

In-vivo and Histopathological Evaluation of Antidiabetic activity of Combination Extract of *Garcinia indica* and *Lannea coromandelica* in STZ-NA induced in Rats

Agarwal Richa*, Arora Pankaj

Lord's University, Alwar, Rajasthan, India

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ABSTRACT

In order to manage type-II diabetes mellitus, the current study assesses the antidiabetic potential of a polyherbal extract made of *Lannea Coromandelica* (Indian Ash Plant) and *Garcinia Indica* (Kokum). Although earlier studies have demonstrated the effectiveness of these herbs alone in the treatment of diabetes, this study concentrated on their combined effects. Streptozotocin-induced diabetic Wistar rats were given different quantities of the polyherbal extract, and the results were compared to a group that received normal antidiabetic medication treatment. According to the results, the polyherbal combination considerably lowered blood glucose levels and lessened the negative symptoms of hyperglycemia. Additionally, rats treated with the polyherbal extract displayed a considerable improvement in body weight, suggesting its potential to ameliorate diabetes-related weight loss. The bioactive substances in these herbal extracts worked together to improve overall physiological function and improve glycemic management. In addition to highlighting the advantages of polyherbal formulations as an alternative to traditional treatment, this study reveals the synergistic therapeutic effects of *Lannea Coromandelica* and *Garcinia Indica* in the management of diabetes.

Keywords: *Antidiabetic Activity, Lannea Coromandelica, Garcinia Indica, Blood Glucose*

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INTRODUCTION

Nearly 6% of people worldwide suffer from diabetes mellitus, one of the most prevalent diseases, and its dynamics are fast shifting in low- and middle-income nations. The International Diabetes Federation (IDF) projects that by 2030, low- and middle-income nations will account for 80% of the world's diabetes population.¹ According to the IDF 2011 study, there are currently 90.0, 61.3, and 23.7 million diabetics in China, India, and the United States of America, respectively.² By 2030, that number might rise to 129.7, 101.2, and 29.3 million. One of the six leading causes of death worldwide, diabetes also results in several systemic problems. Alpha-glucosidase inhibitors, sulfonylureas, biguanides, and thiazolidinediones are examples of glucose-lowering medications used to treat diabetes mellitus, as is hormone treatment (insulin).³ Many research institutes and pharmaceutical corporations are interested in drug development to uncover molecules with strong therapeutic potential and fewer adverse events because the development of an adverse event is one of the problems in the treatment of any systemic condition. 3.47.0% of hospital admissions in the United States are caused by adverse drug reactions, which affect 102.5 percent of patients.⁴

Numerous plants are effective in treating a range of systemic illnesses in traditional medical systems.⁵ One of

the major issues facing the traditional medical system is its lack of full uniformity, although many traditional/Indigenous medical systems are more successful than the modern one.⁶ The ancient literature contains extensive documentation on the idea of polyherbal formulation. The polyherbal formulation offers more and longer-lasting medicinal potential than a single herb.⁷ To create and standardize a polyherbal formulation employing a plant with documented antidiabetic activity and assess its therapeutic benefits in rodents, the current study was designed.

MATERIAL AND METHODS

Herbal drug material Selection and collection

Lannea Coromandelica (Indian Ash Plant) and *Garcinia Indica* (Kokum fruit) were purchased from market. Plants were Identified and Authentication by Botanical Survey of India, Jodhpur, Rajasthan. Herbal products are used to remove the water/moisture content from it by a natural process, i.e., under sunlight.

Experimental Animals

Wistar rats weighing 200–250 g of either sex were acquired. They were kept in polypropylene cages on rodent pellets at a regulated temperature of 22±2°C and acclimated to a 12-hour light/dark cycle. Food and water were freely available until two hours before the trial. The animals were maintained and cared for by the "Committee

*Author for Correspondence: richaagarwal509@gmail.com

Table 1 Effect of PHF on Blood Glucose levels in STZ-nicotinamide induced diabetes

Group (n=5)	Treatment	Blood glucose (mg/dL)				
		Day 1	Day 7	Day 14	Day 21	Day 28
I	Normal control	73.67 ± 1.706	76.83 ± 1.92	80.17 ± 1.77	74.67 ± 3.373	87.33 ± 5.80
II	Diabetic control	243.7 ± 12.73	254.2 ± 15.13 ^{###}	279.5 ± 17.61 ^{###}	311.8 ± 24.17 ^{###}	319.2 ± 23.96 ^{###}
III	Glibenclamide (5 mg/kg)	297.7 ± 26.70	268.7 ± 16.52	223.2 ± 6.45 ^{**}	189.8 ± 15.54 ^{***}	132.7 ± 10.24 ^{***}
IV	PHF (200 mg/kg)	322.5 ± 33.77	314.2 ± 30.27	217.8 ± 10.51 ^{**}	208.3 ± 9.03 ^{***}	207.5 ± 8.21 ^{***}
VI	PHF (400 mg/kg)	288.8 ± 15.75	301.2 ± 21.99	200.3 ± 5.06 ^{***}	185.2 ± 11.87 ^{***}	119.2 ± 6.41 ^{***}

Values are expressed as Mean ± S.E.M (n=6). Where, ^{###} P < 0.001 as compared to normal control; ^{**} P < 0.01, ^{***} P < 0.001 as compared to Diabetic control.

Table 2: Effect of PHF on Body weight, Urine Volume and Urine Sugar in STZ-nicotinamide induced Diabetes

Group (n=6)	Treatment	Initial body weight (g)	Final body weight (g)	Urine Volume (ml/ 24 hr)	Urine glucose
I	Normal control	275.0 ± 10.25	266.7 ± 11.45	20.83 ± 1.24	Nil
II	Diabetic control	216.7 ± 10.54	173.3 ± 8.02 ^{###}	54.33 ± 2.81 ^{###}	+++
III	Glibenclamide (5 mg/kg)	213.3 ± 6.146	203.3 ± 7.149 [*]	25.00 ± 1.34 ^{***}	Nil
IV	PHF (200 mg/kg)	211.7 ± 8.333	185.0 ± 9.91	43.17 ± 1.27 ^{***}	+
VI	PHF (400mg/kg)	216.7 ± 8.433	209.2 ± 8.40 ^{**}	28.33 ± 1.02 ^{***}	Nil

Values are expressed as Mean + S.E.M (n=6). Where, ^{###} P < 0.001 as compared to normal control; ^{*} P < 0.05, ^{**} P < 0.01, ^{***} P < 0.001 as compared to Diabetic control

(+) - Trace elements of sugar and (+++) - more than 2% of sugar

for Control and Supervision of Experiments on Animals (CPCSEA)"s approved criteria. Two hours following the trial, food and water were made available. All animal tests were carried out in compliance with the project proposal no. The establishment's ethical committee on animal experimentation issued ref.no. BRML/IAEC/2024-41.

Induction of Diabetes

Diabetes mellitus condition was induced in overnight fasted Wistar rats by a single intraperitoneal injection of freshly made solutions of STZ which was dissolved in 0.1M ice-cold sodium citrate buffer (pH 4.5) was prepared 15 minutes before injection as compound STZ are unstable in solutions and administered at the dose of 40mg/kg body weight. After 72hours following induction, blood glucose level was measured with strips using glucometer. Rats with blood glucose level above 150mg/dL were considered as diabetes and included in the study.

Drug Treatment Different drug treatment was given to different groups of rats after the induction of diabetes. The treatment includes

Combination of extract in equal quantity (200 mg/kg and 400 mg/kg body wt.; orally)

Glibenclamide (0.5 mg/kg body wt.; peritoneal cavity)

Grouping of Animals

The animals were divided into 5 groups, 6 in each, total no. of animals =30

Group I: Normal control (vehicle only i.e., 0.9 % NaCl).

Group II: Diabetes control (STZ induced 60mg/kg; i.p).

Group III: Diabetes induced+ standard drug (Glibenclamide 5mg/kg; peritoneal cavity)

Group IV: Diabetes induced+ polyherbal extract (200mg/kg; p.o)

Group V: Diabetes induced + polyherbal extract (400mg/kg; p.o)

Preparation of doses The extracts dissolved or suspended in distilled water; their pH was brought to 7.0.

Administration of doses Herbal extracts are administered in a single by gavages using a stomach tube.

Blood samples were collected and at the end of the stipulated period of study, all the animals were sacrificed by anaesthetic inhalation. The pancreas and liver were collected of the rat for histopathological markers.

Evaluation Parameters

Blood Glucose

Except for the normal control and diabetes control groups, the treatment began on the same day and was administered orally for 28 days. Animals in groups had unrestricted access to water and a typical feed throughout this time. Estimates of blood glucose levels were made on days 0, 7, 14, 21 and 28 of the treatment. A glucometer strip and glucometer were used to estimate the glucose levels in the blood that was extracted from the tail vein.

Body Weight

The body weight of rats was recorded on 0 and 28th day of the treatment with the help of electronic balance. Statistical Analyses The data obtained from the different

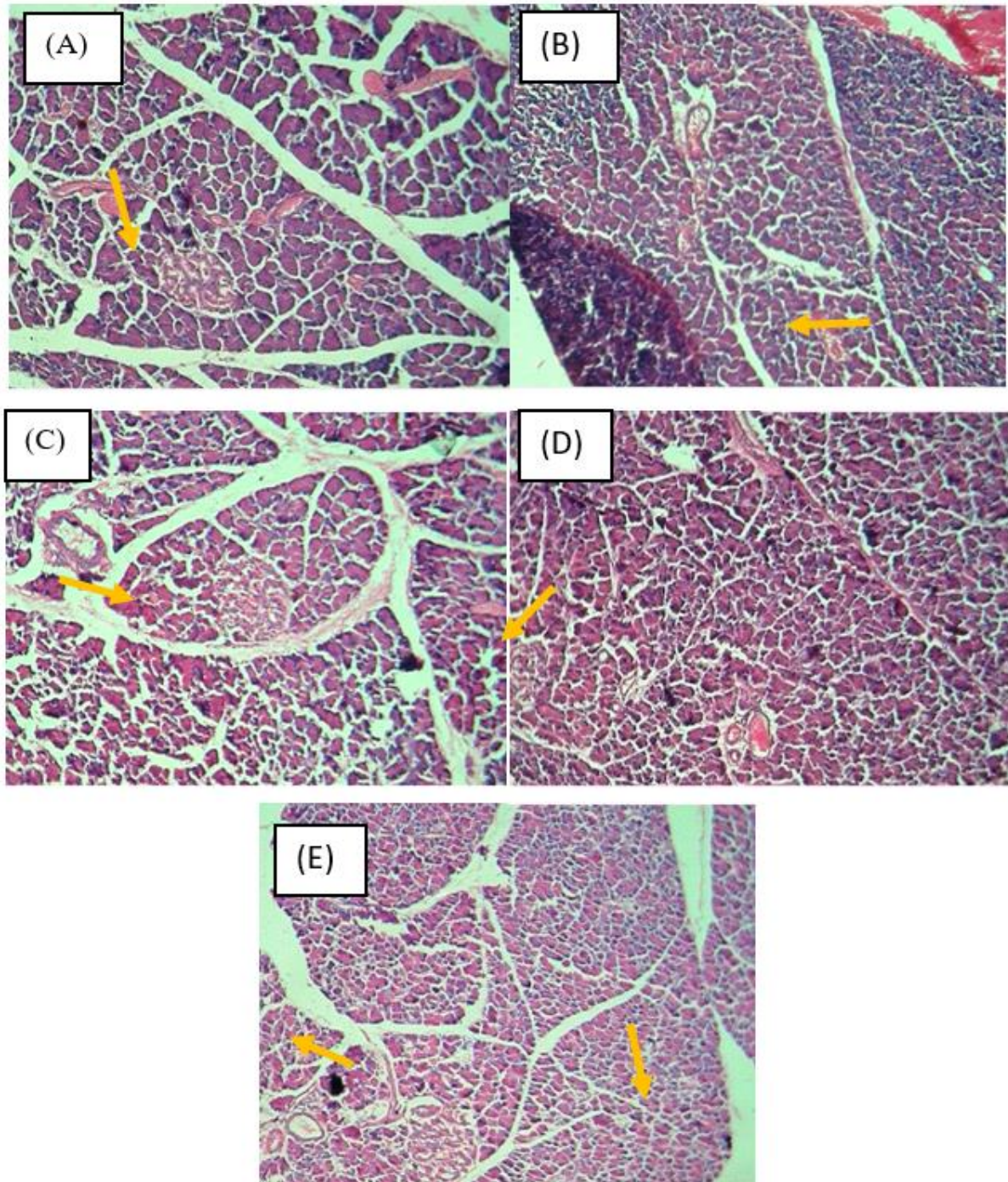


Fig.1: Photomicrograph of pancreas of rats. (A) normal control group; (B) diabetic control group; (C) diabetic + glibenclamide; (D) diabetic + PHF(200 mg/kg); (E) diabetic + PHF (400 mg/kg).

studies and the biochemical estimation is expressed as mean $\pm$ SEM for each group. After this, the statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Dennett's test using SPSS 16.0 window version. Values $p > 0.05$ were considered non-significant, p

RESULTS

The effect of Diabac on fasting blood glucose level of diabetic rats is shown in figure 4. Diabetic rats showed a

significant increase in the fasting blood glucose levels as compared to normal control rats. A significant dose dependent decrease in blood glucose level was observed in diabetic rats after treatment with PHF (200 mg/kg and 400 mg/kg) or glibenclamide as compared to diabetic control rats as shown in Table 1. After 28 Days, highest change in body weight was found in diabetic control group, where diabetic rats exhibited significantly ($P < 0.01$) lower body weight as compared to normal control rats. Treatment with PHF(200 & 400 mg/kg) or

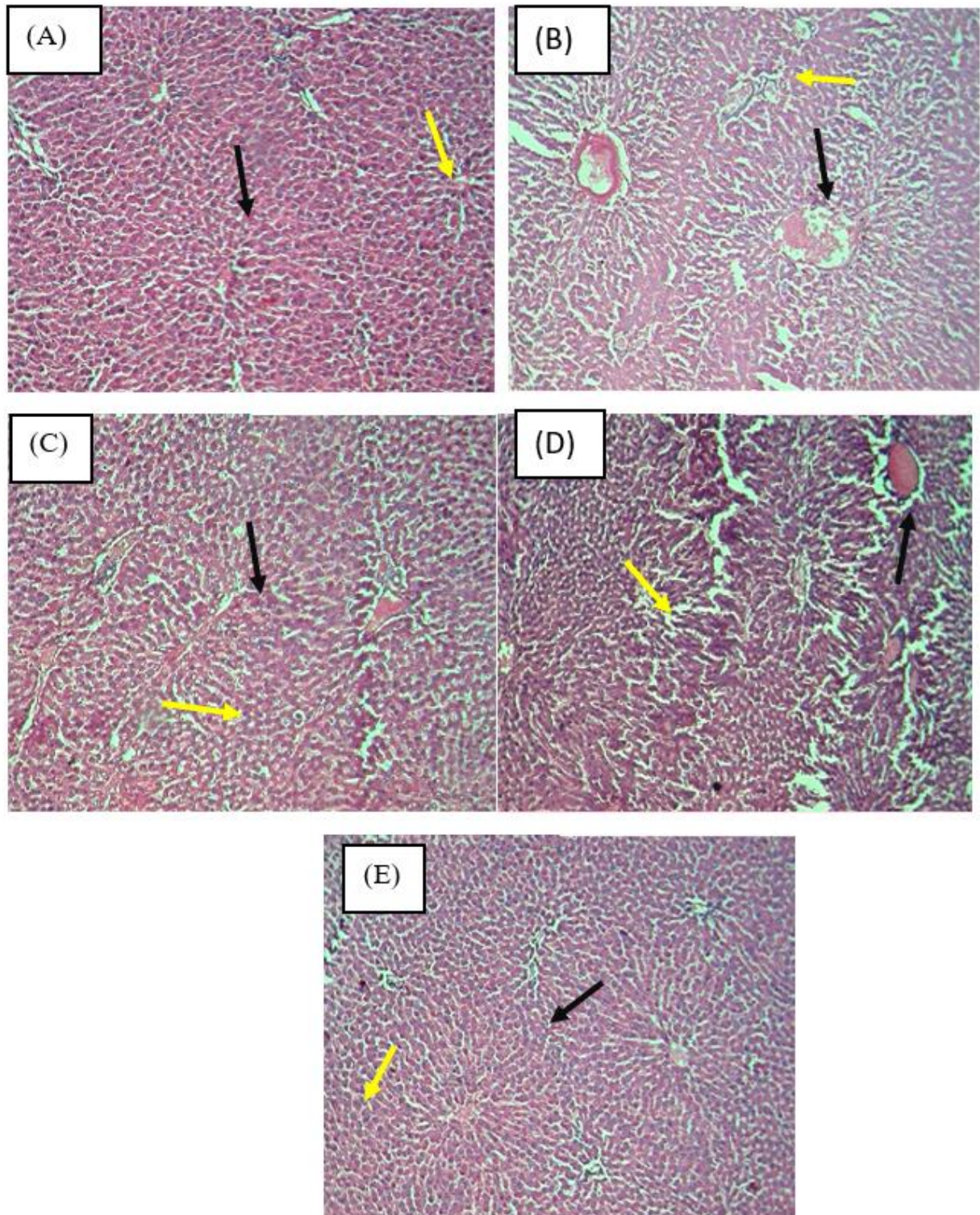


Fig.2: Photomicrograph of Liver of rats. (A) normal control group; (B) diabetic control group; (C) diabetic + glibenclamide; (D) diabetic + PHF(200 mg/kg); (E) diabetic + PHF (400 mg/kg)

glibenclamide (5 mg/kg) could elevate the reduced body weight. Histogram shows that there was a significant ($P < 0.001$) increase in urine volume of the diabetic control (DC) rats as compared to normal control (NC) rats. The treatment with PHF(200 & 400 mg/kg) or glibenclamide (5 mg/kg) showed a significant ($P < 0.001$) decrease urine

volume when compared to diabetic control rats as shown in Table 2.

Effect of PHF on Pancreas histology

Histology showed in Normal group showed a large islet structure surrounded by exocrine gland tissue. No inflammatory cells are seen in the islet. Diabetic Control

refers degeneration of beta cells. Diabetic + Glibenclamide No inflammatory cells are seen in the islet also it shows mild degenerated beta cells. Diabetic + PHF (200 mg/kg Few inflammatory cells are seen in the islet. Diabetic + PHF(400 mg/kg): No inflammatory cells are seen in the islet also seen were congested vascular spaces amidst these cells as shown in figure 1.

Effect of PHF on Liver histology

Normal Contro showed liver parenchyma with intact architecture. Diabetic Control showed liver parenchyma with distorted architecture. There were seen necrotic areas comprising of aggregates of neutrophils and cellular debris (black arrow). Diabetic + glibenclamide showed liver parenchyma with intact architecture. Diabetic +PHF (200 mg/kg) showed liver parenchyma with less intact architecture while Diabetic +PHF (400 mg/kg) showed liver parenchyma with intact. Most of the hepatocytes showed abundant cytoplasm with centrally placed vesicular nucleus and prominent nucleoli (yellow arrow). There were seen regenerative hepatocytes with almost normal liver architecture (black arrow) as shown in figure 2.

DISCUSSION

Hyperglycemia is the hallmark of diabetes, a chronic metabolic disease that is becoming more and more of a global health concern. Environments and genetics play a significant role in the pathophysiology of diabetes.⁸ Approximately 470 million persons worldwide had diabetes in 2022, and by 2045, that figure is expected to increase to 693 million. When diabetes develops, oxidative stress and inflammation are brought on by persistent hyperglycemia.⁹

Furthermore, because of the glucotoxicity effects, which hasten the mortality of diabetes, it is commonly linked to dysfunction and serious clinical consequences, including diabetic retinopathy, nephropathy, neuropathy, and peripheral artery disease. Therefore, reducing the production of reactive oxygen species (ROS) and managing elevated blood glucose levels are crucial for managing diabetes or reducing its consequences.¹⁰ To reduce the incidence of diabetes, several natural products that have been extracted from plant sources may be used as supplements or replacements since they have antioxidant action and fewer adverse effects. Consuming the aforementioned plant components may lower the incidence of chronic illnesses and prevent colon cancer, according to a prior study. The high concentration of monounsaturated fatty acids (MUFA) in these plants, particularly oleic fatty acids, may help reduce inflammation, oxidative stress, mitochondrial dysfunction, and cellular death in hepatocytes.¹¹

Due to its anti-inflammatory, antioxidant, and free radical scavenging properties, *Lannea Coromandelica* and *Garcinia Indica* have been shown in earlier research to improve immunity and decrease lipid peroxidation processes in rats with CCl₄-induced liver injury.¹² We hypothesized its positive impact on diabetes because of its exceptional antioxidant activity.¹³ Thus, the purpose of our

study was to examine the effects of this combination on STZ-induced diabetic rats.

Most mouse strains can be utilized for streptozotocin (STZ)-induced diabetic mellitus (DM), which provides a very quick and economical method that gives researchers studying DM access to a variety of genotypic and phenotypic possibilities that would not otherwise be available.¹⁴ Even though STZ is frequently used in small animal models, there is sometimes little and inconsistent information available about drug preparation, dosage, and administration, the severity and time to onset of DM, and any associated morbidity and death.¹⁵

The beta cells of the pancreatic islets that produce insulin are poisoned by the broad-spectrum antibiotic STZ.¹⁶ It has been used experimentally in a wide range of large and small animal species and is presently utilized clinically to treat metastatic islet cell cancer of the pancreas. Over the years, a great deal of research has been done on how STZ works to deplete β cells.¹⁷ Although streptozotocin's cytotoxicity may be attributed to its functions as a protein alkylating agent and nitric oxide donor, it is generally believed that STZ is absorbed by the cell membrane GLUT2 glucose transporter, causing DNA alkylation and ultimately β cell death. Since STZ enters the cell through GLUT2, its harmful effects are not limited to β cells and can harm other tissues, such as the liver and kidney.¹⁸ In this study, we used Streptozotocin for our experiments in the induction of experimental diabetes mellitus. For induction of experimental diabetes, 60mg/kg of Streptozotocin was injected intravenously STZ in a buffer solution with a pH of 4.0.

Type 2 diabetes (T2D) is a long-term condition characterized by hyperglycemia, or elevated fasting blood glucose (FBG) levels. It is a chronic disorder of the metabolism of lipids and carbohydrates. Ninety percent of people with diabetes globally have type 2 diabetes. T2D has a very high rate of complications compared to other disorders, and it may even be a contributing factor to depression and Alzheimer's disease.¹⁹ It is widely acknowledged that the primary pathophysiology of type 2 diabetes and its consequences is caused by insulin resistance and elevated oxidative stress. The hallmarks of type 2 diabetes include a steady decline in insulin function and malfunctioning of the β -cells, which tries to make up for insulin resistance.²⁰ Reduced antioxidant function and elevated free radical levels are frequently linked to type 2 diabetes. Medicinal plants with minimal side effects have been used for hundreds of years as part of traditional treatments for people with type 2 diabetes.²¹

Medicinal plants have been crucial to the development of novel drugs in recent years. Metformin, the most popular anti-T2D medication, was first created from *Galega officinalis*, a medicinal plant that has been used for several centuries to treat diabetes.²² The limonene and flavonoids found in the aforementioned plants, which are cultivated all over the world, have anti-inflammatory and anti-tumor properties that make them useful for ethnomedical purposes. Because of their ability to scavenge radicals in vivo, natural antioxidants derived from plants are gaining more attention. The type of plants and the extraction

technique determine which bioactive ingredients are extracted from different plants.²³

In this study, the effect of the polyherbal extract on diabetes induced by STZ the anti-diabetic and antioxidant effects of the extract were examined by performing experiments in rat models of T2D. There are several classes of oral antidiabetic medications on the market at the moment.²⁴ These medications would be divided into three categories based on their antidiabetic properties: insulin sensitization, insulin secretion, and extra-pancreatic insulin release. In addition to these effects, medications also dramatically lower the level of glycated hemoglobin. The most commonly administered oral hypoglycemic medication is glibenclamide.²⁵

Chemically, 5-chloro-N- [2- [4 cyclohexyl carbamoyl sulfamoyl] phenyl] ethyl]-2-methoxy benzamide is a sulfonylurea molecule that increases insulin release from beta cells by acting as either pancreatic or extra pancreatic. In hyperglycemic circumstances, glibenclamide works by promoting the generation of insulin from the pancreatic beta cells already present. It exhibits extrapancreatic effects in addition to this direct activity.²⁶ This medication attaches itself to the sulfonylurea receptor 1 (SUR 1), which is a regulatory subunit of the beta cells' ATP-sensitive potassium channels (KATP). This inhibition opens the voltage-dependent calcium channels and depolarizes the cell membrane. It causes beta cells' intracellular calcium concentration to rise, which in turn triggers the release of insulin.²⁷ Therefore, in clinical diabetes, the single medication glibenclamide decreases hepatic glucose synthesis and increases insulin secretion. According to one study, in streptozotocin (STZ)-diabetic rats, sulfonylureas increased the production of insulin from the pancreatic beta cells that were already present. Additionally, this substance shows neuroprotective effects on the neurons in the brain's hippocampus regions.

CONCLUSION

The anti-diabetic qualities of polyherbal extract (*Lannea Coromandelica* and *Garcinia Indica*) were evaluated in this study. Both herbal treatments are beneficial in treating diabetes, according to numerous research. We used combination dosages and compared the results with a standard group in order to examine the combined effects of both herbal drug extractions on type-II Diabetes Miletus. Our results showed how well this test chemical combination worked against diabetes and the negative symptoms of high blood glucose that go along with it. Furthermore, as the animals were treated with the test drugs, their body weight increased and histological markers also shows improvement in the pancreatic cell and liver cell.

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