipolar disorder

Manic Depressed Mixed

Major depression

- Single episode or recurrent With or without melancholia With or without psychotic features

Other specific affective disorders

Cyclothymic disorder Dysthymic disorder

Atypical affective disorders

- Atypical bipolar disorder
- Atypical depression.

2.1.2. Burden of depression

Among the well-known burdens caused by depression are patient suffering, family distress and conflict, impaired cognitive development of young children in cases of postpartum depression and the strikingly increased risk of suicide. More recent studies have examined the impact on functioning and the economic burdens. Patients with depression had functioning scores about the same as those with advanced coronary artery disease, scores that were in turn lower than all other conditions studied, including hypertension, diabetes mellitus, and arthritis. This impairment in functioning, when coupled with the high prevalence, chronic or relapsing course, and frequent early onset, led a group of World Health Organization researchers to conclude that unipolar major depression is the leading cause of disability worldwide. Functional improvement occurs with effective treatment.

When depression co-occurs with other general medical conditions, patient adherence to treatment is worsened, chances for improvement or recovery from the other condition are lessened, and health care costs are further increased. One study in a large group health maintenance organization (HMO) compared two groups of “high utilizes” (i.e., patients whose annual medical expenses were above the HMO median).

Costs for high utilizers who were depressed were $1,500 higher per year than for those who were not depressed. Health care costs in patients with depression and co-occurring medical illness are increased even when the nature and severity of the medical condition are controlled.

2.1.3. Symptoms and signs of depression

physical

- Sleep disturbances-insomnia, oversleeping, waking much earlier than usual
- Changes in appetite or eating: much more or much less

Decreased energy, fatigue Headaches, stomachaches, digestive problems or other physical symptoms that are not explained by other physical conditions or do not respond to treatment

Behavioral/Attitude

- Loss of interest or pleasure in activities that were once enjoyed, such as going out with friends, hobbies, sports, sex, etc.
- Difficulty in concentrating, remembering, or making decisions Neglecting responsibilities or personal appearance.

Emotional

- Persistent sad or "empty" mood, lasting two or more weeks
- Crying "for no reason"
- Feeling hopeless, helpless, guilty or worthless
- Feeling irritable, agitated or anxious
- Thoughts of death or suicide

Depression causes cognitive, psychomotor and other types of dysfunctions (eg, poor concentration, fatigue, loss of sexual desire, menstrual abnormalities) as well as a depressed mood. Other mental symptoms or disorders (eg, anxiety and panic attacks) commonly coexist, sometimes complicating diagnosis and treatment. Patients with all forms of depression are more likely to abuse alcohol or other recreational drugs in an attempt to self-treat sleep disturbances or anxiety symptoms; however, depression is a less common cause of alcoholism and drug abuse than was once thought. Patients are also more likely to become heavy smokers and to neglect their health, increasing their risk of development or progression of other disorders [eg. Chronic obstructive pulmonary disease (COPD)]. Depression may reduce protective immune responses. Depression increases the risk of Myocardial infarction and stroke because cytokines and factors that increase blood clotting are released during depression.

Major depression (unipolar disorder), periods (episodes) that include 5 mental or physical symptoms that last 2 weeks are classified as major depression. Symptoms must include sadness deep enough to be described as despondency or despair (often called depressed mood) or loss of interest or pleasure in usual activities.

2.1.4. Major depression is often divided into subgroups

Psychotic subgroup

It is characterized by delusions, often of having committed unpardonable sins or crimes, harboring incurable or shameful disorders, or of being persecuted. Patients may have auditory or visual hallucinations (e.g., accusatory or condemning voices).

Catatonic subgroup

It is characterized by severe psychomotor retardation or excessive purposeless activity, withdrawal, and, in some patients, grimacing and mimicry of speech (echolalia) or movement (echopraxia).

Melancholic subgroup

It is characterized by loss of pleasure in nearly all activities, inability to respond to pleasurable stimuli, unchanging emotional expression, excessive or inappropriate guilt, early morning awakening, marked psychomotor retardation or agitation, and significant anorexia or weight loss.

Atypical subgroup

It is characterized by a brightened mood in response to positive events and rejection sensitivity, resulting in depressed overreaction to perceived criticism or rejection, feelings of leaden paralysis or energy, weight gain or increased appetite, and hypersomnia.

Dysthymia

Low-level or sub threshold depressive symptoms are classified as dysthymia. Symptoms typically begin insidiously during adolescence and follow a low-grade course over many years or decades (diagnosis requires a course of 2years); dysthymia may intermittently be complicated by episodes of major depression. Affected patients are habitually gloomy, pessimistic, humorless, passive, lethargic, introverted and hypercritical of self and others, and complaining.

2.1.5. Etiology

The exact cause is unknown. Heredity has an uncertain role; depression is more common among 1st degree relatives of depressed patients and concordance between identical twins is high. Hereditary genetic polymorphisms for the serotonin transporter active in the brain may be triggered by stress. People who have a history of child abuse or other major life stresses and have the short allele for this transporter are about twice as likely to develop depression as those who have the long allele. Other theories focus on changes in neurotransmitter levels, including abnormal regulation of cholinergic catecholaminergic (noradrenergic or dopaminergic) and serotonergic (5-hydroxytryptamine) neurotransmission. Neuroendocrine deregulation may be a factor, with particular emphasis on 3 axes: hypothalamic-pituitary-adrenal, hypothalamic – pituitary - thyroid, and growth hormone. Psychosocial factors also seem involved major life stresses, especially separations and losses, commonly precede episodes of major depression however, such events do not usually cause lasting, severe depression except in people predisposed to a mood disorder.

People who have had an episode of major depression are at higher risk of subsequent episodes. People who are introverted and who have anxious tendencies may be more likely to develop a depressive disorder. Such people often lack the social skills to adjust to life pressures. Depression may also develop in people with other mental disorders. Women are at higher risk, but no theory explains the reason, possible factors include greater exposure to or heightened response to daily stresses, higher levels of monoamine oxidase (the enzyme that degrades neurotransmitters considered important for mood) and endocrine changes that occur with menstruation and at

menopause. In postpartum depression, symptoms develop within 4 weeks after delivery; endocrine changes have been implicated, but the specific cause is unknown. Also, thyroid function is more commonly deregulated in women.

In seasonal affective disorder, symptoms develop in a seasonal pattern, typically during autumn or winter; the disorder tends to occur in climates with long or severe winters. Depressive symptoms or disorders may occur with various physical disorders, including thyroid and adrenal gland disorders, benign and malignant brain tumors, stroke, AIDS, Parkinson's disease, and multiple sclerosis. Certain drugs, such as corticosteroids, some ß -blockers, antipsychotics (especially in the elderly), and reserpine, can also result in depressive disorders. Abuse of some recreational drugs (eg: alcohol, amphetamines) can lead to or accompany depression. Toxic effects or withdrawal of drugs may cause transient depressive symptoms [16].

2.1.6. Diagnosis

Diagnosis is based on identifying the symptoms and signs described above. Several brief questionnaires are available for screening. They help elicit some depressive symptoms but cannot be used alone for diagnosis. Severity is assigned by the degree of pain and disability (physical, social, and occupational); duration of symptoms also helps determine severity. The presence of suicidal risk indicates that the disorder is severe. A physician should gently but directly ask patients about any thoughts and plans to harm themselves or others. Psychosis and catatonia indicate severe depression [17].

2.1.7 Biological basis of depression

Monoamine hypothesis

There are many theories and hypothesis regarding the pathophysiology of depression. However, for most of the last 50 years, the biological approach to depression has been dominated by the monoamine hypothesis. This hypothesis proposes that depression is caused by the low levels of one or more functional deficit in monoamine neurotransmitters; serotonin, noradrenaline and dopamine, at key sites in the brain could produce depression. The monoamine hypothesis of depression proposed the brain monoamine neurotransmitters; serotonin, noradrenaline and dopamine could produce depression [17].

The hypothesis could neither explain why up to 2-3 weeks of continued medication was needed to alleviate depressive symptoms, even though monoamine changes often occur within 1-2 days nor why other drugs such as cocaine and amphetamine that enhanced serotonergic or noradrenergic transmission are not effective in treating depression.

Furthermore, the hypothesis could not explain why antidepressants are effective in other disorders, such as social phobia, why other drugs such as tianeptine are active even though they are thought to enhance serotonin reuptake, an effect opposite to the SSRI antidepressants. Neither can the hypothesis explain why the densities of some serotonin (5-HT) receptors are increased by long-term electroconvulsive therapy, one of the most effective treatments of depression.

Nevertheless, despite these serious limitations, the monoamine hypothesis stimulated the development of safer antidepressants like the SSRIs, such as, citalopram, fluoxetine, fluvoxamine, paroxetine and sertraline, the selective noradrenaline reuptake inhibitors and the dual action antidepressants, venlafaxine and milnacipran, that modify both central noradrenergic and serotonergic systems.

Hypothalamic-Pituitary-Adrenal (HPA) Axis:

The association between abnormality of HPA axis and depression has long been known. There were well documented abnormalities of HPA axis function in depressed patients, such as, enhance 24-hour urinary free cortisol and raised serum cortisol levels, impaired dexamethasone suppression and blunting of adrenocortico-trophic hormone (ACTH) release in response to corticotrophin-releasing factor (CRF) challenge. Chronic stress or stressful life events such as loss of parents and child physical or sexual abuse with the onset of depression and its severity. It has been suggested that such events might cause long-lasting alterations in CRF containing neurons, thereby increasing an individual's vulnerability to stressors later in life.

Two hypotheses have been proposed as pathophysiological explanations for the HPA over activity observed in depression. The first involves increased brain CRF driving the HPA axis into "overdrive"; it is suggested that CRF may provide a link between the monoamine and neuroendocrine theories of depression since CRF appears to regulate tyrosine hydroxylase, the rate-limiting enzyme in the synthesis of noradrenaline [17]. The second hypothesis suggests impaired negative feedback at both the pituitary corticotrophin and central glucocorticoid receptor levels, and it is proposed that a primary alteration in glucocorticoid receptor (GR) or mineralocorticoid receptor (MR) number or function may contribute to the pathophysiology of depression. However, a study by Dinan and co-workers suggests there is a switch from CRF to vasopressin (AVP) regulation of

HPA axis during depression, implying that a CRF antagonist would be unlikely to correct the HPA disturbance observed in depression whereas a blockade of AVP receptor might offer a more appropriate pharmacological approach.

Other neuroendocrine axis and non-amine neurotransmitters

It is well documented that hypothyroidism can lead to major depression and could be reversed by thyroxine treatment. There is some evidence of a dysfunction of the hypothalamic-pituitary-thyroid (HPT) axis in major depression. In some patients with major depression, slightly elevated thyroxine (T4) levels, a blunted response of thyroid stimulating hormone (TSH) to thyrotropin-releasing hormone (TRH), and loss of normal nocturnal surge in TSH levels are reported.

Decrease in T4, and free T4, levels after antidepressant treatment is also reported. There are other non-amine neurotransmitters that are hypothesised to be involved in pathophysiology of major depression. Recently, an antagonist of substance P, a co-transmitter with serotonin (neurotransmitter) involved in the transmission of pain, has been reported to have antidepressant qualities in placebo- controlled trials in patients with moderate to severe depression. The involvement of GABA and glutamatergic neurotransmission in pathophysiology of depression is also proposed.

A neurotrophic factor, brain derived neurotrophic factor (BDNF) is also proposed to be involved in pathophysiology of depression. An experimental study demonstrated that centrally administered BDNF attenuated the depressive behavior. In addition, chronic antidepressant treatment has been shown to increase BDNF expression, particularly in the hippocampus.

Neurogenesis and neuroplasticity hypothesis

The neurogenesis hypothesis proposes that (the adult) neurogenesis is impaired in depression and is responsible for the hippocampal structural changes, which are reversible in the remission stage of the disease. It has been shown in some studies that treatment with antidepressants, Electroconvulsive therapy (ECT) and increased physical activity, can stimulate the proliferation of hippocampal progenitor cells, which contribute to the first stage of adult neurogenesis.

Enhanced activity of the serotonergic system has also been reported to improve adult hippocampal neurogenesis. However, in addition to the hippocampus, other regions in the brain also show changes in depression. The alterations in prefrontal neurons and glial cell numbers, and substantially reduced glial cell density in the amygdale that are moderately improved by mood stabilizers, are also reported to occur in major depression. Moreover, in major depression, there are changes in other neurotrophic factors such as BDNF that can influence the neuronal plasticity.

Based on the above factors, severe and chronic disturbance of cellular plasticity, including reduced neurogenesis, forms the basis of the neurogenesis and cellular plasticity hypothesis of major depression. However, whether these changes are the cause or consequence of depression is presently unclear. In addition, the mechanism whereby adult neurogenesis and plasticity could be involved in major depression and how this hypothesis can be applied diagnostically is unclear.

Macrophage theory of depression and role of inflammatory changes

The macrophage theory of depression is the first theory that brings the role of immune system and inflammatory changes into pathophysiology of depression. In this hypothesis, IL-1ß, which is secreted from the macrophages, directly stimulates the CRF secretion in the hypothalamus and induces hyperactivity of the HPA axis. This theory therefore links the immune system with the neuroendocrine and neurotransmitter changes in major depression.

Neurodegeneration hypothesis of depression

The interaction between brain 5-HT level and the activity of its autoreceptors plays a role in mood changes and depression. In major depression, activation of the Inflammatory Response System (IRS) and, increased concentrations of proinflammatory cytokines, prostaglandin E2 and negative immuno-regulatory cytokines in peripheral blood have been reported. Recently, pro-inflammatory cytokines have been found to have profound effects on the metabolism of brain serotonin through the enzyme indoleamine 2,3-dioxygenase (IDO) that metabolizes the tryptophan, the precursor of 5-HT to neurodegenerative quinolinate and neuroprotective kynurenate. The cytokine-serotonin interaction that leads to the challenge between quinolinate and kynurenate in the brain explains the neurodegeneration hypothesis of depression [17].

The cytokine-serotonin interaction through the enzyme IDO that results in tryptophan depletion into kynurenine pathway plays an important role in pathophysiology of depression. Chronic psychological stress or medical illness can result in a rise of pro- inflammatory cytokines and then in tryptophan depletion and lead to depression in the end.

2.1.8. Drugs used in the treatment of depression

Serotonin–norepinephrine reuptake inhibitors (SNRIs) are a class of antidepressant drugs used in the treatment of major depressive disorder (MDD). SNRIs are potent inhibitors of serotonin (5-Hydroxytryptamine, 5-HT) and norepinephrine (NE,noradrenalin) reuptake. These neurotransmitters are known to play an important role in mood. The human serotonin transporter (SERT) and norepinephrine transporter (NET) are membrane proteins that are responsible for the reuptake of serotonin and norepinephrine. Balanced dual inhibition of monoamine reuptake can possibly offer advantages over other antidepressants drugs by treating a wider range of symptoms. SNRIs are second-generation agents, such as selective serotonin reuptake inhibitors (SSRIs) and norepinephrine reuptake inhibitors (NRIs). Over the past two decades, second-generation agents have gradually replaced first-generation agents, such as tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs) as the drugs of choice for the treatment of MDD. This is mainly because of their improved tolerability and safety profile [18].

In1952, antimycobacterial agent isoniazid, was discovered to have psychoactive properties while researched as a possible treatment for tuberculosis. Researchers noted that patients given isoniazid became cheerful, more optimistic, and more physically active. Soon after its development, isoninazid and related substances were shown to slow enzymatic breakdown of norepinephrine, serotonin and dopamine via inhibition of mitochondrial enzyme monoamine oxidase. For this reason, this class of drugs became known as MAOIs. During the time development of distinctively different antidepressant agents was also researched. Imipramine became the first clinically useful TCA. Imipramine was found to affect numerous neurotransmitter systems and to block reuptake of norepinephrine and serotonin from the synapse, therefore increasing the levels of these neurotransmitters. Use of MAOIs and TCAs gave major advances in treatment of depression, but their use was limited by unpleasant side effects and significant safety and toxicity issues [18].

Throughout the 1960s and 1970s the catecholamine hypothesis of emotion and its relation to depression was of wide interest and that the decreased levels of certain neurotransmitters, such as norepinephrine, serotonin, and dopamine might play a role in the pathogenesis of depression. This led to the development of fluoxetine, the first SSRI. The improved safety and tolerability profile of the SSRIs in patients with MDD, compared with TCAs and MAOIs, represented yet another important advance in the treatment of depression [19]. Since the late 1980s SSRIs have dominated the antidepressant drug market.

Today there is increased interest in antidepressant drugs with broader mechanisms of action that may offer improvements in efficacy and fewer adverse effects. In 1993 a new drug was introduced to the US market called venlafaxine, a serotonin-norepinephrine reuptake inhibitor [20].

Venlafaxine was the first compound described in a new class of antidepressive substances called phenylethylamines. These substances are unrelated to TCA and other SSRIs. Venlafaxine blocks the neuronal reuptake of serotonin, noradrenaline, and, to a lesser extent, dopamine in the central nervous system. In contrast with several other antidepressant drugs, venlafaxine can induce a rapid onset of action mainly due to a subsequent norepinephrine reuptake inhibition [21]. Desvenlafaxine is an active metabolite of venlafaxine.

Table - 2: List of drugs used in the treatment of depression

Group	Drug	Usual dose(Daily)
Tricyclics	Amitriptyline	75-150 mg
	Imipramine	75-150 mg
5-HT re-uptake inhibitors	Citalopram	20-60 mg
	Fluoxetine	20 mg
	fluvoxamine	100-300 mg
	Sertraline	50-100 mg
	Paroxetine	
Monoamine oxidase Inhibitors	Phenelzine	60-90 mg
	Tranylcypromine	20-40 mg
	Moclobemide	300-600 mg
Noradrenergic and 5HT re-uptake Inhibitors	Desvenlafaxine	75-375 mg
	Nefazodone	300-600 mg
Selective noradrenaline re-uptake inhibitor	Reboxetine	8-12 mg
Noradrenergic and specific serotonergic Inhibitor	Mirtazapine	15-45mg

2.1.9. Desvenlafaxine

Desvenlafaxine is also known as o-desmethylvenlafaxine, which is an antidepressant of the serotonin-nor-epinephrine reuptake inhibitor class. Desvenlafaxine is a synthetic form of the major metabolite of venlafaxine [22].

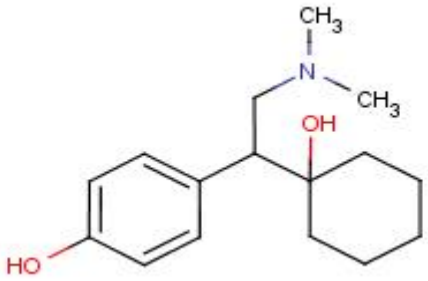


Figure - 1: Structure of desvenlafaxine succinate Molecular Formula : $C16H25NO_2$

Chemical Name : 4-[2-(dimethylamino)-1-(1-hydroxycyclohexyl) ethylphenol.

Molecular weight : 263.375

Category : Anti depressant (serotonin norepinephrin reuptake inhibitor).

Physical properties

Color: White to off-white powder.

State/Form: Solid-crystal.

Description: Desvenlafaxine succinate

Solubility: Soluble in water at 32 mg/mL as the free base (pH dependent)

Melting Point: 124 – 126 °C.

Mechanism of action

Desvenlafaxine is an active metabolite of venlafaxine. Desvenlafaxine is one of several serotonin norepinephrine reuptake inhibitor (SNRIs). This selectively blocks the reuptake of serotonin or norepinephrine. This increase the level of both neurotransmitter in the synapse which is thought to be beneficial in the depressed individuals. It is not have novel mechanism of action, its action is similar to its parent drug venlafaxine. It has been formulated as prolonged release tablet [23].

Desvenlafaxine inhibits the reuptake of serotonin and norepinephrine in the central nervous system. Its affinity for serotonin receptors is less than venlafaxine, while its affinity for norepinephrine is greater than venlafaxine's. *In vitro*, desvenlafaxine has not demonstrated significant affinity for muscarinic-cholinergic, histamine$_1$, or α_1-adrenergicreceptors. Desvenlafaxine reuptake inhibition of serotonin and norepinephrine is not dose related [24].

Metabolism

It is primarily metabolized via glucuronide conjugation and minimally by oxidative metabolism via CYP3A4 enzyme family. At 72 hours about 45% is excreated unchanged in urine. Protein binding of desvenlafaxine is about 11 hours [25].

Clinical uses

- It is used for the treatment of major depressive disorder.
- It is used in the treatment of vasomotor symptoms of menopause.
- It is also used in the anxiety and painful physical symptoms. [26]

Adverse effects

- The most common being nausea, headache, dizziness, dry mouth and diarrhoea.
- Other potential adverse effect are insomnia, increased blood pressure, heart rate and cholesterol level.
- Less common, but more serious adverse effect reported in these trials includes hypertension, QT interval prolongation, and exacerbation of ischemic cardiac disease, elevated lipid and elevated liver enzymes [27, 28].

Table - 3: Pharmacokinetic data of Desvenlafaxine

Pharmacokinetic Parameter	Desvenlafaxine
Half-life	11 hr
Bioavailability	80.5%
Metabolism	CYP3A4
Plasma protein Binding	33%
Excretion	45% excreted unchanged in urine

2.2. Diabetes

2.2.1. Definition

Diabetes mellitus (DM) is a group of metabolic disorders characterized by hyperglycemia and abnormalities in carbohydrate, fat, and protein metabolism. It results from defects in insulin secretion, insulin sensitivity, or both. Chronic micro vascular, macro vascular, neuropathic,nephropathy and retinopathy complications may ensue.

2.2.2. Classification of diabetes

The two most common types of diabetes were insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM), and this nomenclature reflected the need

for insulin to survive. The WHO classification also recognised malnutrition-related diabetes mellitus, other types of diabetes mellitus associated with specific conditions, and gestational diabetes, which is diabetes diagnosed for the first-time during pregnancy

2.2.3. Pathophysiology

Type-1 DM

Type-1 DM accounts for 5% to 10% of all diabetes cases developing in childhood or early adulthood and results from immune mediated destruction of pancreatic β-cells, ensuing in an absolute deficiency of insulin. There is a long preclinical period (up to 9 to 13 years) marked by the presence of immune markers when β-cell destruction is thought to occur. Hyperglycemia occurs when 80% to 90% of β- cells are destroyed. There is a transient remission ("honeymoon" phase) followed by established disease with allied risks for complications and death. The factors that initiate the autoimmune process are unknown, but the process is mediated by macrophages and T lymphocytes with circulating auto antibodies to various β-cell antigens (e.g., islet cell antibody, insulin antibodies).

Type-2 DM

Type-2 DM accounts for as many as 90% of DM cases and is usually characterized by the presence of both insulin resistance and relative insulin deficiency.

- Insulin resistance is manifested by increased lipolysis and free fatty acid production, increased hepatic glucose production, and decreased skeletal muscle uptake of glucose.
- β- Cell dysfunction is progressive and contributes to worsening blood glucose control over time.

Type-2 DM occurs when a diabetogenic lifestyle-excessive calories, inadequate exercise, and obesity is superimposed upon a susceptible genotype.

- Uncommon causes of diabetes (1% to 2% of cases) include endocrine disorders (e.g., acromegaly, Cushing's syndrome), gestational diabetes mellitus (GDM), diseases of the exocrine pancreas (e.g., pancreatitis), and medications (e.g., glucocorticoids, pentamidine, niacin, and α-interferon).
- Impaired fasting glucose and impaired glucose tolerance are terms used to describe patients whose plasma glucose levels are higher than normal but not diagnostic of DM. These disorders are risk factors for developing DM and cardiovascular disease and are associated with the insulin-resistance syndrome.
- Microvascular complications include retinopathy, neuropathy, and nephropathy.
- Macrovascular complications include coronary heart disease, stroke, and peripheral vascular disease.

2.2.4. Clinical presentation

Type-1 diabetes mellitus

- Individuals with type-1 DM are often thin and are prone to develop diabetic ketoacidosis if insulin is withheld or under conditions of severe stress with an excess of insulin counter regulatory hormones.
- Between 20% and 40% of patients present with diabetic ketoacidosis after several days of polyuria, polydipsia, polyphagia, and weight loss.

Type-2 diabetes mellitus

- Patients with type 2 DM are often asymptomatic and may be diagnosed secondary to unrelated blood testing. However, the presence of complications may indicate that they have had DM for several years.
- Lethargy, polyuria, nocturia, and polydipsia can be present on diagnosis; significant weight loss is less common.

2.2.5. Diagnosis

Screening for type 2 DM should be performed every 3 years in all adults beginning at the age of 45. Testing should be considered at an earlier age and more frequently in individuals with risk factors (e.g., family history of DM, obesity, signs of insulin resistance).

- The recommended screening test is fasting plasma glucose (FPG).
- Normal FPG is less than 100 mg/dL (5.6 mmol/L).
- Impaired fasting glucose is defined as FPG of 100 to 125 mg/dL (5.6 to 6.9 mmol/L).
- Impaired glucose tolerance is diagnosed when the 2-hour post load sample of the oral glucose tolerance test is between 140 and 199 mg per dL (7.8 to 11.0 mmol/L).

Pregnant women should undergo risk assessment for GDM at their first prenatal visit and proceed with glucose testing if at high risk (e.g., positive family history, personal history of GDM, marked obesity, or member of a high-risk ethnic group).

2.2.6. Criteria for the diagnosis of diabetes mellitus

- Symptoms of diabetes plus casual [a] plasma glucose concentration ≥ 200 mg/dL (11.1 mmol/L)
- Fasting [b]plasma glucose≥126 mg/ dL (7.0 mmol/L)
- 2-Hour post load glucose ≥ 200 mg/dL (11.1 mmol/L) during an OGTT [c]

In the absence of unequivocal hyperglycemia, these criteria should be confirmed by repeat testing on a different day. The third measure (oral glucose tolerance test; OGTT) is not recommended for routine clinical use.

a- Casual is defined as any time of day without regard to time since last meal. The classic symptoms of diabetes include polyuria, polydipsia, and unexplained weight loss.

b- Fasting is defined as no caloric intake for at least 8 hours.

c-The test should be performed as described by the World Health Organization, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.

Table - 4: Glycemic Goals of Therapy

Biochemical Index	ADA	ACE and AACE
Hemoglobin A1C	<7% [a]	≤6.5%
Preprandial plasma glucose	90–130 mg/dL (5.0–7.2 mmol/L)	<110 mg/dL (6.1 mmol/L)
Postprandial plasma glucose	<180 mg/dL[b] (<10 mmol/L)	<140 mg/dL (<7.8 mmol/L)

AACE- American Association of Clinical Endocrinologists

ACE-American College of Endocrinology

ADA- American Diabetes Association

DCCT- Diabetes Control and Complications Trial

a- Referenced to a nondiabetic range of 4–6% using a DCCT-based assay. More stringent glycemic goals (i.e., a normal A1C, <6%) may further reduce complications at the cost of increased risk of hypoglycemia (particularly in those with type 1 diabetes).

b- Postprandial glucose measurements should be made 1–2 hours after the beginning of the meal, generally the time of peak levels in patients with diabetes.

2.2.7. Treatment

General approach

Near-normal glycemia reduces the risk of micro vascular disease complications, but aggressive management of traditional cardiovascular risk factors (i.e., smoking cessation, treatment of dyslipidemia, intensive blood pressure control, antiplatelet therapy) is needed to reduce macro vascular disease risk.

Appropriate care requires goal setting for glycemia, blood pressure, and lipid levels; regular monitoring for complications; dietary and exercise modifications; appropriate self-monitoring of blood glucose (SMBG); and appropriate assessment of laboratory parameters.

Nonpharmacologic therapy

Medical nutrition therapy is recommended for all patients. For individuals with type-1 DM, the focus is on regulating insulin administration with a balanced diet to achieve and maintain a healthy body weight. A meal plan that is moderate in carbohydrates and low in saturated fat, with a focus on balanced meals is recommended. In addition, patients with type-2 DM often require caloric restriction to promote weight loss. Bedtime and between-meal snacks are not usually needed if pharmacologic management is appropriate. Aerobic exercise can improve insulin resistance and glycemic control in most patients and may reduce cardiovascular risk factors, contribute to weight loss or maintenance, and improve well-being. Exercise should be started slowly in previously sedentary patients. Older patients and those with atherosclerotic disease should have a cardiovascular evaluation prior to beginning a substantial exercise program.

Pharmacologic therapy

Insulin and Other Injectable Preparations

Regular insulin has a relatively slow onset of action when given subcutaneously, requiring injection 30 minutes prior to meals to achieve optimal postprandial glucose control and to prevent delayed postmeal hypoglycemia.

Lispro, aspart, and **glulisineinsulin** are analogs that are more rapidly absorbed, peak faster, and have shorter durations of action than regular insulin. This permits more convenient dosing within 10 minutes of meals (rather than 30 minutes prior), produces better efficacy in lowering postprandial blood glucose than regular insulin in type 1 DM, and minimizes delayed postmeal hypoglycemia.

Table - 5: List of insulin injectable preparations	
Duration of action	Examples
Rapid-acting insulins	Humalog (insulin lispro) NovoLog (insulin aspart) Apidra (insulin glulisine)
Short-acting insulins	HumulinR(regular) Novolin R (regular)
Intermediate-acting insulins (NPH)	Humulin N ,Novolin N
Long-acting insulins	Lantus (insulin glargine) Levemir (insulin detemir)
Premixed insulins	Humalog Mix 50/50 (50% neutral protamine lispro, 30% aspart)

Neutral protamine hagedorn (NPH) is intermediate-acting. Variability in absorption, inconsistent preparation by the patient, and inherent pharmacokinetic differences may contribute to a labile glucose response, nocturnal hypoglycemia, and fasting hyperglycemia.

Glargine and Detemir are long-acting "peakless" human insulin analogs that result in less nocturnal hypoglycemia than NPH insulin when given at bedtime.

In type-1 DM, the average daily insulin requirement is 0.5 to 0.6 units/kg Requirements may fall to 0.1 to 0.4 units/kg in the honeymoon phase. Higher doses (0.5 to 1 unit/kg) are warranted during acute illness or ketosis. In type 2 DM, a dosage range of 0.7 to 2.5 units/kg is often required for patients with significant insulin resistance.

- Hypoglycemia and weight gain are the most common adverse effects of insulin.
- Treatment of hypoglycemia is as follows:

Glucose : (10 to 15 g) given orally is the recommended treatment in conscious patients.

Dextrose IV may be required in individuals who have lost consciousness.

Glucagon: 1 g intramuscular, is the treatment of choice in unconscious patients when IV access cannot be established.

Exenatide is a synthetic analog of exendin-4, a 39-amino acid peptide isolated from the saliva of the Gila monster that enhances glucose dependent insulin secretion and reduces hepatic glucose production. It also decreases appetite and slows gastric emptying, which may reduce caloric intake and cause weight loss. It significantly decreases postprandial glucose excursions but has only a modest effect on FPG values. The average A1C reduction is approximately 0.9%. The most common adverse effects are nausea, vomiting, and diarrhea. The initial dose is 5 mcg subcutaneously twice daily, titrated to 10 mcg twice daily in 1 month if needed and as tolerated. It should be injected 0 to 60 minutes before the morning and evening meals. Exenatide should be used as adjunctive therapy in patients who have not achieved adequate glycemic control despite treatment with metformin, a sulfonylurea, and/or a thiazolidinedione.

Pramlintide is a synthetic analog of amylin, a neurohormone cosecreted From β-cells with insulin. Pramlintide suppresses inappropriately high postprandial glucagon secretion, reduces food intake (which can cause weight loss), and slows gastric emptying. The average A1C reduction is approximately 0.6%, but optimization of concurrent insulin therapy may result in further A1C decreases. Pramlintide decreases prandial glucose excursions but has little effect on FPG concentrations. Its main advantage is in type 1 DM, where it helps stabilize wide, postprandial glycemic swings. The most common adverse effects are nausea, vomiting, and anorexia. It does not cause hypoglycemia when used alone, but it is indicated only in patients receiving insulin, so hypoglycemia can occur. If a prandial insulin dose is used, it should be reduced by 30% to 50% when pramlintide is started to minimize severe hypoglycemic reactions. In type 2 DM, the starting dose is 60 mcg subcutaneously prior to major meals; the dose is titrated up to 120 mcg per dose as tolerated and as warranted based on postprandial plasma glucose levels. In type 1 DM, dosing starts at 15mcg prior to each meal, titrated up to a maximum of 60 mcg prior to each meal if tolerated and warranted.

Sulfonylureas

Sulfonylureas exert a hypoglycemic action by stimulating pancreatic secretion of insulin. All sulfonylureas are equally effective in lowering blood glucose when administered in equipotent doses. On average, the A1C will fall by 1.5% to 2% with FPG reductions of 60 to 70 mg/dL (3.3 to 3.9mmol/L). Tolbutamide, glibenclaimide, glipizide, bumetanide are included in sulphonylureas.

The most common side effect is hypoglycemia, which is more problematic with long half-life drugs. Individuals at high risk include the elderly, those with renal insufficiency or advanced liver disease, and those who skip meals, exercise vigorously, or lose a substantial amount of

weight. Weight gain is common; less common adverse effects include skin rash, hemolytic anemia, GI upset, and cholestasis. Hyponatremia is most common with Chlorpropamide but has also been reported with Tolbutamide.

The recommended starting doses should be reduced in elderly patients who may have compromised renal or hepatic function. Dosage can be titrated every 1 to 2 weeks (longer interval with chlorpropamide) to achieve glycemic goals.

Short-Acting Insulin Secretagogues (Meglitinides)

Similar to sulfonylureas, meglitinides lower glucose by stimulating pancreatic insulin secretion, but insulin release is glucose dependent and diminishes at low blood glucose concentrations. Hypoglycemic risk appears to be less with meglitinides than with sulfonylureas. The average reduction in A1C is about 0.8% to 1%. These agents can be used to provide increased insulin secretion during meals (when it is needed) in patients who are close to glycemic goals. They should be administered before each meal (up to 30 minutes prior). If a meal is skipped, the medication should also be skipped.

Repaglinide (Prandin) is initiated at 0.5 to 2 mg with a maximum dose of 4 mg per meal (up to four meals per day or 16 mg/day).

Nateglinide (Starlix) dosing is 120 mg three times daily before each meal. The dose may be lowered to 60 mg per meal in patients who are near goal A1C when therapy is initiated.

Biguanides

Metformin is the only biguanide available in the United States. It enhances insulin sensitivity of both hepatic and peripheral (muscle) tissues. This allows for increased uptake of glucose into these insulin-sensitive tissues. Metformin consistently reduces A1C levels by 1.5% to 2%, FPG levels by 60 to 80 mg/dL, and retains the ability to reduce FPG levels when they are very high (>300 mg/dL). It reduces plasma triglycerides and low-density lipoprotein (LDL) cholesterol by 8% to 15% and modestly increases high density lipoprotein (HDL) cholesterol (2%). It does not induce hypoglycemia when used alone.

- Metformin should be included in the therapy for all type 2 DM patients (if tolerated and not contraindicated) because it is the only oral anti hyperglycemic medication proven to reduce the risk of total mortality and cardiovascular death.
- The most common adverse effects are abdominal discomfort, stomach upset, diarrhea, anorexia, and a metallic taste. These effects can be minimized by titrating the dose slowly and taking it with food. Extended release metformin (Glucophage XR) may reduce some of the GI side effects. Lactic acidosis occurs rarely and can be minimized by avoiding its use in patients with renal insufficiency (serum creatinine 1.4 mg/dL or greater in women and 1.5 mg/dL or greater in men), congestive heart failure, or conditions predisposing to hypoxemia or inherent lactic acidosis. Metformin should be discontinued 2 to 3 days prior to IV radiographic dye studies and withheld until normal renal function has been documented post study.

Metformin immediate-release is usually initiated at 500 mg twice daily with the largest meals and increased by 500 mg weekly until glycemic goals or 2,000 mg/day is achieved. Metformin 850 mg can be dosed once daily and then increased every 1 to 2 weeks to a maximum of 850 mg three times daily (2,550 mg/day).

*Metformin extended-release (*Glucophage XR) can be initiated with 500 mg with the evening meal and increased by 500 mg weekly to a maximum dose of 2,000 mg/day. Administration two to three times a day may help minimize GI side effects and improve glycemic control. The 750-mg tablets can be titrated weekly to the maximum dose of 2,250 mg/day.

Thiazolidinediones (Glitazones)

These agents activate PPAR-ɣ, a nuclear transcription factor important in fat cell differentiation and fatty acid metabolism. PPAR-ɣ agonists enhance insulin sensitivity in muscle, liver, and fat tissues indirectly. Insulin must be present in significant quantities for these actions to occur [29].

When given for about 6 months, Pioglitazone and Rosiglitazone reduce A1C values by about 1.5% and FPG levels by about 60 to 70 mg/dL at maximal doses. Maximal glycemic-lowering effects may not be seen until are given early in the disease course when sufficient ɣ-cell function and hyperinsulinemia are present.

Pioglitazone decreases plasma triglycerides by 10% to 20%, whereas Rosiglitazone tends to have no effect. Pioglitazone does not cause significant increases in LDL cholesterol, whereas LDL cholesterol may increase by 5% to 15% with rosiglitazone.

Fluid retention may occur, perhaps as a result of peripheral vasodilation and/or improved insulin sensitization with a resultant increase in renal sodium and water retention. A dilutional anemia may result, which does not require treatment. Edema is reported in 4% to 5% of patients when glitazones are used alone or with

other oral agents. When used in combination with insulin, the incidence of edema is about 15%.

Glitazones are contraindicated in patients with New York Heart Association Class III and IV heart failure and should be used with great caution in patients with Class I or II heart failure or other underlying cardiac disease. Weight gain is dose related, and an increase of 1.5 to 4 kg is not uncommon. Rarely, rapid gain of a large amount of weight may necessitate discontinuation of therapy. Weight gain positively predicts a larger A1C reduction but must be balanced with the potential adverse effects of long term weight gain. Several case reports of hepatotoxicity with Pioglitazone or Rosiglitazone have been reported, but improvement in alanine aminotransferase (ALT) was consistently observed upon drug discontinuation. Baseline ALT should be obtained prior to therapy and then periodically thereafter at the practitioner's discretion. Neither drug should be started if the baseline ALT exceeds 2.5 times the upper limit of normal. The drugs should be discontinued if the ALT is more than 3 times the upper limit of normal.

Rosiglitazone has been associated with an increased risk of myocardial ischemic events such as angina or myocardial infarction in several studies. Although causality has not been conclusively established, the FDA has required that a "black box" warning be added to the labeling. A new long term study to evaluate potential cardiovascular risks is planned. Rosiglitazone (Avandia) is initiated with 2 to 4 mg once daily. The maximum dose is 8 mg/day. A dose of 4 mg twice daily can reduce A1C by 0.2% to 0.3% more than a dose of 8 mg taken once daily.

Pioglitazone (Actos) is started at 15 mg once daily. The maximum dose is 45 mg/day.

Glucosidase Inhibitors

These agents prevent the breakdown of sucrose and complex carbohydrates in the small intestine, thereby prolonging the absorption of carbohydrates. The net effect is a reduction in the postprandial glucose concentrations (40 to 50 mg/dL) while fasting glucose levels are relatively unchanged (about 10% reduction). Efficacy on glycemic control is modest, with average reductions in A1C of 0.3% to 1%. Good candidates for these drugs are patients who are near target A1C levels with near-normal FPG levels but high postprandial levels.

The most common side effects are flatulence, bloating, abdominal discomfort, and diarrhea, which can be minimized by slow dosage titration. If hypoglycemia occurs when used in combination with a hypoglycemic agent (sulfonylurea or insulin), oral or parenteral glucose (dextrose) products or glucagon must be given because the drug will inhibit the breakdown and absorption of more complex sugar molecules (e.g., sucrose).

Acarbose (Precose) and Miglitol (Glyset) are dosed similarly. Therapy is initiated with a very low dose (25 mg with one meal a day) and increased very gradually (over several months) to a maximum of 50 mg three times daily for patients weighing 60 kg or more, or 100 mg three times daily for patients above 60 kg. The drugs should be taken with the first bite of the meal so that the drug is present to inhibit enzyme activity.

Dipeptidyl Peptidase-IV Inhibitors

Dipeptidyl peptidase-IV inhibitors prolong the half-life of an endogenously produced glucagon-like peptide-1. These agents partially reduce the inappropriately elevated glucagon post prandially and stimulate glucose dependent insulin secretion. The average reduction in A1C is approximately 0.7% to 1% at a dose of 100 mg/day.

These drugs are well tolerated, weight neutral, and do not cause GI side effects. Mild hypoglycemia appears to be the only significant adverse effect, but long-term safety data are limited.

Sitagliptin (Januvia) is usually dosed at 100 mg orally once daily. In patients with renal impairment, the daily dose should be reduced to 50 mg (creatinine clearance 30–50 mL/min) or 25 mg (creatinine clearance <30 mL/min).

Vildagliptin was not approved in the United States at the time of this writing (June 2008). The usual dose is expected to be similar to Sitagliptin.

Pharmacotherapy of type-1 diabetes mellitus

- All patients with type I DM require insulin, but the type and manner of delivery differ considerably among individual patients and clinicians.
- Therapeutic strategies should attempt to match carbohydrate intake with glucose-lowering processes (usually insulin) and exercise. Dietary intervention should allow the patient to live as normal a life as possible.
- The timing of insulin onset, peak, and duration of effect must match meal patterns and exercise schedules to achieve near-normal blood glucose values throughout the day.
- A regimen of two daily injections that may roughly approximate physiologic insulin secretion is split-mixed injections of a morning

dose of NPH insulin and regular insulin before breakfast and again before the evening meal. This assumes that the morning NPH insulin provides basal insulin for the day and covers the midday meal, the morning regular insulin covers breakfast, the evening NPH insulin gives basal insulin for the rest of the day, and the evening regular insulin covers the evening meal. Patients may be started on 0.6 units/kg/day, with two-thirds given in the morning and one-third in the evening. Intermediate-acting insulin (e.g., NPH) should comprise two-thirds of the morning dose and one-half of the evening doses.

2.3. Pioglitazone

Pioglitazone is a new thiazolidinedione compound used in the treatment of type-2 diabetes. It is reported to be extensively metabolised by CYP enzymes in the liver[30]. CYP enzymes are essential in oxidative drug metabolism, being involved in approximately 80% of oxidative drug metabolism and accounting for almost 50% of the overall elimination of commonly used drugs[31] . Pioglitazone [-5-[[4-[2-(5-ethyl-2-pyridinyl) ethoxy]phenyl]methyl]-2,4-] thiazolidinedione monohydrochloride] is a thiazolidinedione class antidiabetic drug that acts primarily by decreasing insulin resistance. The thiazolidinediones (or "glitazones") were introduced in the late 1990s as an adjunctive therapy for type 2 diabetes.

2.3.1. Mechanism of action

Pioglitazone decreases insulin resistance via its action at the peroxisome proliferator activated receptor subtype gamma (PPAR-γ) .It is not an insulin secretagogue and does not therefore cause hypoglycaemia when used alone. Its most common clinically important adverse effect is fluid retention, which can lead to or exacerbate heart failure and pulmonary oedema [32].

In vitro studies have suggested that pioglitazone is metabolized by multiple CYP enzymes, mainly by CYP2C8, CYP2C9 and CYP3A4.Furthermore, the relative contribution of different CYP enzymes *in vivo* and the effects of CYP enzyme induction and CYP2C8 inhibition on the metabolism of pioglitazone in humans are unknown. Pioglitazone is reported to inhibit both CYP2C8 and CYP3A4 [33] enzymes *in vitro*, but its inhibitory effect on CYP2C8 activity *in vivo* has not been investigated.

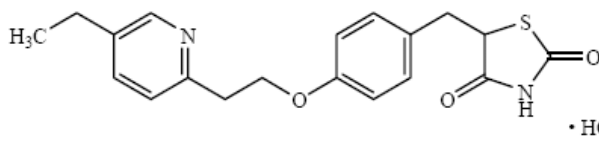


Figure – 2: Structure of Pioglitazone.

The first member of the class, Troglitazone (RezulinR), was rapidly withdrawn from the market because of fulminant drug-induced hepatitis. The other two compounds Pioglitazone (ActosR) and Rosiglitazone (AvandiaR) appear to be devoid of idiosyncratic liver toxicity.

2.3.2. Pharmacodynamics and clinical use

Pioglitazone is a ligand for peroxisome proliferator activated receptor-γ (PPAR-γ), a member of the nuclear receptor superfamily. Once activated by Pioglitazone, PPAR-γ forms a heterodimer with the retinoid X receptor. The heterodimer then binds to specific DNA sequences and regulates the target genes involved in the metabolism of glucose and lipids. The activation of PPAR-γ leads to increased insulin sensitivity in hepatic, fat and skeletal muscle cells, thereby inhibiting hepatic gluconeogenesis and increasing peripheral glucose uptake. However, the exact mechanism remains obscure. Pioglitazone is not an insulin secretagogue, and it is dependent on the presence of insulin to exert its effects. In addition to its action on glucose metabolism, pioglitazone promotes the differentiation of adipocytes, which leads to redistribution of lipid from visceral to subcutaneous deposits.

In Europe, Pioglitazone is approved as monotherapy and in combination with metformin or sulphonylurea for treatment of patients with type-2 diabetes. Pioglitazone is taken orally once daily at a dosage of 15 or 30 mg/day, with titration to 45 mg/day if necessary. At maximal dose, Pioglitazone decreases glycosylated haemoglobin value (HbA1c) on average by 1-1.5%. The blood glucose lowering effect of pioglitazone develops gradually over weeks, with a maximal decrease in blood glucose being reached after 10-14 weeks.

3.3.3. Pharmacokinetics and drug interactions

Pioglitazone is rapidly absorbed from the gastrointestinal tract, with peak plasma concentrations (C_{max}) observed within two hours. Food slightly delays the time to peak concentration (t_{max}), but does not alter the extent of absorption. The mean absolute oral bioavailability of pioglitazone is 83%. Pioglitazone is extensively (97% to over 99%) bound to plasma proteins, primarily to albumin, and has a rather small apparent volume of distribution (0.63 l/kg).

Table - 6: Summary of the pharmacokinetics of Pioglitazone

Rapidly absorbed	**t_{max} 2–5 hours**
Linear kinetics	Over range 2–60 mg

Terminal plasma half-life	5–6 hours (pioglitazone) 16–23 hours (metabolites)
Bioavailability	83%
Plasma protein binding	> 97%
Dual route of excretion	> 55% via bile

In humans, Pioglitazone is extensively metabolised by hydroxylation and oxidation to five primary metabolites (M-I, M-II, M-IV, M-V and M-VI), and by further oxidation of M-IV to a secondary metabolite M-III. In addition, four minor metabolites (M-VII to M-X) have been identified *in vitro* and in animals The hydroxy derivates of Pioglitazone, M-II and M-IV, and the keto derivate M-III have been shown to be pharmacologically active in animal models. M-IV and M-III are the principal metabolites found in human serum, and at steady state they comprise approximately 75-80% of the total AUC of active compounds. M-II is found at relatively low concentrations in plasma, and it does not significantly contribute to total active compounds. M-IV and M-III are highly (>98%) bound in plasma proteins. The mean elimination half-life of pioglitazone ranges from 3 to 9 hours, but the half-life of the active metabolites M-IV and M-III is considerable longer (26-28 hours), which is likely to contribute to an extended pharmacological activity that allows for once daily administration of Pioglitazone.

Following oral administration, approximately 15-30% of the Pioglitazone dose is recovered in the urine. However, renal elimination of the parent Pioglitazone is negligible, and the drug is primarily excreted as metabolites and their conjugates. The main compound excreted in urine is M-V. Age and gender have no significant effect on the pharmacokinetics of Pioglitazone. Overall, the pharmacokinetics of Pioglitazone in patients with type 2 diabetes is similar *In vitro* studies with HML have suggested that multiple CYP isoenzymes (1A1, 1A2, 2C8, 2C9, 2C19, 2D6 and 3A4) are involved in the metabolism of Pioglitazone, with the most important isoenzymes reported to be CYP2C8, CYP2C9 and CYP3A4 . CYP2C8 and CYP3A4 are reported to contribute approximately 40% and less than 20%, respectively, to the metabolism of Pioglitazone and M-IV.

However, the information on the contribution of different CYP isoforms to the metabolism of pioglitazone seems to be based on unpublished data, and the experimental systems in which these results were obtained, have not been described. In addition, information from different sources appears to be discrepant. For example, the Finnish product information has previously stated that the metabolism of pioglitazone occurs predominantly via CYP3A4 and CYP2C9, whereas the US label states that the major CYP isoforms involved are CYP2C8 and CYP3A4. Prior to this thesis, no published studies have been available on the effects of CYP enzyme inducers or CYP2C8 inhibitors on the pharmacokinetics of pioglitazone *in vivo*.

Pioglitazone has been reported to both inhibit (testosterone 6β-hydroxylation, Ki 11.8 μM) and induce CYP3A4 activity *in vitro*. In healthy volunteers, Pioglitazone has not altered CYP3A4 marker activity (urinary excretion ratio of 6β-hydroxycortisol to cortisol) or affected the pharmacokinetics of CYP3A4 substrate Simvastatin significantly, suggesting that Pioglitazone is not a significant inducer or inhibitor of CYP3A4 *in vivo*. Pioglitazone has also been reported to inhibit CYP2C9 (tolbutamide hydroxylation, Ki32.1 μM) *in vitro*, but it has not altered the pharmacokinetics of drugs metabolized mainly by CYP2C9 (warfarin, glibenclamide, glipizide) *in vivo*. *In vitro*, pioglitazone has most potently inhibited CYP2C8 (paclitaxel 6α-hydroxylation, Ki 1.7 μM; amodiaquine N-deethylation, IC50 11.7 μM). However, no studies have been published on the effect of Pioglitazone on the pharmacokinetics of CYP2C8 substrate drugs in humans. Pioglitazone has been reported to inhibit OATP1B1 and OATP1B3 *in vitro*, suggesting that Pioglitazone could also be a substrate for these transporters. To date, the effect of genetic polymorphism in CYP2C8 or drug transporters on Pioglitazone metabolism has not been studied.

3.3.4 Adverse effects

Common adverse effects of pioglitazone include weight gain, fluid retention and plasma volume expansion, which can produce mild dilutional anaemia, peripheral oedema and can lead to or exacerbate heart failure. The underlying mechanism of fluid retention has not been fully elucidated, but it appears to be a dose-related

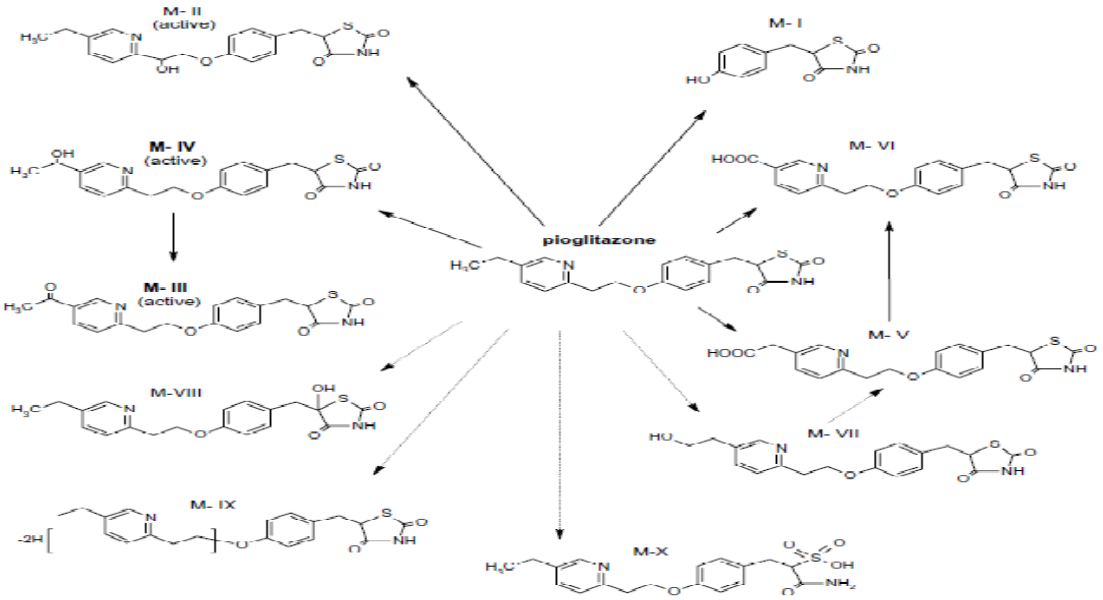


Figure – 3: Metabolism of Pioglitazone in humans and minor metabolites of Piogliazone identified *in vitro* to that in healthy volunteers.

class effect of thiazolidinediones. The risk of oedema and heart failure is higher when thiazolidinediones are combined with insulin. The manufacturer of pioglitazone reports an incidence of peripheral oedema of 4.8% for monotherapy and 5.9-7.2% for combination therapy with metformin or sulphonylurea, increasing to 15.3% for combination treatment with insulin. In a recently published large study, oedema in the absence of heart failure was experienced by 21% of pioglitazone recipients versus 13% of placebo recipients, and heart failure occurred significantly more oft en in pioglitazone recipients (11%) than in placebo recipients (8%; P < 0.0001) .

Heart failure and insulin therapy are contraindications to the use of thiazolidinediones in Europe. Unlike troglitazone, pioglitazone has rarely been associated with hepatotoxicity or elevated liver enzymes. There have been a few reports of liver injury with pioglitazone, but direct causality has not been established. Recent studies have suggested that exposure to thiazolidinediones has important eff ects on bone. In rodent models, activation of PPAR-γ by thiazolidinediones has stimulated adipocyte formation from the mesenchymal precursor cells at the expense of the formation of osteoblasts, resulting in bone loss. Recent data from an observational study in older adults with diabetes suggest that thiazolidinedione use is associated with an increased rate of bone loss in women.

Another randomized, controlled study found that short-term rosiglitazone treatment inhibits bone formation and accelerates bone loss in healthy postmenopausal women. In addition, a safety analysis of a large, controlled clinical trial revealed that significantly more female patients who received rosiglitazone experienced fractures than did females who received either metformin or glibenclamide .The majority of the fractures observed in the rosiglitazone group were in the humerus, hand and foot. The number of women with hip or spine fractures was low and did not differ between the groups.

2.4. Drug interactions

Drug interactions occur when the effect and/or concentration of a drug is modified by another substance, including a concomitant treatment, over-the-counter medication, food, alcohol, or tobacco, among others.1,2 Clinically significant drug-drug interactions (DDIs) are defined as events in which the safety or effectiveness of one medication is substantially altered by another drug or substance. These DDIs can result in potentially dangerous, and sometimes fatal, adverse events (AEs). In addition, DDIs can result in reduced effectiveness of treatment or can lead to AEs that, although not serious, are bothersome for patients.

In both cases, the interaction ultimately results in negative long-term outcomes and increased healthcare utilization and costs[31]. Compared with healthy individuals, or patients with an acute illness who require short-term treatment with one medication, patients with chronic illnesses, such as major depressive disorder (MDD), have more opportunities to experience DDIs, because antidepressants are often prescribed for months, if not years. In

addition, patients with depressive disorders typically have comorbid symptoms that require administration of concomitant medications.

2.4.1. Pharmacokinetics and drug interactions

Pharmacokinetics explores the time course of a drug and its metabolites in the body Pharmacokinetics is often divided into absorption and disposition. Absorption describes the movement of a drug from the site of administration to the circulatory system[34].Orally administered drugs are absorbed from the gastrointestinal tract and carried via the portal vein to the liver before entering the systemic circulation. The term bioavailability refers to the proportion of the administered drug that reaches the systemic circulation and is available to have an effect. Disposition is divided into distribution and elimination. Distribution involves the transfer of drugs between plasma and tissues, and elimination the loss of drugs from the body. Most drugs are eliminated by the liver into bile or by the kidneys into urine.

Drug interactions are an important aspect of clinical drug treatment. Drug interactions can lead to severe side effects and such interactions have even resulted in early termination of drug development, refusal of approval and withdrawal from the market [35] .Therefore, in addition to clinicians, also the pharmaceutical industry and regulatory authorities have paid increasing attention to drug-drug interactions.

In pharmacokinetic drug interactions, the absorption, distribution, metabolism or excretion of a drug is altered. Many clinically important pharmacokinetic drug interactions are based on inhibition or induction of CYP enzymes. The characteristics of various CYP enzymes and their involvement in the metabolism of commonly used drugs are now quite well established. Active membrane transporters have also been increasingly recognized to play an important role in pharmacokinetics and drug interactions [36] .This knowledge may provide a basis for better understanding and predictability of pharmacokinetic drug interactions.

2.4.2. Drug metabolism

Drugs are eliminated either unchanged by the process of excretion or via biotransformation to metabolites. Most drugs are lipid-soluble, which promotes their passage through biological membranes and enables access to their site of action. Most lipophilic compounds are, however, eliminated poorly unless they are metabolized to more polar compounds. The metabolites are usually inactive or less active than the parent drug. However, some metabolites may have enhanced activity (prodrugs)or toxic effects. Drug biotransformation reactions can be classified into phase I functionalisation reactions or phase II conjugation reactions. Phase I reactions introduce a functional group on the parent compound by oxidation, reduction or hydrolysis reactions, many of which are catalysed by the CYP system and require NADPH as a cofactor. Phase II reactions lead to the formation of a covalent linkage between a functional group of the parent drug or phase I metabolite and an endogenous compound and include glucuronidation, sulfation, acetylation and methylation reactions.

The enzyme systems involved in phase I reactions are located mainly in the endoplasmic reticulum, while phase II conjugating enzymes are located in both the cytoplasm and the endoplasmic reticulum [37] . The liver is the principal organ of drug metabolism, although other organs, such as the gastrointestinal tract, kidneys, lung and skin, can have significant metabolic capacity [37].

A notable portion of a drug may be metabolised in the intestine or liver before entering the systemic circulation, which can significantly limit the oral bioavailability of a drug (first-pass metabolism). Metabolic capacity can vary markedly between individuals, leading to differences in drug response and adverse effects among patients. The variability in metabolic capacity is multifactorial; gender, polymorphism of drug-metabolising enzymes, smoking, dietary factors and other drugs can all affect drug metabolism.

2.4.3. CYP enzymes

Cytochrome P450 enzymes are a family of haeme-containing mono-oxygenases that play a major role in phase I metabolism in humans[38] .In addition to participating in the metabolism of drugs and other xenobiotics, CYP enzymes have an important role in the biosynthesis and degradation of many endogenous compounds such as arachidonic acid and eicosanoids, steroid hormones, cholesterol and bile acid, vitamin D and retinoic acid. The name cytochrome P450 comes from the wavelength of light (450 nm) absorbed by the pigment (P) in the enzymes when the haeme iron is reduced and bound to carbon monoxide[39]. In humans, 57 cytochrome P450 genes arranged in 18 families have been identified, of which only the CYP1, CYP2 and CYP3 families seem to contribute to the metabolism of drugs CYP families are further divided into subfamilies and specific isoenzymes[40].

All isoenzymes in the same family have at least 40% amino acid similarity, and those in the same subfamily have at least 55% amino acid similarity. Individual CYP enzymes are designated by a family number (e.g. CYP**2**C8), a subfamily letter (CYP2**C**8) and a number for an individual enzyme within the

subfamily (CYP2C**8**)[41].Many *in vitro* studies of CYP-mediated drug metabolism in humans are conducted using human liver microsomes (HLM). Upon homogenisation and centrifugation of liver tissue, the endoplasmic reticulum is fragmented to microvesicles, which are referred to as microsomes. The microsomes contain several drug-metabolising enzymes, including the CYP enzymes, flaving-containing mono-oxygenases (FMO) and UDP-glucuronosyltransferases.

There are two members of the CYP1A subfamily in humans: CYP1A1 and CYP1A2. CYP1A1 is found primarily in extrahepatic tissues, most notably in the lung and placenta CYP1A2[43]. is mainly a hepatic enzyme, and it accounts for about 12-18% of all CYP enzymes in the liver [44]. CYP1A enzymes are inducible by xenobiotics, such as polycyclic aromatic hydrocarbons (PAH) found in cigarette smoke and grilled food, and the induction is mainly mediated by the aryl hydrocarbon receptor (AhR) .Rifampicin has increased, for example, the clearance of CYP1A2 substrate mexiletine by over 60% [45]but compared with CYP3A4, CYP1A2 seems to be only weakly induced by rifampicin in humans[46] CYP1A2 is important in the metabolism of many drugs, including caffeine, clozapine, theophylline and tizanidine[47]. Fluvoxamine and ciprofloxacin are strong inhibitors of CYP1A2 *in vivo* .Furafylline is used as a selective CYP1A2 probe inhibitor *in vitro*[48] Marked interindividual variability has been reported in the activity of CYP1A2 in Humans [48]. Although the *CYP1A2* gene shows structural polymorphism, its importance in explaining variability in CYP1A2 activity is unclear.

2.4.3.1 CYP2A6

CYP2A6 is the first form of human CYP enzyme in the CYP2 family. CYP2A6 is predominantly hepatic enzyme, and it constitutes approximately 4-8% of the total liver CYP content [49].Coumarine and nicotine are specific substrates of CYP2A6, and methoxsalen is a potent inhibitor of CYP2A6 .CYP2A6 is highly polymorphic and its genotype has been associated with, for example, smoking habits [50]. CYP2A6 may be inducible by antiepileptic drugs [51] .

2.4.3.2 CYP2B6

CYP2B6 has recently received more attention as a clinically important enzyme in drug metabolism[52]. It is highly polymorphic[53], and it may represent up to 6% of the total CYP content in the liver [54]. It is expressed at lower levels in some extrahepatic tissues[55].Clinically used substrate drugs for CYP2B6 include bupropion, cyclophosphamide, propofol, nevirapine and efavirenz [56] .CYP2B6 is inducible by, for example, rifampicin [57] .Clopidogrel and ticlopidine are potent inhibitors of CYP2B6[58-60]

3.4.3.3 CYP2C subfamily

The human CYP2C subfamily comprises four members: CYP2C8, CYP2C9, CYP2C18 and CYP2C19. Of these, CYP2C8, CYP2C9 and CYP2C19 are of clinical importance and are collectively responsible for the metabolism of about 20% of clinically used drugs CYP2C[61] enzymes are expressed mainly in the liver, where they account for approximately 20% of the total CYP content [62], but they are expressed at a significant level also in the small intestine [63] .Each member of the CYP2C subfamily is genetically polymorphic [64].

The importance of CYP2C8 in drug metabolism is being increasingly recognised, and it has a major role in the metabolism of a growing number of substrates, including paclitaxel, repaglinide, rosiglitazone, pioglitazone, cerivastatin, amiodarone, amodiaquine, chloroquine and arachidonic acid [65]. Some overlapping substrate specificity appears to exist between CYP2C8 and CYP3A4 in, for instance, the metabolism of carbamazepine, cerivastatin and repaglinide [66] .CYP2C8 constitutes about 7% of total microsomal CYP content in the liver, and CYP2C8 protein has been detected in several extrahepatic tissues as well[67].CYP2C8 is inducible by rifampicin, phenobarbital and dexamethasone *in vitr*[67] Montelukast is a very selective and potent CYP2C8 inhibitor *in vitro* . Gemfibrozil (gemfibrozil glucuronide) and trimethoprim inhibit CYP2C8 both *in vitro* and *in vivo* CYP2C9 is the predominant CYP2C enzyme in both the intestine and the liver [68] CYP2C9 is estimated to be responsible for the metabolism of up to 15% of all drugs that undergo phase I metabolism, and its substrates include S-warfarin, phenytoin, losartan, fluvastatin, sulphonylurea antidiabetic drugs and several NSAIDs [69] .Sulfaphenazole is a selective and potent CYP2C9 inhibitor. Clinically significant inhibition may occur also with coadministration of amiodarone, fluconazole, miconazole, voriconazole and certain other sulphonamides. CYP2C9 activity *in vivo* is inducible by rifampicin. Genetic polymorphism of CYP2C9 affects warfarin, phenytoin and sulphonylurea drug dose requirements and has been associated with an increased risk of bleeding complications during warfarin treatment[70].

The most common allelic variants with reduced catalytic activity are *CYP2C9*2* and *CYP2C9*3*, and they have allele frequencies of 11% and 7%, respectively, in Caucasians [71]. CYP2C19 metabolises proton pump inhibitors, some antidepressants, diazepam, proguanil and propranolol, among others[72] . Omeprazole has been

used as a probe inhibitor of CYP2C19 both *in vitro* and *in vivo*. Other drugs, such as fluoxetine, fluvoxamine, ticlopidine and isoniazid, can also inhibit the metabolism of CYP2C19 substrate drugs .Rifampicin and artemisinin have been identified as inducers of CYP2C19.Approximately 3-5% of Caucasians and up to 20% of Asian populations are poor metabolisers of CYP2C19 substrates .CYP2C19 genotype has been shown to affect the efficacy of proton pump inhibitor treatments [73].

2.4.3.4 CYP2D6

CYP2D6 is the only functionally active isoenzyme in the CYP2D subfamily in humans. Although it constitutes only 2-5% of total CYP content in the liver, it is responsible for up to 25% of the metabolism of known drugs[(74)] .At lower levels, CYP2D6 is expressed in extrahepatic tissues, including the gastrointestinal tract and brain. CYP2D6 is polymorphically expressed, with four existing phenotypes that define the rate of drug metabolism by CYP2D6; poor metabolizes (PM), who lack the functional enzyme, intermediate metabolisers (IM), who have at least one partially defi cient allele, extensive metabolisers (EM), who have two normal alleles, and ultrarapid metabolisers (UM), who have multiple gene copies. Up to 10% of Caucasians are poor metabolisers of CYP2D6 CYP2D6 substrates include many tricyclic antidepressants[75], selective serotonin reuptake inhibitors (SSRIs), neuroleptics, beta-blockers and opiates. Quinidine, paroxetine, fluoxetine, fluvoxamine, moclobemide, flecainide and terbinafine are potent inhibitors of CYP2D6.In contrast to all other CYP enzymes involved in drug metabolism, CYP2D6 is not known to be inducible.

2.4.3.5 CYP2E1

CYP2E1 accounts for about 14-17% of hepatic CYP content. This isoenzyme has mainly toxicological relevance, since it can bioactivate many compounds to carcinogens and reactive metabolites .It takes part in the metabolism of ethanol, disulfiram, paracetamol, anaesthetics such as halothane, enflurane, isoflurane and sevoflurane, and chlorzoxazone, which serves as a probe of CYP2E1 activity Ethanol and isoniazid are known inducers of CYP2E1. Disulfiram is reduced by CYP2E1 to diethyldithiocarbamate (DDC), which is used as an *in vitro* inhibitor of CYP2E1.

3.4.3.6 CYP3A subfamily

CYP3A enzymes are the most important oxidative enzymes in human drug metabolism. They have been estimated to participate in the metabolism of 40-50% of all drugs. The isoforms of CYP3A in humans include CYP3A4, CYP3A5, CYP3A7 and CYP3A43. CYP3A4 is the most abundant CYP enzyme in both the small intestinal mucosa and the liver, where it accounts for almost 30% of total CYP content. Its substrates include the calcium-channel blockers nifedipine, felodipine, diltiazem and verapamil, the HMG-CoA reductase inhibitors atorvastatin, lovastatin and simvastatin, the HIV protease inhibitors, the PDE5 inhibitors such as sildenafi l, and the benzodiazepines alprazolam, midazolam and triazolam.

Hepatic and intestinal CYP3A4 can be induced by several drugs, such as carbamazepine, phenytoin and rifampicin, and St. John's wort (*Hypericum Perforatum*). There are many known potent inhibitors of CYP3A4, including the azole antifungals ketoconazole, itraconazole and voriconazole, the macrolide antibiotics clarithromycin, erythromycin and troleandomycin, the calcium-channel blockers diltiazem and verapamil, the HIV protease inhibitors and grapefruit juice. Although the activity of CYP3A4 varies greatly, its population distribution is unimodal and genetic polymorphisms do not appear to explain the inter individual variation.

CYP3A5 is significantly expressed in only about 10-20% of Caucasian livers. It is also found in extra hepatic tissues and is the dominant CYP3A form in the human kidney. In individuals expressing CYP3A5, the contribution relative to the total hepatic CYP3A seems to range from 17% to 50% . CYP3A7 is present primarily in foetal tissues, representing about 50% of the total CYP in foetal liver. It is also expressed in some adult livers. The substrate specificities of CYP3A5 and CYP3A7 are, in general, similar to that of CYP3A4. Variable expression of CYP3A5 and CYP3A7 may account in part for the variation in the metabolism of CYP3A4 substrates. CYP3A43 is expressed in relatively high levels in the prostate and testes, but its expression in the liver is low. The functional role and substrate specificity of CYP3A43 are currently unknown.

2.4.4 Induction and inhibition of CYP enzymes

Induction has been suggested to be an adaptive process in which prolonged exposure to drugs or other chemicals causes an up-regulation in the amount of enzymes that are capable of metabolizing the inducing agent. Since induction affects the rate of protein synthesis (or degradation), a steady state with respect to induction is generally reached in two to three weeks. The disappearance of the induction effect (wash-out period) after discontinuation of the inducing agent can also take several weeks. Induction of drug-metabolising enzymes may increase the elimination and reduce the bioavailability of the substrate drug, and correspondingly, decrease the drug's plasma concentration. In contrast, for drugs that are metabolized to active or reactive metabolites,

induction can lead to enhanced drug effects or toxicity .Inhibition of drug-metabolising enzymes can lead to increased plasma concentration of the substrate drug, and thus, exaggerated and prolonged pharmacological effects.

This increases the likelihood of adverse effects and drug toxicity, especially with drugs that are extensively metabolised and have a narrow therapeutic index, unless appropriate dose reductions are made. In the case of prodrugs requiring metabolic activation, inhibition can reduce the clinical efficacy of the substrate drug. Contrary to induction, inhibition may occur immediately after one or two doses of the inhibitor.

3.4.4.1 Mechanisms of induction

Induction of drug-metabolising enzymes is mainly mediated by intracellular nuclear receptors. These include the pregnane X receptor (PXR), the constitutive androstane receptor (CAR) and the aryl hydrocarbon receptor (AhR). PXR and CAR mediate the induction of CYP2 and CYP3 enzymes, but phase II conjugative enzymes and drug transporters can also be induced. The mechanism of induction involves binding of the inducing agent to PXR or CAR. The complex then forms a heterodimer with the retinoid X receptor (RXR), which in turn binds to DNA-responsive element and enhances the transcription of the target gene .PXR and CAR seem to have flexible and overlapping binding specificities, and they can activate each other's target genes. Human PXR is activated by a wide range of structurally diverse chemicals, such as rifampicin, ritonavir and hyperforin, and phenobarbital has been shown to activate human CAR. Both PXR and CAR are abundantly expressed in the liver and intestine, with little expression appearing in other tissues.

Polycyclic aromatic hydrocarbon compounds found in tobacco smoke and grilled food induces drug-metabolising enzymes by binding to the AhR. This complex, together with another protein, AhR nuclear translocator (Arnt), increases enzyme expression by binding to the target gene's responsive element. This mechanism activates mainly CYP1A1 and CYP1A2, but the concentrations of glutathione S-transferase and UDPglucuronosyltransferase enzymes are also increased. Other known nuclear receptors, including the farnesoid X receptor (FXR) and the peroxisome proliferators activated receptor (PPAR), have also been shown to take part in regulating the expression of drug disposition genes .Contrary to nuclear receptor-mediated induction; ethanol can induce CYP2E1 by stabilisation of the enzyme, which results in accumulation of CYP2E1.

2.4.5 Mechanisms of inhibition

The clinically relevant mechanisms of CYP inhibition can be divided into reversible and irreversible inhibition (mechanism-based inactivation. Based on enzyme kinetics, reversible inhibition can be categorised into competitive, uncompetitive, noncompetitive and mixed inhibition.

In competitive inhibition, the inhibitor binds to the enzyme at the same site as the substrate and subsequently blocks the substrate binding; the substrate and inhibitor compete for the enzyme's active site. This type of inhibition can be overcome by increasing concentrations of the substrate. Competitive inhibitors oft en have similar structures as the substrate drugs. In uncompetitive inhibition, the inhibitor binds effectively only to the enzyme-substrate complex and makes the complex catalytically inactive.

In noncompetitive inhibition, the inhibitor binds to the enzyme with the same affinity in both unbound and substrate-bound forms, reducing its catalytic activity. As a result, the extent of inhibition depends only on the concentration of the inhibitor. In mixed inhibition, the inhibitor can bind with different affinities to the enzyme or to the enzyme-substrate complex, thus interfering with the substrate binding and vice versa. High substrate concentrations can reduce but not overcome mixed inhibition. In irreversible inhibition, drugs with reactive functional groups are metabolised by CYP enzymes to reactive intermediates that covalently modify the CYP enzyme, and inhibition cannot therefore be reversed. Because metabolic activation is required, these drugs are oft en called mechanism-based inactivators or suicide substrates. Binding of an irreversible inhibitor to the enzyme can be prevented by competition with a substrate or a reversible inhibitor.

2.4.6 Drug transporters

It has become increasingly evident that active drug transport systems influence the pharmacokinetics of many drugs by controlling their movement into and out of cells. Transporters work in concert with drug-metabolising enzymes, and it is thus oft en appropriate to consider together the impact of CYP mediated drug metabolism and transporter-mediated drug efflux and uptake when making assessments of drug pharmacokinetics. Drug interactions can occur when induction or inhibition of drug transporters alter e.g. intestinal absorption, proximal renal-tubular excretion, biliary excretion or penetration across the blood-brain barrier of substrate drugs.

The best-known drug transport systems that play a role in drug interactions are the P-glycoprotein (MDR1, multidrug resistance protein 1) and human organic anion transporting polypeptides (OATPs).

The P-glycoprotein is a transmembrane protein that operates as an efflux pump to export drugs out of cells. It facilitates excretion of substances into urine, bile and intestinal lumen (i.e. reduced absorption) and prevents excess accumulation in the brain. There is overlapping specificity between CYP3A4 and P-glycoprotein substrates and inhibitors. Substrate drugs for P-glycoprotein include anticancer drugs, HIV protease inhibitors, steroids, digoxin, quinidine, cyclosporine and loperamide. Quinidine, erythromycin, verapamil and itraconazole are known inhibitors of P-glycoprotein. P-glycoprotein is inducible by, for example, rifampicin and St. John's wort .OATPs are a class of transmembrane proteins that are expressed in human intestine, liver, kidney and brain tissue .In contrast to the P-glycoprotein, OATPs act as drug uptake pumps, transporting drugs into cells. Substrates for members of the OATP subfamily include bile salts, hormones, HMG-CoA reductase inhibitors, digoxin and methotrexate.

Many agents that affect P-glycoprotein function also affect OATP activity. Coordinate activity of both drug uptake and efflux transporters may determine the net absorption and subsequent elimination of a drug. In addition to drug-induced changes in P glycoprotein and OATP activity, these transporters also exhibit genetic polymorphism.

Of the 12 million people in the U.S. afflicted with NIDDM, nearly 40% are currently being treated with oral hypoglycemic agents [(2);] it is clearly imperative to seek optimal treatment for these patients.

Patients with type 2 diabetes often use several drugs concurrently [1]and are therefore prone to harmful drug-drug interactions. Many clinically important drug interactions are related to cytochrome P450 (CYP) enzymes, whose activity can be suppressed (inhibition) or enhanced (induction) by different drugs.

2.5 Co-existence and relation between diabetes and depression:

Depression is one of the most common illnesses affecting in excess of 10-15% of the population at some time in their lives.[8]It is a chronic illness that affects mood, thoughts, physical health and behavior of any individual and has been estimated to affect up to 21% of the world's population. Depression will be the second largest killer after heart disease by 2020-studies show depression is a contributory factor to fatal coronary disease.[9, 10] Antidepressants are used to elevate mood in depressive illness.

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose level and disturbance in carbohydrate, proteins and fat metabolism and increased risk of complications from vascular diseases. There are estimated 143 million people worldwide suffering from diabetes and the number may probably doubled by the year 2030.[11]In India the prevalence rate of diabetes is estimated to be 1-5%.[12]

Depression and diabetes are common conditions that often coexist and may clinically interact with each other. Depression has a negative impact on medication adherence[13] .The disorders are managed clinically by administering numbers of drugs for long duration. In such a scenario, there is a possibility that one drug may alter the effects of other drugs. The study conducted by Raval *et al* regarding the prevalence and determinants of depression in type 2 diabetes patients concluded the high prevalence of depression in patients with type2 diabetes mellitus also there was 41 per cent prevalence of depression among the diabetic subjects.[14] Diabetic patients have a 20% higher risk of depression than the general population as treatment with antidepressant drugs can directly interfere with blood glucose levels or may interact with hypoglycaemic agents.[15]

Desvenlafaxine an antidepressant of class selective serotonin norepinephrine reuptake inhibitor is an o-desmethylvenlafaxine, a synthetic form of major metabolite of venlafaxine. It has been formulated as prolonged release tablet.[16]It is primarily metabolized via glucuronide conjugation and minimally by oxidative metabolism via CYP3A4 enzyme family. At 72 hours about 45% is excreted unchanged in urine. The mean plasma elimination half-life ($t_{1/2}$) of Desvenlafaxine is approximately11 hours [17, 18]

Pioglitazone is a thiazolidinedione that specifically reduces insulin resistance in type-2 diabetes. It has proved to be an effective antihyperglycaemic agent, reducing fasting plasma glucose and displaying dose-dependent, long-term reductions in glycosylated haemoglobin (HbA1C) [19].Pioglitazone undergoes extensive hepatic metabolism, predominantly via the CYP2C8 system. Secondary pathways include CYP3A4, CYP2C9 and CYP1A1/2. Pioglitazone has absolute bioavailability of 83% with terminal half life of 5-6 hours and 97% protein bound.[19]

It is found that both the drugs are metabolized by common enzymes CYP3A4. We are expecting that Pioglitazone may also alter the effects of Desvenlafaxine by altering the pharmacokinetic parameters similar to other drugs metabolized by common way. The possibility of these two drugs to interact is by alteration in the absorption site, replacement at protein binding site (distribution) also at metabolism site and elimination site. Any

alteration on the effect of it is harmful to the patient since, it may affect the mutual depression of the patient. The study on antidepressant activity will help to predict the extent of interaction whereas the study on pharmacokinetics parameters will help to predict the mechanism of action of drug interaction and also help to support the pharmacodynamic interaction. So it is very much significant and justified to conduct experiment on animals to verify the drug-drug interaction between pioglitazone and desvenlafaxine**.**

3. MATERIALS AND METHODS

3.1. Material selection

3.1.1. Experimental animals and treatment

Housing

Healthy adult male albino rabbits, healthy albino rats weighing 2.8 to 3.5 kg, 160-180g, respectively were selected for the experiment. Rabbits were housed in stainless steel cages with a fenestrated floor to allow feces to drop through into a pan and were provided with regular rabbit chow. Rats are housed in separate clean cages. The bedding material of the cages rats were removed and replaced thrice a week with fresh materials as often as necessary to keep the animals clean and dry. The animals were provided with distilled water ad libitum throughout the experiment. The rats were fed with standard pelleted diet. The animals were acclimatized to standard laboratory conditions of temperature (25±30C) and maintained on 12:12 hour natural light: dark cycle.

The animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).The study protocol was approved by Institutional Animal Ethics Committee (IAEC), Liveon Biolabs Private limited, Tumkur, Reg. no. 1610/RO/C/12/CPCSEA.

Animal identification: Cage cards were utilized to identify the strain of animal, sex, and number, name of the principal investigator and title of research protocol. Temporary identification of individual animal was accomplished by dyeing the fur.

3.1.2. Chemicals

- **Desvenlafaxine:** Pure sample of Desvenlafaxine was procured as a gift sample from Micro labs, Hosur, Tamil Nadu.
- **Pioglitazone:** Pure sample of Pioglitazone was procured as a gift sample from Micro labs, Hosur, Tamil Nadu.
- **5-Hydroxytryptophan:** Yarrow Chem Products, Mumbai.
- **Normal saline:** Lifusion alkem pharma
- **Tween-80:** Merck specialties pvt. ltd.
- **Surgical spirit:** Karnatka Fine Chem
- Distilled water.

All the chemicals used were of analytical grade.

3.1.3. Apparatus

- High performance Liquid Chromatography Agilent 1200 series model L-7100.
- Laboratory Centrifuge Remi R8C.
- Electronic balance.Ohous corp. pine brooki, NJ USA AR0640-N13123
- Rabbit holder, Disposable syringe, 22 & 26 gauge Needles, Spatula, Sterilized cotton, Oral cannula, Variable micropipette [0-100 µL], Volumetric flask, Measuring cylinder, Mortar and pestle, Eppindroff tubes, Centrifuge tubes, Refrigerator, Membrane [Nylon-65] syringe filter.

3.2. Methods

3.2.1. Preparation of Desvenlafaxine standard solution

25 mg of standard DVS taken into 100 ml volumetric flask containing 60 ml distilled water, sonicated for 15 minutes, diluted up to volume 100 ml with water and filtered discarded first 5 ml. filtrate 25 ml transferred to 50 ml volumetric flask and complete the volume with acetonitrile (Concentration 125µg/ml).

3.2.2. Preparation of Pioglitazone standard solution

30mg of pioglitazone pure sample was dissolved in 1% of Tween-80, which was prepared by triturating 1ml of surfactant Tween-80 in little quantity of water and make up in 100ml volumetric flask with distilled water. Stock solution was prepared to get 3mg/ml concentration.

3.2.3. Preparation of 5-hydroxytryptophan standard solution

5-Hydroxytryptophan was dissolved in saline (0.9%) after triturating with 10% Tween-80. Final volume was made up in volumetric flask using saline. Stock solution was prepared to get 2mg/ml concentration.

3.2.4. General procedures

Method for oral administration of drug in rabbits

The rabbits were fixed in wooden stalls and an oral gag was placed in between the jaws and held in

position by holding upper and lower jaw using the left hand. One end of the feeding tube was moistened with glycerin and introduced into the mouth through the central hole of the gag. One end of the feeding tube was pushed slowly such that it enters the esophagus. The other end of the feeding tube was connected to a syringe, 2-3 ml of distilled water was administrated initially to ensure that the incubation is in right position.

The drug solution was administrated similarly and this was followed by 3ml-distilled water to ensure the administration of correct dose of the drug. The oral feeding tube was then gently removed and the animal was removed from the wooden stall immediately and is tilted with its head down, which prevents the entry of any fluid into the respiratory tract. The gag and the oral feeding tube were cleaned.

Blood sampling technique in rabbit

Blood samples were collected from the marginal ear vein. For this, the rabbits were kept in wooden stalls, with their heads-protruding out. The marginal ear vein was located by gentle trucking and tapping of the ear, this made the vein more visible. After disinfecting the site, 22 gauge needles was inserted into the ear vein and blood was collected from the hub of the needle directly into a blood collecting tube. A cotton ball was pressed on the top of the injection site before pulling out the needle, and clip was applied on the top of the cotton ball for 2-3 minutes to stop further bleeding.

Method for collection of serum

The collected blood and was kept aside for30-40 minutes. The serum was obtained by centrifuging the blood sample at 3000 RPM for 15-20 minutes. The transparent supernatant liquid (serum) obtained is transferred into a clean dry 5 ml eppindrof tubes.

Estimation of desvenlafaxine by HPLC method

Matoga, M., F Pehourcq, K. Titier, F. Dumora and C. Jarry h reported the estimation of serum desvenlafaxine using High Performance Liquid Chromatography [HPLC]. It is a sensitive, accurate and reproducible method for the estimation of desvenlafaxine in plasma or serum. In this method, the estimation of desvenlafaxine is carried out using a stationary phase Qdalisil BDS C_{18} 4.6x150 mm, 5µ, UV detection at a wavelength of 229 nm. A Aligent 1220 LC HPLC instrument with a double beam ultraviolet detector set at 229 nm was used. Chem station software was used for data handling. The column used was Qdalisil BDS C_{18} 4.6x150 mm, 5 µ. The 20 ml of the serum was injected into the column. Flow rate was maintained at 1.0ml/min. The chromatogram was recorded and used for quantification.

The amount of desvenlafaxine present in serum is calculated using the following equation:

3.3. Experimental procedure

3.3.1. Effect of pioglitazone treatment on pharmacokinetic parameters of desvenlafaxine in healthy albino rabbits.

Four male albino rabbits weighing between 2 to 2.5 kg were taken and marked suitably. Rabbits were fasted for 18 hours before commencing the experiment. During this period, the rabbits were allowed to take adequate water. Fasting was continued till the completion of the experiment. After fasted for 18 hours, the blood was collected (at '0' hour) before the administration of desvenlafaxine. Later all the rabbits received desvenlafaxine (30mg/kg, p.o.) solution orally, the time of administration was noted.

Blood samples were collected thereafter at prefixed time intervals i.e. 0.1, 2, 4, 8 and 12 hours after dosing in blood collection tube and kept aside for 30-40minutes. Serum samples were obtained after centrifugation at 3000 r.p.m. for 15-20minutes. The transparent supernatant liquid (serum) obtained was transferred into a clean dry eppindroff tubes. Serum samples were stored at -20°C for analysis.

After blood collection animals were left for a washout period of 15 days with normal diet. The next part of this experiment was conducted on the same group of animals. All the rabbits received pioglitazone (3mg/kg, p.o.) orally once a day for one week. On the 7^{th} day, 6 hours after administration of the drug, the rabbits were fasted for 18 hours. On the 8^{th} day, pioglitazone (3mg/kg, p.o.) was administered orally to all the animals, the time of administration was noted. After 60minutes of pioglitazone administration, desvenlafaxine (30mg/kg, p.o.) was given orally. Blood samples were collected in a blood collection tube at prefixed time intervals i.e. 0,1, 2, 4, 8, and12 hours after desvenlafaxine dosing, serum was separated from blood and stored at -20°C for analysis.

The serum concentration of desvenlafaxine was estimated by High Performance Liquid Chromatography method.[70] Desvenlafaxine estimation method: in this method, the estimation of desvenlafaxine is carried out using a reversed phase $C_{18\ column}$ (Gracesmart RP-18), mobile phase (Acetonitrile/0.25 M potassium phosphate (pH 2.7) 30:70 v/v) and UV detection at a wavelength of 235 nm. Flow rate maintained at 1.0 ml/min.

The results are tabulated in table 7, and depicted in figure 4.

The serum concentration of desvenlafaxine before and after treatment pioglitazone were applied to software Ramkin to calculate pharmacokinetic parameters like AUC_{0-t}, $AUMC_{0-t}$, C_{max}, T_{max}, $t_{1/2}$ and MRT. The results are presented in **table 8**, and the changes in pharmacokinetic parameters are depicted in **figure 5**.

The chromatogram obtained for standard desvenlafaxine are depicted in **figure 6**, and the chromatogram obtained for the serum desvenlafaxine at 4th, hour of day 1 (i.e. before pioglitazone treatment) are depicted in **figure 7**. The chromatograms obtained for serum desvenlafaxine concentration at 4th hour of day 21 (after pioglitazone treatment) are depicted in **figure 8**.

Evaluation

Pharmacokinetic data of desvenlafaxine was measured assuming complete oral absorption. All the experimental results were expressed as mean ±SEM and assessed by student't' test using parametric statistics, IBM PC version 1.01, London software, INC. A value of $P<0.05$ was considered statistically significant.

3.3.2. Effect of pioglitazone treatment on antidepressant activity of desvenlafaxine in healthy albino rats

3.3.2.1. Despair swim test

Purpose and rationale

Behavioral despair was proposed as a model to test for antidepressant activity by Porsolt, et al. It was suggested that mice or rats forced to swim in a restricted space from which they cannot escape are induced to a characteristic behavior of immobility. This behavior reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression.[71]

Experimental procedure

Six Male albino rats weighing between 160-180 grams were used. All rats were brought to the laboratory one day before the experiment and were housed separately in cages with free access to food and water. Rats are individually forced to swim inside a vertical Plexiglas cylinder (height: 40cm; diameter: 18cm) containing 15cm of water maintained at 250C.

Rats placed in the cylinder for the first time are initially highly active, vigorously swimming in circles, trying to climb the wall or diving to the bottom. After 2–3 min activity begins to subside and to be interspersed with phases of immobility or floating of increasing length. After 5–6 min immobility reaches a plateau where the rats remain immobile for approximately 80% of the time. After 15 min in the water the rats are removed and allowed to dry in a heated enclosure (32 °C) before being returned to their home cages. They are again placed in the cylinder 24 h later and the total duration of immobility is measured during a 5 min test.

An animal is judged to be immobile whenever it remains floating passively in the water in a slightly hunched but upright position, its nose just above the surface.

In the first part of experiment, animals were administered with desvenlafaxine (30 mg/kg, p.o.) The time of the drug administration was noted for all the animals. The animals were forced to swim and the duration of immobility was measured for a duration of 5 min. at 30th min, 1st , 2nd , 4th and 6th hour after drug administration. All the rats were left for washout period of 15 days.

In the next part of the experiment, the same groups of animals after a gap of 15 days were administered with pioglitazone (3mg/kg, p.o.) once a day for one week. On the 8th day, pioglitazone (3mg/kg, p.o.) were administered to all the animals, and the time of administration was noted. After 60minutes of Pioglitazone administration, desvenlafaxine (30mg/kg, p.o.) was administered, the test was repeated and the total duration of immobility for duration of 5 min was measured at 1st, 2nd, 4th, 8th, and 16th hour after desvenlafaxine administration. The results obtained are tabulated in **table 9**, depicted in **figure 9.**

Evaluation

The data are expressed as mean ± SEM for each treatment group. The data obtained from each response measures were subjected to students't' test using parametric statistics, IBM PC version. 1.01. London. A value of $P<0.05$ was considered statistically significant.

3.3.2.2. Serotonin syndrome method [71, 72]

Purpose and rationale

Compounds which stimulate serotonin receptors or which increases dramatically the serotonergic transmission in the CNS cause a series of behavioral changes in rats which is called the serotonin syndrome such as head weaving, increased locomotion, forepaw treading, tremor, hind limb abduction, flat posture and lower lip retraction. With increasing knowledge about the subtypes of serotonin receptors these symptoms were defined to be associated with 5-HT receptors and their specific agonists.

The behavioral motor syndrome (5-HT syndrome) can be elicited by injecting, the serotonin precursor L-5-hydroxytryptophan, 5-HT agonists like 5-methoxy-N,N dimethyltryptamine (5-MeODMT) or quipazine. 5-HT-releasing compounds like p-

chloroamphetamine or fenfluramine have also been shown to induce the 5-HT syndrome.

The serotonin syndrome in rats has been used to study the interaction of drugs with central 5-HT system of rats. It is also used for the screening of the psychoactive drugs and this method offers several advantages like this is fast, require no elaborate equipment and provide information on CNS permeability etc.

Experimental procedure

Six male albino rats weighing between 160-180 grams were selected and housed in cage with free access to food and water. In the first part of experiment, rats were administered with desvenlafaxine (30mg/kg, p.o.) .The time of the drug administration was noted for all the animals. After 30minutes of desvenlafaxine administration, 5-hydroxytryptophan (5-HTP) (50 mg/kg, i.p.) was administered to all the rats. Each rat was scored during 0, 15, 30, 45 and 60 minute after the injection. The severity of the symptoms were scored as following scale, forepaw treading (0=absent; 1=weak; 2=continuous), head weaving (0=absent; 1=weak; 2=continuous),hind limb abduction (0=absent; 1=present),. All the rats were left for washout period of 15 days.

In the next part of the experiment, the same animals after a gap of 15 days were administered with pioglitazone (3mg/kg, p.o.) once a day for one week. On the 8th day, pioglitazone (3mg/kg, p.o.) was administered to all the animals, and the time of administration was noted. After 60 minutes of pioglitazone administration, desvenlafaxine (30mg/kg, p.o.) was administered. Again after 30minutes of desvenlafaxine administration, 5-hydroxytryptophan (50mg/kg, i.p.) was administered to all the rats.

Severities of symptoms were scored as mentioned earlier. The results obtained are tabulated in the **table 10,** depicted in **figure 10.**

Evaluation

The data are expressed as mean ± SEM for each treatment group. The data obtained from each response measures were subjected to students't' test using parametric statistics, IBM PC version. 1.01. London. A value of P<0.05 was considered statistically significant.

RESULT AND DISCUSSION

Table 7: Data showing the serum concentration of desvenlafaxine before and after pioglitazone treatment in healthy albino rabbits

Time (hours)	Serum desvenlafaxine concentration in µg /ml (Mean ± SEM)	
	Desvenlafaxine (30 mg/kg, p.o.)	Desvenlafaxine (30 mg/kg, p.o.) + Pioglitazone (3mg/kg, p.o.)
0	0.0 ± 0.0	0.0 ± 0.0
1	103.67 ± 1.21	105.56 ± 2.0
2	93.27 ± 1.73	93.36 ± 1.70
4	73.97 ± 2.06	81.36 ± 2.13 *
8	64.53 ± 2.41	68.56 ± 0.08
16	35 ± 1.88	42.26 ± 0.54 *

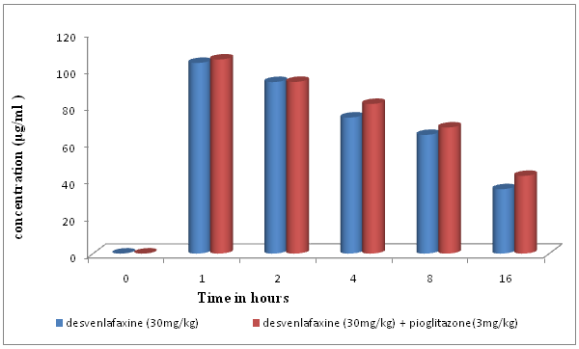
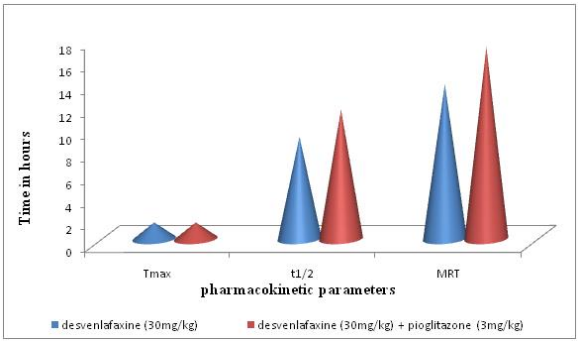


Fig 7: Graphical representation showing the serum concentration desvenlafaxine before and after pioglitazone treatment in healthy albino rabbits



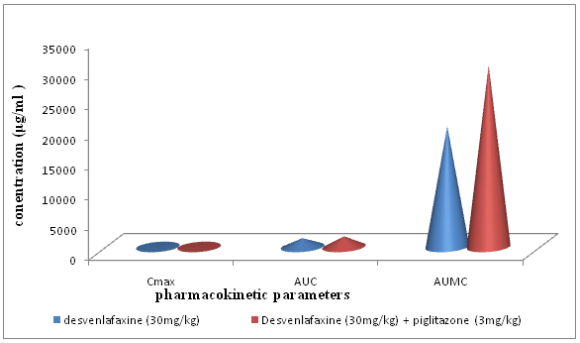


Fig. 8: Graphical representation of the effect of pioglitazone on the pharmacokinetic parameters of desvenlafaxine in healthy albino rabbits

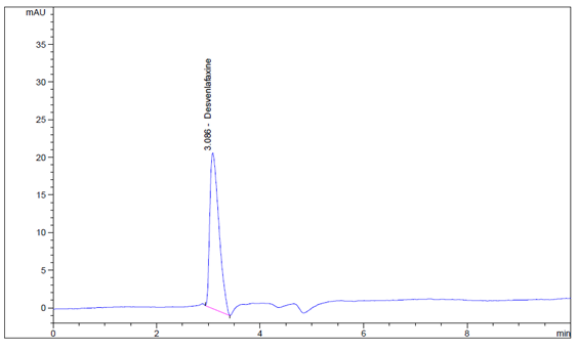


Fig 9: Chromatogram of serum desvenlafaxine (4th hour of 1st day of treatment)

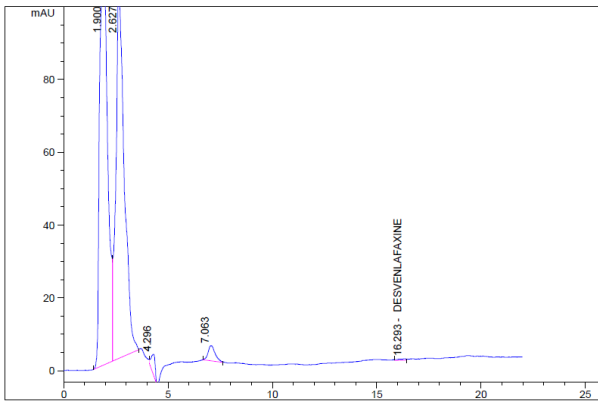


Fig 10: Chromatogram of serum desvenlafaxine (4th hour of 1st day of treatment)

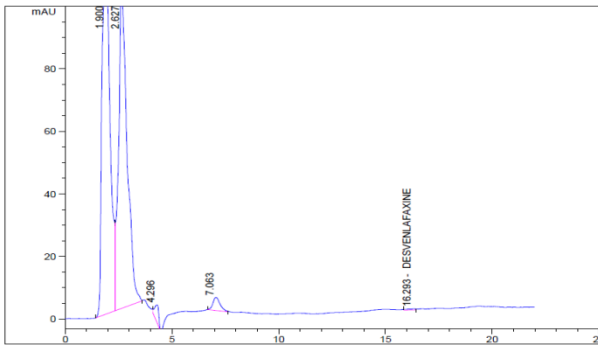


Fig 11: Chromatogram of serum desvenlafaxine concentration after pioglitazone treatment at 4th hour

Table 9: Data showing the immobility time of desvenlafaxine before and after pioglitazone treatment in healthy albino rats by despair swim test

Time Intervals (hour)	Duration of immobility time in seconds (Mean ± SEM)	
	Drug treatment	
	Desvenlafaxine (30 mg/kg, p.o.)	Desvenlafaxine (30 mg/kg, p.o.) + Pioglitazone (3mg/kg, p.o.)
1	91.33 ± 1.26	89.4 ± 0.56
2	96.16 ± 0.66	94.66 ± 0.99
4	91.66 ± 0.49	98.0 ± 0.80*
8	89 ± 0.58	84 ± 0.52
16	86 ± 2.05	82.7 ± 0.84

*P<0.05 = Significant.

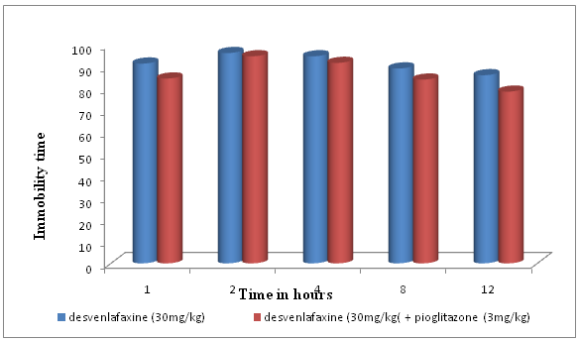


Fig 9: Graphical representation of immobility time of desvenlafaxine before and after pioglitazone treatment in albino rats by despair swim test.

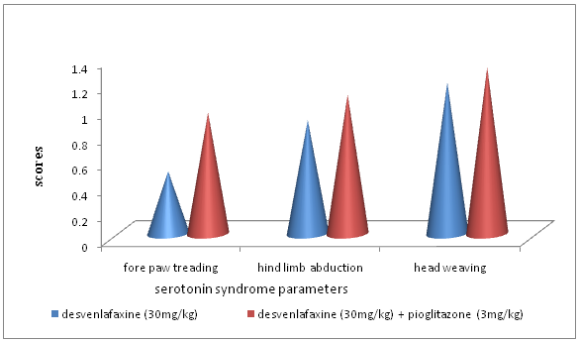


Fig 10: Graphical representation of effect of pioglitazone treatment on desvenlafaxine in healthy albino rats by serotonin syndrome test

4.1 Results Effect of pioglitazone treatment on the pharmacokinetic parameters before and after desvenlafaxine in healthy albino rabbits

Desvenlafaxine is well absorbed after oral administration, concentration of 103.67 mcg/ml is observed at 1st hour and it is the peak effect. It started declining at 2nd hour i.e. 93.27 mcg/ml, 4th hour 73.97 mcg/ml, 8th hour 64.53 mcg/ml and a sustained concentration of 35 mcg/ml at 16th hour.

Desvenlafaxine concentration after pioglitazone treatment for one week did not show significant increase at 1st and 2nd hour however there is significant increase in concentration at 4th ,8th and 16th hour after pioglitazone treatment.

The pharmacokinetic parameters in Table4 revealed that pioglitazone treatment for one week failed to show any significant changes in T_{max} and C_{max} of desvenlafaxine.

AUC AND AUMC of desvenlafaxine increased from 1452.37 to 1769 µg/ml/hr and 20022.57 to 30159.33 µg/ml/hr respectively.

The terminal half life of desvenlafaxine was increased from 9.07 to 11.47 hours due to pioglitazone treatment. MRT of desvenlafaxine was increased to 17.03 hour from 13.73 hour by pioglitazone.

The effect of pioglitazone treatment on the antidepressant effect of desvenlafaxine in healthy albino rats by despair swim test

Desvenlafaxine exhibited immobility time of 91.33 seconds at 1st hour and maximum of 96.16 at 2nd hour. Immobility time of 86 seconds was observed at 16th hour of treatment.

In next phase, pioglitazone treatment for one week did not show significant increase in the immobility duration at 1st, 2nd ,8th , and 16th hour of treatment .The significant increase in the immobility duration is observed at 4th hour of pioglitazone treatment followed by desvenlafaxine.

The effect of pioglitazone treatment on the antidepressant activity of desvenlafaxine by serotonin syndrome

Desvenlafaxine (30 mg/kg, p.o.) exhibited fore paw treading 0.0 at 15th second its peak effect was noted 1.0 at 60th second and 0.33 and 0.66 at 30 and 45th second respectively in one minute after drug administration.

Pioglitazone (3 mg/kg, p.o.) treatment for one week increases the fore paw treading score in healthy albino rats slightly to 0.3, 0.83, 1.16 and 1.6 at 15th, 30th, 45th and 60th seconds after administration of desvenlafaxine on the 8th day.

Hind limb abduction has a slight increasing effect. Desvenlafaxine (30 mg/kg, p.o.) exhibited hind limb abduction 0.50 at 15th seconds its peak effect was noted 1.62 at 60th seconds and 1.0 and 1.25 at 30th and 45th seconds in respectively.

Pioglitazone (3 mg/kg, p.o.) treatment for one week shown slight increase in the hind limb abduction in healthy albino rats to 0.43, 0.5,0.75,1.75 at 15th , 30th , 45th and 60th seconds respectively after 30 mg/kg, p.o. dose of desvenlafaxine at 8th day.

Desvenlafaxine (30 mg/kg, p.o.) exhibited head weaving score 0.75 at 15th seconds its peak effect was noted 1.75 at 60th second and 1.0 and 1.25 at 30th and 45th seconds respectively.

Pioglitazone (3 mg/kg, p.o.) treatment for one week increases the hind limb abduction in healthy albino rats to 0.85, 1.2, 1.35, 1.83 at 30 mg/kg, p.o. dose of desvenlafaxine at 15th ,30th ,45th and 60th seconds respectively. Serotonin syndrome is associated with increased serotonergic activity in the central nervous system (CNS). Serotonin syndrome is a potentially fatal complication of serotonergic drug therapy. Usually, serotonin syndrome occurs with the concomitant use of two serotonergic drugs.

4.2 Discussion

Depression is one of the most common illnesses affecting in excess of 10-15% of the population at some time in their lives. It is a chronic illness that affects mood, thoughts, physical health and behavior of any individual and has been estimated to affect up to 21% of the world's population. Depression will be the second largest killer after heart disease by 2020- studies show depression is a contributory factor to fatal coronary disease. Antidepressants are used to elevate mood in depressive illness.

Depression and diabetes are common conditions that often coexist and may clinically interact with each other. Depression has a negative impact on

medication adherence[13] .The disorders are managed clinically by administering numbers of drugs for long duration. In such a scenario, there is a possibility that one drug may alter the effects of other drugs. The study conducted by Raval *et al* regarding the prevalence and determinants of depression in type 2 diabetes patients concluded the high prevalence of depression in patients with type2 diabetes mellitus also there was 41 per cent prevalence of depression among the diabetic subjects.[14] Diabetic patients have a 20% higher risk of depression than the general population as treatment with antidepressant drugs can directly interfere with blood glucose levels or may interact with hypoglycaemic agents.[15]

Both the depression and diabetes have to be controlled by taking various therapeautic agents varying from 3 to 10. Many of these drugs have common enzymes for metabolism or highly bound to plasma protein hence, likely to alter the pharmacokinetics of other drugs. The possibility of drug-drug interaction is very high since no of drugs consumed are more for long time. The present study is planned to evaluate possible interference by diabetic drug pioglitazone on antidepressant desvenlafaxine. Any changes in the concentration of desvenlafaxine are fatal to the patient as higher concentration leads to CNS depression and lower concentration to uncontrolled depression.

The pioglitazone treatment for one week did not change the concentration of desvenlafaxine at 1st and 2nd hour, later there is slight increase in concentration at 4th , 8th and 16th hour treatment. The result indicates the possible interference by pioglitazone on the metabolism of desvenlafaxine.

The two primary oxidative metabolites of desvenlafaxine in human liver microsomes were formed by N-demethylation and hydroxylation on the benzyl ring of the molecule; CYP2C9 and CYP3A4 produced these metabolites *in vitro*. Based on the relative amounts of CYP isozymes in liver microsomes, the major isozyme involved in the oxidative metabolism of desvenlafaxine was determined to be CYP3A4. Multiple human UGT isoforms were shown to be involved in the metabolism of desvenlafaxine to desvenlafaxine-O-glucuronide, including UGT1A1, UGT1A3, UGT2B4, UGT2B15, and UGT2B17.

Pioglitazone is a thiazolidinedione that specifically reduces insulin resistance in type-2 diabetes. It has proved to be an effective antihyperglycaemic agent, reducing fasting plasma glucose and displaying dose-dependent, long-term reductions in glycosylated haemoglobin (HbA1C) [19].Pioglitazone undergoes extensive hepatic metabolism, predominantly via the CYP2C8 system. Secondary pathways include CYP3A4, CYP2C9 and CYP1A1/2. Pioglitazone has absolute bioavailability of 83% with terminal half life of 5-6 hours and 97% protein bound.[19]

On co-administration of pioglitazone and desvenlafaxine C_{max} and T_{max} did not change. AUC of desvenlafaxine has increased from 1452 to 1769 on pioglitazone treatment. Both the results indicate that there is no interference or negligible interference on absorption of desvenlafaxine by pioglitazone. The $t_{1/2}$ and $_{MRT}$ have increased from 9.07 to 11.47 and 13.73 to 17.03 respectively. These results indicate the presence of desvenlafaxine in the blood for long time. The probable reason is delay in metabolism of desvenlafaxine by pioglitazone, since both the drugs are metabolized by common enzymes CYP3A4 and CYP2C9.

Antidepressant activity of desvenlafaxine by pioglitazone was evaluated using two animal model models viz. Despair swim test and serotonin syndrome.

Rodents forced to swim in a narrow space from which there is no escape adopt, after an initial period of vigorous activity, a characteristic immobile posture, moving only when necessary to keep their heads above the water. The animals' immobility was interpreted as indicating they had learned that escape was impossible and had adopted an immobile position to conserve energy, viewed anthropomorphically as if they had given hope of escaping from this stressful situation[76].Immobility was therefore given the name behavioral despair.

The immobility time due to desvenlafaxine treatment did not change significantly by pioglitazone except at 4th hour. There is significant increase observed at 4th hour may be due to the accumulation of desvenlafaxine. This result is in accordance with pharmacokinetic parameters observed earlier part of experiment.

Serotonin syndrome is associated with increased serotonergic activity in the central nervous system (CNS). Serotonin

5. CONCLUSION

The following conclusions can be drawn from the experiment results observed during the study of pioglitazone (3 mg/kg, p.o.) treatment for 7 days showed changes in pharmacokinetic parameters of desvenlafaxine like AUMC, t1/2, and MRT of desvenlafaxine in healthy albino rabbits.

Pioglitazone (3 mg/kg, p.o.) treatment for 7 days showed significant increase in immobility time of desvenlafaxine tested by despair swim test.

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