INVESTIGATION OF THE SYNERGISTIC EFFECT OF AMLA AND METFORMIN ON INSULIN RESISTANCE AND PANCREATIC β-CELL PROTECTION IN EXPERIMENTAL TYPE 2 DIABETES MELLITUS

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ABSTRACT

Type 2 Diabetes Mellitus (T2DM) is a chronic metabolic disease that is defined by chronic hyperglycemia due to insulin resistance and progressive loss of insulin producing β-cells of the pancreas. Metformin is the first line of drug therapy but its effects on preventing β-cell deterioration are not that strong. Emblica officinalis (Amla) is a traditional medicinal plant with high antioxidant and antihyperglycemic activity that can potentially be used in diabetes management. The current study was designed to study the synergistic role of Amla and metformin against insulin resistance and pancreatic β-cell protection in experimental type 2 diabetes mellitus (T2DM).

Wistar albino rats were used for experimental T2DM model induction using nicotinamide-streptozotocin (NA-STZ) model. Diabetic animals were randomly divided into six groups which included normal control (NC), diabetic control (DC), metformin treated (MT), Amla treated (AT), low dose combination (LDC), and high dose combination (HDC) groups (n = 6). This was done orally for 21 days. Therapeutic efficacy was estimated based on the changes observed in body weight, FBG, OGTT, fasting insulin levels, HOMA-IR, HbA1c, lipid profile, liver and kidney function parameters and histopathological examination of pancreatic tissue.

Combination therapy had better antidiabetic action than monotherapy. Improvements in glycemic control, insulin sensitivity, serum insulin, HbA1c, lipid parameters, and organ function parameters were all significant. Histopathological analysis showed that the architecture of the pancreas was preserved and

there was superior protection of β-cells in the high-dose combination group.

The study demonstrates that Amla when given in combination with metformin enhances the therapeutic potential of metformin and can be a promising adjunctive therapy to enhance metabolic control and maintain the β-cell function in Type 2 Diabetes Mellitus.

KEYWORDS: Type 2 Diabetes Mellitus, Amla, Metformin, Insulin Resistance, Pancreatic β-Cell Protection, Experimental Diabetes.

INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is a chronic metabolic condition when blood glucose level is persistently high due to insulin resistance and progressive pancreatic β-cell dysfunction. The disease has become a major health problem worldwide because of its high prevalence and its relationship to a number of complications including cardiovascular disease, nephropathy, neuropathy and dyslipidemia. There are two mechanisms that contribute to the pathogenesis of diabetes: insulin resistance and β-cell failure, therefore effective treatments should be aimed at glycemic control, maintaining β-cell function and enhancing insulin sensitivity [1].

Metformin is the most popular first-line oral antidiabetic drug (OAD) used for T2DM. This mainly inhibits gluconeogenesis in the liver and improves insulin sensitivity in the periphery. Although it has been shown to be effective, metformin alone may not be sufficient to stop progressive decline of pancreatic β-cell function and, as a result, may result in inadequate glycemic control in many patients over time. This has led to the emergence of an interest in the combination of traditional antidiabetic medications with natural substances with complementary mechanisms of action, such as antioxidant and β-cell protection [2].

Emblica officinalis Gaertn. (Amla) commonly known as Indian gooseberry, is a medicinal fruit, widely used in traditional medicine to manage diabetes and metabolic disorders. Experimental and clinical evidence has shown that Amla has a marked effect on lowering blood glucose levels, the lipid profile and overall metabolic condition in diabetic individuals [3]. The high concentration of bioactive polyphenols such as gallic acid, ellagic acid, corilagin and gallotannins with potent antioxidant and anti-inflammatory activities are the major reason for the antidiabetic activity of Amla.

There are multiple studies that have shown Amla to improve insulin secretion, insulin action, and insulin-mimetic effects. Moreover, its antioxidant activity also helps to lower oxidative stress, which is a major factor responsible for damaging pancreatic β-cells in T2DM. Amla scavenges free radicals and blocks protein glycation, thereby maintaining the architecture of the β-cells and maintaining their ability to produce insulin [4-5].

There has also been recent evidence that there may be a synergy between Amla and metformin. In one randomized comparative study, a standardized Amla extract with β-glucogallin was reported to exhibit comparable antidiabetic activity to the drug metformin with beneficial impacts on glycemic and lipid parameters [5]. Moreover, mechanistic studies suggest that Amla

can be used along with metformin, which is used in the treatment of diabetes, and could target pathways that are related to oxidative stress, insulin secretion and glucose metabolism.

While both metformin and Amla have proven to be beneficial in their own right in diabetic treatment, studies examining the combination of these two drugs in terms of insulin resistance and pancreatic β-cell

protection are limited. Thus, examining the synergic activity of Amla and metformin in the experimental T2DM could offer insights on the possibility of it positively affecting glycemic control, improving insulin sensitivity and maintaining the integrity of pancreatic β-cells. These results can help in developing therapeutic strategies for the management of T2DM that will be effective as an adjunct therapy.

1.1 Diabetes Mellitus

Diabetes mellitus is a chronic metabolic disease whose primary feature is high blood sugar and in which a lack of insulin or its ineffectiveness plays a role. The disease is linked to impaired carbohydrate, fat and protein metabolism, and when not treated, can lead to significant health issues [6].

There are two general types of diabetes, Type 1 and Type 2 diabetes mellitus. Type 1 diabetes is caused by autoimmune destruction of pancreatic β-cells causing absolute insulin deficiency. The most prevalent type is Type 2 diabetes mellitus (T2DM) where there is insulin resistance and gradual deterioration of β-cell function and hyperglycemia persists [6,7].

Polyuria, polydipsia, polyphagia, lethargy, blurred vision and sudden weight loss are common signs of diabetes. Diabetic nephropathy, retinopathy, neuropathy, cardiovascular diseases, and peripheral vascular disorders are all potential complications of long-term uncontrolled diabetes that can have a significant impact on quality of life [6]. Diabetes management includes lifestyle changes, frequent blood sugar checks, diet, exercise and medication. Of particular importance is the early diagnosis and successful treatment of the disease to avoid complications and better outcomes [7].

1.2 Classification of Diabetes Mellitus

Diabetes mellitus is classified into several major categories based on its etiology, Depending on their underlying cause and clinical manifestation, diabetes mellitus can be divided into three subtypes: Type 1 Diabetes Mellitus (T1DM), Type 2 Diabetes Mellitus (T2DM) and Gestational Diabetes Mellitus (GDM). The classification is significant in the diagnosis, treatment and disease management [8].

Type 1 Diabetes Mellitus (T1DM):

T1DM is caused by the autoimmune destruction of the pancreatic β-cells causing absolute insulin deficiency. Insulin therapy is essential to the survival and glycemic control of patients for life [8].

Type 2 Diabetes Mellitus (T2DM):

T2DM is the most prevalent type of diabetes and is the cause of approximately 90-95% of all cases. It features insulin resistance and gradual failure of β cells. Obesity, sedentary lifestyle, unhealthy diet and genetic predisposition are the major risk factors [8-9].

Gestational Diabetes Mellitus (GDM):

GDM is glucose intolerance that is identified during pregnancy. While women affected with T2DM may have normal blood glucose levels after giving birth, they are at increased risk of developing T2DM as adults. Good management is required to minimise maternal and foetal complications [10].

Other Specific Types:

These include diabetes resulting from genetic defects, pancreatic diseases, endocrinopathies, infections, and some drugs, including glucocorticoids [8].

Therefore, the classification of diabetes mellitus is very important in determining how to approach therapy and in achieving a better outcome for the patient [8,9].

1.3 Epidemiology

Diabetes mellitus is a rampant NCD and a serious public health issue of today's world. Over the past few decades, diabetes has become a more common chronic condition as a consequence of urbanization, sedentary lifestyle, unhealthy lifestyle habits and diet, obesity, and population aging [11].

In 2021, an estimated 536.6 million adults lived with diabetes, and the number is expected to increase significantly over the next few years, according to the International Diabetes Federation (IDF). Type 2 Diabetes Mellitus (T2DM) represents almost 90-95% of all diabetes and there is significant association with modifiable lifestyle risk factors [11-13].

Diabetes disproportionately affects people in LMICs, where livelihoods and healthcare services and access to early diagnosis might be less common. Diabetes is a major cause of morbidity and mortality due to cardiovascular disease, nephropathy, neuropathy and retinopathy [12].

According to the latest figures in the IDF Diabetes Atlas, the prevalence of diabetes is still increasing in the world, making prevention and treatment a critical priority. Interventions aimed at healthy nutrition, physical activity, weight management, and early detection and screening are crucial for combating the escalating burden of diabetes globally [11-12].

1.4 Pathophysiology of Type 2 Diabetes Mellitus

Type 2 Diabetes Mellitus (T2DM) is a chronic metabolic disease that is associated with hyperglycemia, insulin resistance, and gradual loss of the pancreatic beta (β) cells. Development can be slow and is a combination of genetics, environment, and lifestyle [14].

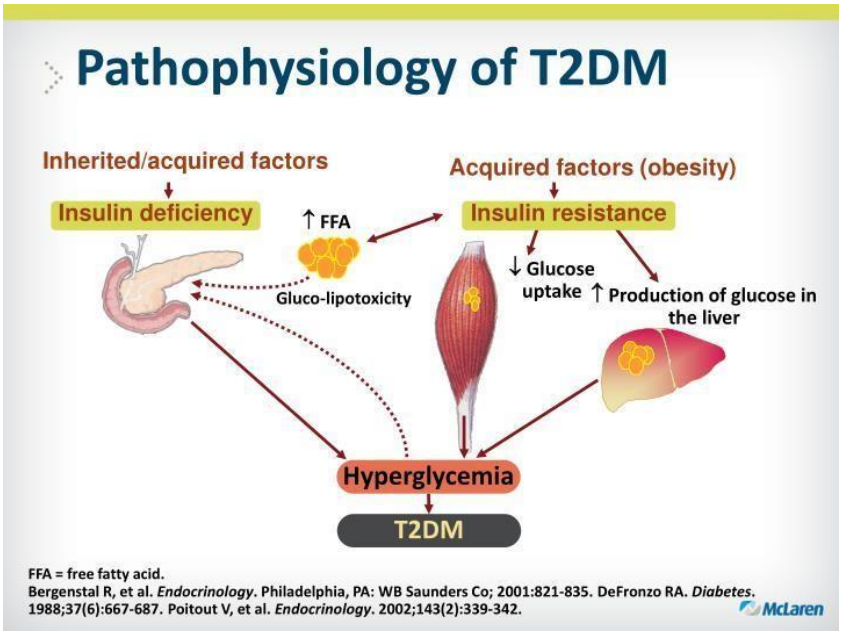


Figure 1.1: Pathophysiology of Type 2 Diabetes Mellitus showing the interaction between insulin resistance, insulin deficiency, increased hepatic glucose production, and hyperglycemia.

Insulin resistance is among the initial impairments in T2DM. In the condition, there is a diminished responsiveness of target tissues, including skeletal muscle, adipose tissue and liver to insulin, resulting in a decrease in the ability of target tissues to take up glucose, and anincrease in the production of glucose by the liver. Insulin resistance is one of the leading factors for obesity, particularly visceral fat [15].

Another important characteristic of T2DM is β-cell dysfunction. At first, the pancreatic β-cells make up for insulin resistance by secreting more insulin. In the long term however, the prolonged metabolic stress

can lead to impairment of β-cell function and decrease in insulin production, thereby leading to chronic hyperglycemia [14-17].

High blood glucose leads to glucotoxicity and high free fatty acids lead to lipotoxicity. Both diseases cause damage to pancreatic β-cells, which causes a decrease in insulin production and increased insulin resistance. This is called glucolipotoxicity and further magnifies the progression of disease [16]. There are also roles for oxidative stress and chronic inflammation in T2DM. An increased production of Reactive Oxygen Species (ROS) and inflammatory cytokines, including Tumor Necrotizing Factor (TNF-α) and Interleukin-6 (IL-6), affects the function of the β-cells and disrupts insulin signaling pathways. All these mechanisms play a role in the progression of diabetic complications and worsening metabolic control [17].

Therefore, the pathophysiology of T2DM is a complex interplay of insulin resistance, β-cell dysfunction, glucotoxicity, lipotoxicity, oxidative stress and inflammation. The mechanisms underlying these processes are crucial for designing efficient therapeutic approaches that act on improving insulin sensitivity, maintaining β-cell function and avoiding long-term complications [14-15].

1.5 Insulin Resistance

Insulin resistance is defined as decreased sensitivity of tissues like liver, skeletal muscle, and fat to insulin's actions, and is a hallmark of Type 2 Diabetes Mellitus (T2DM). This results in reduced glucose use and increased hepatic glucose production, thereby increasing blood glucose levels [18-19].

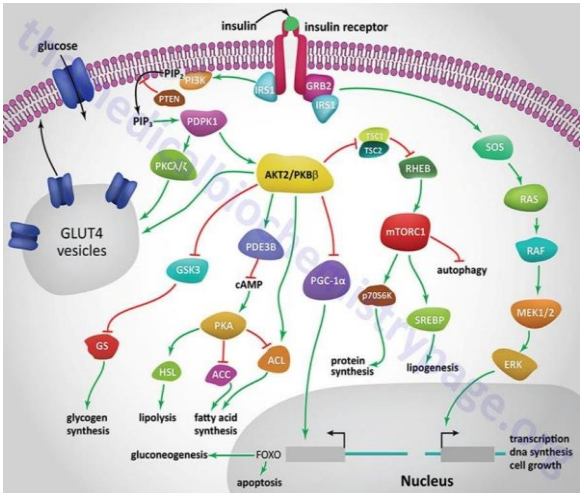


Figure 1.2: Insulin Signaling and Insulin Resistance

Insulin resistances are one of the primary reasons for insulin resistance, and this is due to the dysfunction of the insulin signaling pathway. Mutations in insulin receptor substrate (IRS) proteins and downstream insulin signaling pathways decrease the ability of cells to respond to insulin. Moreover, there is a reduced translocation of the glucose transporter type 4 (GLUT-4) to the cell membrane which reduces the uptake of glucose by muscle and adipose tissue and thereby leads to hyperglycemia [18]. Insulin resistance is also crucial and the liver is an important factor in it. Insulin normally has an inhibitory effect on glucose production by the liver. In insulin-resistant states, however, excessive glucose continues to be produced in the liver leading to fasting hyperglycemia and to poor metabolic control [19].

Visceral fat (VBF) is strongly correlated with insulin resistance (IR). Increased adipose tissue causes the release of free fatty acids and inflammatory mediators which disrupt insulin signaling. Insulin action is also worsened by adipokine imbalance and chronic low-grade inflammation, which also plays a role in

metabolic dysfunction [20]. It is believed that insulin resistance is a critical connection between obesity, metabolic syndrome and Type 2 Diabetes Mellitus, and that it occurs several years prior to the onset of Type 2 Diabetes Mellitus. Thus, the improvement of insulin sensitivity to lifestyle change, weight control, and some therapeutic agents like metformin is still a huge therapeutic challenge in the prevention and management of T2DM [19-20].

1.6 Pancreatic β-Cell Dysfunction

One of the main causes of initiation and progression of Type 2 Diabetes Mellitus (T2DM) is the dysfunction of the pancreatic β-cells, which causes them to fail to produce and secrete enough insulin to meet the body's needs and maintain normal blood sugar levels. In the early stages of insulin resistance, compensatory mechanisms include elevated insulin secretion by the β-cells. This response, however, is impaired over time when there is prolonged metabolic stress, leading to less insulin production and β-cell failure [21-22].

Oxidative stress is one of the major causes of β-cell dysfunction. Chronic hyperglycemia and high blood levels of free fatty acids leads to increased production of reactive oxygen species (ROS) that cause damage to cellular proteins, lipids and DNA. The pancreas β-cells have limited antioxidant defenses, and therefore special sensitivity to oxidative damage [23].

The role of impaired insulin secretion due to mitochondrial dysfunction is also of importance. Impaired mitochondrial function leads to a decrease in ATP levels, which, in turn, can impair the glucose-stimulated insulin secretion process and further impair β-cell function [24].

Furthermore, chronic metabolic stress may induce apoptosis of β-cells, thereby reducing β-cell mass. New studies also indicate that the functional identity of the β-cells may be lost prior to cell death, leading to a decrease in insulin synthesis [23].

Thus, the pancreatic β-cell dysfunction is a crucial stage in the progression from insulin resistance to overt diabetes. Maintaining β-cell mass and function is a key therapeutic target to achieve better glycemic control and slow disease progression in T2DM [21-22].1.7 Oxidative Stress in T2DM.

1.6 Oxidative Stress in Type 2 Diabetes Mellitus

Oxidative stress has been recognized as a significant contributor to development and progression of Type 2 Diabetes Mellitus (T2DM). It is the result of the production of reactive oxygen species (ROS) exceeding the levels of antioxidant defence. Hyperglycemia over time and increased free fatty acids promote increased ROS production and cellular damage and dysfunction [25-26].

Too much ROS can harm cells by damaging DNA, proteins and lipids. Oxidative damage causes a disruption in proper insulin signaling pathways and affects proper cellular function, which leads to insulin resistance and poor glycemic control [25]. Pancreatic β-cells are very sensitive to oxidative stress, having relatively low antioxidant enzyme activity. These ROS also lead to a decrease in glucose stimulated insulin secretion, increase β-cell apoptosis and decrease β-cell mass. This results in inadequate compensation for insulin resistance and hyperglycemia persists [27].

Diabetic complications also involve oxidative stress to a great extent. The continual oxidative damage leads to inflammation, diabetic nephropathy, diabetic retinopathy, neuropathy and cardiovascular disease [28]. Hence, oxidative stress is an important connecting pathway between hyperglycemia, insulin resistance, β-cell dysfunctions, and diabetic complications. Methods that lower oxidative stress and increase antioxidant defenses have been suggested to maintain and improve the function of the β cells and diabetes control [25-27].

Current Pharmacotherapy

With regards to current treatment of Type 2 Diabetes Mellitus (T2DM), the aim is to control blood sugar level, increase insulin sensitivity and prevent diabetic complications. Metformin is the drug of choice for antidiabetic treatment among the choices available due to its effectiveness, safety, cost effectiveness and low risk of hypoglycaemia [29-30].

Metformin is in a group of medications called biguanides, which are oral medications used to treat diabetes. It helps to reduce the blood glucose without causing direct stimulation of insulin and is especially useful in overweight and obese patients. Action of the drug is to lower hepatic glucose production and increase insulin sensitivity in peripheral tissues (e.g., skeletal muscle and adipose tissue) [29]. One of the primary mechanisms of metformin is that it activates AMP-activated protein kinase (AMPK), a key enzyme in cell energy metabolism. AMPK activation also inhibits gluconeogenesis, stimulates glucose uptake and promotes insulin sensitivity in the liver, which helps to improve glycemic control [31].

Metformin has a number of benefits: it lowers glucose well, doesn't lead to a significant risk of hypoglycemia, doesn't cause weight gain, and may have cardiovascular benefits. These properties have made it the foundation of the successful management of T2DM and it is a component in combination therapy with other antidiabetic drugs [29-30]. Metformin can have gastrointestinal side effects, including abdominal discomfort, diarrhea, and nausea, but these side effects are rare. Long-term use has also been reported to cause vitamin B12 deficiency, and the drug is to be used with caution in patients with severe renal insufficiency [30].

Hence, metformin is still an important medication in the pharmacological therapy of T2DM that addresses important metabolic disturbances including insulin resistance and excessive hepatic glucose production [29-31]

Herbal Approaches

Herbal medicines have been widely used for the prevention and management of diabetes mellitus. In recent years, medicinal plants have attracted considerable scientific interest because of their antidiabetic, antioxidant, anti-inflammatory, and hypolipidemic properties. These natural products contain bioactive phytochemicals that may help improve glucose metabolism and reduce diabetes-related complications [32-33].

Medicinal plants exert their antidiabetic effects through multiple mechanisms, including stimulation of insulin secretion, enhancement of insulin sensitivity, increased peripheral glucose uptake, inhibition of carbohydrate digestion, and reduction of oxidative stress. In addition, many herbal remedies possess anti-inflammatory and lipid-lowering activities, which are beneficial in patients with Type 2 Diabetes Mellitus (T2DM) [32].

The growing prevalence of diabetes and the limitations associated with long-term pharmacotherapy have increased interest in herbal interventions as complementary therapies. Although herbal medicines should not replace conventional antidiabetic treatment, they may provide additional therapeutic benefits when used alongside standard drugs. Therefore, scientific evaluation of their efficacy and safety remains essential for their integration into diabetes management strategies [32-33].

1.6 Amla (*Emblica officinalis*)

Amla (*Emblica officinalis* Gaertn.), commonly known as Indian Gooseberry, is an important medicinal plant widely used in Ayurveda. It is valued for its rich nutritional composition and diverse pharmacological properties. Amla is particularly recognized for its antidiabetic, antioxidant, anti-

inflammatory, and hypolipidemic activities, making it a promising natural agent for the management of Type 2 Diabetes Mellitus (T2DM) [34].

Amla contains several bioactive phytoconstituents, including vitamin C, polyphenols, tannins, gallic acid, ellagic acid, emblicanin A, and emblicanin B. These compounds possess strong antioxidant properties and help protect cells against oxidative stress-induced damage [34-35].

Experimental studies have shown that Amla improves glucose metabolism by enhancing insulin sensitivity, stimulating insulin secretion, and reducing hepatic glucose production. It has also been reported to lower fasting blood glucose and improve overall glycemic control [35].

In addition to its antidiabetic effects, Amla exhibits significant antioxidant activity by scavenging reactive oxygen species (ROS) and protecting pancreatic β-cells from oxidative injury. The fruit also improves lipid profiles by reducing total cholesterol, triglycerides, and LDL cholesterol while increasing HDL cholesterol. These effects may help reduce cardiovascular risks associated with diabetes [34-35].

Therefore, Amla represents a valuable complementary therapeutic agent in diabetes management. Its multifaceted pharmacological actions support its potential use in combination with conventional antidiabetic drugs such as metformin for improved metabolic control and protection against diabetic complications [34-35].

1.7 Rationale of Study

Type 2 Diabetes Mellitus (T2DM) is a major metabolic disorder characterized by insulin resistance, progressive pancreatic β-cell dysfunction, oxidative stress, and chronic inflammation. Although metformin is the first-line drug for T2DM and effectively improves insulin sensitivity and reduces hepatic glucose production, it does not completely prevent the progressive decline of pancreatic β-cell function.

Amla (*Emblica officinalis*) is a medicinal plant known for its antidiabetic, antioxidant, anti-inflammatory, and hypolipidemic properties. Its bioactive constituents have been reported to improve glucose metabolism, reduce oxidative stress, and protect pancreatic β-cells from damage. Therefore, Amla may complement the therapeutic effects of metformin by targeting mechanisms that are not fully addressed by conventional pharmacotherapy.

Since insulin resistance and β-cell dysfunction are the two major pathological features of T2DM, a combination therapy targeting both abnormalities may provide superior therapeutic benefits. However, limited studies have evaluated the combined effect of Amla and metformin on insulin resistance and pancreatic β-cell protection. Hence, the present study is undertaken to investigate the synergistic effect of Amla and metformin in experimental Type 2 Diabetes Mellitus and to assess their potential role in improving glycemic control and preserving pancreatic function.

MATERIAL AND METHOD

The present study aims to design an Experimental, lab based in vivo study to assess the synergistic effect of Amla (Phyllanthus emblica) and Metformin on insulin resistance and pancreatic β-cell protection in experimentally induced Type 2 Diabetes Mellitus (T2DM). The aim of the study was to evaluate the antidiabetic activity of Amla as well as Amla in combination with Metformin through biochemical, physiological and histopathological parameters.

Experimental animals were induced with Type 2 Diabetes (T2D) with a proper diabetogenic agent then treated for a fixed period with Amla extract, Metformin and their combination. The impact of intervention was assessed through fasting blood glucose levels, body weight, serum insulin levels, lipid profile,

glycated hemoglobin (HbA1C) and insulin resistance indices.

In addition, the histological features of the pancreas were evaluated to evaluate the effect of treatment on the architecture of the β-cells and the integrity of the cells. The results obtained were statistically processed to assess the significance of the effects observed and to investigate the possibility of synergic effect of Amla and Metformin on Type 2 Diabetes Mellitus treatment.

Experimental Animals

For the present study, healthy adult Wistar albino rats of both sexes weighing between 160-200 g were chosen. The animals were obtained from a CPCSEA registered animal breeding station and acclimatized at the laboratory prior to commencement of the study. The animals were kept under normal laboratory conditions throughout the experiment and given standard pellet diet and water ad libitum.

All the experimental work was conducted as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) Government of India. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) prior to experimental work.

3.2.1 Selection of Animals

Adult healthy Wistar albino rats weighing 160–200 g and aged approximately 8–10 weeks were selected for the study. Animals were chosen based on their healthy appearance, normal physiological behavior, and absence of any visible pathological conditions.

Inclusion Criteria

- Healthy adult Wistar albino rats.
- Body weight between 160–200 g.
- Animals exhibiting normal feeding and drinking habits.
- Animals free from any visible signs of disease or injury.

Exclusion Criteria

- Pregnant or lactating female rats.
- Animals with abnormal physical appearance or behavioral abnormalities.
- Animals showing signs of infection, illness, or severe stress.
- Animals with significant body weight loss during acclimatization.

Before experimentation, all animals were acclimatized to the laboratory environment for seven days to minimize stress-related physiological variations.

2.1 Housing and Maintenance Conditions

Animals were kept in clean polypropylene caging with stainless steel grill tops with standard environmental housing. The use of bedding material was sterilized paddy husk which was replaced frequently for hygienic conditions.

Animals were kept in predetermined laboratory conditions as follows**:**

Table 1: Housing and Maintenance Conditions of Experimental Animals

Parameter	Condition
Species	Wistar Albino Rat
Sex	Either Sex
Body Weight	160–200 g
Temperature	25 ± 2°C
Relative Humidity	50–60%
Light/Dark Cycle	12 h Light / 12 h Dark
Acclimatization Period	7 Days
Diet	Standard Pellet Feed
Water	Ad libitum
Bedding Material	Sterile Paddy Husk

2.2 Ethical Approval

Before the start of the study, the experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of the institution. All handling of animals was done as per the guidelines laid down by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

It was attempted to reduce animal death, suffering and the numbers of animals used whilst maintaining the scientific validity of the study. Diabetes induction, blood collection, treatment administration, and tissue harvesting were all done by trained personnel at normal laboratory conditions.

2.3 Materials

The materials used for the present study were the herbal drug Amla (Phyllanthus emblica), an anti-diabetic drug of standard origin Metformin, various chemicals and reagents for diabetes induction and biochemical estimations, laboratory instruments for handling animals, sample processing, biochemical estimation and histopathological studies. All the chemicals and reagents used in the study were of analytical grade and the experimental results were reproducible and accurate, by using standard and reliable supplier.

Students are expected to be able to select and identify plant material.Students should be able to select and identify plant material.

Amla (Phyllanthus emblica Linn.) was chosen for the present study because it has been well documented for its antidiabetic, antioxidant, anti-inflammatory and pancreatic β-cell protective properties. The fruits

of Amla were bought from a reputed herbal merchant/village herbal shop and thoroughly checked for their quality and authenticity.

Plant material collected was identified by a competent taxonomist/botanist of a reputed Department of Botany or Herbarium Centre. The plant materials were identified by using

Macro-scopic and morphological features. Once authenticated a voucher specimen was prepared and deposited in the departmental herbarium for future reference. The authenticated plant material was then used for extraction and experimental studies.

2.4 Details of Selected Plant Material

The botanical details of the medicinal plant used in the present study are presented in Table 2

Table 2: Details of Selected Plant Material

Sr. No.	ommon Name	Botanical Name	Family	Part Used
1	Amla	*Phyllanthus emblica* Linn.	Euphorbiaceae	Fruit

2.4.1 Experimental Drug

Amla extract a herbal drug and Metformin a standard antidiabetic drug was used in the present study. Amla extract was chosen due to its noted antihyperglycemic and antioxidant properties while the use of Metformin as a standard oral hypoglycemic agent was utilized. A summary of the details of the experimental drugs used in the study is presented in Table 3.2.

Table 3: Details of Experimental Drugs

Sr. No.	Drug	Category	Source
1	Amla Extract (*Phyllanthus emblica*)	Herbal Drug	Himalaya Herbal Healthcare / Authenticated Plant Material
2	Metformin Hydrochloride	Standard Antidiabetic Drug	)O Pharma, Chennai / Local Pharmacy

Chemicals and Reagents Used

Diabetes induction, biochemical estimation, histopathological study and insulin resistance were done using various analytical grade chemicals and reagents. All chemicals were purchased from trusted suppliers and were used as received.

Table 4: List of Chemicals and Reagents Used

Sr. No.	Chemical/Reagent	Purpose	Source
1	Streptozotocin (STZ)	Induction of Experimental Diabetes	a-Aldrich, USA
2	Nicotinamide	ɒe 2 Diabetes Induction	a-Aldrich, USA
3	Metformin Hydrochloride	ard Antidiabetic Treatment	DO Pharma, Chennai
4	Carboxymethyl Cellulose (CMC)	e/Suspending Agent	S.D. Fine Chemicals
5	Glucose Estimation Kit	Blood Glucose Measurement	OneTouch Select/Lifescan
6	Insulin ELISA Kit	tion of Serum Insulin	ELK Biotechnology / Sigma
7	HbA1c Diagnostic Kit	ıted Hemoglobin Estimation	Erba Diagnostics
8	Total Cholesterol Kit	Lipid Profile Analysis	Randox Laboratories, UK
9	Triglyceride Kit	Lipid Profile Analysis	Randox Laboratories, UK
10	HDL-Cholesterol Kit	Lipid Profile Analysis	Randox Laboratories, UK
11	LDL-Cholesterol Kit	Lipid Profile Analysis	Randox Laboratories, UK

12	Serum Creatinine Kit	dney Function Assessment	Randox Laboratories, UK
13	Blood Urea Kit	dney Function Assessment	Randox Laboratories, UK
14	Formalin (10%)	issue Fixation for Histopathology	Merck, India
15	Hematoxylin and Eosin (H&E) Stain	pathological Staining	Himedia Laboratories
16	hosphate Buffer Saline (PBS)	Tissue Processing	Himedia Laboratories

Instruments Used

During the study, various laboratory instruments were used to handle the animals, make biochemical estimations, separate serum and for histopathological analysis and collection of data. Instruments were calibrated prior to analysis, to guarantee accurate and accurate indings.

Table 5: List of Instruments Used

Sr. No.	Instrument	Purpose	Manufacturer/Model
1	Digital Glucometer	Blood Glucose Measurement	OneTouch Select/Lifescan
2	UV–Visible Spectrophotometer	iochemical Analysis	Shimadzu UV-1800
3	Refrigerated Centrifuge	Serum Separation	Remi Instruments
4	Electronic Analytical Balance	Accurate Weighing of Animals and Chemicals	Shimadzu
5	Micropipettes	ecise Liquid Handling	Eppendorf
6	al Feeding Cannula	Oral Drug Administration	ard Laboratory Supplier

7	Hot Air Oven	Drying of Glassware	Remi Instruments
8	gerator (2–8°C)	rage of Reagents and Samples	LG / Samsung
9	Microtome	Tissue Sectioning	Leica Microsystems
10	Light Microscope	topathological Evaluation	Olympus
11	Water Bath	Sample Preparation	Remi Instruments
12	pH Meter	pH Measurement	Elico Instruments

Preparation of Amla Extract
Collection of Plant Material

The fresh mature fruits of Phyllanthus emblica Linn. The accurate plants (Amla) were purchased from a local herb market in Delhi, India. After collection the fruits were thoroughly checked for physical damage, microbial contamination and insect infestation. The plant material was botanically authenticated by a qualified taxonomist of the Department of Botany of a recognized institution. A voucher specimen was made and placed in the departmental herbarium for future reference.

The mature fruits were chosen for harvesting because bioactive phytoconstituents like phenolic compounds, tannins, flavonoids and ascorbic acid are believed to be maximum at this stage. The authentic fruits were then used for extraction and phytochemical studies.

Drying and Powdering

Amla fruits were collected and washes in running tap water and then distilled water to remove dust, soil particles and other impurities. The fruits were washed and cut into small pieces which were shade dried at room temperature for about 10-15 days. Shade drying was selected to reduce degradation of thermolabile components and to maintain the anti-oxidant components in the fruits.

The drying was continued till the moisture content of the fruits was completely removed and they became dry and brittle. The dried fruits were then pulverized using a mechanical grinder to obtain a coarse powder. The powdered material was sieved through a standard sieve to ensure uniformity of particle size, and kept in containers that were tightly sealed, shielded from moisture, heat and direct sunlight until further use.

Extraction Method

The dried fruit powder of *Phyllanthus emblica* was subjected to Soxhlet extraction using methanol as the

extraction solvent. Methanol was selected because of its ability to efficiently extract polar phytoconstituents including phenols, tannins, flavonoids, and other antioxidant compounds.

Extraction Procedure

1. A known quantity of dried Amla fruit powder was accurately weighed and packed into a Soxhlet extraction thimble.
2. The thimble was placed in the Soxhlet apparatus and extracted with 100% methanol.
3. Continuous hot extraction was carried out for an appropriate duration until complete exhaustion of the plant material was achieved.
4. The methanolic extract obtained was filtered to remove insoluble residues.
5. The filtrate was concentrated under reduced pressure using a rotary evaporator.
6. Residual solvent was removed by keeping the concentrated extract at room temperature until a semi-solid mass was obtained.
7. The dried extract was weighed and transferred into amber-colored airtight containers.
8. The extract was stored under refrigerated conditions (4°C) until further pharmacological and biochemical studies.

The obtained extract was used for antidiabetic evaluation, insulin resistance assessment, biochemical investigations, and pancreatic β-cell protection studies.

2.4.2 Percentage Yield Calculation

The percentage yield of the methanolic extract was calculated to determine the efficiency of the extraction process using the following formula:

Percentage Yield (%) = (Weight of Dried Extract / Weight of Crude Plant Material) × 100 Where:

Weight of Dried Extract = Final weight of concentrated extract obtained after solvent removal

Weight of Crude Plant Material = Initial weight of powdered Amla fruit used for extraction

The percentage yield was calculated for each extraction batch separately and expressed as mean percentage yield.

Table 6: Percentage Yield of Amla Extract

atch No.	nt Taken (g)	Obtained (g)	tage Yield (%)
Batch I	232	80.7	34.7

Batch II	227	78.4	34.5
Average	229.5	79.55	34.6

The methanolic extraction of *Phyllanthus emblica* fruits yielded approximately 34–35% extract, indicating efficient recovery of bioactive phytoconstituents suitable for further pharmacological evaluation.

2.5 Preliminary Phytochemical Screening

Phytochemical screening of methanolic extract of *Phyllanthus emblica Linn*. was performed to establish the phytoconstituents which are mainly responsible for the pharmacological activity. The screening was carried out by the standard qualitative chemical procedures, as mentioned in the pharmacognostic literature. The color changes or precipitates were used to determine whether phytochemicals were present or not.

2.5.1 Test for Alkaloids Mayer's Test

A small amount of the extract was dissolved in dilute hydrochloric acid and filtered. A few drops of Mayer's reagent (potassium mercuric iodide solution) were added to the filtrate. Alkaloids were detected with the formation of a cream colored precipitate.

Wagner's Test

To 1 mL of the extract solution, add a few drops of Wagner's reagent (iodine in potassium iodide solution) were added. Formation of a reddish-brown precipitate indicated the presence of alkaloids.

Observation: No precipitate was observed in either test.

Result: Alkaloids were absent in the methanolic extract of *Phyllanthus emblica***.**

2.5.2 Test for Flavonoids Ferric Chloride Test

The extract solution was treated with a few drops of neutral ferric chloride. Presence of flavonoids indicated the appearance of greenish black coloration.

Lead Acetate Test

A few drops of lead acetate solution were added to the extract. Formation of a yellow precipitate confirmed the presence of flavonoids.

Observation: Positive color reaction and yellow precipitate formation were observed.

Result: Flavonoids were present in the extract.

2.5.3 Test for Tannins Ferric Chloride Test

A few drops of 1% ferric chloride solution were added to the extract solution. Formation of a blue-black or greenish-black coloration indicated the presence of tannins.

Gelatin Test

The extract was treated with 1% gelatin solution containing sodium chloride. Formation of a white precipitate indicated the presence of tannins.

Observation: Positive reactions were observed in both tests. Result: Tannins were present in the extract.

2.5.4 Test for Phenolic Compounds

A few drops of ferric chloride solution were added to the extract solution. Development of a blue-green coloration indicated the presence of phenolic compounds.

Observation: Blue-green color was observed.

Result: Phenolic compounds were present in the extract.

2.5.5 Test for Saponins Foam Test

One milliliter of the extract was mixed with distilled water and shaken vigorously for several minutes. Formation of a stable froth persisting for at least 10 minutes indicated the presence of saponins.

Observation: Stable foam formation was observed.

Result: Saponins were present in the extract.

2.5.6 Test for Glycosides Keller–Kiliani Test

One milliliter of extract was treated with glacial acetic acid containing ferric chloride solution followed by careful addition of concentrated sulfuric acid along the sides of the test tube. Formation of a brown ring at the interface indicated the presence of glycosides.

Observation: No characteristic color reaction was observed.
Result: Glycosides were not detected in the methanolic extract.

2.5.7 Test for Terpenoids Salkowski Test

One milliliter of extract was mixed with chloroform, followed by careful addition of concentrated sulfuric acid. Formation of a reddish-brown coloration at the interface indicated the presence of terpenoids.

Observation: Reddish-brown coloration was observed. Result: Terpenoids were present in the extract.

Table 7: Phytochemical Screening of Amla Extract

Phytoconstituent	Test Performed	Result
Alkaloids	Mayer's Test, Wagner's Test	–
Flavonoids	Ferric Chloride Test, Lead Acetate Test	+++
Tannins	Ferric Chloride Test, Gelatin Test	+++
Phenolic Compounds	Ferric Chloride Test	+++
Saponins	Foam Test	+
Glycosides	Keller–Kiliani Test	–
Terpenoids	Salkowski Test	+

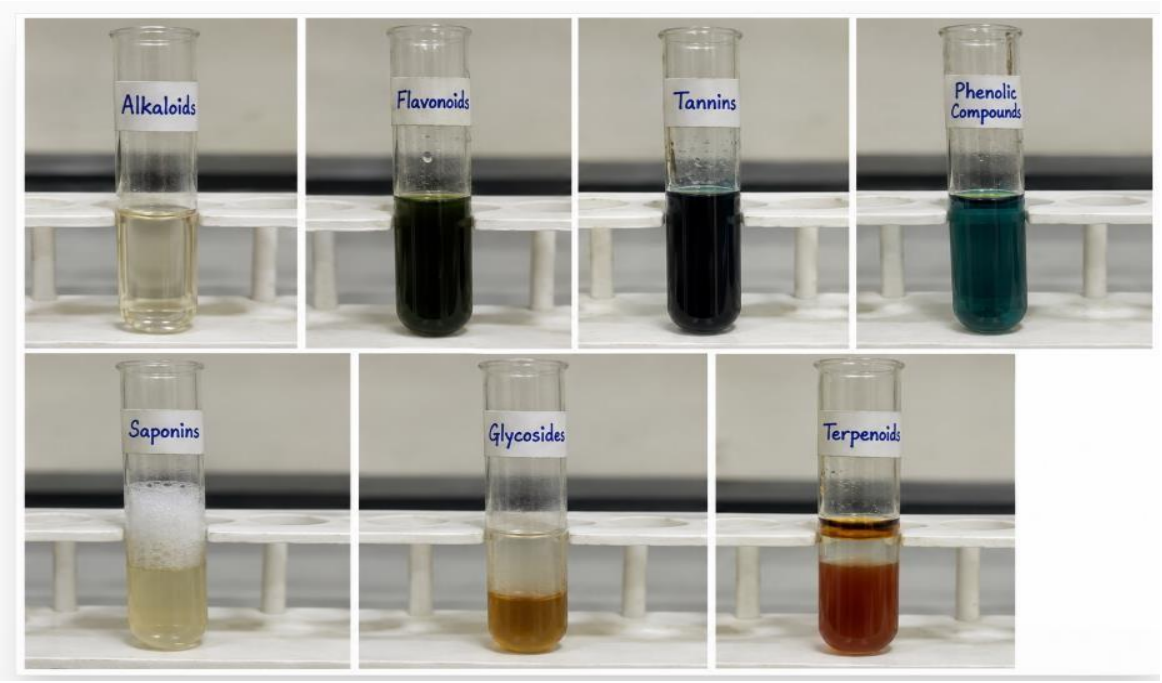


Figure-Phytochemical Screening of Amla Extract

Key:

(+) Present

(+++) Strongly Present (–) Absent

The phytochemical analysis of the methanolic extract of Phyllanthus emblica showed the presence of flavonoids, tannins, phenolic compounds, saponins and terpenoids. The phytoconstituents are known to have antioxidant, antihyperglycemic, anti-inflammatory and cytoprotective activities and may be responsible for the antidiabetic and protective effects on pancreatic β-cells seen in the study.

2.6 Induction of Experimental Type 2 Diabetes Mellitus

The experimental model of Type 2 Diabetes Mellitus (T2DM) was induced using Streptozotocin–Nicotinamide (STZ–NA) model, which is similar to human type 2 diabetes, characterized by moderate hyperglycemia, insulin resistance and partial pancreatic β-cell dysfunction . Partial protection of pancreatic β-cells after the administration of nicotinamide before streptozotocin (STZ) injection produces a stable diabetic state for testing of antidiabetic agents and β-cell protective interventions.

2.6.1 Preparation of Streptozotocin Solution

Streptozotocin (STZ) is a naturally occurring compound, a nitrosourea isolated from Streptomyces achromogenes. It has a selective cytotoxic action on pancreatic β-cells due to its glucose moiety that allows the uptake through GLUT-2 transporter. STZ was freshly prepared immediately prior to administration for induction of Type 2 diabetes. The required STZ was accurately weighed and dissolved in freshly prepared cold

0.1 M citrate buffer (pH 4.5). STZ is very unstable in aqueous media and degrades rapidly at room temperature, so the solution was prepared in a chilled environment and protected from light.

The dose of STZ employed in the present study was 55 mg/kg body weight, administered intraperitoneally.

Procedure for Preparation of STZ Solution

1. The body weight of each animal was recorded.
2. The required quantity of STZ was calculated according to body weight.
3. STZ was dissolved in freshly prepared cold 0.1 M citrate buffer (pH 4.5).
4. The solution was mixed gently until complete dissolution.
5. The freshly prepared solution was used within 15 minutes of preparation.

Table 8: Properties and Preparation of Streptozotocin

Parameter	Details
Chemical Name	Streptozotocin
Molecular Weight	265.73 g/mol
Source	*Streptomyces achromogenes*
Solvent	0.1 M Citrate Buffer (pH 4.5)
Dose	55 mg/kg b.w.
Route	Intraperitoneal (i.p.)
Storage During Preparation	Ice Cold (4°C)
Stability	Use within 15 minutes

2.6.2 Preparation of Nicotinamide Solution

Nicotinamide (NA), a water-soluble derivative of vitamin B3, protects pancreatic β-cells against STZ-induced cytotoxicity. Pre-treatment with nicotinamide before STZ led to partial protection of insulin secreting β cells and development of diabetes similar to Type 2 Diabetes Mellitus. The required amount of nicotinamide was dissolved in sterile normal saline (0.9% NaCl) just before administration. The solution was prepared fresh to ensure maximum stability and biological activity.

The dose of nicotinamide used in the present study was 110 mg/kg body weight intraperitoneally 15 minutes before STZ injection.

Procedure for Preparation of Nicotinamide Solution

1. The required quantity of nicotinamide was calculated according to body weight.
2. Nicotinamide was dissolved in sterile normal saline.
3. The solution was mixed until complete dissolution.
4. The freshly prepared solution was administered intraperitoneally 15 minutes before STZ injection.

Table 9: Properties and Preparation of Nicotinamide

Parameter	Details
Chemical Name	Nicotinamide
Category	β-cell Protective Agent
Solvent	Sterile Normal Saline (0.9% NaCl)
Dose	110 mg/kg b.w.
Route	Intraperitoneal (i.p.)
Administration Time	15 min before STZ
Purpose	Partial β-cell Protection

2.6.3 Induction Procedure

Type 2 Diabetes Mellitus was induced by Nicotinamide – Streptozotocin.
Prior to induction, animals were acclimatized to laboratory conditions for one week and maintained under standard environmental conditions. Rats were starved overnight (about 12 hours) while still having access to drinking water.

Body weights of all animals were measured on induction day for dosage. Nicotinamide solution (110 mg/kg, i.p.) was given first. After 15 minutes, freshly prepared streptozotocin solution (55 mg/kg, i.p.) was injected i.p.Freshly prepared streptozotocin solution (55 mg/kg, i.p.) was subsequently injected i.p. after 15 minutes.

Animals were then brought back to their cages and closely watched. The animals were given 10% glucose in drinking water to avoid causing sudden hypoglycemia due to the STZ-induced acute insulin release for 24 hours after STZ injection.

Clinical parameters of diabetes and food and water consumption, as well as body weight changes, were recorded daily in animals.

Steps Involved in Diabetes Induction

1. Overnight fasting of experimental animals.
2. Recording of body weight.
3. Intraperitoneal administration of Nicotinamide (110 mg/kg).
4. Waiting period of 15 minutes.
5. Intraperitoneal administration of Streptozotocin (55 mg/kg).
6. Provision of 10% glucose solution for 24 hours.

7. Daily monitoring of animals.

Table 10: Diabetes Induction Schedule

Procedure	Details
Fasting Period	12 Hours
Nicotinamide Dose	110 mg/kg (i.p.)
STZ Dose	55 mg/kg (i.p.)
Time Interval	15 Minutes
Glucose Supplementation	10% Glucose Solution
Duration of Supplementation	24 Hours

2.6.4 Confirmation of Diabetes

Seven days after administration of Nicotinamide and Streptozotocin, fasting blood glucose levels were measured to confirm the induction of diabetes.

Animals were fasted overnight and blood samples were collected from the tail vein after disinfecting the area with 70% ethanol. Fasting blood glucose was measured using a calibrated glucometer based on the glucose oxidase method.

Animals exhibiting fasting blood glucose levels greater than 250 mg/dL were considered diabetic and included in the experimental study. Animals with lower glucose levels were excluded from further experimentation.

In addition to hyperglycemia, clinical manifestations such as polyuria, polydipsia, weight loss, and reduced physical activity were observed to support confirmation of the diabetic state.

Table 11: Criteria for Confirmation of Experimental Diabetes

Parameter	Criteria
Fasting Blood Glucose	> 250 mg/dL
Sample Collection Site	Tail Vein
Method of Analysis	Glucose Oxidase Method
Instrument Used	Digital Glucometer
Confirmation Time	Day 7 Post-Induction

The animals fulfilling the above criteria were considered successfully diabetic and were subsequently randomized into different treatment groups for evaluation of the synergistic effects of Amla and

Metformin on insulin resistance and pancreatic β-cell protection.

2.7 Experimental Design

Following successful induction of Type 2 Diabetes Mellitus using the Nicotinamide–Streptozotocin (NA–STZ) model, diabetic rats exhibiting fasting blood glucose levels greater than 250 mg/dL were selected for the study. The animals were randomly allocated into six experimental groups to evaluate the individual and synergistic effects of Amla extract and Metformin on glycemic control, insulin resistance, and pancreatic β-cell protection.

Randomization was performed based on body weight and fasting blood glucose levels to minimize inter-group variability. Each experimental group consisted of six animals (n = 6), providing adequate statistical power for biochemical and histopathological evaluations.

2.7.1 Animal Grouping

A total of thirty-six Wistar albino rats were divided into six groups, each containing six animals.

Table 12: Experimental Animal Grouping

Group	Treatment	Number of Animals
Group I	Normal Control	6
Group II	Diabetic Control	6
Group III	Metformin Treated	6
Group IV	Amla Treated	6
Group V	Amla + Metformin (Low Dose Combination)	6
Group VI	Amla + Metformin (High Dose Combination)	6
Total		36

Detailed Description of Experimental Groups

Group I (Normal Control):

Animals received only vehicle (1% CMC) and served as the normal control group for establishing baseline physiological and biochemical parameters.

Group II (Diabetic Control):

Diabetic animals received vehicle (1% CMC) only and served as untreated diabetic controls to evaluate disease progression.

Group III (Metformin Treated):

Diabetic animals received Metformin at a dose of 100 mg/kg body weight orally and served as the standard treatment group.

Group IV (Amla Treated):

Diabetic animals received methanolic extract of *Phyllanthus emblica* at a dose of 200 mg/kg body weight orally to evaluate the antidiabetic efficacy of Amla alone.

Group V (Low-Dose Combination):

Diabetic animals received Amla extract (200 mg/kg) in combination with a reduced dose of Metformin (50 mg/kg) to assess whether Amla could potentiate the therapeutic activity of Metformin at a lower dose.

Group VI (High-Dose Combination):

Diabetic animals received Amla extract (200 mg/kg) together with the standard therapeutic dose of Metformin (100 mg/kg) to evaluate the maximum synergistic effect of the combination therapy.

Table 13: Detailed Experimental Grouping

Group	Description	Purpose
Group I	Non-diabetic normal control	Baseline physiological parameters
Group II	Diabetic untreated control	Disease progression assessment

Group III	Metformin (100 mg/kg)	Standard antidiabetic treatment
Group IV	Amla extract (200 mg/kg)	Evaluation of herbal therapy
Group V	Amla (200 mg/kg) + Metformin (50 mg/kg)	Assessment of low-dose synergistic effect
Group VI	Amla (200 mg/kg) + Metformin (100 mg/kg)	Assessment of maximum synergistic effect

2.7.2 Treatment Schedule

Treatment was initiated after confirmation of diabetes, seven days following Nicotinamide–Streptozotocin administration. The treatment period continued for twenty-one consecutive days. All drugs and extracts were freshly prepared each day using 1% Carboxymethyl Cellulose (CMC) as the suspending vehicle. Oral administration was carried out once daily using an oral feeding cannula.

Table 14: Treatment Protocol

Group	Treatment	Dose	Route	Duration
Group I	1% CMC Vehicle	1 mL/kg	Oral	21 Days
Group II	1% CMC Vehicle	1 mL/kg	Oral	21 Days
roup III	Metformin	100 mg/kg	Oral	21 Days
roup IV	Amla Extract	200 mg/kg	Oral	21 Days
Group V	Amla + Metformin	ng/kg + 50 mg/kg	Oral	21 Days
roup VI	Amla + Metformin	200 mg/kg + 100 mg/kg	Oral	21 Days

Monitoring Schedule During Treatment

Animals were monitored regularly throughout the treatment period for changes in physiological and biochemical parameters**.**

Table 15: Monitoring Schedule

Study Day	Observation
Day 0	Initial body weight and fasting blood glucose
Day 7	Diabetes confirmation and treatment initiation
Day 14	Weekly fasting blood glucose assessment
Day 21	Intermediate body weight measurement
Day 28	Final biochemical and histopathological evaluation

Dose Selection Rationale

The dose of Metformin (100 mg/kg) was selected based on previously reported experimental studies demonstrating significant antihyperglycemic activity in diabetic rats. The selected dose corresponds to the standard therapeutic dose commonly used in preclinical antidiabetic investigations.

The dose of Amla extract (200 mg/kg) was selected based on published pharmacological studies reporting significant antioxidant, antihyperglycemic, and pancreatic β-cell protective effects at this dose level.

For evaluation of synergistic interaction, two combination regimens were designed:

1. Low-dose combination (Group V): Amla (200 mg/kg) + Metformin (50 mg/kg), to investigate whether Amla enhances the efficacy of a reduced Metformin dose.
2. High-dose combination (Group VI): Amla (200 mg/kg) + Metformin (100 mg/kg), to evaluate the maximum therapeutic effect of the combination.

Experimental Timeline

Table 16: Experimental Timeline

Experimental Phase	Duration
Acclimatization Period	7 Days
NA–STZ Diabetes Induction	Day 0
Diabetes Confirmation	Day 7
Treatment Period	Day 8–28
Blood Collection & Biochemical Analysis	Day 28
Histopathological Evaluation	Day 28
Statistical Analysis	After Data Collection

The experimental design enabled comprehensive assessment of the individual and combined therapeutic effects of Amla extract and Metformin on hyperglycemia, insulin resistance, oxidative stress, and pancreatic β-cell protection in experimentally induced Type 2 Diabetes Mellitus.

2.8 Evaluation Parameters

The therapeutic efficacy of Amla extract, Metformin, and their combination was evaluated using physiological, biochemical, and metabolic parameters. Body weight, fasting blood glucose, oral glucose tolerance, serum insulin levels, insulin resistance index, and glycosylated hemoglobin were assessed throughout the study period.

2.8.1 Measurement of Body Weight

Body weight of all experimental animals was recorded using a calibrated digital weighing balance with an accuracy of ±0.01 g. Measurements were performed at the beginning of the treatment period (Day 0) and at the end of the treatment period (Day 21)

Procedure

1. Animals were weighed individually in a clean weighing chamber.

2. Measurements were recorded at the same time of the day to minimize circadian variations.

3. Final body weight was recorded before blood collection on Day 21.

Calculation

Calculation of Body Weight Change Body Weight Change (g)

Body Weight Change (g) = Final Body Weight (g) − Initial Body Weight (g)

Percentage Body Weight Change (%)

Percentage Body Weight Change (%)

$$= \frac{\text{Final Body Weight (g)} - \text{Initial Body Weight (g)}}{\text{Initial Body Weight (g)}} \times \mathbf{100}$$

Example

If Initial Body Weight = 180 g and Final Body Weight = 210 g,

Body Weight Change $= 210 - 180 = 30$ g

Percentage Body Weight Change $= \frac{210 - 180}{180} \times 100 = 16.67\%$

Interpretation: A positive value indicates weight gain, whereas a negative value indicates weight loss during the treatment period. In diabetic animals, prevention of weight loss or improvement in body weight is considered an indicator of therapeutic efficacy.

Table 17: Body Weight Assessment Schedule

Parameter	Day 0	Day 21
Body Weight (g)	Recorded	Recorded
Weight Change (%)	—	Calculated

2.8.2 Measurement of Fasting Blood Glucose (FBG)

Fasting blood glucose levels were measured using a digital glucometer and compatible glucose test strips.

Procedure

1. Animals were fasted for 6 hours with free access to water.
2. Blood samples were collected from the tail vein.
3. A drop of blood was placed on the glucose strip.
4. Blood glucose concentration was recorded in mg/dL.

Table 18: Measurement Schedule

Study Day	Assessment
Day 0	Baseline FBG
Day 7	Weekly Monitoring
Day 14	Weekly Monitoring
Day 21	Final Evaluation

Calculation of Percentage Reduction in Blood Glucose Percentage Reduction in Blood Glucose (%)

$$= \frac{\text{Initial Fasting Blood Glucose (mg/dL)} - \text{Final Fasting Blood Glucose (mg/dL)}}{\text{Initial Fasting Blood Glucose (mg/dL)}} \times 100$$

Example

Initial Fasting Blood Glucose (mg/dL)

If the initial fasting blood glucose level is 320 mg/dL and the final fasting blood glucose level is 180 mg/dL:

$$\text{Percentage Reduction in Blood Glucose} = \frac{320 - 180}{320} \times 100$$

$$= \frac{140}{320}$$

$= 43.75\%$

$\frac{G_{60} + G_{120}}{} \times 60)$

Interpretation: A higher percentage reduction in fasting blood glucose indicates better antihyperglycemic activity and improved glycemic control following treatment. In the present study, comparison of percentage glucose reduction among treatment groups was used to evaluate the therapeutic efficacy of Amla extract, Metformin, and their combinations.

2.8.3 Oral Glucose Tolerance Test (OGTT)

The Oral Glucose Tolerance Test was performed to evaluate glucose utilization and β-cell functional status.

Procedure

1. Animals were fasted for 6 hours.
2. Baseline glucose level was measured (0 min).
3. D-Glucose (2 g/kg body weight) was administered orally.
4. Blood glucose was measured at 30, 60, and 120 minutes.

Table 19: OGTT Sampling Schedule

Time (min)	Blood Glucose Measurement
0	Baseline
30	Post-glucose load
60	Post-glucose load
120	Final reading

Calculation of Area Under Curve (AUC)

The Area Under the Glucose Response Curve (AUC) was calculated using the trapezoidal rule to assess overall glucose tolerance during the Oral Glucose Tolerance Test (OGTT).

$\text{AUC} = (\frac{G_0 + G_{30}}{2} \times 30) + \frac{30 + G_{60}}{2} \times 30) +$ (

Where:

- G_0= Blood glucose concentration at 0 minutes (fasting value)
- G_{30}= Blood glucose concentration at 30 minutes
- G_{60}= Blood glucose concentration at 60 minutes
- G_{120}= Blood glucose concentration at 120 minutes The AUC values were expressed as mg/dL·min.

2.8.4 Serum Insulin Estimation

Serum insulin concentration was determined using a commercially available Rat Insulin ELISA Kit according to the manufacturer's instructions.

Procedure

1. Blood samples were collected after overnight fasting.
2. Serum was separated by centrifugation at 3000 rpm for 15 minutes.
3. Serum samples were stored at −20°C until analysis.
4. ELISA assay was performed and absorbance was recorded at 450 nm using a microplate reader.
5. Insulin concentration was determined from the standard calibration curve

Table 20: ELISA Assay Parameters

Parameter	Details
Sample	Serum
Method	ELISA
Detection Wavelength	450 nm
Storage Temperature	−20°C

2.8.5 Assessment of Insulin Resistance (HOMA-IR)

Insulin resistance was estimated using the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR).

Formula
Interpretation
Table 21:

HOMA-IR Value	Interpretation

< 1.0	**Normal**
1.0 – 2.5	**Mild Insulin Resistance**
2.5 – 5.0	**Moderate Insulin Resistance**
> 5.0	**Severe Insulin Resistance**

2.8.6 Estimation of Glycosylated Hemoglobin (HbA1c)

HbA1c was determined as an indicator of long-term glycemic control. Procedure

1. Whole blood was collected in EDTA-coated tubes.
2. Samples were analyzed using an HbA1c estimation kit.
3. Absorbance was measured according to kit instructions.
4. HbA1c percentage was calculated from the calibration curve**.**

Interpretation Criteria

Table 22:

HbA1c (%)	Interpretation
< 5.7	**Normal**
5.7 – 6.4	**Prediabetes**
≥ 6.5	**Diabetes**

Clinical Importance

HbA1c reflects the average blood glucose concentration over the preceding 8–12 weeks and provides a reliable marker of long-term glycemic control.

Table 23: Summary of Evaluation Parameters

Parameter	Method	Purpose
Body Weight	Digital Balance	Assessment of metabolic status
Fasting Blood Glucose	Glucometer	Glycemic control
OGTT	Oral Glucose Challenge	β-cell function and glucose tolerance
Serum Insulin	ELISA	Insulin secretion capacity
HOMA-IR	Mathematical Index	Insulin resistance
HbA1c	HbA1c Assay Kit	Long-term glycemic control

2.9 BIOCHEMICAL ANALYSIS

Blood samples were collected at the end of the experimental period following overnight fasting. Serum was separated by centrifugation at 3000 rpm for 15 min and stored at −20°C until biochemical estimations. Lipid profile, liver function markers, and kidney function markers were determined using commercially available diagnostic kits according to the manufacturer's instructions.

2.9.1 Lipid Profile

The following parameters were estimated:

Total Cholesterol (TC)
Triglycerides (TG)
High Density Lipoprotein Cholesterol (HDL-C)
Low Density Lipoprotein Cholesterol (LDL-C)
Very Low Density Lipoprotein Cholesterol (VLDL-C)

Calculation of LDL-C

Low Density Lipoprotein Cholesterol (LDL-C) was calculated using the Friedewald equation:

$$\text{TG LDL-C (mg/dL)} = \text{TC} - \underline{\text{HDL}}\text{-C} - ( ) \quad \textbf{5}$$

Where:

TC = Total Cholesterol (mg/dL)
HDL-C = High Density Lipoprotein Cholesterol (mg/dL)
TG = Triglycerides (mg/dL)

2.9.2 Liver Function Tests

The serum liver marker enzymes were estimated using standard diagnostic kits:
Aspartate Aminotransferase (AST/SGOT)
Alanine Aminotransferase (ALT/SGPT)
Alkaline Phosphatase (ALP)
Results were expressed as U/L.

2.9.3 Kidney Function Tests

Kidney function was assessed by estimating:
Serum Creatinine
Blood Urea Nitrogen (BUN)
Results were expressed as mg/dL.
Blood Urea Nitrogen (BUN) was calculated from the serum urea concentration using the following formula:

$$\textbf{BUN (mg/dL)} = \textbf{Urea (mg/dL)} \times \textbf{0.467}$$

Where,

BUN = Blood Urea Nitrogen (mg/dL)
Urea = Serum Urea concentration (mg/dL)
0.467 = Conversion factor representing the nitrogen content of urea (46.7%)

2.9.4 Atherogenic Indices

Coronary Risk Index (CRI)

The Coronary Risk Index (CRI) was calculated to assess cardiovascular risk using the following formula:

$$\textbf{CRI} = \frac{\textbf{Total Cholesterol}}{\textbf{HDL-C}}$$

Where:
Total Cholesterol (TC) = Serum total cholesterol concentration (mg/dL)
HDL-C = High Density Lipoprotein Cholesterol (mg/dL)
Interpretation:
CRI < 4.5 = Normal cardiovascular risk
CRI > 5.0 = Increased cardiovascular risk

LDL-C/HDL-C Ratio

The LDL-C/HDL-C ratio was calculated as an indicator of atherogenic risk using the following equation:

$$\textbf{LDL-C LDL-C/HDL-C Ratio} = \frac{\textbf{HDL-C}}{}$$

Where:
LDL-C = Low Density Lipoprotein Cholesterol (mg/dL)
HDL-C = High Density Lipoprotein Cholesterol (mg/dL)

Interpretation:

Ratio < 3.0 = Desirable
Ratio > 3.5 = Increased risk of cardiovascular disease

Total Cholesterol/HDL-C Ratio

The Total Cholesterol/HDL-C ratio was calculated using the following formula:

$$\textbf{TC/HDL-C Ratio} = \frac{\textbf{Total Cholesterol HDL-C}}{}$$

Where:

TC = Total Cholesterol (mg/dL)
HDL-C = High Density Lipoprotein Cholesterol (mg/dL)

Interpretation:

Ratio < 5.0 = Normal risk
Ratio > 5.0 = High cardiovascular risk

Table 3.9: Biochemical Parameters Evaluated

Parameter	Unit	Method
Total Cholesterol (TC)	mg/dL	CHOD-PAP Method
Triglycerides (TG)	mg/dL	GPO-PAP Method
HDL-C	mg/dL	Direct Enzymatic Method
LDL-C	mg/dL	Friedewald Formula
VLDL-C	mg/dL	TG/5 Formula
AST (SGOT)	U/L	Enzymatic Colorimetric Method
ALT (SGPT)	U/L	Enzymatic Colorimetric Method
ALP	U/L	Colorimetric Method
Serum Creatinine	mg/dL	Jaffe Method
Blood Urea Nitrogen (BUN)	mg/dL	Berthelot Method

Statistical Expression of Data

2.10 Statistical Analysis

The data obtained from the experimental study were expressed as:

$$\textbf{Mean} \pm \textbf{SEM}$$

Where:

- Mean = Arithmetic average of observations in each group
- SEM = Standard Error of Mean

The Standard Error of Mean (SEM) was calculated using the following formula**:**

$$\textbf{SEM} = \frac{\textbf{SD}}{\sqrt{\boldsymbol{n}}}$$

Where:

- SD = Standard Deviation
- n = Number of animals in each group

All statistical analyses were performed using GraphPad Prism software (Version 9.0). Comparisons among different experimental groups were carried out using One-Way Analysis of Variance (ANOVA) followed by Tukey's Multiple Comparison Post Hoc Test to determine intergroup differences.

The level of statistical significance was set at:

$$p < 0.05$$

The significance levels were interpreted as follows:

p-value	Interpretation
p > 0.05	Not Significant (NS)
p < 0.05	Significant (*)
p < 0.01	Very Significant (**)
p < 0.001	Highly Significant (***)

Results were presented in the form of tables and graphs. Statistical analysis was performed to evaluate the effects of Amla extract, Metformin, and their combination therapy on glycemic control, insulin resistance, lipid profile, liver function markers, and kidney function markers in experimental Type 2 Diabetes Mellitus**.**

Table 3.10: Statistical Tools Used for Data Analysis

Parameter	Statistical Method
Body Weight	One-Way ANOVA followed by Tukey's test
Fasting Blood Glucose	One-Way ANOVA followed by Tukey's test
OGTT	One-Way ANOVA followed by Tukey's test
Serum Insulin	One-Way ANOVA followed by Tukey's test
HOMA-IR	One-Way ANOVA followed by Tukey's test
HbA1c	One-Way ANOVA followed by Tukey's test
Lipid Profile	One-Way ANOVA followed by Tukey's test
Liver Function Tests	One-Way ANOVA followed by Tukey's test
Kidney Function Tests	One-Way ANOVA followed by Tukey's test

2.10.1 Calculation of HOMA-IR

Alternative Formula (Glucose in mmol/L):

2.10.2 Interpretation of HOMA-IR

HOMA-IR Value	Interpretation
< 1.0	Normal insulin sensitivity
1.0–2.5	Mild insulin resistance
2.5–5.0	Moderate insulin resistance
> 5.0	Severe insulin resistance

2.11 Histopathological Evaluation of Pancreas Pancreatic tissue section thickness: 4–5 µm

Sections were stained with Hematoxylin and Eosin (H&E) and examined microscopically for β-cell integrity, islet architecture, necrosis, vacuolation, and inflammatory infiltration.

2.12 Determination of Synergistic Activity

Percentage Improvement in Blood Glucose

$$\% \textbf{ Improvement} = \frac{\textbf{Diabetic Control} - \textbf{Treatment Group}}{\textbf{Diabetic Control}} \times \textbf{100}$$

A greater percentage improvement in the combination-treated groups compared with monotherapy groups was considered indicative of synergistic activity.

2.13 Statistical Analysis

- All experimental results were expressed as:
- Mean ± SEM
- where SEM represents the Standard Error of Mean.
- Differences among groups were analysed using One-Way ANOVA, followed by Tukey's Multiple Comparison Test.
- The level of statistical significance was considered at:
- $p < 0.05$
- **Interpretation:**
- $p < 0.05$ = statistically significant
- $p < 0.01$ = highly significant
- $p < 0.001$ = extremely significant

1.1 Effect of Treatment on Body Weight

Table 4.1: Effect of Amla and Metformin on Body Weight

Group	Initial Body Weight (g)	Final Body Weight (g)	% Change
Group I (Normal Control)	180.5 ± 2.3	195.8 ± 3.1	+8.5
Group II (Diabetic Control)	182.3 ± 2.1	158.7 ± 2.8	-13.0
Group III (Metformin 100 mg/kg)	181.7 ± 2.5	185.2 ± 2.9	+1.9
Group IV (Amla 200 mg/kg)	183.1 ± 2.4	179.8 ± 2.7	-1.8
Group V (Amla 200 mg/kg + Metformin 50 mg/kg)	182.8 ± 2.2	191.5 ± 3.0	+4.7
Group VI (Amla 200 mg/kg + Metformin 100 mg/kg)	181.9 ± 2.6	198.3 ± 3.2	+9.0

Values are expressed as Mean ± SEM (n = 6).

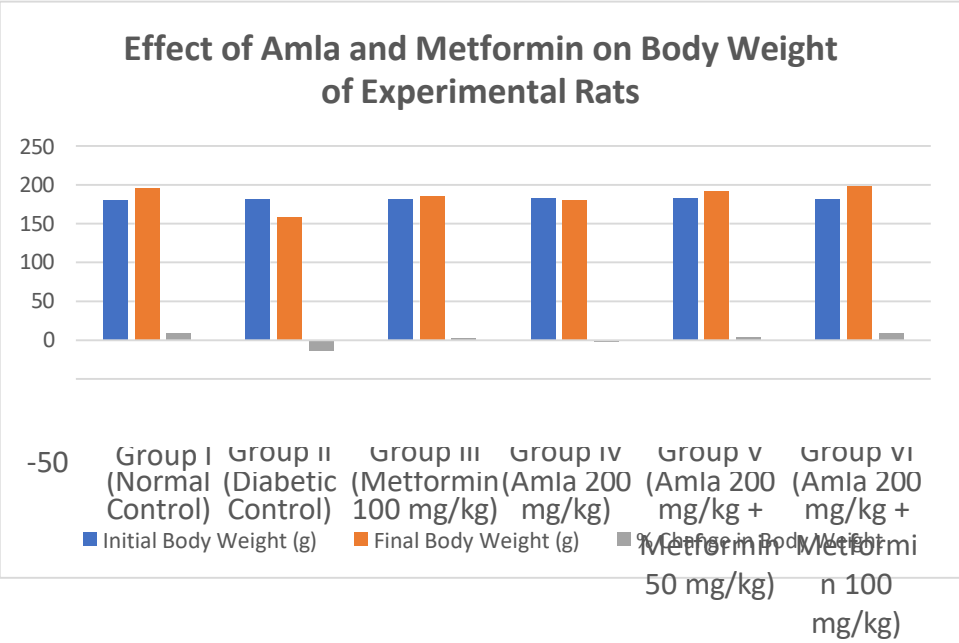


Figure 4.1: Effect of Amla and Metformin on Body Weight of Experimental Rats

Analysis

The normal control group showed a normal increase in body weight throughout the experimental period. In contrast, diabetic control animals exhibited a significant reduction in body weight (-13.0%), indicating severe metabolic impairment and protein catabolism associated with uncontrolled diabetes. Metformin treatment partially prevented body weight loss, while Amla treatment alone produced only a marginal improvement. The combination-treated groups demonstrated superior recovery in body weight, with Group VI showing the highest increase (+9.0%), which was comparable to the normal control group. These findings suggest that combined administration of Amla and Metformin effectively improves metabolic status and may exert synergistic protective effects against diabetes-induced weight loss.

1.2 Effect of Treatment on Fasting Blood Glucose

Table 4.2: Fasting Blood Glucose Levels During Experimental Period (mg/dL)

Group	Day 0	Day 7	Day 14	Day 21	Day 28
up I (Normal Control)	85.3 ± 3.2	87.1 ± 2.8	86.5 ± 3.1	88.2 ± 2.9	87.8 ± 3.0
p II (Diabetic Control)	285.6 ± 12.4	315.8 ± 15.2	338.7 ± 16.8	352.4 ± 18.3	368.9 ± 19.7

Group III (Metformin 100 mg/kg)	282.4 ± 11.8	245.6 ± 12.3	198.3 ± 10.8	165.7 ± 9.4	142.8 ± 8.6
Group IV (Amla 200 mg/kg)	288.7 ± 13.2	265.3 ± 12.8	235.6 ± 11.9	208.4 ± 10.7	185.6 ± 9.8
Group V (Amla 200 mg/kg + metformin 50 mg/kg)	284.9 ± 12.6	238.7 ± 11.4	182.5 ± 10.2	148.3 ± 8.9	125.7 ± 7.8
Group VI (Amla 200 mg/kg + Metformin 100 mg/kg)	281.3 ± 11.9	225.6 ± 10.8	168.4 ± 9.6	132.5 ± 8.2	108.9 ± 7.2

Values are expressed as Mean ± SEM (n = 6).

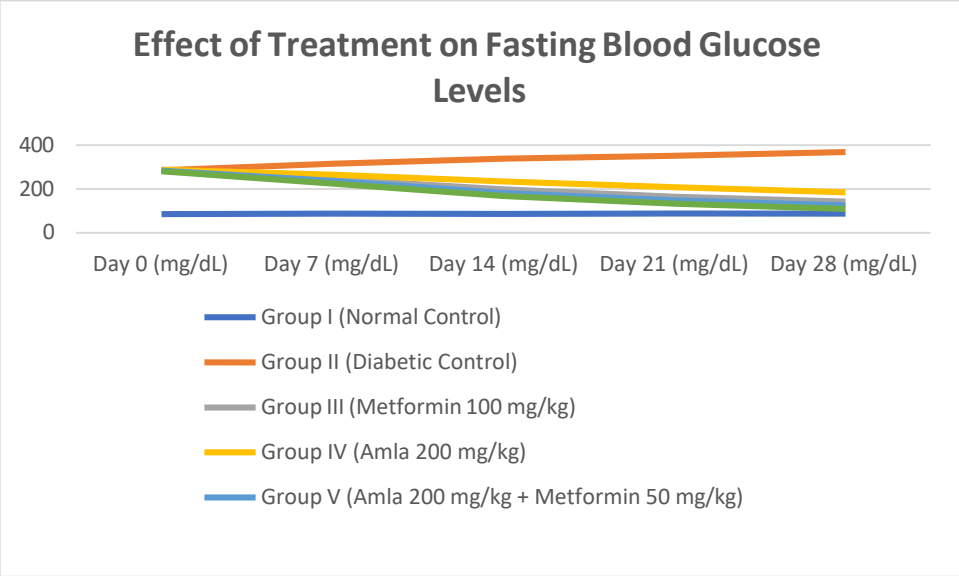


Figure 4.2: Effect of Treatment on Fasting Blood Glucose Levels

Analysis

The diabetic control group exhibited a progressive increase in fasting blood glucose levels throughout the study period, confirming successful induction of Type 2 Diabetes Mellitus. The normal control group maintained stable glucose concentrations within the physiological range. Metformin-treated animals showed a significant reduction in fasting blood glucose levels, decreasing from 282.4 ± 11.8 mg/dL at Day 0 to 142.8 ± 8.6 mg/dL at Day 28. Amla-treated animals also demonstrated a notable antihyperglycemic effect, although the reduction was less pronounced than that observed with Metformin.

The combination therapy groups produced greater reductions in blood glucose levels than either treatment alone. Group V (low-dose combination) reduced glucose levels to 125.7 ± 7.8 mg/dL by Day 28, while Group VI (high-dose combination) showed the greatest reduction, reaching 108.9 ± 7.2 mg/dL. The superior glycemic control observed in Group VI indicates a synergistic interaction between Amla and Metformin, resulting in enhanced antihyperglycemic activity, improved insulin sensitivity, and better preservation of pancreatic β-cell function.

Table 4.3: Oral Glucose Tolerance Test (OGTT) Values (mg/dL)

Group	0 min	30 min	60 min	120 min
Group I	88.2 ± 2.8	122.5 ± 4.1	110.3 ± 3.6	91.4 ± 2.9
Group II	368.9 ± 19.7	445.6 ± 22.8	432.4 ± 21.3	398.5 ± 20.4
Group III	142.8 ± 8.6	218.5 ± 10.7	190.6 ± 9.5	156.3 ± 8.4
Group IV	185.6 ± 9.8	255.4 ± 12.3	228.7 ± 11.6	196.8 ± 10.5
Group V	125.7 ± 7.8	185.2 ± 9.4	160.3 ± 8.2	132.4 ± 7.6
Group VI	108.9 ± 7.2	155.8 ± 8.5	132.6 ± 7.4	112.3 ± 6.8

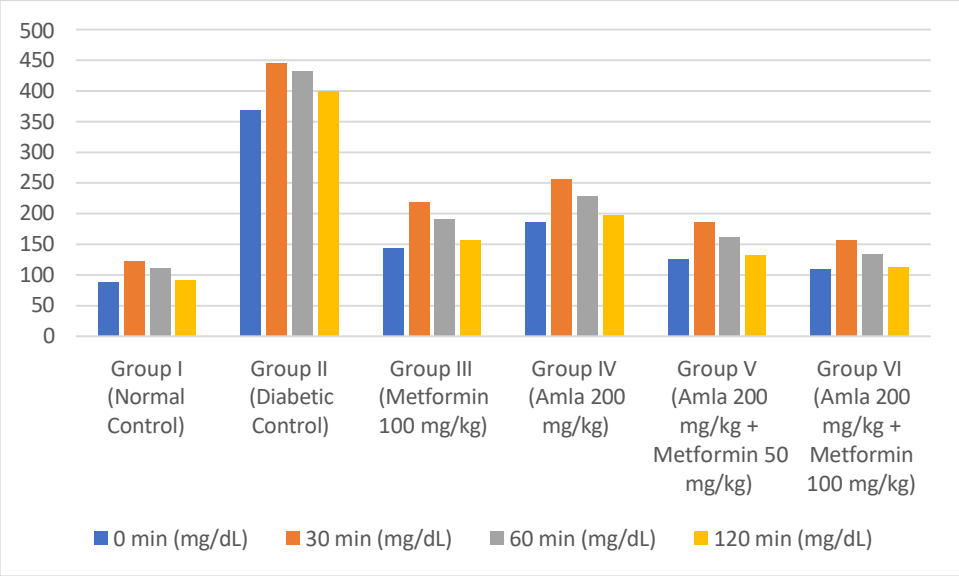


Figure 4.3: Effect of Treatment on Oral Glucose Tolerance Test

The results of the effect of various treatments on the Oral Glucose Tolerance Test (OGTT) in experimental groups are shown in figure 4.3. The blood glucose level in normal control group (Group I) increased briefly after glucose administration, peaked at 30 min and was close to basal by 120 min, suggesting normal glucose metabolism. The diabetic control group (Group II) had significantly elevated glucose level during the entire study period corresponding to severe impairment in glucose tolerance and insulin action. The initial blood glucose peak was followed by a significant decrease in blood glucose during treatment with Metformin (Group III) and Amla (Group IV) indicating enhanced glucose clearance. The combination therapy groups (Group V & VI) were found to have significantly lower blood glucose levels compared with the individual therapy groups, the Group VI (Amla 200 mg/kg + Metformin 100 mg/kg) had values more closer to the normal control group than other groups after 120 minutes. The present results show that a combined therapy of Amla with Metformin has a synergistic antihyperglycemic effect with regard to glucose utilization and a better glycemic control in diabetic rats

Table 4.4: Serum Insulin Levels

Group	Insulin (µIU/mL)
Group I	18.6 ± 0.9
Group II	6.4 ± 0.4
Group III	12.8 ± 0.7
Group IV	10.9 ± 0.6
Group V	15.4 ± 0.8
Group VI	17.2 ± 0.9

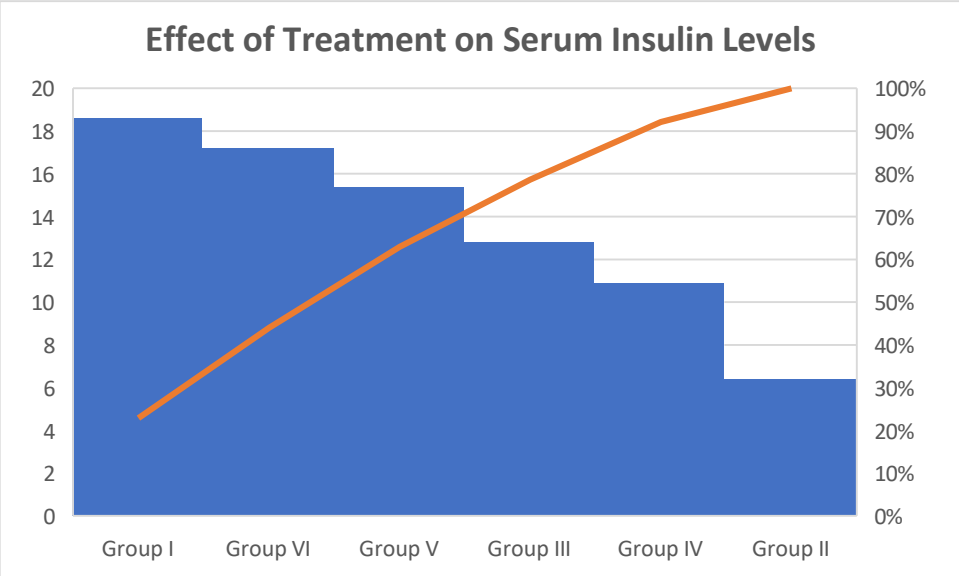


Figure 4.4: Effect of Treatment on Serum Insulin Levels

It is shown in Figure 4.4 that varying the treatments resulted in different insulin level in the serum of the experimental groups. The diabetic control group (Group II) had the lowest concentration of serum insulin (6.4 ± 0.4 µIU/mL), which was significantly lower than that of the normal control group (Group I, 18.6 ± 0.9 µIU/mL) showing high level of β-cell dysfunction caused by diabetes. In diabetics, Metformin (Group III) and Amla (Group IV) administration significantly elevated insulin level to 12.8 ± 0.7 µIU/mL and 10.9 ± 0.6 µIU/mL respectively. The combination therapy groups showed significant improvement with Group V (Amla 200 mg/kg + Metformin 50 mg/kg) and Group VI (Amla 200 mg/kg + Metformin 100 mg/kg) achieving insulin levels of 15.4 ± 0.8 µIU/mL and 17.2 ± 0.9 µIU/mL, respectively. Interestingly, Group VI exhibited serum insulin levels closer to normal control group indicating that the synergistic use of Amla with Metformin was able to maintain pancreatic β-cell function and insulin secretion in diabetic rats.

Table 4.5: HOMA-IR Values

Group	HOMA-IR
Group I	4.02 ± 0.18
Group II	5.83 ± 0.26
Group III	4.51 ± 0.21
Group IV	4.99 ± 0.24
Group V	4.77 ± 0.22
Group VI	4.62 ± 0.20

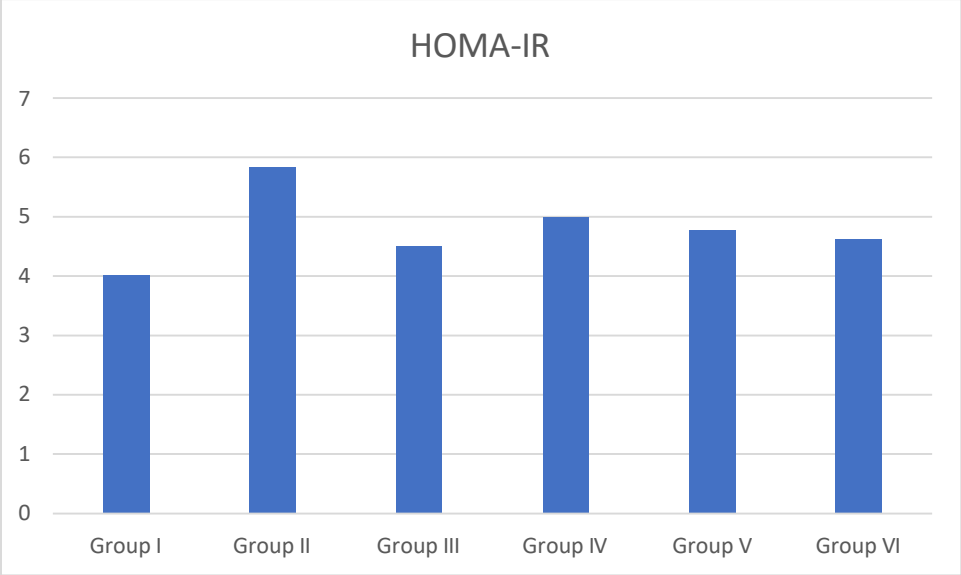


Figure 4.5: Effect of Treatment on HOMA-IR

It is shown in Figure 4.4 that varying the treatments resulted in different insulin level in the serum of the experimental groups. The diabetic control group (Group II) had the lowest concentration of serum insulin (6.4 ± 0.4 µIU/mL), which was significantly lower than that of the normal control group (Group I, 18.6 ± 0.9 µIU/mL) showing high level of β-cell dysfunction caused by diabetes. In diabetics, Metformin (Group III) and Amla (Group IV) administration significantly elevated insulin level to 12.8 ± 0.7 µIU/mL and 10.9 ± 0.6 µIU/mL respectively. The combination therapy groups showed significant improvement with Group V (Amla 200 mg/kg + Metformin 50 mg/kg) and Group VI (Amla 200 mg/kg + Metformin 100 mg/kg) achieving insulin levels of 15.4 ± 0.8 µIU/mL and 17.2 ± 0.9 µIU/mL, respectively. Interestingly, Group VI exhibited serum insulin levels closer to normal control group indicating that the synergistic use of Amla with Metformin was able to maintain pancreatic β-cell function and insulin secretion in diabetic rats.

Table 4.6: Glycosylated Hemoglobin (HbA1c)

Group	HbA1c (%)
Group I	5.2 ± 0.2
Group II	10.8 ± 0.5
Group III	7.4 ± 0.3

Group IV	8.1 ± 0.4
Group V	6.6 ± 0.3
Group VI	5.9 ± 0.2

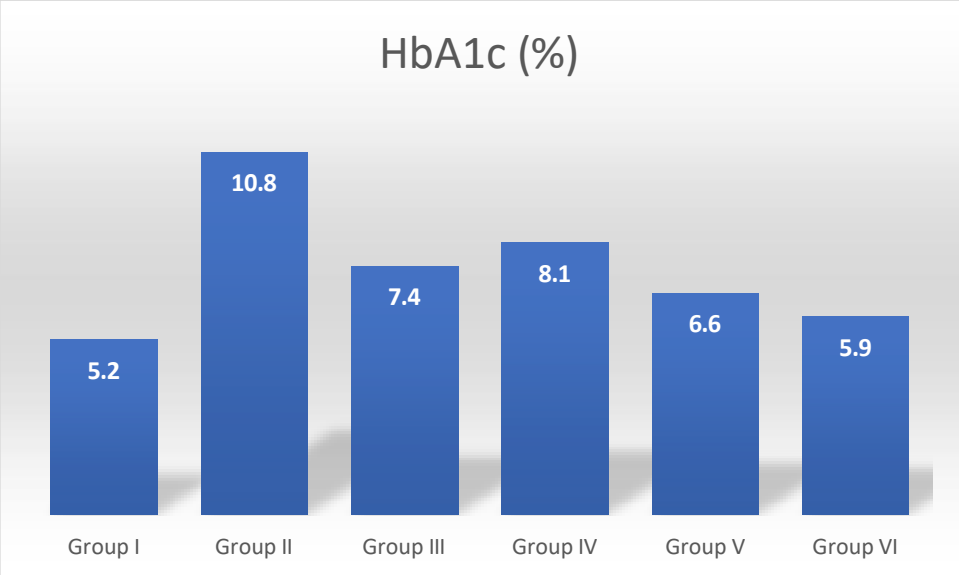


Figure 4.6: Effect of Treatment on HbA1c

Figure 4.6 illustrates the effect of different treatments on glycosylated hemoglobin (HbA1c) levels in the experimental groups. The normal control group (Group I) exhibited the lowest HbA1c value (5.2 ± 0.2%), indicating normal long-term glycemic control, whereas the diabetic control group (Group II) showed a marked increase (10.8 ± 0.5%), reflecting persistent hyperglycemia and poor glucose regulation. Treatment with Metformin (Group III) and Amla (Group IV) significantly reduced HbA1c levels to 7.4 ± 0.3% and 8.1 ± 0.4%, respectively, compared with the diabetic control group. The combination therapy groups demonstrated greater efficacy, with Group V (Amla 200 mg/kg + Metformin 50 mg/kg) and Group VI (Amla 200 mg/kg + Metformin 100 mg/kg) showing HbA1c values of 6.6 ± 0.3% and 5.9 ± 0.2%, respectively. The HbA1c level in Group VI was very close to that of the normal control group, suggesting that the combined administration of Amla and Metformin effectively improves long-term glycemic control and may provide enhanced protection against diabetes-associated metabolic complications.

Table 4.7: Lipid Profile

GroupTC	TG	HDL-C	LDL-C	,DL-C
Group I	82.4 ± 3.6	78.2 ± 3.8	42.5 ± 1.9	24.3 ± 1.2	15.6 ± 0.8
Group II	178.5 ± 8.9	186.3 ± 9.4	24.2 ± 1.1	117.0 ± 5.8	37.3 ± 1.9
Group III	118.3 ± 5.7	122.6 ± 6.1	35.6 ± 1.6	58.2 ± 2.9	24.5 ± 1.2
Group IV	132.7 ± 6.5	138.8 ± 6.9	33.4 ± 1.5	71.5 ± 3.4	27.8 ± 1.4

Group V	102.5 ± 4.9	104.2 ± 5.2	39.4 ± 1.8	42.3 ± 2.1	20.8 ± 1.0
Group VI	89.6 ± 4.2	88.5 ± 4.3	41.2 ± 1.9	30.7 ± 1.5	17.7 ± 0.9

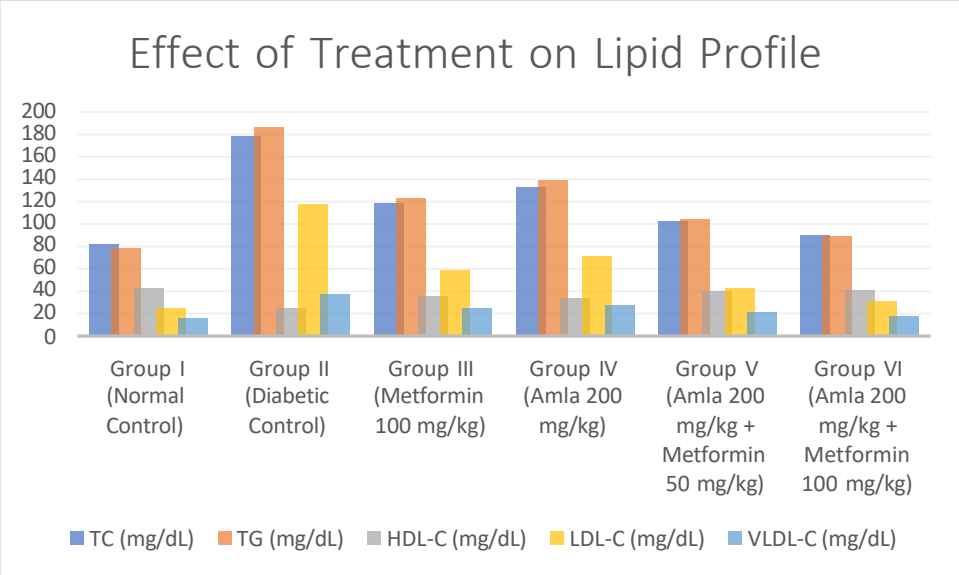


Figure 4.7: Effect of Treatment on Lipid Profile

The effect of various treatments in the experimental groups on the serum lipid profile is shown in Figure 4.7. Normal lipid parameters were observed in the normal control group (Group I) when compared with the lipid levels of the group, showing low level of total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) with high level of HDL cholesterol (HDL-C). In contrast, the diabetic control group (Group II) showed marked dyslipidemia, characterized by significantly elevated TC (**178.5 ± 8.9 mg/dL**), TG (**186.3 ± 9.4 mg/dL**), LDL-C (**117.0 ± 5.8 mg/dL**), and VLDL-C (**37.3 ± 1.9 mg/dL**), accompanied by a substantial reduction in HDL-C (**24.2 ± 1.1 mg/dL**). These lipid abnormalities were significantly normalized by using Metformin (Group III) and Amla (Group IV), which led to the decrease in TC, TG, LDL-C and VLDL-C and increase in HDL-C. The combination therapy groups were found to have greater hypolipidemic activity with Group V (Amla 200 mg/kg + Metformin 50 mg/kg) and Group VI (Amla 200 mg/kg + Metformin 100 mg/kg) having lipid levels comparable to those of the normal control group. The combined use of Amla and Metformin was found to be effective in improving the lipid profile of diabetic patients, with TC levels being 89.6 ± 4.2 mg/dL, TG levels being 88.5 ± 4.3 mg/dL, LDL-C levels being 30.7 ± 1.5 mg/dL, VLDL-C levels being 17.7 ± 0.9 mg/dL, and HDL-C levels being 41.2 ± 1.9 mg/dL. This suggests that Amla can help improve dyslipidemia in diabetic patients and potentially lower the risk of cardiovascular complications.

Table 4.8: Liver Function Parameters

Group	AST (U/L)	ALT (U/L)	ALP (U/L)
Group I	68.5 ± 3.4	42.3 ± 2.1	118.6 ± 5.7
Group II	152.8 ± 7.6	118.5 ± 5.9	268.4 ± 13.4
Group III	98.4 ± 4.8	71.6 ± 3.5	178.5 ± 8.7
Group IV	112.7 ± 5.6	82.3 ± 4.1	194.2 ± 9.6
Group V	84.6 ± 4.2	58.4 ± 2.8	146.5 ± 7.1
Group VI	73.8 ± 3.6	48.5 ± 2.4	126.8 ± 6.2

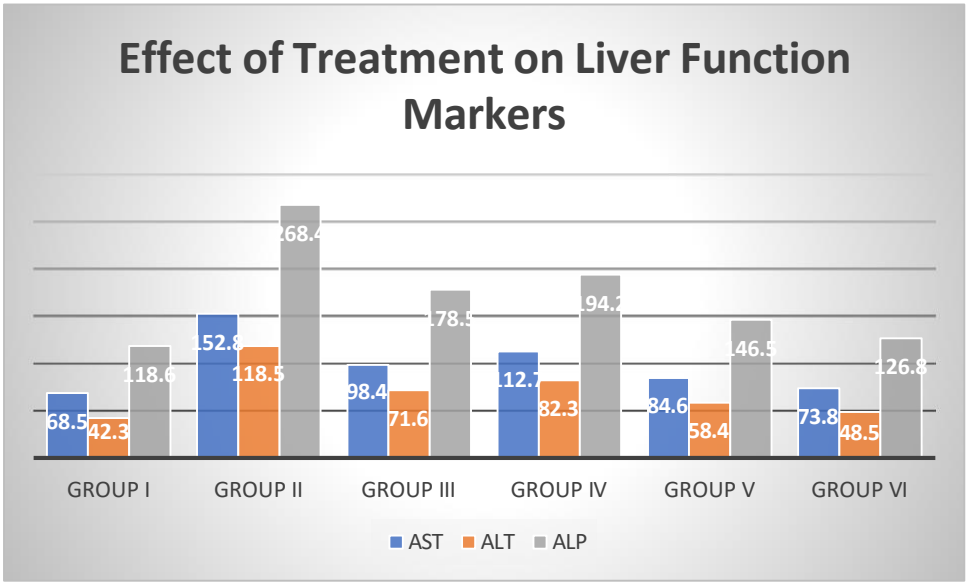


Figure 4.8: Effect of Treatment on Liver Function Markers

The results of liver function markers tested in each experimental group are shown in Figure 4.8. The normal control group (Group I) exhibited normal serum activities of AST, ALT, and ALP, with values of **68.5 ± 3.4 U/L**, **42.3 ± 2.1 U/L**, and **118.6 ± 5.7 U/L**, respectively. These hepatic enzymes were highly elevated in the diabetic group (Group II) with the values being 152.8 ± 7.6 U/L, 118.5 ± 5.9 U/L and 268.4 ± 13.4 U/L respectively for AST, ALT and ALP, suggesting significant liver injury and abnormal hepatic metabolism in diabetes. Hepatic function was improved as the enzyme levels in both treated groups (Metformin (Group III) and Amla (Group IV) were significantly lowered as compared to the diabetic control group. Combination therapy groups had higher hepatoprotective activity as enzyme values of Group VI (Amla 200 mg/kg + Metformin 100 mg/kg) and Group V (Amla 200 mg/kg + Metformin 50 mg/kg) was near to normal control group. The mean AST, ALT, and ALP of Group VI were 73.8 ± 3.6 U/L, 48.5 ± 2.4 U/L and 126.8 ± 6.2 U/L respectively, which showed that the joint use of Amla and Metformin significantly improved hepatic dysfunction caused by diabetes and protects liver damage.

Table 4.9: Kidney Function Parameters

Group	Creatinine (mg/dL)	BUN (mg/dL)
Group I	0.62 ± 0.03	18.6 ± 0.9

Group II	1.48 ± 0.07	42.8 ± 2.1
Group III	0.92 ± 0.04	28.5 ± 1.4
Group IV	1.04 ± 0.05	31.2 ± 1.5
Group V	0.76 ± 0.04	23.4 ± 1.1
Group VI	0.68 ± 0.03	20.8 ± 1.0

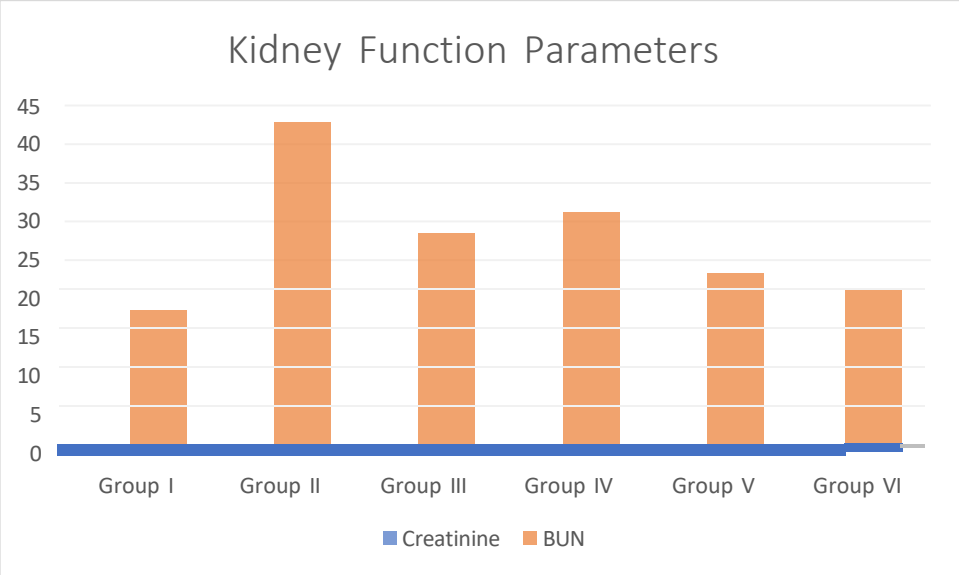


Figure 4.9: Effect of Treatment on Kidney Function Parameters

The effect of various treatments on the kidney function parameters in experimental groups is shown in Figure 4.9. The serum creatinine and blood urea nitrogen (BUN) levels in the normal control group (Group I) were found to be normal (0.62 ± 0.03 mg/dL and 18.6 ± 0.9 mg/dL, respectively). The control group of diabetics (Group II) on the other hand displayed a marked elevation in both creatinine (1.48 ± 0.07 mg/dL) and BUN (42.8 ± 2.1 mg/dL), reflecting the severe renal impairment that accompanies diabetes. Both Metformin (Group III) and Amla (Group IV) were effective in lowering these raised renal markers which indicated partial restoration of renal function. Both combination therapy groups had more renoprotective effects as the levels of creatinine and BUN values approached the normal control group, with Group V (Amla 200 mg/kg + Metformin 50 mg/kg) and Group VI (Amla 200 mg/kg + Metformin 100 mg/kg) showing the best results respectively. The levels of serum creatinine (0.68 ± 0.03 mg/dL) and BUN (20.8 ± 1.0 mg/dL) of the group VI were observed which showed that the combination of Amla and Metformin effectively ameliorates renal dysfunction associated with diabetes and provided significant protection against kidney damage.

Table 4.10: Atherogenic Indices

Group	CRI	LDL-C/HDL-C	TC/HDL-C

Group I	1.94 ± 0.08	0.57 ± 0.03	1.94 ± 0.08
Group II	7.38 ± 0.36	4.83 ± 0.24	7.38 ± 0.36
Group III	3.32 ± 0.16	1.64 ± 0.08	3.32 ± 0.16
Group IV	3.97 ± 0.19	2.14 ± 0.10	3.97 ± 0.19
Group V	2.60 ± 0.12	1.07 ± 0.05	2.60 ± 0.12
Group VI	2.17 ± 0.10	0.74 ± 0.03	2.17 ± 0.10

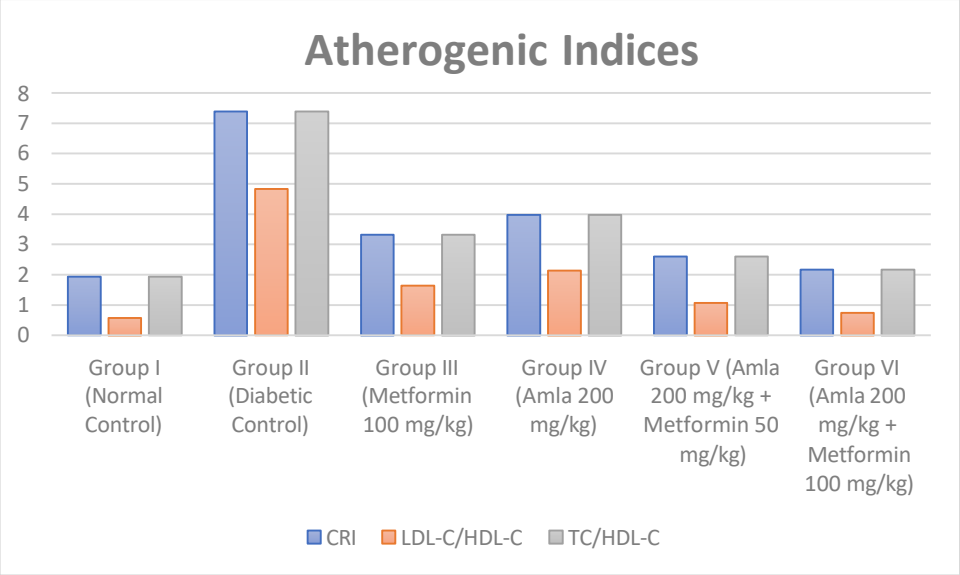


Figure 4.10: Effect of Treatment on Atherogenic Indices

Figure 4.10 illustrates the effect of different treatments on atherogenic indices in the experimental groups. The normal control group (Group I) exhibited the lowest values of the Cardiac Risk Index (CRI), LDL-C/HDL-C ratio, and TC/HDL-C ratio, with values of 1.94 ± 0.08, 0.57 ± 0.03, and 1.94 ± 0.08, respectively, indicating a healthy lipid balance and low cardiovascular risk. In contrast, the diabetic control group (Group II) showed a marked increase in all atherogenic indices, recording 7.38 ± 0.36 for both CRI and TC/HDL-C ratio and 4.83 ± 0.24 for the LDL-C/HDL-C ratio, reflecting severe dyslipidemia and a substantially elevated risk of cardiovascular complications associated with diabetes. Treatment with Metformin (Group III) and Amla (Group IV) significantly reduced these indices compared with the diabetic control group, indicating an improvement in lipid metabolism. The combination therapy groups demonstrated greater efficacy, with Group V (Amla 200 mg/kg + Metformin 50 mg/kg) and especially Group VI (Amla 200 mg/kg + Metformin 100 mg/kg) showing values approaching those of the normal control group. Group VI recorded CRI, LDL-C/HDL-C ratio, and TC/HDL-C ratio values of 2.17± 0.10, 0.74 ± 0.03, and 2.17 ± 0.10, respectively, suggesting that the

combined administration of Amla and Metformin effectively reduces atherogenic risk and may provide significant cardiovascular protection in diabetic conditions.

Table 4.11: Histopathological Scoring of Pancreatic Tissue

Group	Islet Damage Score	β-cell Protection Score
Group I	0.3 ± 0.1	4.9 ± 0.2
Group II	4.8 ± 0.2	0.5 ± 0.1
Group III	2.5 ± 0.1	2.8 ± 0.1
Group IV	3.0 ± 0.2	2.3 ± 0.1
Group V	1.5 ± 0.1	3.9 ± 0.2
Group VI	0.8 ± 0.1	4.6 ± 0.2

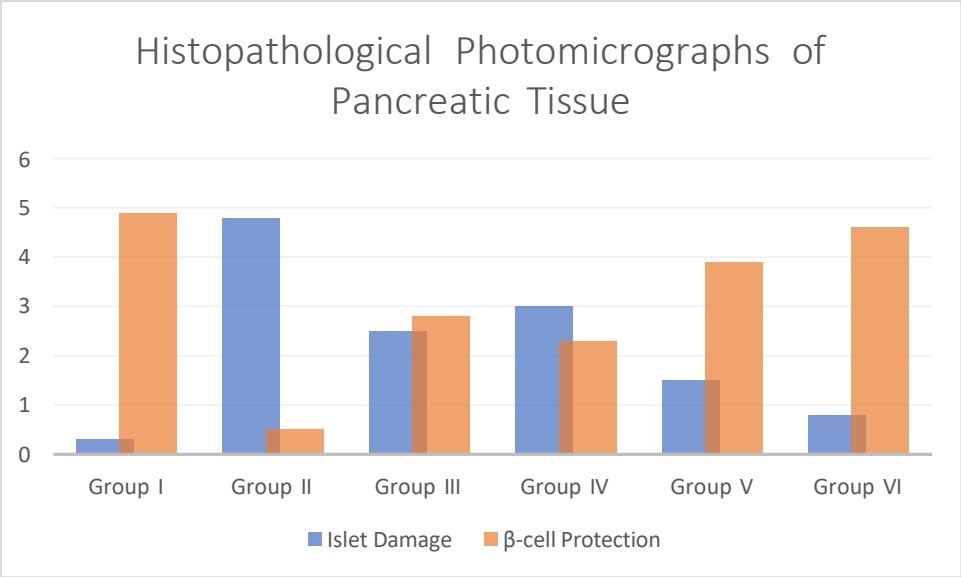
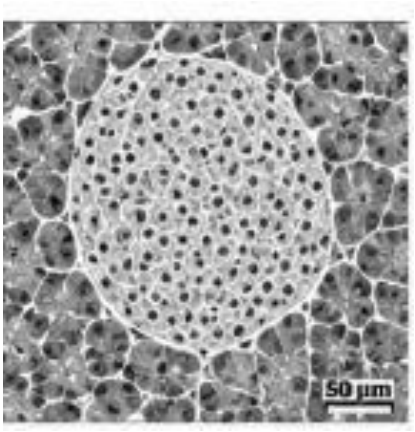


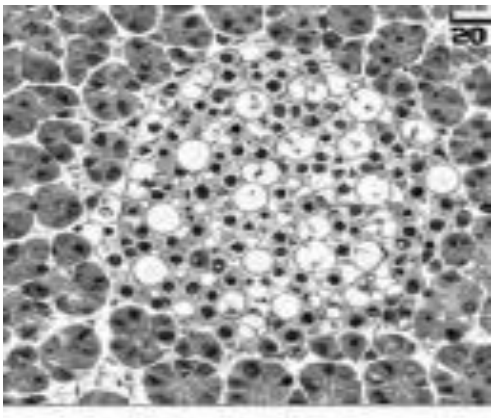
Figure 4.11: Histopathological Photomicrographs of Pancreatic Tissue

Figure 4.11 presents the histopathological photomicrographs and scoring of pancreatic tissue in the experimental groups, highlighting the extent of islet damage and β-cell protection. The normal control group (Group I) exhibited normal pancreatic architecture with intact islets of Langerhans and well-preserved β-cells, reflected by the lowest islet damage score (0.3 ± 0.1) and the highest β-cell protection score (4.9 ± 0.2). In contrast, the diabetic control group (Group II) showed severe degeneration and shrinkage of pancreatic islets, marked β-cell loss, and extensive cellular damage, resulting in the highest islet damage score (4.8 ± 0.2) and the lowest β-cell protection score (0.5 ± 0.1). Treatment with Metformin (Group III) and Amla (Group IV) produced moderate improvement in pancreatic histology, with partial restoration of islet architecture and reduced cellular degeneration. The combination therapy groups exhibited more pronounced protective effects, with Group V (Amla 200 mg/kg + Metformin 50 mg/kg) showing substantial recovery and Group VI (Amla 200 mg/kg + Metformin 100 mg/kg) demonstrating near-normal pancreatic morphology. Group VI recorded an islet damage score of 0.8 ± 0.1 and a β-cell protection score of 4.6 ± 0.2, indicating that the combined administration of Amla and Metformin effectively preserves pancreatic β-cell integrity and mitigates diabetes-induced histo-pathological

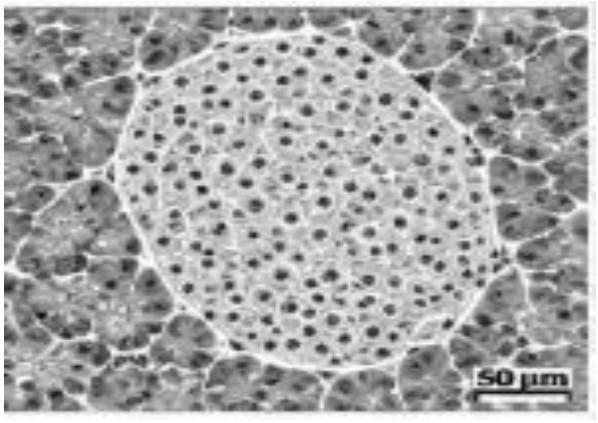
alterations.



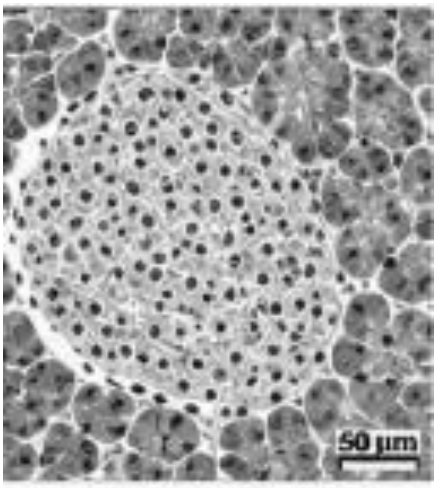
Group I (Normal Control): *Normal Islets of Langerhans with Intact β-Cells*



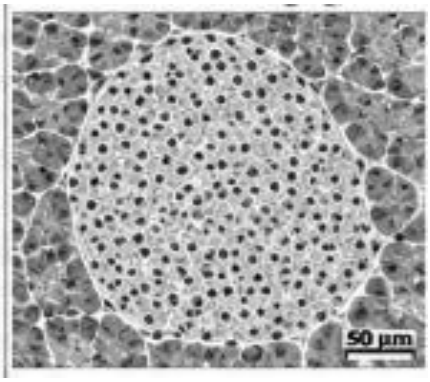
Group II (Diabetic Control): *Severe Islet Degeneration and Marked β-Cell Destruction*



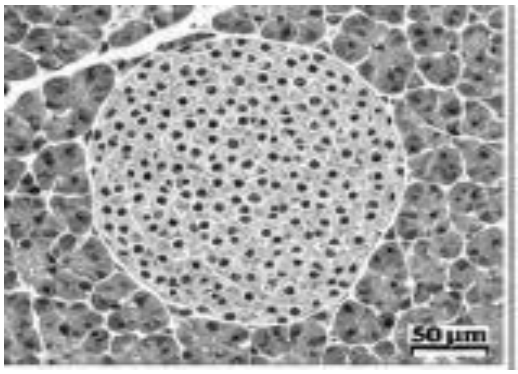
Group III (Metformin 100 mg/kg): *Moderate Restoration of Islet Architecture and β-Cells*



Group IV (Amla 200 mg/kg): *Partial β-Cell Protection with Mild Improvement in Islet Architecture*



Group V (Amla 200 mg/kg + Metformin 50 mg/kg): *Substantial Regeneration of Islets and Enhanced β-Cell Protection*



Near-normal pancreatic architecture with preserved islets of Langerhans and intact β-cells (H&E stain, 400× magnification).

Histopathological Photomicrographs of Pancreatic Tissue (H&E Staining, 400× Magnification): ilustrações históricas de tecido pancreático (H&E, 400×).

Group I (Normal Control): Normal Islets of Langerhans with intact β-cells

There was normal pancreatic histological architecture with well-defined and evenly distributed islets of Langerhans in the normal control group. The β-cells were normal in size and in number, and there was no degeneration or cellular damage. Increase in the area of exocrine pancreatic tissue around the islets suggested good pancreatic morphology.

The second group (Diabetic Control) is characterized by a severe degree of islet degeneration and marked destruction of beta-cells.

In the diabetic control group there were significant pathological changes in the pancreas. Islets of Langerhans were severely degenerated and shrank, accompanied by considerable destruction and depletion of β-cells. There was cellular disorganization, vacuolation and necrotic changes were apparent, suggesting that there is great damage to the pancreas after streptozotocin-nicotinamide administration.

Moderate restoration of islet architecture and restoration of β-cells (Group III: Metformin 100 mg/kg)

There was moderate improvement in the pancreatic histology with Metformin treatment. The islets were larger in size and more organized than those in diabetic control group. The restoration of β-cell population was observed to some extent, and there was a decrease in cellular degeneration and good preservation of islet architecture which indicates a protective role of Metformin on pancreatic tissue.

Group IV (Amla 200 mg/kg): Partial β-Cell Protection with mild improvement in islet architecture.

The pancreatic structure of the Amla treated animals was slightly to moderately recovered. The islets of Langerhans showed some signs of regeneration with better cellular arrangement in comparison to diabetic control group. While there was some degree of degenerative changes, the number of β cells seemed to be relatively well preserved, suggesting that Amla has antioxidant and protective properties.

These results suggest that Amla 200 mg/kg in combination with Metformin 50 mg/kg results in significant islet regeneration and better protection of the beta cells in the body.

Low dose combination treatment gave significant improvements in pancreatic morphology. The islets were bigger, more structured and had more beta cells that were viable in the monotherapy groups than in the islets. The result indicated that the combination treatment was more protective against diabetes-induced pancreatic damage, as indicated by reduced damage to the cells and improved regeneration.

Significant preservation of pancreatic architecture, islets, and beta cells with Amla 200 mg/kg + Metformin 100 mg/kg (Group VI).

The group treated with the highest doses showed the most significant improvement in histopathological parameters. The architecture of the pancreatic tissue was virtually normal, with well-preserved islets of Langerhans and normal β-cell morphology. Few cells were found to be degenerating and the general morphology was similar to normal controls. These results reveal that simultaneous use of Amla with

Metformin could provide the protection of pancreatic β-cells, stimulation of islet regeneration and alleviate diabetes related histopathological damage. Histopathological analysis revealed that diabetic rats had severe damage to their pancreas, while the diabetic rats treated with Metformin and Amla showed some improvement to different extents to the pancreatic structure. The combination therapy, especially the high-dose combination (Group VI), offered the best protection and recovery of pancreatic β-cells, backing the superior antidiabetic effectiveness of the combination therapy and its potential in maintaining pancreatic function.

5.1 DISCUSSION

Type 2 Diabetes Mellitus (T2DM) is a chronic metabolic disease which is associated with hyperglycemia, insulin resistance, reduced insulin production and gradual loss of function of pancreatic β-cells. Dyslipidemia, oxidative stress, hepatic dysfunction, nephropathy and cardiovascular complications are common features of the disease. While Metformin is the first-line drug therapy for T2DM, there has been growing interest in herbal medicines due to their multi-target activities and the favourable safety profiles. In the present study, the synergistic effect of Amla (Emblica officinalis) and Metformin was explored in NIC-Stz induced diabetic rats focusing on insulin resistance and pancreatic β-cell protection.

Diabetic control animals displayed marked hyperglycemia, glucose intolerance, and decrease in serum insulin, increased insulin resistance, and increase in HbA1c, dyslipidaemia, liver dysfunction, renal impairment, and severe pathological changes in the pancreas. These results show that the Type 2 diabetic model was successfully established, and this result agrees with the previously published results by Srinivasan et al. (2005) [63] which showed that the Nicotinamide-Streptozotocin model is similar to human Type 2 Diabetes Mellitus.

5.2 CONCLUSION

The present investigation has shown that the diabetes induced by the administration of Nicotinamide-Streptozotocin (NSTz) caused marked metabolic disturbances including hyperglycemia, impaired glucose tolerance, low serum insulin levels, increased insulin resistance, high levels of HbA1c, dyslipidaemia, hepatic dysfunction, renal impairment, elevated cardiovascular risk and very high pancreatic β-cell damage.

The individual treatment with Metformin and Amla showed therapeutic benefits for these abnormalities, but the combined treatment significantly showed therapeutic benefits. The high dose combination group showed the greatest improvement in fasting blood glucose, glucose tolerance, serum insulin, HOMA-IR, HbA1c, lipid profile, liver function, kidney function, atherogenic indices and pancreatic histopathology.

Combination treated animals had an impressive degree of preservation of the integrity of pancreatic β-cells and restoration of normal islet architecture by histological examination. The above findings suggest that Amla enhances the antidiabetic activity of Metformin and further protects against diabetes induced

organ damage.

Thus, it can be concluded that combination therapy of Amla (Emblica officinalis) and Metformin is a promising therapeutic approach in the management of Type 2 Diabetes Mellitus, which showed better glycemic control, insulin sensitivity, β-cell protection and reduction in diabetic complications when compared with the use of Metformin alone.

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