

Evaluation of Therapeutic Effects of Rasagiline in Diabetic Sprague Dawley Rats



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ABSTRACT

INTRODUCTION

Diabetes mellitus is a collection of metabolic diseases with both micro and macrovascular consequences. It is one of the leading causes of death worldwide. Many people are unable to reach their target HbA1c even with the availability of numerous medications. There has been a case report of rasagiline-induced extreme hypoglycemia.

OBJECTIVE

The purpose of this study was to investigate how glimepiride & rasagiline affected FBGL & blood insulin levels in diabetic animal models, as well as to ascertain the effect of rasagiline on body weight, and serum insulin levels. A comparison of the combined effects of sulfonylureas and rasagiline was also proposed.

METHODOLOGY

Adult male Sprague Dawley rats were induced with diabetes mellitus with Alloxan, Animals were grouped into normal control group, diabetic group, glimepiride, low dose rasagiline, high dose rasagiline, rasagiline + glimepiride. The groups received the drugs by gavage for 14 days. Body weight and FBGL were assessed on days 0, 7, & 14. Serum insulin was tested using the ELISA technique on day 14. Mathematical formulas were used to calculate HOMA-B, HOMA-IR, and QUICKI.

RESULTS

Rasagiline had a dose-dependent hypoglycemic effect, lowering the FBGL by 15% and 31%, respectively, at 0.1 mg/kg and 0.25 mg/kg. Glimepiride and HR rasagiline had similar effects. Rasagiline alone and glimepiride together reduced FBGL by 49% and 56%, respectively. All treatment groups showed a considerable increase in serum insulin levels. Compared to the control group of diabetics, glimepiride and rasagiline both markedly enhanced mathematical measures of β -cell activity and insulin resistance, such as HOMA-B, HOMA-IR, and QUICKI.

CONCLUSION

In comparison to disease control, rasagiline is found to dramatically improve β -cell activity, boost insulin production, and decrease FBGL. Compared to glimepiride alone, rasagiline and glimepiride combined show a potentiating impact on FBGL and HOMA-B.

CHAPTER 1

INTRODUCTION

1.1 Diabetes Mellitus:

A group of metabolic disorders known as diabetes mellitus are characterized by persistently high blood sugar levels caused by deficiencies in the action or secretion of insulin, or both. This condition disrupts the metabolism of macromolecules, leading to a compromised ability of the body to regulate and respond to hormones, ultimately impacting the maintenance of proper blood glucose levels. (1) It is rising to an alarming epidemic level.

1.2 Symptoms and signs of Diabetes Mellitus:

T1DM commonly presents with the classic signs of diabetes, including increased thirst, increased hunger, and increased urine. It is characterized by a swift onset of severe hyperglycemia. These symptoms also occur in T2DM when hyperglycemia reaches very elevated levels. T1DM or long-term undiagnosed T2DM are the main causes of significant weight loss. Diabetes symptoms that may go undiagnosed include bodily pain, weariness, restlessness, and unexplained weight loss. Mild or slowly developing symptoms could also escape attention.(2)

1.3 Global Trends in Diabetes Prevalence:

Recent studies suggest that the occurrence of diabetes in adults is anticipated to rise from 4% in 1995 to around 6.4% by the year 2025. This shift is anticipated to be significant, with a 42% increase in developed countries, surging from 51 to 72 million, and a 170% surge in developing nations, escalating from 84 to 228 million. It is predicted that by 2025, there would be close to 380 million persons worldwide with diabetes, up from 194 million in 2003. (3) Notably, India, China, and the USA are anticipated to be the country's most severely impacted by this epidemic in 2025.(4) The global diabetes report from the World Health Organization (WHO) highlights that in many regions, the lack of effective policies to create environments conducive to healthy lifestyles, combined with restricted access to high-quality healthcare, leads

to inadequate attention to the prevention and treatment of the disease, especially among those with limited financial resources.(5)

Previously, diabetes was believed to primarily afflict wealthier individuals in urban settings. However, factors like urbanization, shifts in dietary habits, and increasingly sedentary lifestyles have led to its prevalence extending to middle and low-income countries, such as Pakistan. Prior to 2018, the sole comprehensive national diabetes survey conducted in Pakistan dated back to 1999, with its findings published in 2007. This survey revealed a type 2 diabetes prevalence rate of 11% based on the oral glucose tolerance test (OGTT). Additionally, different provinces of Pakistan were separately assessed for the prevalence of type 2 diabetes as part of the same survey.(6)

1.4 Types of Diabetes Mellitus:

A collection of disorders with consistently high blood sugar levels are together referred to as diabetes mellitus. The differences in the mechanisms underlying the development of these forms of diabetes serve as the basis for their classification.

Type 1 Diabetes Mellitus (T1DM), previously referred to as insulin-dependent or juvenile-onset diabetes, is an autoimmune condition in which pancreatic beta cells are targeted and destroyed by activated CD4⁺ and CD8⁺ T cells, along with macrophages infiltrating the pancreatic islets.(7) It commonly develops during childhood or early adulthood, typically before the age of 35.

On the other hand, Type 2 Diabetes Mellitus (T2DM) is marked by insufficient insulin synthesis and secretion resulting from insulin resistance. T2DM is further categorized into two subgroups: diabetes with and without obesity. In obese T2DM patients, resistance to endogenous insulin typically arises from changes in cell receptors, often associated with abdominal fat distribution. On the other hand, Non-obese T2DM is defined by post-receptor insulin resistance combined with inadequate insulin production and secretion.

Gestational diabetes mellitus (GDM) occurs when diabetes develops during pregnancy and typically resolves after the pregnancy ends. During pregnancy, women experience notable fluctuations in blood glucose levels and may go through prolonged fasting periods. Furthermore, a transient condition of insulin resistance is brought on

by the placenta's function in raising insulin secretion and decreasing insulin sensitivity at the end of the first trimester.(4)

1.5 Diagnosis of diabetes & its management

Early detection of diabetes and prediabetes is crucial, utilizing the recommended HbA1c criteria for various types, except gestational diabetes. (8) The American Diabetes Association (ADA) recommends using fasting glucose levels as a standard method for diabetes screening, although postprandial blood sugar, random blood sugar, and glucose tolerance tests are also utilized to assess blood sugar levels. A diabetes diagnosis can be made if at least one of the following conditions is met:

- Presence of diabetes symptoms (such as excessive urination, thirst, or unexplained weight loss) along with a random plasma glucose level of ≥ 11.1 mmol/L (200 mg/dL).
- A FBGL, after at least 8 hours of fasting, between 70-110 mg/dL.

The WHO classification is consistent with the American Diabetes Association (ADA) in terms of the clinical stages of blood glucose regulation (normoglycemia, impaired glucose tolerance/impaired fasting glucose (IGT/IFG), and diabetes) as well as the underlying types of diabetes mellitus. However, the WHO classification also includes a category formerly known as gestational impaired glucose tolerance (GIGT) and gestational diabetes mellitus (GDM), with fasting glucose at ≥ 7.0 mmol/L (126 mg/dL) and/or 2-hour glucose at ≥ 7.8 mmol/L (140 mg/dL) after a 75-g oral glucose tolerance test (OGTT).(9)

As diabetes advances, it causes tissue and vascular damage, resulting in severe complications like retinopathy, neuropathy, nephropathy, cardiovascular issues, and ulcerations. Individuals with long-standing type 1 diabetes are prone to microvascular complications and macrovascular conditions, including coronary artery disease, heart disease, and peripheral vascular disease. Large vessel atherosclerosis is more common in those with type 2 diabetes and is frequently linked to obesity, hypertension, and hyperlipidemia. Many people with T2DM have end-stage renal disease or cardiovascular problems.(9)

Maintaining blood glucose levels as close to normal as possible while avoiding hypoglycemia is the main goal of diabetes care. Achieving this goal involves five key components: education, physical activity, dietary management, oral medications, and/or insulin, often used together.(4)

In contrast to relying solely on lifestyle interventions, each individual pharmacological agent used as a singular treatment increased the proportion of patients achieving HbA1c target levels below 7% by two to three times. The decision on which pharmacological agents to select for each patient should involve a collaborative process of shared decision-making.(10) Oral anti-diabetic drugs are administered when patients do not improve with first therapies and continue to have high blood glucose levels and glycated hemoglobin ≥ 6.0 . The four main types of anti-diabetic drugs include: (a) biguanides, such as metformin, which reduce glucose production in the liver; (b) insulin secretagogues, like sulfonylureas, which stimulate insulin release from the pancreas; (c) insulin sensitizers, such as thiazolidinediones, which increase insulin sensitivity in peripheral tissues; and (d) insulin/insulin analogs, which provide exogenous insulin through recombinant forms.(4)

CHAPTER-II

LITERATURE REVIEW

2.1. Understanding Diabetes and its types:

Diabetes mellitus exists in two forms: Type 1 (T1DM) and Type 2 (T2DM). T1DM is an autoimmune disorder that affects pancreatic cells, resulting in reduced or impaired insulin production. In contrast, T2DM is caused by pancreatic beta cell dysfunction, which prevents the body from effectively utilizing insulin.(11) Maintaining consistent control of blood sugar levels, coupled with addressing concurrent conditions like high blood pressure and elevated lipid levels, continues to be a crucial aspect of the successful care for individuals diagnosed with T2DM.(12) For more than 50 years, medical professionals have acknowledged the occurrence of hyperglycemia during pregnancy, which typically resolves after childbirth. However, there is no consistent global agreement on the specific hyperglycemic levels that should qualify for a diagnosis of Gestational diabetes mellitus (GDM) and, consequently, warrant treatment during pregnancy.(13) GDM is significant for two main reasons, regardless of the diagnostic standards or how they evolve over time. First of all, it raises the possibility of pregnancy complications, including hypertensive diseases for the mother and problems like fetal development and obesity for the fetus. Second, a diagnosis of GDM designates a population of women and their offspring who are at an increased risk of obesity, diabetes, and early cardiovascular disease over the long term.(14) GDM is closely linked to a higher chance of maternal diabetes and cardiovascular disease in later life.(13)

2.2. "Ominous Octet" in T2DM

"Ominous octet" represents eight major biological factors that contribute to high blood sugar levels in T2DM. These factors can occur individually or together and include reduced insulin production in the pancreas, excessive glucagon release from the pancreas, increased glucose production in the liver, brain dysfunction affecting insulin response, enhanced fat breakdown (lipolysis), increased glucose reabsorption in the kidneys, reduced hormone effectiveness in the small intestine

(incretin effect), impaired glucose uptake in muscles, liver, and fat tissue. One or more of these important pathways are the focus of current blood sugar management therapies.(15)

2.3. Understanding the Structure and Function of Insulin in Diabetes Mellitus

The two chains of insulin, a protein hormone, composed of 51 amino acids, are the A chain, which has 21 amino acids, and the B chain, which has 30 amino acids connected by two disulphide linkages.

The short arm of chromosome 11 houses the genetic blueprint for insulin, located within pancreatic β -cells. The synthesis of insulin starts with a precursor known as pre-proinsulin, consisting of 100 amino acids, which features a signal peptide, the B chain, a connecting (C) peptide, and the A chain. Through a series of modifications after translation, pre-proinsulin undergoes processing to become its mature form, ultimately attaining its final structure and functionality.(16,17) This hormone, which is produced by the pancreatic β -cells, is essential for controlling the body's glucose balance. T1 and T2 DM are metabolic diseases marked by elevated blood sugar levels that are brought on by β -cells' insufficient synthesis of insulin. Even though T1DM is not linked to obesity, individuals with T1DM may experience the development of obesity & insulin resistance due to prolonged insulin therapy. This situation may necessitate the use of combination therapies for effective treatment.(18)

2.4. Insulin Resistance and Hyperinsulinemia's Contribution to Obesity and Type 2 Diabetes:

The body's reduced capacity to effectively use insulin in critical tissues such as the liver, adipose (fat) tissue, and skeletal muscle is a hallmark of obesity. One of the main characteristics of obesity is this reduced insulin sensitivity, often known as insulin resistance. (19) This resistance prompts an increased demand for insulin, leading to adaptive changes in pancreatic β -cells. Both the mass and function of these cells are heightened to release an adequate amount of insulin, thereby sustaining normal blood glucose levels. The phenomena of insulin hypersecretion and the development of hyperinsulinemia are the outcomes of this compensatory mechanism.

Elevated insulin levels are part of a harmful cycle that leads to the metabolic dysregulations seen in type 2 diabetes and obesity.(20,21)

2.5. Regulation of Insulin Secretion and Therapeutic Considerations in Diabetes Mellitus

There is strong evidence that the mechanisms governing the secretion and production of insulin are meticulously regulated in β -cells, given its critical role in preserving glucose homeostasis. Glucose is the primary physiological factor regulating the translation of mRNA and the transcription of the insulin gene.(22,23) Insulin replacement is necessary for people with T1DM, and this usually means either continuous subcutaneous insulin infusion or several daily injections of basal and prandial insulin.(24)

In a global survey involving 1250 healthcare professionals (HCPs), 75.5% expressed a willingness to pursue more aggressive treatments if not for the associated risk of hypoglycemia with insulin. (25) Additionally, in a survey of 708 insulin-naïve individuals with Type 2 Diabetes (T2D), "problematic hypoglycemia" emerged as one of the most commonly reported reasons for avoiding insulin therapy. Both HCPs and people with diabetes share concerns about the potential burden of insulin regimens, with 54.5% of HCPs (out of 1250) and 27.6% of individuals with diabetes (out of 1530) highlighting challenges related to "taking insulin at prescribed times or with meals every day."(26,25)

2.6. Insulin Therapy Options in Diabetes Management

In the United States, two rapid-acting insulin formulations have been approved: insulin lispro suspension and insulin aspart solution.(27) Both are analogs of human insulin that, unlike regular human insulin, do not form self-aggregates in solution. This allows them to be absorbed more rapidly.(27,28,29) Insulin lispro is backed by several studies affirming its efficacy in diabetes patients. A major concern with insulin therapy is the risk of hypoglycemia, especially during sleep. Several studies have investigated the prevalence of low blood sugar in people with T1DM using insulin lispro along with NPH or ultralente as the basal insulin. These studies consistently show that insulin lispro is associated with a reduced frequency of hypoglycemia, particularly during the night.(30,31,32) The utilization of rapid-acting

insulins presents several benefits. First of all, insulin can be administered closer to mealtimes due to its faster start of effect. Second, improved regulation of postprandial blood glucose levels is made possible by a faster time to peak activity. The decreased chance of hypoglycemia at night is an additional advantage. Rapid-acting insulin's shorter half-life, which necessitates several daily injections to maintain adequate glycemic control, is a major drawback.(33)

Regular insulin (Humulin® R and Novolin® R), buffered regular insulin, and pig regular insulin are the four varieties of short-acting insulins. There are benefits and drawbacks to using daily insulins. Positively, they give versatility when used with other forms of insulin, such as NPH, Lente, or ultralente. A twice-daily dosage plan might potentially be possible due to their greater duration of effect. The elevated risk of hypoglycemia, particularly when paired with longer-acting insulins, is a significant drawback. The potential for hypersensitive reactions, especially with pork insulin, is another issue.(33)

Intermediate-acting insulin analogs generally having a duration of 10–16 hours, a peak effect between 6–10 hours, and a commencement of action within 1-2 hours. Examples include NPH and lente (from the Latin "lentus," meaning slow). In NPH insulin, the addition of protamine slows its absorption, while in insulin lente, zinc is added to achieve the same effect of delayed absorption.(34)

There are 2 long-acting insulins: insulin glargine and ultralente. These insulin analogs usually initiate their action about 2 hours after administration, displaying a peak action that may sometimes be absent or occurs within a span of 6-20 hours. These insulins are characterized by a prolonged activity duration of up to 36 hours. A strategy to extend insulin activity involves the design of analogs with a higher concentration of positively charged amino acids. This alteration is intended to raise the isoelectric point of insulin, bringing it closer to a near-neutral pH.(35) This method helps reduce the solubility of insulin at a neutral pH after injection, leading to slower absorption into the bloodstream. Additionally, some long-acting insulin formulations include protamine or zinc to further prolong the absorption time. Insulin detemir has a modified B chain, with threonine removed at B30 and myristic acid (a 14-C fatty acid) attached to lysine at B29.(36,37)

Every diabetic has a different insulin dosage that works best for them. For those with T1DM to survive, insulin therapy is crucial. The appropriate moment to begin and the type of regimen to adopt are still unknown, despite indications that intensive insulin

therapy can assist persons with T2DM attain optimal glycemic control (HbA1c <7.0%). To reduce hypoglycemia and its related problems, a simple insulin regimen is advised. Insulin regimen simplicity seeks to improve patient satisfaction by reducing complications and disturbances to lifestyle.(33)

2.7. Oral Medications for Type 2 Diabetes Management

Managing T2DM poses a global challenge due to therapeutic inertia, a barrier that obstructs the achievement of optimal glycemic control, particularly in patients requiring insulin therapy. Various factors contribute to this problem, spanning the patient, physician, and systemic levels. These factors include misconceptions about insulin therapy, limited expertise in primary care for managing insulin regimens, financial limitations, and a lack of time, resources, and motivation to improve the effective use of insulin.(38)

The primary categories of oral medications for managing diabetes encompass biguanides, sulfonylureas, meglitinide, thiazolidinedione (TZD), dipeptidyl peptidase 4 (DPP-4) inhibitors, sodium-glucose cotransporter (SGLT2) inhibitors, α -glucosidase inhibitors. (15)

Metformin is the prototype medication that belongs to the biguanide class. They work by increasing the body's sensitivity to natural insulin, decreasing intestinal glucose absorption, and reducing the liver's generation of glucose. This class achieves a reduction in hepatic glucose output by diminishing gluconeogenesis and promoting glycolysis. Additionally, biguanides elevate insulin signaling by enhancing insulin receptor activity. (11) Abdominal discomfort, along with other gastrointestinal side effects such as bloating and diarrhea, is not uncommon when starting metformin. These symptoms often improve if the dosage is gradually increased. Some patients find the extended-release version of the medication more manageable. However, approximately 10% of individuals cannot tolerate metformin due to its gastrointestinal adverse effects. The most severe complication linked to metformin use is lactic acidosis, an infrequent occurrence (0.03 cases per 1000 patients-years) with potential lethality, usually manifesting in the context of other serious acute illnesses.(39)

Biguanides decrease insulin concentrations, improve insulin sensitivity, and lower fasting plasma sugar; nevertheless, their effectiveness is restricted when insulin is not present. The decline in glucose levels in people with non-insulin-dependent diabetes mellitus (NIDDM) is mostly attributed to a decrease in hepatic glucose production and an increase in peripheral glucose absorption. Biguanides have the advantage of preventing hypoglycemia and having a beneficial effect on blood lipids, which is beneficial for those with type II diabetes and hyperlipidemia. Although there is conflicting data about biguanides' potential benefits for heart-related conditions, most patients tolerate their frequent gastrointestinal side effects.(40) According to the results of this retrospective cohort study, which included 21,996 patients, people who were recently prescribed metformin as their only treatment had a lower risk of cardiovascular events, major hypoglycemic episodes, and all-cause mortality than people who were recently prescribed sulfonylureas as their only treatment.(41,42)

Sulfonylureas, such as tolbutamide, glipizide, and glyburide, function by inhibiting K_{ATP} channels, thereby restricting gluconeogenesis in the liver. The primary side effects associated with these medications are hypoglycemia and hypersensitivity reactions.(15) Sulfonylureas may be prescribed by physicians in place of metformin for patients with impaired renal function. This is because doctors are familiar with sulfonylureas, they are affordable, and they effectively reduce blood sugar levels.(41)

The Thiazolidinedione (TZDs) category, represented by rosiglitazone and pioglitazone, was initially regarded as an innovative choice for both primary and secondary treatments for T2DM upon their introduction in the late 1990s. They received acclaim for achieving glycemic control and physiological outcomes comparable to, and sometimes exceeding, other established initial treatments like metformin. TZDs demonstrated insulin-sensitizing effects by directly activating PPARs, particularly the α subtype. Activation of PPAR α by TZDs has been demonstrated to enhance insulin sensitivity in peripheral tissues. This activation also induces adipogenesis, resulting in reduced endogenous glucose production and postprandial gluconeogenesis. Additionally, it leads to increased clearance of glucose during fasting and postprandial states, resulting in lower levels of blood glucose and insulin. Furthermore, there are reported alterations in β -cell function, as well as changes in cholesterol levels, triglyceride levels, and inflammatory markers. The

prevalent adverse effects observed in individuals undergoing TZD therapy include weight gain, edema, and hepatotoxic events. Concerns regarding cardiovascular safety have been raised, particularly for rosiglitazone, over the years, particularly in relation to congestive heart failure (CHF), myocardial infarction (MI), and elevated mortality associated with adverse cardiovascular events.(43)

Dipeptidyl-peptidase 4 (DPP4) inhibitors have been utilized in the treatment of T2DM. Instead of lowering glucose levels by nature, these inhibitors work to prevent hyperglycemia by altering the amounts of endogenous substrates, which happens when DPP4's enzymatic activity is inhibited. Glucagon-like peptide 1 (GLP1) is currently thought to be the main factor influencing the therapeutic effect of DPP4 inhibitors. As a known physiological substrate of DPP4, GLP1 levels rise after DPP4 inhibition in vivo, which is associated with improved glucose homeostasis.(44) DPP-4 inhibitors have lower hypoglycemia risk than sulfonylureas, but may cause: Hypersensitivity, pancreatitis, liver issues, and skin/subcutaneous tissue problems.(45)

Researchers examining cells in the intestinal lining identified two separate types of glucose transporters: Glucose transporters (GLUTs) and SGLTs. GLUTs function as passive transporters, allowing the substrate to move along its concentration gradient without requiring energy. Conversely, SGLTs operate as secondary-active transporters, leveraging the energy generated by one substrate moving down its concentration gradient to facilitate the uphill transport of the second substrate. This mechanism couples the transport of glucose to the sodium gradient directed inward.(46) SGLT inhibitors include canagliflozin, dapagliflozin and empagliflozin. They exhibit a distinctive mode of action. They function by preventing glucose and sodium from being reabsorbed in the kidney's proximal tubule, which causes them to be expelled through urine. These behaviors help people with diabetes lose weight, lower their blood pressure, and drop their glucose levels.(47) There is evidence that dapagliflozin and canagliflozin increase the risk of blood acidity. The only medication among them that shown a noticeably increased incidence of UTIs was dapagliflozin. Furthermore, empagliflozin was linked to a higher incidence of bladder cancer.(48)

Alpha-glucosidase inhibitors (AGIs) are oral antidiabetic agents that modulate postprandial glycemia by reversibly inhibiting intestinal alpha-glucosidases, enzymes responsible for carbohydrate hydrolysis. This mechanism delays glucose absorption, mitigating postprandial blood glucose excursions and enhancing glycemic control. Notable AGIs include acarbose, miglitol, and voglibose.(49) The primary side effects frequently associated with AGIs are gastrointestinal disturbances. Alpha-glucosidase inhibitors (AGIs) are associated with gastrointestinal side effects, including flatulence (78%), diarrhea, and abdominal pain, resulting from colonic fermentation of undigested carbohydrates. Acarbose, specifically, has rare hepatotoxic potential. However, these adverse effects tend to decrease in severity over time and can be alleviated by initiating treatment with a low dose and gradual titration. (50) Even with progress in diabetes treatment options, attaining optimal glycemic control remains a common challenge. The efficacy of intensive diabetes treatment regimens is often compromised by two major drawbacks: hypoglycemia and weight gain. These adverse effects can lead to decreased patient adherence, reduced treatment acceptance, and ultimately, suboptimal glycemic control.(51,52) A complex web of hormones, including glucagon from α -cells, insulin and amylin from pancreatic β -cells, and gastrointestinal peptides like GLP-1 and GIP, are involved in maintaining glucose homeostasis. Dysregulation of this hormonal axis contributes to the pathophysiology of diabetes, highlighting the potential for multifaceted therapeutic interventions.(53)

Alongside insulin, the 37-amino acid peptide hormone amylin is produced and stored in pancreatic β -cells. Upon glucose stimulation, amylin is co-released with insulin, modulating glucose homeostasis through suppression of glucagon secretion, delayed gastric emptying, and enhanced satiety.(54) There is a synchronized rise and decrease in insulin and amylin levels. The complimentary functions of insulin and amylin help to control blood nutrition levels. Amylin is lacking in diseases such as T1DM, while amylin levels are comparatively low in T2DM, which requires insulin.(53)

Pramlintide, a synthetic amylin analog, exhibits equivalent potency and stability. Administered via pre-prandial subcutaneous injection, pramlintide is indicated for T1DM and insulin-treated T2DM, mimicking amylin's glucoregulatory effects, including delayed gastric emptying, glucagon suppression, and enhanced satiety,

without inducing weight gain, thereby complementing insulin therapy.(55)

Pramlintide exhibits a multifaceted mechanism of action, regulating postprandial glycemia in diabetic individuals through: gastroparesis, delaying gastric emptying, enhanced satiety signaling, suppression of inappropriate postprandial glucagon secretion. By reducing blood glucose fluctuations and improving overall glycemic management, these combined actions help to enhance postprandial glucose control.(56,57,58)

In addition to modest improvements in glycemic control (HbA1c) and weight loss, it has been demonstrated that pramlintide reduces postprandial glucose excursions, (59)reducing the risk of blood sugar spikes after meals. (60)and improvements in surrogate markers of cardiovascular risk and oxidative stress (61,62) The most frequently reported side effect of pramlintide is mild to moderate nausea, typically diminishing within four weeks. Nausea can be mitigated through a gradual upward titration of the dose and is less prevalent in patients with T2DM.(63,64,65)

2.8. Role of Dopamine in Glucose and Energy Homeostasis

Dopamine neurons exhibit a more extensive distribution compared to other monoamines, being found in various regions such as the hypothalamus, olfactory bulb, midbrain substantia nigra, ventral tegmental area, periaqueductal gray, and retina.(66) Dopamine is a monoamine neurotransmitter that is produced by dopaminergic neurons located in the arcuate nucleus of the hypothalamus and the ventral tegmental region of the substantia nigra in the midbrain. (67) Dopamine is found outside of the central nervous system in the kidneys, where it aids in diuresis, natriuresis, and renal vasodilation. (68)

Dopamine receptors are widely dispersed throughout the brain and have important modulatory effects on drive, motivation, motor skills, and cognitive processes. The discovery of five genes responsible for coding various dopamine receptor subtypes, categorized as either D1- (including D1 and D5) or D2-like has enabled the illustration of distinct receptor functions within specific neurocircuits.(69)

Excitation (via the opening of sodium channels) or inhibition (via the opening of potassium channels) are the two possible outcomes of activation of D1-like receptors (D1 and D5). On the other hand, D2-like activation (D2, D3, and D4) usually results in the target neuron being inhibited.(66)

Dopaminergic drugs regulate glucose and energy homeostasis by influencing both central and peripheral systems. Both the endocrine and autonomic nerve systems are affected by these.(70) Increased plasma free fatty acid levels, hyperglycemia, increased hunger, and obesity are all linked to elevated noradrenaline levels in the hypothalamus.(71) Since noradrenaline secretion is decreased when D2 receptors (D2Rs) are activated, dopamine agonists may have metabolic advantages by inhibiting the sympathetic nervous system.(72)

It is thought that via altering brain signals, the timed release of dopamine causes metabolic alterations, such as insulin sensitization, which in turn lowers increased serotonin and noradrenaline activity.(70) Moreover, dopamine acts as an autocrine or paracrine negative regulator of glucose-stimulated insulin secretion because pancreatic beta cells express both D2 receptors and vesicular monoamine transporter 2 (VMAT2), which is responsible for vesicular dopamine loading and storage.(73) Research on animals has shown that insulin and dopamine interact in the striatum and ventral tegmental region (VTA).(74) It seems that the correct control of the mesocorticolimbic system requires a complicated and time-sensitive interaction between insulin and dopamine.(75)

Insulin receptors are distributed across various regions of the brain, with their primary functions extensively studied in the hypothalamus.(76)

The significance of insulin's role beyond maintaining homeostasis in the hypothalamus has garnered increased attention, particularly concerning its influence on reward processing, eating behavior, and overall metabolism. As a result, insulin's interaction with the dopaminergic circuitry is now recognized as pivotal.(74)

2.9. Bromocriptine's role in Diabetes Management

Bromocriptine, an ergot alkaloid-derived sympatholytic agent, exhibits therapeutic efficacy in Parkinson's disease, acromegaly, and hyperprolactinemia through its

postsynaptic dopamine receptor agonism and serotonin modulation. By targeting dopamine receptors in the nigrostriatal tract, bromocriptine mitigates Parkinsonian symptoms, while its regulation of the tuberoinfundibular pathway addresses hormonal dysregulation in acromegaly and hyperprolactinemia.(77) DM is acknowledged as a risk factor for PD. It has been noted that the development of DM in PD patients is linked to more severe PD symptoms. Although dopamine does not play a specific role in blood glucose and lipid metabolism, diabetes may worsen the effects of dopamine depletion in Parkinson's disease (PD). However, the precise mechanisms by which dopamine influences blood glucose through its receptors remain unclear.(78)

Metabolic control pathways are regulated by the central nervous system (CNS) through dopaminergic signaling. Interestingly, drugs like antipsychotics that block dopamine function are linked to metabolic issues, weight gain, insulin resistance, and dyslipidemia.(79) On the other hand, adding bromocriptine to dopamine agonist therapy has been suggested as a way to enhance energy and glucose metabolism. This tactic aims to activate the central nervous system's dopaminergic pathways, which govern metabolic regulation.(80) Bromocriptine exerts its pharmacological effects primarily by activating D2Rs and inhibiting D1 receptors in the central nervous system.(81) Additionally, bromocriptine exhibits actions on serotonin receptors, such as 5-HT_{2B} receptor partial agonism and 5-HT_{2A} receptor inhibition.(82)

A literature search identified eight clinical trials assessing the effectiveness of bromocriptine, with a focus on comparing its efficacy to other leading medications currently used as first-line treatments. Clinical improvement in reducing HbA_{1c} was observed as the once-daily dosage of bromocriptine quick release (BCQR) increased progressively in treatment-naïve patients with DM. RR-bromocriptine; Cycloset is a rapid release form of bromocriptine. The administration of Cycloset to patients who did not respond to treatment with sulfonylurea, metformin, and at least one other oral hypoglycemic agent showed a notable enhancement in HbA_{1c} levels with a gradual escalation in the once-daily dosage of cycloset. After four weeks, BCQR was also shown to improve blood glucose levels. Furthermore, the benefits of using bromocriptine as an add-on therapy to metformin were evident, demonstrating substantial positive changes in glycated hemoglobin, FPG, and postprandial plasma glucose (PPPG) when compared to metformin alone.(83)

The bromocriptine treatment group showed a drop in both glycated hemoglobin and FPG levels in a randomized controlled double-blind study, while the placebo group showed no changes. In particular, the bromocriptine-treated group's HbA1c concentration dropped from 9.9 ± 0.3 to $9.5 \pm 0.2\%$ ($p=0.06$), and their FPG level dropped from 10.59 ± 0.42 to 9.06 ± 0.41 mmol/l ($p=0.01$). Conversely, the FPG levels in the placebo group increased from 10.69 ± 0.52 to 10.6 ± 0.57 mmol/l, whereas the glycated hemoglobin levels increased from 10.2 ± 0.3 to $11.3 \pm 0.6\%$.(84)

Our hypothesis is that the best results could be obtained by using bromocriptine early in the course of the illness, possibly in combination with other possible treatments that alter the course of the illness, including metformin, thiazolidinediones, or GLP-1 agonists. (77) A systematic review that illustrated the importance of a once-daily schedule for bromocriptine was conducted, highlighting not only its benefits in glycemic control and cardiovascular risk reduction but also emphasizing its significance for improved adherence. (85)

Patients with diabetes frequently mention various factors that contribute to non-adherence to prescribed medication regimens. These factors include concerns about the cost of medications, forgetfulness in taking them, comorbid depression, and worries about potential issues such as weight gain, hypoglycemia, or other adverse effects.(86)

Managing patients with type-2 diabetes remains a challenging task for healthcare providers, despite the array of medications traditionally employed, largely due to the multitude of adverse effects associated with them. A novel class of drugs has recently garnered attention for their potential in exhibiting antidiabetic effects. These medications target various receptors, including serotonin and dopamine receptors, offering new avenues for treatment.

In relation to these considerations, bromocriptine presents advantages compared to other diabetes therapies, both new and existing. The once-daily dosing schedule of bromocriptine is particularly beneficial for patients who may struggle with medication adherence due to forgetfulness. This simplified dosing regimen easily integrates into

most daily routines, potentially enhancing overall adherence to the prescribed medication.(85)

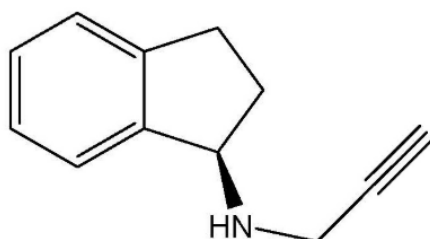
Nausea, hypoglycemia, and hypotension are acknowledged as potential adverse effects of bromocriptine. Nausea, the most commonly reported, is typically temporary. It tends to be more frequent in individuals prescribed higher doses and those with a history of poor tolerance to the medication. While reducing the dose can mitigate nausea, ensuring adherence to treatment and adjusting the dosage remain problematic for some patients.(87)

Hypoglycemia and hypotension are uncommon side effects, typically mild and temporary during the initial adjustment phase. The majority of hypotensive incidents were observed in individuals taking antihypertensive medications, particularly if their initial systolic blood pressure was below 110 mm Hg. Additionally, somnolence, decreased sensation, depression, and anxiety have been documented as potential adverse effects. There's a possibility of exacerbating syncopal migraines in individuals with a history of such migraines.(87) There is an imperative need to transition patients from bromocriptine to alternative medications due to the adverse side effects encountered.

2.10 Rasagiline:

2.10.1 Structure:

Rasagiline, also known as N-propargyl-1-(R)-aminoindan, functions as a robust and irreversible monoamine oxidase type B inhibitor(MAO-B.) (88)



Rasagiline

(89)

Figure 2.1 Chemical Structure of Rasagiline

2.10.2 Clinical Effects:

It has demonstrated efficacy when used alone to treat early-stage Parkinson's disease (PD)(90) and as an adjunct to L-dopa therapy in advanced stages of the disease.(91,92) The therapeutic effects shown in animal models of Parkinson's disease are thought to be due to the increase in striatal extracellular dopamine levels brought about by rasagiline's suppression of MAO-B activity. The improvement in motor function linked to rasagiline treatment is probably facilitated by this mechanism. (93) Rasagiline's inhibitory action is irreversible.(94) Rasagiline has also shown neuroprotective effects in live animal models of neuronal degeneration, (95)as well as in instances of focal ischemia(96) and head trauma. Rasagiline's neuroprotective effects happen independently of the suppression of MAO-B activity.(88) Indeed, cell lines and primary neurons that solely express the MAO-A isoenzyme have shown some of rasagiline's neuroprotective benefits. Additionally, rasagiline's (S)-enantiomer, which is devoid of MAO-inhibitory action, has demonstrated protective properties both in vitro and in vivo.

(97) A crucial aspect of rasagiline's mechanisms involves the activity of its metabolite, 1-amino-indan (AI).(98) 1-amino-indan improves motor function and increases striatal dopamine transmission in mouse models of Parkinson's disease (PD).(99) These effects were not influenced by MAO inhibition, despite AI being a mild inhibitor of MAO-B(100). Rather, they relied on the existence of dopaminergic terminals.

Rasagiline is quickly absorbed when given orally by humans, and it takes less than 0.7 hours for it to reach its maximal plasma concentration (C_{max}). The degree of platelet MAO-B inhibition shown in healthy volunteers is significantly correlated with the logarithm of C_{max}. Rasagiline is significantly metabolised by the liver; less than 1% of the dosage is eliminated unaltered in the urine.(93) Rasagiline tablets containing 1 mg are approved for use as a primary treatment for idiopathic Parkinson's disease in Europe, or as a complement to levodopa in individuals who experience end-of-dose motor fluctuations.(93) It is recommended that adults aged 18 and up take 1 mg once daily, either with or without levodopa. Rasagiline is prescribed as a stand-alone treatment in the United States at a dose of 1 mg once daily. The

recommended starting dose for this supplementary medication is 0.5 mg once day, with the potential to increase to 1 mg once daily if the intended clinical improvement is not achieved.(101)

Negative effects observed in patients taking rasagiline as supplementary therapy typically happened with a frequency and severity comparable to those in individuals receiving either a placebo or entacapone. Furthermore, cognitive and behavioral adverse events showed similarities between the groups receiving rasagiline 1mg once daily and those receiving a placebo. (102)

2.10.3 Potential Hypoglycemic Effects:

The information provided in an article demonstrates that MAO inhibitors can either enhance or impede insulin release in laboratory conditions, depending on their concentration levels. The article suggested that the increase in insulin secretion triggered by glucose may be linked to the MAO inhibitory properties of the medications. Conversely, the inhibition observed at concentrations exceeding 1×10^{-3} M is attributed to a different mechanism that has yet to be identified.(103) The role of monoamine oxidase (MAO) inhibitors appears perplexing initially. In prior studies,(104) we demonstrated that MAO inhibitors, within concentrations ranging from 1 to 10 mM, could inhibit insulin secretion in response to high glucose concentration. Similar findings were reported by Lundquist using pargyline(105). However, contrasting reports indicate that MAO inhibitors failed to inhibit insulin secretion and, in some cases, even stimulated it.(106)

An inadvertent hypoglycemia side effect of rasagiline was documented in a 2017 case study. The case concerned a 25-year-old woman, from Dubai, who was 18 years old when she received a Parkinson's disease (PD) diagnosis. In addition to carbidopa + levodopa + entacapone 25 mg/100 mg/200 mg twice daily, she was receiving treatment with a daily 2 mg rotigotine patch. (107) There were no other health issues affecting the patient.(107)

In September 2015, she moved to rasagiline 1 mg since she was worried about possible future negative effects from this combination. The patient had a fainting episode and passed out at work three days after starting the medicine. When her capillary blood sugar was checked in the ambulance, it was 48 mg/dl, which is low.

She was given 50% dextrose intravenously to treat this, and this totally eliminated her symptoms. After 24 hours of observation, she was sent home before a thorough evaluation could be completed because of social concerns that affected her follow-up care. There had never been any hypoglycemia episodes in her life. Furthermore, she was not taking any additional prescriptions in addition to those listed above.(107) Because monoamine oxidase inhibitors (MAOIs) have been shown to cause hypoglycemia in the past, there was a high suspicion that rasagiline was the source of the patient's symptoms based on the previously mentioned criteria. Rasagiline was thus stopped, and more testing was done to rule out any other possible underlying reasons. Results from imaging tests, including ultrasounds of the belly and pelvis and magnetic resonance imaging of the pancreas, were normal. After being closely monitored during her hospital stay, the patient gradually stopped having hypoglycemia episodes within a week, with the lowest blood sugar level ever recorded being 78 mg/dl. The patient saw remarkable improvement after stopping rasagiline; she no longer experienced symptomatic hypoglycemia, and her at-home blood sugar readings ranged between 70 and 110 mg/dl.(107)

While MAO-B metabolizes dopamine and phenylethylamine, MAO-A works by oxidatively deaminating dopamine, serotonin, and norepinephrine. When MAOIs inhibit these enzymes, serotonin, dopamine, and norepinephrine levels rise, which adds to the antidepressant benefits of MAOIs. (Kaplan HI 1995) Certain compounds within this class, like tranylcypromine and phenelzine, non-selectively inhibit both MAO enzymes, while others like selegiline and rasagiline selectively target MAO-B. Previous data has suggested that non-selective MAO inhibitors may exacerbate hypoglycemia. (108)

According to reports, rasagiline has neuroprotective properties that make it useful for treating PD. There is no research on rasagiline's antidiabetic properties. A 2017 case study demonstrated rasagiline's unintentional hypoglycemia side effect. It is necessary to further assess the cause-and-effect relationship between the two.

2.11 Hypothesis:

Rasagiline lowers plasma glucose by increasing insulin levels, so it has an antidiabetic effect.

2.12 Aims and Objectives:

1. Induction of diabetes.
2. To determine the effect of rasagiline and glimepiride on body weight in diabetic animal models.
3. To determine the effect of rasagiline on FBGL in diabetic animal models.
4. To determine the effect of rasagiline on serum insulin levels in diabetic animal models.
5. Compare the effects of glimepiride and rasagiline on serum insulin levels in diabetic animal models.
6. Compare the effects of glimepiride and rasagiline on fasting blood sugar in diabetic animal models.

2.13 Study Benefits:

If the study shows promising results, this investigation could add a possible anti-diabetic alternative to the present regimens. DM patients who also have additional concomitant conditions, such Parkinson's disease.

CHAPTER III

MATERIALS & METHODS:

3.1 Study Design:

Experimental study (randomized control study) was designed and it was approved by “Ethical Review committee” and “Board of Advance Studies and Research (BASR)” Superior University Lahore, Letter no. SC/BASR/2023/39-11 on 17th- May-2023.

3.2 Study Setting:

The study was conducted at Superior University's Faculty of Medical & Dental Sciences, Department of Pharmacology.

3.3 Duration of Study:

The laboratory work was started in July 2023 and completed in October 2023. Rest of the time was utilized in evaluation of data and compilation of results.

3.4 Sample Size:

Thirty-six healthy adult male Sprague dawley rats.

Thirty-six animals divided in 6 groups (n=6), calculated by using following formula.

$$n = \frac{(Z_{1-\beta} + Z_{1-\alpha/2})^2 (\sigma_1^2 + \sigma_2^2)}{(\mu_1 - \mu_2)^2} (109)$$

$Z_{1-\beta}$ = 90% power of study (1.28)

$Z_{1-\alpha/2}$ = 5% level of significance (1.96)

μ_1 = 533 mg/dl – BSL in Alloxan treated rats

μ_2 = 444 mg/dl – BSL in Alloxan + *Hyppophae rhamnoids* treated rats

σ_1 = 24.31 – Standard deviation in BSL of Alloxan treated rats

σ_2 = 20.68 – Standard deviation in BSL of Alloxan + *Hyppophae rhamnoids* treated rats.

3.5 Sampling Technique:

Simple Random sampling was done by balloting method to minimize bias and ensure validity of results.

3.6 Duration of Study:

The study was started in June 2023 and completed in November 2023.

3.7 Drugs & Chemicals:

Alloxan monohydrate (Sigma Aldrich, USA, cat. no. A7413), amantadine HCl (Brookes Pharmaceutical, Karachi, Pak), glimepiride (PharmEvo, Karachi, Pak), Rat Insulin ELISA INS BT-LAB kit (Bioassay Technology Laboratory, UK Cat. No. E0707Ra) and nylon syringe filters (Membrane Solutions, Washington, USA) were procured from local vendor. Freestyle Optium Neo glucometer (Abbot Diabetes Care Ltd, UK, cat. No. ART30533), its strips (cat. no. ART30680-115) and metallic gavage tubes were procured from local market.

3.8 Methodology:

3.8.1 Selection of animals:

Adult healthy male Sprague dawley rats measuring 150-180 gm were purchased from University of Health Sciences (UHS) Lahore. The animals were kept in Vivarium Superior University, Lahore.

Adequate housing conditions were maintained in polypropylene cages with iron mesh tops. Wood shaving was used for bedding. Temperature was maintained at $24^{\circ}\text{C}\pm 2$. Rats were fed with rodent chow and water *ad libitum*. The criteria outlined in the World Medical Association (WMA) made at Helsinki (2008) regarding the ethical principles for medical research involving experiment in animals were fulfilled.(110)

3.8.2 Data Collection Procedure:

The drugs and chemicals used in the experiment included Alloxan monohydrate Sigma Aldrich. Syringe microfilters 0.2 microns (Membrane Solutions) were purchased. Rasagiline and glimepiride of pharmaceutical grade was purchased. Rat insulin kits from Boehringer-Mannheim were bought. Evocheck Glucometer and its strips were acquired from local market. Glucose kits compatible with the Selectra USA were used.

Alloxan Monohydrate was prepared fresh in ice-cold normal saline. Animals were kept in fasting state for 10-12 hours. Intraperitoneal dose of 150mg/kg (in 1ml solution) was injected to all animals except the negative control group. Aseptic measures were strictly observed and syringe microfilters 0.2 microns (membrane solutions) were used to inject the drug intraperitoneally. Alloxan is well-known to induce severe hypoglycemia because it causes a surge release of Insulin. This was eschewed by giving the rats 10% dextrose water for 48 hours. (111)All the procedures were kept aseptic.

After 72 hours, a blood sample was drawn from the tail vein to determine the FBGL. (112)A FBGL more than 200mg/dl was labelled as diabetic and were divided into groups randomly.

3.8.3 Groups:

3.8.3.1 G-I (Normal Control):

This group was given 1ml of vehicle. Intraperitoneally, animals were given 1ml normal saline. 1ml distilled water was given by gavage for 2 weeks.

3.8.3.2 G-II (Negative Control):

Diabetic animals without any drug treatment received 1ml distilled water by gavage daily for 2 weeks.

3.8.3.3 G-III (Positive Control):

Diabetic group was given glimepiride 0.1mg/kg in 1 ml distilled water daily for 2 weeks.

3.8.3.4 G-IV (Rasagiline low dose):

Diabetic group was given rasagiline 0.1mg/kg dissolved in 1ml distilled water for 2 weeks daily. ^[22]

3.8.3.5 G-V (Rasagiline High dose):

Diabetic group was given rasagiline 0.25mg/kg dissolved in 1ml distilled water for 2 weeks daily.

3.8.3.6 G-VI (Glimepiride and Rasagiline):

Diabetic group received glimepiride 0.1mg/kg and rasagiline 0.1mg/kg dissolved in 1 ml distilled water daily by gavage for 2 weeks.

3.9 Blood Sampling and parameters:

3.9.1 Body Weight:

On Days 0, 7, and 14, the weights of all animals were recorded.

3.9.2 Blood Sampling:

Blood samples were obtained on Day 15 (one day) after the completion of experiment. Animals were kept in fasting state for 12 hours. Rats were anesthetized with Ether and blood was withdrawn by the cardiac puncture.

3.9.3 Fasting Blood Sugar:

It was measured as baseline before giving alloxan, 3 days after alloxanization and then weekly till the end of study. The readings were taken after 12 hours of fasting by tapping tail vein and using a pre-calibrated portable glucometer. (113)

3.9.4 Serum Insulin Levels:

Blood sample of 2ml was obtained by cardiac puncture on day 15 (1 day after last rasagiline administration) after 12 hours of overnight fasting. Serum was separated and stored at -20°C. Insulin levels were measured by Enzyme-Linked Immunosorbent Assay (ELISA) method using commercially available kit.(113)

The kit used was manufactured by Bioassay Technology Laboratory with Cat. No. E0707Ra, Lot 202306007 manufactured on 2023/6/9 with an expiry date of 2024/6/8.

3.9.4.1 Assay Principle:

The kit employed the ELISA technique. In order to facilitate the binding of INS from the sample to these antibodies, the plate was pre-coated with Rat INS antibody.

Subsequently, biotinylated Rat INS Antibody was added, attaching to the INS within the sample. This was followed by the introduction of Streptavidin-HRP, which bound to the Biotinylated INS antibody. Any unbound Streptavidin-HRP was then eliminated through a washing step. Afterwards, a substrate solution was introduced, resulting in the development of color proportional to the concentration of Rat INS present. The reaction was halted by adding an acidic stop solution, and the absorbance was measured at 450 nm.(114)

Several precautions were advised, including allowing the kit and samples to equilibrate to room temperature naturally before use, strictly adhering to instructions, promptly resealing the kit after removing strips to maintain integrity, ensuring the cleanliness and disposable use of pipette tips and plate sealers to prevent contamination, avoiding mixing reagents from different batches, protecting substrate solution B from light exposure, and wearing protective gear when handling the acidic stop solution.

Instructions for specimen collection varied by type:

- For serum: clotting was allowed for 10-20 minutes, then centrifuged and the supernatant collected.

- For plasma: EDTA or heparin was used as an anticoagulant, followed by centrifugation and collection of the supernatant.(114)
- For urine/ascites/cerebrospinal fluid: collection was done using a sterile tube, followed by centrifugation and collection of the supernatant.(114)
- For cell culture supernatant: sterile tubes were used for collection, followed by centrifugation, collection of the supernatant, and, if necessary, subjecting cells to freeze-thaw cycles to release internal components.
- For tissue: rinsing with ice-cold PBS, homogenization, and centrifugation were performed to obtain the supernatant.

3.9.4.2 Reagent Preparation:

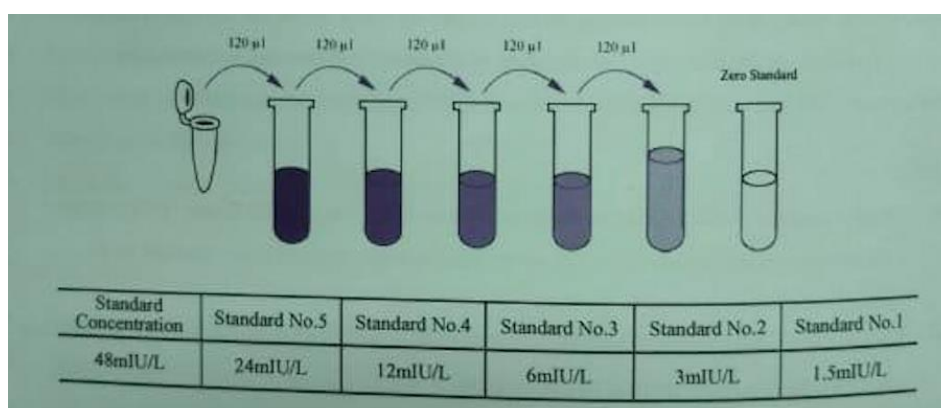
Reagent Preparation steps were as follow;

- Prior to use, all reagents were allowed to come to room temperature.
- Standard Reconstitution: To make a 24 mIU/L standard stock solution, 120 μ L of the standard (48 mIU/L) was reconstituted with 120 μ L of standard diluent. Before producing additional dilutions, the standard was left to sit for fifteen minutes while being gently stirred. By serially diluting the standard stock solution (24 mIU/L) at a 1:2 ratio with standard diluent, duplicate standard points were created, yielding solutions of 12 mIU/L, 6 mIU/L, 3 mIU/L, and 1.5 mIU/L. The standard diluent served as the zero standard (0 mIU/L). Any remaining solution was stored frozen at -20°C and used within one month.

Dilution of standard solutions were suggested as follows:

24 mIU/L	Standard No. 5	120 μ L Original standard + 120 μ L standard diluent
12 mIU/L	Standard No. 4	120 μ L Standard No. 5 + 120 μ L standard diluent
6 mIU/L	Standard No. 3	120 μ L Standard No. 4 + 120 μ L standard diluent
3 mIU/L	Standard No. 2	120 μ L Standard No. 3 + 120 μ L standard diluent
1.5 mIU/L	Standard No. 1	120 μ L Standard No. 2 + 120 μ L standard diluent

(114)



- To make 500 ml of 1x Wash Buffer, 20 ml of Wash Buffer Concentrate 25x was diluted with deionized or distilled water. Crystals that had developed in the concentrate were carefully stirred until they were totally dissolved.

3.9.4.3 Assay Procedure:

1. Before being used, all reagents, standard solutions, and samples were made according to the directions and allowed to come to room temperature. Room temperature was used for the experiment.
2. After figuring out how many strips were needed for the assay, the strips were put into the appropriate frames. Unused strips were kept between 2 and 8°C.
3. The standard well was filled with 50 µl of standard. Note: Since biotinylated antibody was already present in the standard solution, it was not added to the standard well.
4. After adding 40 µl of the sample to each sample well, 10 µl of the anti-INS antibody was added to each sample well. Next, 50 µl of streptavidin-HRP was added to both the sample and standard wells (apart from the blank control well). After fully mixing the plate and applying a sealer, it was incubated at 37°C for 60 minutes.
5. After removing the sealer, wash buffer was used to wash the plate five times. For every wash, 300 µl of wash buffer was absorbed in each well for 30 to 60 seconds. Each well underwent five washes with wash buffer after being aspirated or decanted for automated washing. Paper towels or some absorbent substance was used to blot the plate.
6. After adding 50 µl of substrate solution A to each well, 50 µl of substrate solution B was applied to each well. After applying a fresh sealer, the plate was left in the dark at 37°C for ten minutes.
7. Each well instantly changed from blue to yellow once 50 µl of Stop Solution was applied.

8. Within ten minutes of injecting the stop solution, the optical density of each well was promptly measured using a microplate reader set to 450 nm.

3.9.4.4 Calculation of Results:

The mean OD for each standard was plotted on the vertical (Y) axis against the concentration on the horizontal (X) axis to create a standard curve. The curve that best matched the graph's data points was then drawn. Ideally, computer-based curve-fitting software was used for these calculations, and regression analysis was used to determine the best fit line.

3.9.5 HOMA-B and HOMA-IR:

The Homeostasis Model Assessment (HOMA) is a method used to quantify insulin resistance (HOMA-IR) and β -cell function (HOMA-B) from fasting glucose and insulin or C-peptide concentrations. The formula described below was implemented to drive HOMA-B and HOMA-IR.

$$\text{HOMA-IR} = \frac{\text{Fasting Insulin (mIU/L)} \times \text{Fasting Glucose (mmol/ml)}}{22.5(115)}$$

$$\text{HOMA-B} = \frac{20 \times \text{Fasting Insulin (mIU/L)}}{\text{Fasting Glucose (mmol/ml)} - 3.5(115)}$$

3.9.6 QUICKI

The Quantitative Insulin Sensitivity Check Index (QUICKI) is another method used to assess insulin sensitivity. The formula for QUICKI is:

$$\text{QUICKI} = \frac{1}{\log(\text{Fasting Insulin}(\mu\text{U/mL})) + \log(\text{Fasting Glucose}(\text{mg/dL}))}(116)$$

3.10 Statistical Analysis:

- Version 23 of the Statistical Package for Social Sciences (SPSS) was used to do the statistical analysis.
- The mean plus standard deviation (SD) was used to display the data.

- To evaluate statistically significant differences in mean values between the control and experimental groups, a one-way ANOVA was conducted and post-hoc Tukey's tests were conducted.
- Statistical significance was defined as a P-value of less than 0.05.

CHAPTER-IV

RESULTS

4.1 Effect of Rasagiline on Body Weight in Alloxan Induced Diabetic Rats

On days 0, 7, and 14 of the treatment, the animals were weighed on a digital scale. Group I (NC) had an average body weight $\pm$ standard deviation (S.D.) of 196.83 ± 11.77 g (range 182-214 g) on day 0, while groups II (DC), III (GC), IV (LR), and VI (R+G) had respective average body weights of 197.5 ± 15.43 g (range 182-218 g), 201.8 ± 12.36 g (range 186-220 g), and 198 ± 12.42 g (range 186-218 g). All groups were in the same homogenous subset, according to the ANOVA results, which revealed there were no notable distinctions between the groups. ($p = 0.913$).

Following 7 days of therapy, group I (NC) saw an increase in average body weight $\pm$ S.D. to 225.16 ± 13.64 g (range 208-247 g), while group II (DC) saw a drop to 171.2 ± 14.02 g (range 146-186 g). Group IV (LR) achieved 213.5 ± 17.67 g (range 191-236 g), Group V (HR) reached 208.33 ± 17.69 g (range 188-238 g), Group VI (R+G) reached 212.5 ± 9.39 g (range 208-227 g), and Group III (GC) reached 229.67 ± 12.59 g (range 216-248 g). ANOVA revealed significant differences between the groups. ($p = 0.000$). Groups II (DC) and IV (LR) had considerably lower weights than group I (NC) ($p = 0.000$), whereas group III (GC) had significantly greater weights than group V (HR) ($p = 0.035$), according to post-hoc tests. (Table 4.1) (Fig 4.1).

By day 14, the trends remained similar. (Figure 4.1) Group I (NC) had an average weight of 258.8 ± 23.9 g (range 228-287 g), group II (DC) weighed 158.5 ± 19.99 g (range 128-182 g), group III (GC) was 254 ± 15.67 g (range 244-281 g), group IV (LR) had 226.5 ± 11.51 g (range 215-243 g), group V (HR) weighed 218.5 ± 19.58 g (range 197-251 g), and group VI (R+G) averaged 227.5 ± 7.96 g (range 216-236 g). Groups II (DC), IV (LR), and V (HR) had significantly lower weights than group I (NC) ($p = 0.000$), with group IV (LR) showing no significant difference from group II (DC) ($p = 0.110$). Group V (HR) showed no significant weight difference compared to group IV (LR), and group VI (R+G) had an insignificant weight difference compared to groups I (NC) and III (GC). Homogeneity analysis showed four distinct subsets, with group IV (LR) having an intermediate body weight.

Table: 4.1 Effect of Rasagiline on Body Weight (g) in Alloxan Induced Diabetic Rats (n=6)

Groups	Body weight (grams) Mean $\pm$ S.D.		
	Day-0	Day-7	Day-14
Group-I (NC)	196.83 $\pm$ 11.77	225.16 $\pm$ 13.64	258.8 $\pm$ 23.9
Group-II (DC)	197.5 $\pm$ 15.43	171 $\pm$ 14.02	158.5 $\pm$ 19.99 *
Group-III (GC)	201.83 $\pm$ 12.36	229.67 $\pm$ 12.6 #	254.0 $\pm$ 15.67 #
Group-IV (LR)	198 $\pm$ 12.42	213.5 $\pm$ 17.67 *	226.5 $\pm$ 11.51
Group-V (HR)	197.5 $\pm$ 12.19	208.33 $\pm$ 17.69 #	218.5 $\pm$ 19.58
Group-VI (R+G)	198.33 $\pm$ 12.19	212.5 $\pm$ 9.39 #	227.5 $\pm$ 7.96

Results are expressed as Mean $\pm$ S.D. Significant difference from group-I (NC) is denoted as * while significant difference from group-II (DC) is denoted as # (p-value <0.05).

Where: NC= Normal Control, DC= Disease Control, GC= Glimepiride Control (0.1 mg/kg), LR= Low Dose Rasagiline (0.1 mg/kg), HR= High Dose Rasagiline (0.25mg/kg), R+G= Rasagiline (0.1mg/kg) + Glimepiride (0.1 mg/kg)

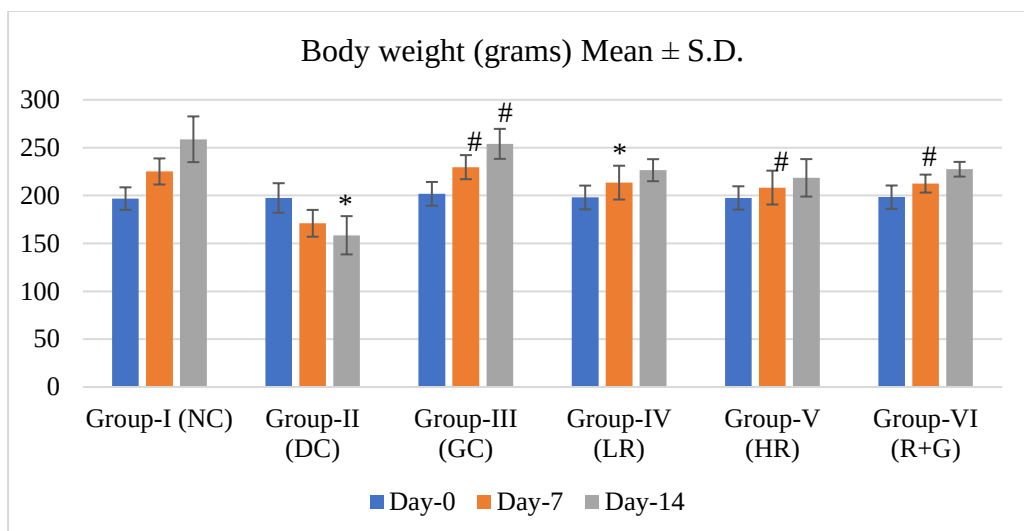


Fig: 4.1. Effect of Rasagiline on Body Weight (g) in Alloxan Induced Diabetic Rats (n=6)

Body weight is expressed as Mean $\pm$ S.D. Significant difference from group-I (NC) is denoted as * while significant difference from group-II (DC) is denoted as # (p-value <0.05).

Where: NC= Normal Control, DC= Disease Control, GC= Glimepiride Control (0.1 mg/kg), LR= Low Dose Rasagiline (0.1 mg/kg), HR= High Dose Rasagiline (0.25mg/kg), R+G= Rasagiline (0.1mg/kg) + Glimepiride (0.1 mg/kg)

4.2 Effect of Rasagiline on Fasting Blood Glucose Levels in Alloxan Induced Diabetic Rats.

In the normal control group, animals were injected intraperitoneally with 1 ml of normal saline, while the other rats received alloxan monohydrate (150 mg/kg) dissolved in 1 ml of normal saline.(117) Three days later, on day-0 of the treatment, animals that successfully developed diabetes (FBGL >200 mg/dl) were assigned to five different groups. Group-I (NC) had a mean FBGL of 106.83 ± 7.63 mg/dl (range: 98 to 117 mg/dl) compared to group-II (DC) with 315.17 ± 34.5 mg/dl (range: 266 to 365 mg/dl), group-III (GC, 0.1 mg/kg) with 316.83 ± 48.36 mg/dl (range: 250 to 384 mg/dl), group-IV (LR, 0.1 mg/kg) with 308 ± 41.12 mg/dl (range: 248 to 371 mg/dl), group-V (HR, 0.25 mg/kg) with 313 ± 39.63 mg/dl (range: 256 to 374 mg/dl), and group-VI (R+G) with 306.8 ± 36.9 mg/dl (range: 266 to 358 mg/dl).

FBGLs were measured on days 7 and 14. (Table 4.2) On day-7, group-I (NC) maintained normal levels of 107 ± 7.87 mg/dl (range: 97 to 119 mg/dl), while group-II (DC) continued to have elevated FBGL at 312.83 ± 37.55 mg/dl (range: 276 to 384 mg/dl). The remaining groups showed significant decreases in FBGL: group-III (GC) dropped to 135.67 ± 23.79 mg/dl (range: 113 to 178 mg/dl), group-IV (LR) to 263.17 ± 21.05 mg/dl (range: 245 to 300 mg/dl), group-V (HR) to 213.83 ± 19.6 mg/dl (range: 184 to 241 mg/dl), and group-VI (R+G) to 160.5 ± 24.29 mg/dl (range: 130 to 194 mg/dl). The combination of rasagiline and glimepiride (R+G) reduced FBGL to normal levels faster than any other treatment.

By day-14, group-I (NC) had an FBGL of 106.67 ± 7 mg/dl (range: 99 to 116 mg/dl), while group-II (DC) remained hyperglycemic with 311.17 ± 45.91 mg/dl (range: 261 to 391 mg/dl). Group-III (GC) dropped to 122.17 ± 12.32 mg/dl (range: 106 to 139 mg/dl), group-IV (LR) to 245.33 ± 26.36 mg/dl (range: 213 to 284 mg/dl), group-V (HR) to 180.5 ± 12.44 mg/dl (range: 165 to 196 mg/dl), and group-VI (R+G) to 133.3 ± 13.44 mg/dl (range: 116 to 149 mg/dl).

Group-II (DC) showed persistent hyperglycemia, with a significant difference in FBGL compared to group-I (NC) (p-value 0.000). Group-III (GC) saw a return to normal FBGL levels, and there was no significant difference between group-I and group-III at day-7 (p-value 0.320) or day-14 (p-value 0.832). (Figure-4.2)

Rasagiline, in both low (0.1 mg/kg) and high doses (0.25 mg/kg), significantly reduced glucose levels compared to the disease control group (DC), with p-values of 0.000 on both day-7 and day-14. However, on day-7, FBGLs in groups IV (LR) and V (HR) remained significantly higher than those in group-I (NC) (p-values 0.000 and 0.007, respectively). By day-14, only group-V (HR) had FBGL levels comparable to group-I (NC) (p-value 0.389), while group-IV (LR) still had significantly higher FBGL (p-value 0.000). The difference between group-IV (LR) and group-V (HR) indicates a dose-dependent increase in rasagiline's glucose lowering effect.

Group-VI (R+G) showed no significant difference in FBGL from group-I (NC) at day-7 (p-value 0.992). By day-14, the combination therapy had reduced FBGL even lower than group-I (NC), though the difference was not statistically significant (p-value 0.992). (Figure-4.2)

Table: 4.2. Effect of Rasagiline on Fasting Blood Glucose Levels (mg/dl) in Alloxan Induced Diabetic Rats (n=6)

Groups	Fasting Blood Glucose Levels (mg/dl) Mean $\pm$ S.D.		
	Day-0	Day-7	Day-14
Group-I (NC)	106.83 $\pm$ 7.63	107 $\pm$ 7.87	106.67 $\pm$ 7
Group-II (DC)	315.17 $\pm$ 34.5 *	312.83 $\pm$ 37.55 *	311.17 $\pm$ 45.91 *
Group-III (GC)	316.83 $\pm$ 48.36 *	135.67 $\pm$ 23.79 #	122.17 $\pm$ 12.32 #
Group-IV (LR)	308 $\pm$ 41.11 *	263.17 $\pm$ 21.04 *#	245.33 $\pm$ 26.35 *#
Group-V (HR)	313 $\pm$ 39.63 *	213.83 $\pm$ 19.61 *#	180.5 $\pm$ 12.43 #
Group-VI (R+G)	306 $\pm$ 38 *	160.5 $\pm$ 24.29 #	133.33 $\pm$ 13.44 #

Results are expressed as Mean $\pm$ S.D. Significant difference from group-I (NC) is denoted as * while significant difference from group-II (DC) is denoted as # (p-value <0.05).

NC= Normal Control, DC= Disease Control, GC= Glimepiride Control (0.1 mg/kg), LR= Low Dose Rasagiline (0.1 mg/kg), HA= High Dose Rasagiline (0.25 mg/kg), R+G= Rasagiline (0.1mg/kg) + Glimepiride (0.1 mg/kg)

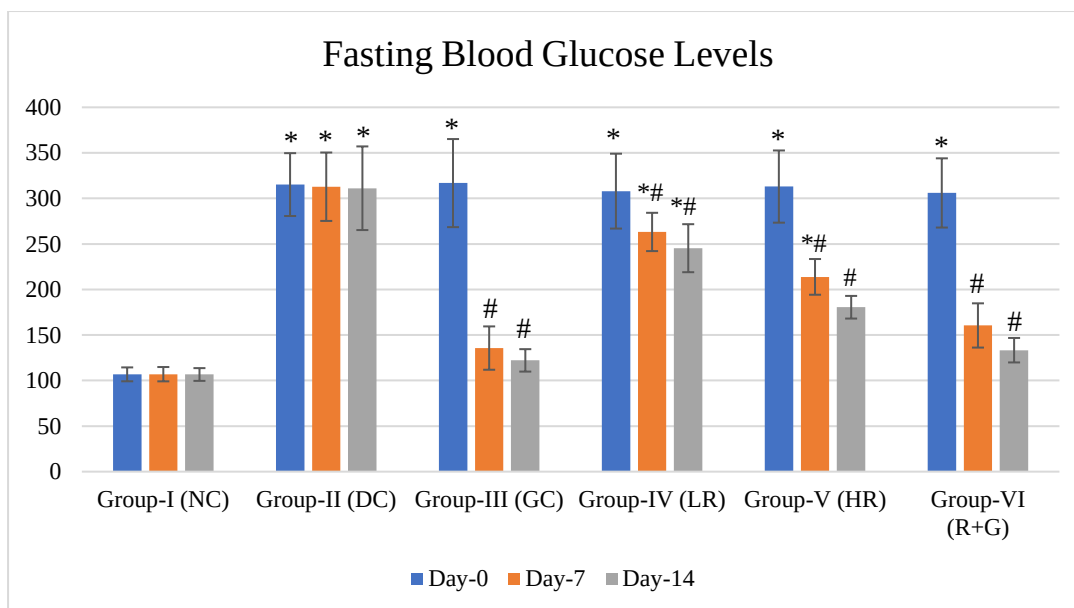


Fig:4.2 Effect of Rasagiline on Fasting Blood Glucose Levels (mg/dl) in Alloxan Induced Diabetic Rats (n=6)

Results are expressed as Mean $\pm$ S.D. Significant difference from group-I (NC) is denoted as * while significant difference from group-II (DC) is denoted as # (p-value <0.05).

NC= Normal Control, DC= Disease Control, GC= Glimepiride Control (0.1 mg/kg), LR= Low Dose Rasagiline (0.1 mg/kg), HA= High Dose Rasagiline (0.25 mg/kg), R+G= Rasagiline (0.1mg/kg) + Glimepiride (0.1 mg/kg)

4.3 Effect of Rasagiline on Serum Insulin Levels in Alloxan Induced Diabetic Rats

On day-14, blood samples were collected via cardiac puncture and stored in serum tubes. The samples were then centrifuged to extract the serum, which was used to measure insulin levels using a Rat Insulin ELISA kit. The optical density of the serum samples was compared with control samples using a Synergy HTX microplate ELISA reader at a wavelength of 450 nm. The results were recorded in an MS Excel worksheet, as shown in Fig. 4.1. A free trial version of GraphPad Prism 8 was utilized to interpolate the normal plot of control samples and determine insulin levels from the optical density data.

The mean serum insulin level in group-I (NC) was 10.84 ± 0.15 mIU/L, ranging from 10.81 to 11.00 mIU/L. Group-II (DC) showed a significant decrease in insulin levels, with a mean of 6.53 ± 0.23 mIU/L (range: 6.13 to 6.77 mIU/L, p-value 0.000).

All treatment groups had significantly higher serum insulin levels compared to group-II (DC), with a p-value of 0.000. Group-III (GC) had the highest serum insulin levels at 11.67 ± 0.36 mIU/L, ranging from 11.44 to 12.21 mIU/L, and it was the only group with insulin levels significantly higher than group-I (NC) (p-value 0.005).

In group-IV (LR), the mean serum insulin level was 7.97 ± 0.20 mIU/L (range: 7.70 to 8.26 mIU/L), which was lower than in group-I (NC) (p-value 0.000). Group-V (HR) and group-VI (R+G) had insulin levels slightly different from group-I (NC), with estimated values of 9.60 ± 0.51 mIU/L (range: 9.02 to 10.34 mIU/L) and 11.14 ± 0.49 mIU/L (range: 10.51 to 11.88 mIU/L), respectively (Table 4.3, Fig 4.5, Appendix 3, 5, and 6).

Table: 4.3 Effect of Rasagiline on Serum Insulin Levels (mIU/L) in Alloxan Induced Diabetic Rats (n=6)

Groups	Serum Insulin (mIU/L)
Group-I (NC)	10.84 ± 0.15
Group-II (DC)	6.53 ± 0.23 *
Group-III (GC)	11.67 ± 0.36 *#
Group-IV (LR)	7.97 ± 0.20 *#
Group-V (HR)	9.60 ± 0.51 #
Group-VI (R+G)	11.14 ± 0.49 #

Results are expressed as Mean ± S.D. Significant difference from group-I (NC) is denoted as * while significant difference from group-II (DC) is denoted as # (p-value <0.05).

NC= Normal Control, DC= Disease Control, GC= Glimepiride Control (0.1 mg/kg), LR= Low Dose Rasagiline (0.1 mg/kg), HR= High Dose Rasagiline (0.25mg/kg), R+G= Rasagiline (0.1mg/kg) + Glimepiride (0.1 mg/kg)

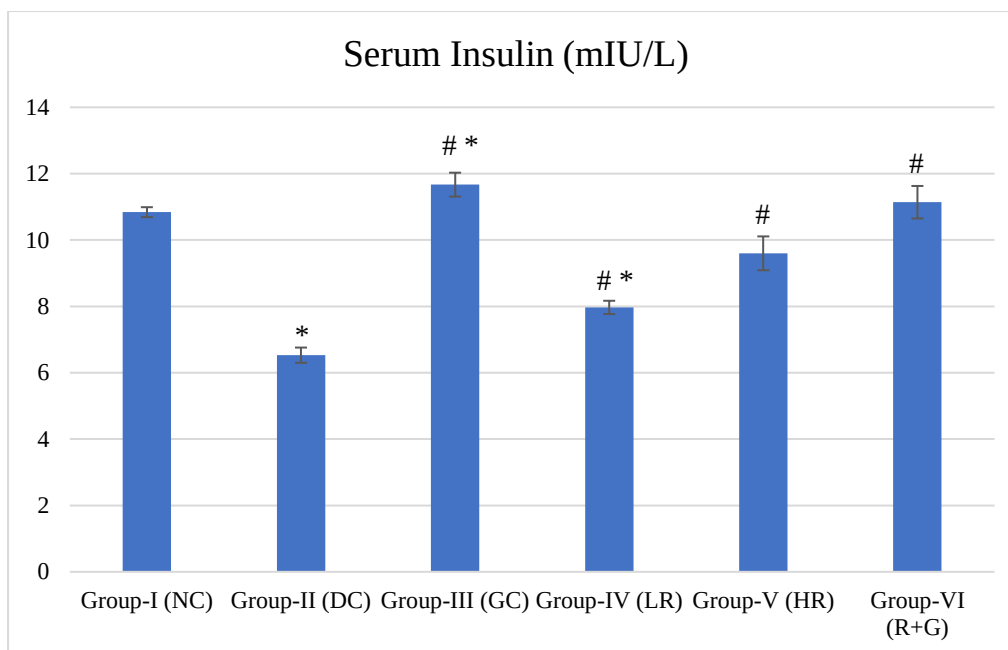


Fig: 4.5 Effect of Rasagiline on Serum Insulin Levels (mIU/L) in Alloxan Induced Diabetic Rats (n=6)

Results are expressed as Mean $\pm$ S.D. Significant difference from group-I (NC) is denoted as * while significant difference from group-II (DC) is denoted as # (p-value <0.05).

NC= Normal Control, DC= Disease Control, GC= Glimepiride Control (0.1 mg/kg), LR= Low Dose Rasagiline (0.1mg/kg), HR = High Dose Rasagiline (0.25mg/kg), R+G= Rasagiline (0.1mg/kg) + Glimepiride (0.1 mg/kg)

4.4 Effect of Rasagiline on HOMA-B in Alloxan Induced Diabetic Rats

The Homeostatic Model Assessment of β -cell function (HOMA-B) was calculated using fasting blood glucose levels (FBGL) on day 14 and serum insulin levels. (115)

It reflects the capacity of pancreatic β -cells to release insulin in response to glucose. In group I (NC), the HOMA-B index was 91.25 ± 13.86 (range: 73.44 to 105.79). In group II (DC), the HOMA-B value significantly decreased to 9.73 ± 1.8 (range: 7.19 to 12.32), which is only about 10% of that in group I (NC) (Table 4.4; Fig 4.6, Appendix-3).

The HOMA-B indices for treatment groups III, IV, V, and VI were 73.37 ± 14.16 (range: 57.82 to 96.66), 16.02 ± 2.39 (range: 13.07 to 19.84), 29.71 ± 3.73 (range: 24.56 to 34.44), and 58.85 ± 11.61 (range: 46.08 to 76.76), respectively (Table 4.4; Fig 4.6, Appendix-3). All treatment groups showed a significant improvement in the HOMA-B index compared to group II (DC) (p-value 0.000), except group IV (LR) (p-value 0.862). Group VI (R+G) had a significantly lower HOMA-B index compared to group III (GC) (Appendices 5 & 6).

Table: 4.4 Effect of Rasagiline on HOMA-B in Alloxan Induced Diabetic Rats (n=6)

Groups	HOMA-B
Group-I (NC)	91.25 ± 13.86
Group-II (DC)	9.73 ± 1.79 *
Group-III (GC)	73.37 ± 14.16 #
Group-IV (LR)	25.74 ± 2.39*
Group-V (HR)	29.4 ± 3.73 *#
Group-VI (R+G)	57.07 ± 11.61 #

Results are expressed as Mean ± S.D. Significant difference from group-I (NC) is denoted as * while significant difference from group-II (DC) is denoted as # (p-value <0.05).

NC= Normal Control, DC= Disease Control, GC= Glimepiride Control (0.1 mg/kg), LR= Low Dose Rasagiline (0.1mg/kg), HR = High Dose Rasagiline (0.25mg/kg), R+G= Rasagiline (0.1mg/kg) + Glimepiride (0.1 mg/kg)

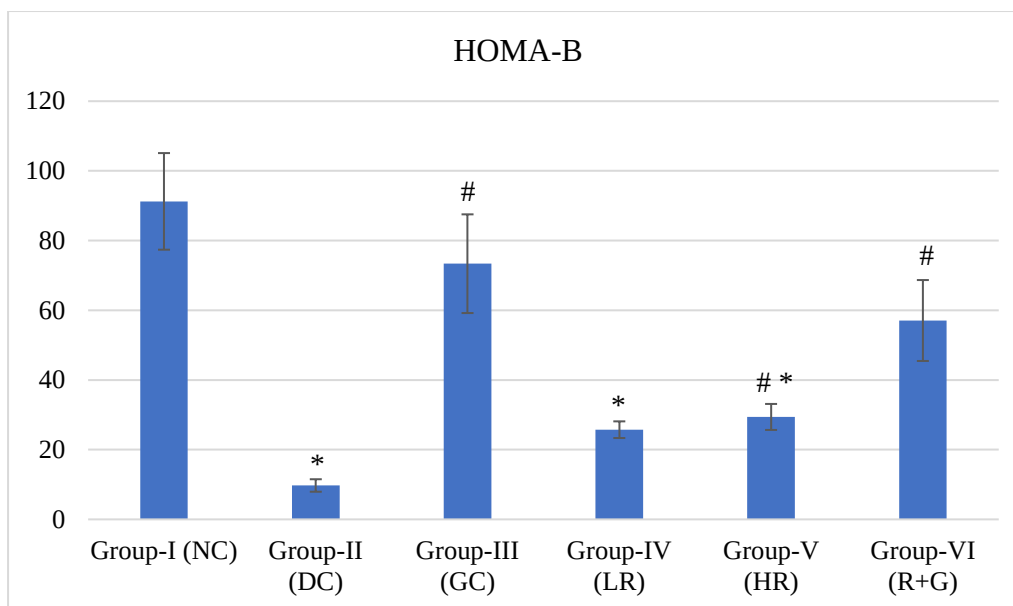


Fig: 4.6 Effect of Rasagiline on HOMA-B in Alloxan Induced Diabetic Rats (n=6)

Results are expressed as Mean $\pm$ S.D. Significant difference from group-I (NC) is denoted as * while significant difference from group-II (DC) is denoted as # (p-value <0.05).

NC= Normal Control, DC= Disease Control, GC= Glimepiride Control (0.1 mg/kg), LR= Low Dose Rasagiline (0.1mg/kg), HR = High Dose Rasagiline (0.25mg/kg), R+G= Rasagiline (0.1mg/kg) + Glimepiride (0.1 mg/kg)

4.5 Effect of Rasagiline on HOMA-IR and QUICKI in Alloxan Induced Diabetic Rats

The HOMA-IR was calculated using serum insulin levels and FBGL.(115) The HOMA-IR values reflect the degree of insulin resistance in peripheral tissues. Group I (NC) had a mean HOMA-IR of 2.86 ± 0.18 , with a range from 2.58 to 3.10, while Group II (DC) demonstrated a significantly elevated HOMA-IR of 5.01 ± 0.733 (range: 4.34 to 6.33).

For the treatment groups (III–VI), the mean HOMA-IR values were: 3.53 ± 0.454 (range: 3.02 to 4.19), 4.83 ± 0.539 (range: 4.31 to 5.63), 4.28 ± 0.342 (range: 3.86 to 4.80), and 3.67 ± 0.427 (range: 3.17 to 4.23), respectively (Table 4.5, Fig 4.7, Appendix-3).

Group IV (LR) had a significantly higher HOMA-IR compared to Group I (NC) (p-value 0.000) but was not significantly different from Group II (DC) (p-value 0.156). Groups III (GC), V (HR), and VI (R+G) showed significantly lower HOMA-IR values when compared to Group II (DC), with p-values of 0.000, 0.001, and 0.000, respectively (Appendices 5 & 6).

Groups III, V, and VI demonstrated significantly lower HOMA-IR values compared to Group II, suggesting that the treatments administered in these groups effectively reduced insulin resistance to varying extents. In contrast, Group IV (LR) exhibited minimal improvement, as its HOMA-IR value remained similar to that of the diabetic control, indicating that this treatment was the least successful in lowering insulin resistance. The p-values for Groups III, V, and VI were all below 0.001, confirming that these treatments had a statistically significant effect on reducing insulin resistance, with the most notable recovery observed in Group III (GC) and Group VI (R+G).

The QUICKI is another derived formula to observe the response of insulin.(118) Group I (NC) had a mean QUICKI value of 0.3266 ± 0.00318 , with a range between 0.32 and 0.33, indicating normal insulin sensitivity. In contrast, Group II (DC) exhibited significantly lower QUICKI values of 0.3028 ± 0.00548 (range 0.29 to

0.31), suggesting marked insulin resistance in the diabetic control group (p-value 0.000). (Table 4.5, Fig 4.8, Appendix-3).

The treatment groups showed varying degrees of improvement in QUICKI values. Group III (GC) had a QUICKI of 0.3173 ± 0.0055 (range 0.31 to 0.32), Group IV (LR) had 0.3041 ± 0.00437 (range 0.30 to 0.31), Group V (HR) had 0.3089 ± 0.00330 (range 0.30 to 0.31), and Group VI (R+G) recorded 0.3155 ± 0.03 (range 0.31 to 0.32). Groups III and VI did not show a significant difference in QUICKI values compared to the normal control (Group I), indicating a strong recovery in insulin sensitivity. However, Group IV's QUICKI was higher than Group II (DC) but remained significantly lower than all other treatment groups (p-value 0.000), implying limited improvement in insulin sensitivity. Group V had a QUICKI significantly lower than Group I, but higher than Group II, suggesting a moderate improvement in insulin resistance (p-values 0.004, 0.000, and 0.067, respectively).

The QUICKI results show that Group II (DC) had significantly lower values compared to the normal control (Group I), indicating high insulin resistance. Groups III (GC) and VI (R+G) exhibited QUICKI values close to the normal control, suggesting effective improvement in insulin sensitivity. Group IV (LR) showed only slight improvement, with its QUICKI significantly lower than all other treatment groups, indicating limited effectiveness. Group V (HR) showed moderate improvement, with QUICKI higher than the diabetic control but lower than the normal control. Overall, Groups III and VI demonstrated the best recovery in insulin sensitivity.

Table: 4.5 Effect of Rasagiline on HOMA-IR and QUICKI in Alloxan Induced Diabetic Rats (n=6)

Groups	HOMA-IR	QUICKI
Group-I (NC)	2.86 ± 0.19	0.327 ± 0.00318
Group-II (DC)	5.01 ± 0.73 *	0.302 ± 0.00548 *
Group-III (GC)	3.53 ± 0.45 #	0.316 ± 0.00555 #
Group-IV (LR)	4.8 ± 0.54 *	0.304 ± 0.00437 *#
Group-V (HR)	4.3 ± 0.34 *#	0.308 ± 0.00330 *#
Group-VI (R+G)	3.7 ± 0.43 #	0.315 ± 0.00939 #

Results are expressed as Mean ± S.D. Significant difference from group-I (NC) is denoted as * while significant difference from group-II (DC) is denoted as # (p-value <0.05).

NC= Normal Control, DC= Disease Control, GC= Glimepiride Control (0.1 mg/kg), LR= Low Dose Rasagiline (0.1mg/kg), HR = High Dose Rasagiline (0.25mg/kg), R+G= Rasagiline (0.1mg/kg) + Glimepiride (0.1 mg/kg)

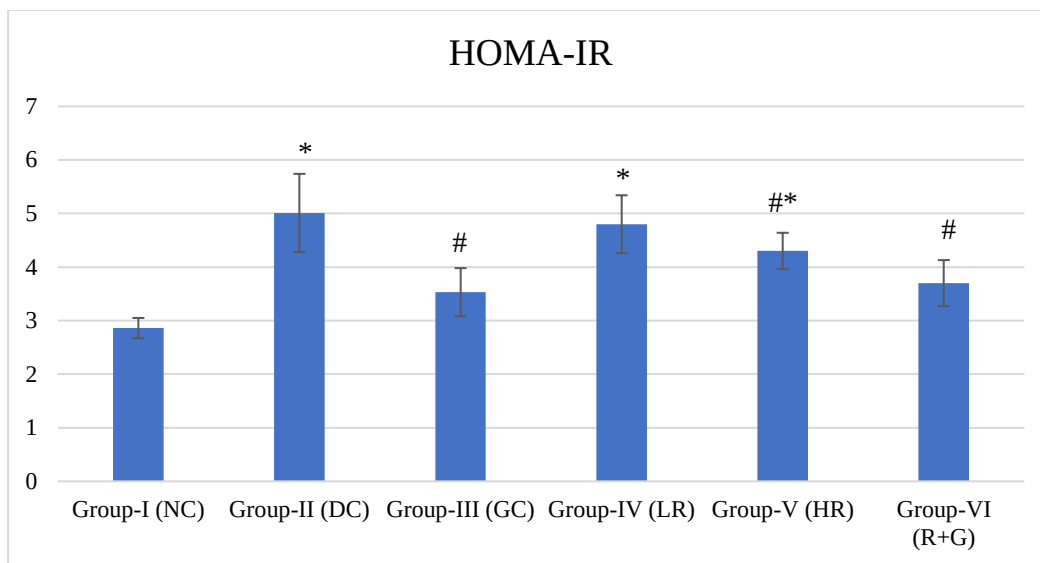


Fig: 4.7 Effect of Rasagiline on HOMA-IR in Alloxan Induced Diabetic Rats (n=6)

Results are expressed as Mean $\pm$ S.D. Significant difference from group-I (NC) is denoted as * while significant difference from group-II (DC) is denoted as # (p-value <0.05).

NC= Normal Control, DC= Disease Control, GC= Glimepiride Control (0.1 mg/kg), LR= Low Dose Rasagiline (0.1mg/kg), HR = High Dose Rasagiline (0.25mg/kg), R+G= Rasagiline (0.1mg/kg) + Glimepiride (0.1 mg/kg)

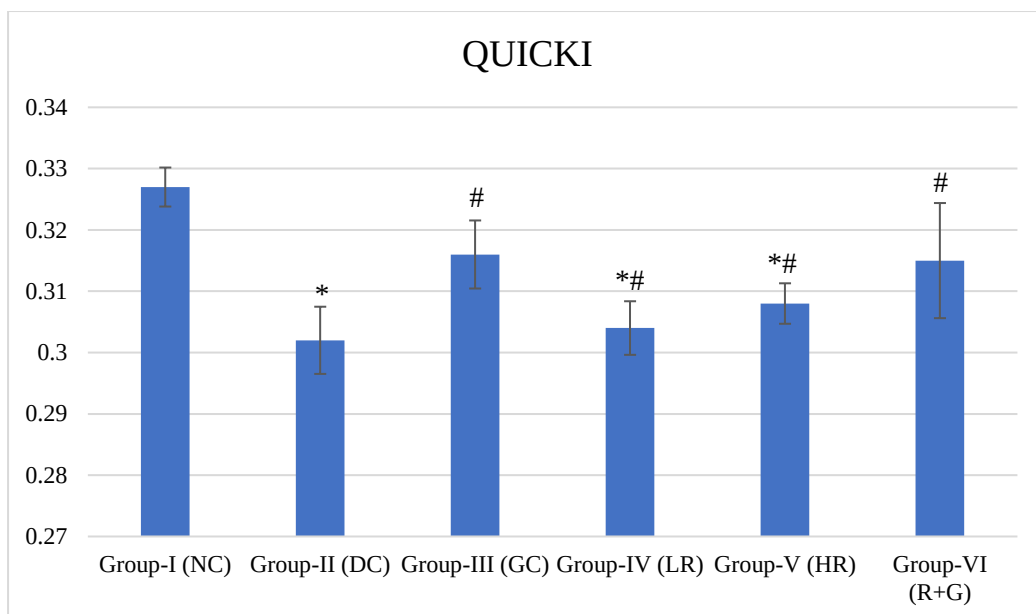


Fig: 4.8 Effect of Rasagiline on QUICKI in Alloxan Induced Diabetic Rats (n=6)

Results are expressed as Mean $\pm$ S.D. Significant difference from group-I (NC) is denoted as * while significant difference from group-II (DC) is denoted as # (p-value <0.05).

NC= Normal Control, DC= Disease Control, GC= Glimepiride Control (0.1 mg/kg), LR= Low Dose Rasagiline (0.1mg/kg), HR = High Dose Rasagiline (0.25mg/kg), R+G= Rasagiline (0.1mg/kg) + Glimepiride (0.1 mg/kg)

CHAPTER-V

DISCUSSION

The chronic metabolic disease known as diabetes mellitus is typified by hyperglycemia brought on by deficiencies in either insulin activity or secretion, or both. (119) It is one of the major health issues affecting the entire planet, and new medication treatments are required. So, in the current investigation, the anti-diabetic action of rasagiline was studied. According to Hattori et al., it has historically been used as a selective monoamine oxidase-B inhibitor in Parkinson's disease patients' treatment. (120) Assays on FBG level, the serum levels of insulin concentration, weight change of body, and HOMA-B, HOMA-IR, and QUICKI indexes in diabetic Sprague Dawley rats have been observed.

DM was induced in animal model with no reported mortality. Alloxan 150mg/kg body weight was given intraperitoneally. The study was carried out at Superior University's Department of Pharmacology, Faculty of Medical & Dental Sciences. The laboratory work was started in July 2023 and completed in October 2023. There was no mortality. On third day, animals having FBGL more than 200mg/dl were enrolled for our study. FBGL recorded after induction of diabetes on Day 3 was very sharply above 200 mg/dL in all diabetic groups thus establishing that diabetes had indeed been successfully induced in these animals. The results of Bukhari et al. 2024, who noted comparable increases in glucose levels in rodents after alloxan-induced diabetes, are consistent with this notable rise in fasting blood glucose levels.(121)

Body weight changes are significant markers of the catabolic condition caused by diabetes mellitus. The DC group exhibited substantial body weight loss with a 34% decrease on Day 7 and a 39% decrease by Day 14, indicating severe tissue loss due to hyperglycemia. Interestingly, rasagiline exhibited dose-dependent effects on body weight. The low dose rasagiline group, LR, demonstrated a notable reversal of weight loss with a 19% increase by Day 7 and a 30% increase by Day 14. The high dose rasagiline group, HR, also showed significant improvements with a 17% increase by Day 7 and a 27% increase by Day 14, showcasing rasagiline's potential to counteract the weight loss associated with diabetes.

In the comparison group, GC, there was a 25% increase in weight by Day 7 and a 37% increase by Day 14. The combined treatment group, R+G, which involved both rasagiline and glimepiride, showed an even more pronounced improvement with a 20% increase in weight by Day 7 and a 30% increase by Day 14. Body weight increased by 14% in a span of 21 days in a study as demonstrated by Wijianto et al.(122) These results underline the varying impacts of rasagiline, glimepiride, and their combinations on reversing the weight loss effects of diabetes, providing compelling evidence of their therapeutic potentials. (Fig 4.1) (Appendix 5). The percentages of body increase is almost same in HR, LR and GC.

Although the effect of rasagiline on body weight has not yet been investigated. But a drug with similar mechanism of action; bromocriptine, a D2 agonist, is known to have positive effects on parameter of body weight. A study by Gaziano et al, shows that by lowering body weight, bromocriptine in type 2 diabetes lowers cardiovascular risk. (123)

The study on rasagiline's efficacy in glucose metabolism highlighted distinct outcomes across different dosage and combination groups, monitored over a span of 14 days. By Day 7, the DC group demonstrated a remarkable 65% increase in FBL control, a trend that sustained through Day 14, underscoring its robust effectiveness. In contrast, the GC group, used for comparison, showed a substantial decline in effectiveness, with FBL control decreasing by 56% on Day 7 and further dropping to 61% by Day 14 as also demonstrated by Wijianto et al., that glimepiride lowers blood glucose level to 16% in a span of 21 days. (122) The lower dose rasagiline group (LR) saw a 15% decrease in FBL management effectiveness by Day 7, which expanded to 21% by Day 14, suggesting that lower doses may be less effective. More notably, the higher dose rasagiline group (HR) experienced a 31% decrease in effectiveness at Day 7, which escalated to a 42% decrease by Day 14, indicating a significant yet dose-dependent impact.

The combination treatment group (R+G), which included both rasagiline and glimepiride, encountered a 49% reduction in FBL in comparison to disease control by Day 7, further reducing to 57% by Day 14. (Table 4.2) It justified the potentiality of rasagiline in moderating glucose metabolism in diabetic conditions (124). This

research work marks the first demonstration of the summation effect of rasagiline and glimepiride, offering a novel therapeutic approach for diabetes treatment.

One such widely recognized drug from the second generation of sulfonylurea is a well-established stimulator for insulin release from pancreatic β -cells, namely glimepiride, which was also used as a positive control in this study. By Day 7, an excellent reduction of FBL values was observed in the group of treatment with glimepiride that was maintained till the end of Day 14. (p-value= .000) Glimepiride, on binding to sulfonylurea receptor 1 (SUR1) that is represented in conjunction with the ATP-sensitive potassium channels, causes membrane depolarization, subsequently causing stimulus for insulin secretion (125)

When compared to the disease control group, the rasagiline and glimepiride-treated group's FBG levels significantly decreased over the trial's duration. By Day 7, the high dose rasagiline group's FBGL was decreased by 31%, almost as much as the group getting glimepiride. (p-value = 0.962) By Day 14, FBL levels remained decreased by 42% in HR group and 61% in GC group while R+G group showing 57% decrease in FBGL almost similar to GC group. (Appendix 2) (Table 4.2) (Figure 4.2) This suggests that rasagiline is at least as effective as glimepiride in lowering blood glucose but through separate mechanisms.

Although research on rasagiline's impact on fasting blood glucose levels is still pending, studies have demonstrated that comparable medications, such as the D2 agonist bromocriptine, dramatically decreased HbA1c and FPG in individuals with type 2 diabetes. (83)

Serum insulin induction was notably higher in the groups treated with rasagiline and glimepiride compared to the disease control group, with particular emphasis on dosage and combination efficacy. The serum insulin level significantly dropped by 39% in the DC group. This aligns with the recent study by Pirzada et al., which demonstrated that alloxan-induced diabetes leads to a decrease in serum insulin levels, contributing to altered biochemical parameters. (126) The group treated with glimepiride demonstrated 21% increase in insulin levels in humans as shown in study by Mary Korytkowski et al. (127) The low dose rasagiline group (LR) achieved an

18% increase, while the high dose rasagiline group (HR) reported a substantial 32% increase in insulin levels, suggesting dose-dependent efficacy. The combined rasagiline and glimepiride group (R+G) showed an impressive 41% increase in serum insulin levels, indicating a synergistic effect that potentially enhances the overall insulin response more than individual treatments. (Table 4.3) (Figure 4.3) (Appendix 3)

This pattern suggests that both rasagiline and glimepiride, especially in higher doses or combined forms, can significantly elevate insulin secretion or maintain β -cell function, aligning closely with the levels observed in the solely glimepiride-treated group. (P-value = 0.000) (Appendix 5)

Our results coincide with the case report by Tamami Oshige et al. shown that patients with T2DM can drop their blood sugar levels by taking traditional-type bromocriptine. (128) These findings underscore rasagiline's effect to mimic or even enhance the therapeutic outcomes typically associated with glimepiride, offering adjuvant therapy for diabetes management.

The HOMA-B index, a significant marker of β -cell function, displayed substantial improvements across several treatment groups, as opposed to the dramatic 89% decrease observed in the DC (disease control) group. The GC (Glimepiride control) group showed a notable 86% increase in HOMA-B, highlighting the effectiveness of glimepiride in boosting β -cell functionality. These results are supported by the recent study done by Sahin et al., demonstrating protective effect of glimepiride against β -cell damage through antioxidant activities. (129) Meanwhile, the LR group exhibited a 62% increase and the HR group a 67% increase in β -cell activity, confirming rasagiline's dose-dependent efficacy. The combination therapy group (R+G), which received both rasagiline and glimepiride, demonstrated an impressive 82% increase in HOMA-B. (Table 4.4)

The notable increases in HOMA-B across the rasagiline-treated groups support the possible protective or restorative effects of rasagiline on β -cells, though higher doses might be necessary for optimal β -cell regeneration, as indicated by the less substantial change in the low-dose group.

The disease control (DC) group surprisingly showed a 42% increase in HOMA-IR. Conversely, treatment groups exhibited reductions in HOMA-IR, indicating improved insulin sensitivity. The glimepiride control (GC) group experienced a 29% reduction in HOMA-IR, showcasing glimepiride's effectiveness on improving insulin sensitivity as supported by recent study done by Sheikh et al.(130) Similarly, the low dose rasagiline (LR) group saw a 5% decrease, and the high dose rasagiline (HR) group recorded a 14% reduction in HOMA-IR, reflecting rasagiline's possible benefits, albeit more significantly at higher dosages. Moreover, the combination therapy group (R+G), which included both rasagiline and glimepiride, demonstrated a 26% decrease. (Table 4.5) These findings emphasize the variability in treatment outcomes, with rasagiline and glimepiride both showing prospect in improving insulin sensitivity, particularly when used in combination.

According to a research by Tamami Oshige et al., after taking bromocriptine, HOMA-IR, which primarily measures insulin resistance in the liver, dropped by 20%, from 2.4 to 1.9.(128)

This is further enhanced by the QUICKI index results, whereby the rasagiline groups were seen to have drastically improved their insulin sensitivity. The HR group had a QUICKI value of 0.308 ± 0.00330 , almost as the same value of the GC group (0.302 ± 0.00548), therefore rasagiline has strong capability in improving insulin sensitivity. (Table 4.5)

Glimepiride is a second-generation sulfonylurea with a well explained mechanism of action through the stimulation of the release of insulin from pancreatic β -cells. It was thus used as a positive control in this study. There were significant reductions in FBL and improvements in the parameters of insulin sensitivity in the glimepiride-treated group, the GC. On Day 14, the groups of glimepiride and high-dose rasagiline were similar to each other in most parameters like FBGL, HOMA-B, and insulin levels. It means that rasagiline may exert antidiabetic effects like glimepiride but the exact mechanism of action may be explored in future studies.

Rasagiline's glycemic effect has yet not been worked on but a D2 Agonist, bromocriptine is known to have anti-diabetic effects along with improvement in insulin levels. A case report by Tamami Oshige et al. shown that patients with T2DM

can drop their blood sugar levels by taking traditional-type bromocriptine. Additionally, by inhibiting lipolysis, bromocriptine reduces insulin resistance.(128)

CHAPTER-VI

CONCLUSION

In rats with alloxan-induced diabetes, the results showed that rasagiline significantly reduced blood sugar levels when compared to glimepiride. Its capacity to lower FBL, raise insulin levels in the blood, and improve β -cell and HOMA-B function points to it as a novel therapeutic option for diabetes. Furthermore, rasagiline is a helpful medication to attenuate the barrier of insulin resistance due to its ability to reduce HOMA-IR, QUICKI, and insulin resistance. The entire explanation of rasagiline's therapeutic success in the treatment of diabetics requires longer-term research and human clinical trials.