Biochemical Evaluation of Antidiabetic Efficacy of a newly synthesized Metformin - Resveratrol Aldehyde Complex Studied High Fat Diet Fed - Low Dose Streptozotocin - Induced Experimental Diabetes in Rats

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Abstract

The present study was aimed to systematically evaluate the antidiabetic efficacy of a newly synthesized Metformin - Resveratrol Aldehyde complex (Met-Res-Aldehyde complex) in high fat diet fed - low-dose streptozotocin-induced experimental type 2 diabetes mellitus in rats. Based on the results obtained through toxicity and dosage fixation studies, the diabetic group of rats were orally administered 5 mg/kg b.w./rat/day of the Met-Res-Aldehyde complex for a period of 30 days. The antidiabetic efficacy of the complex was assessed by measuring the levels of vital biochemical indices such as fasting blood glucose, plasma insulin, C-peptide, haemoglobin, glycosylated hemoglobin, total protein, urea, uric acid, and creatinine. HOMA-IR values were also determined. The presence of sugar in the urine was analyzed. To understand the possible mechanism of action of the synthesized complex, the activity of carbohydrate metabolizing enzymes such as hexokinase, pyruvate kinase, lactate dehydrogenase, glucose-6-phosphatase, fructose 1,6-bisphosphatase, and glucose-6-phosphate dehydrogenase was assayed in the liver and kidney tissues. The liver glycogen content and the activity of glycogen regulatory enzymes such as glycogen synthase and glycogen phosphorylase were assayed. Metformin was used as a reference drug to compare the effectiveness of the newly synthesized complex. The results obtained evidenced the non-toxic as well as hypoglycemic potential of the newly synthesized Met-Res-Aldehyde complex. The complex acts through the regulation of activity of carbohydrate and glycogen metabolizing enzymes in maintaining the normoglycemia in experimental type 2 diabetic rats.

Keywords: HFD- diet fed- Low dose STZ-inducedT2DM, Metformin-Resveratrol -Aldehyde complex, Carbohydrate metabolism, Glycogen metabolism, Regulatory enzymes.

Introduction

According to the WHO Constitution established in 1946, health is a basic right for all, regardless of age, gender, race, religion, political belief, or economic or social status, and the government's commitment and liability is to certify the health of their people [1]. However, deterioration of health due to ageing, establishment of diseases, morbidity, and subsequent mortality are the requisite laws of natural history that cannot be evaded but can only be deferred. Immature mortality due to communicable diseases such as smallpox, cholera, etc., is totally eradicated due to the serendipitous advancement of timely and honored antibiotics and vaccines. However, the four common behavioral risk factors, namely tobacco use, excessive alcohol intake, malnutrition, and lack of exercise, are accountable for about 80% of premature mortalities due to non-communicable diseases (NCDs), which mainly include cardiovascular diseases, cancer, obstructive pulmonary disease, and diabetes. Due to their relatively large populations, low- and middle-income countries account for nearly 80% of premature deaths related to NCDs [2, 3]. Prevention and control of NCDs has emerged as a global priority in the Sustainable Development Goals (SDG). Most of the NCDs share a number of behavioral risk factors that are unlikely to occur in isolation; rather, they characteristically congregate and interact to exponentially raise the risks of NCDs. [4, 5]. Though diabetes mellitus (DM) was scheduled next to the other three potential NCDs, it shares most of the primary as well as secondary complications of the other three NCDs [6, 7] and is the second largest negative total effect on global health-adjusted life expectancy worldwide [8, 9]. DM is one of the largest global public health struggles, imposing a heavy global burden on public health issues in terms of socio-economic development in recent decades, and will continue to ascend in the next few decades unless otherwise instantaneous remedial measures are initiated to restrain its dominance [10, 11].

Diabetes is a multi-factorial, multi-systemic, metabolic, and endocrine disorder that arises due to either deficiency (T1DM) and /or inefficiency of insulin (T2DM). T1DM is due to the selective destruction of insulin-secreting pancreatic β-cells of the pancreas [12]. T2DM accounts for more than 95% of the total diabetic population and is a heterogeneous disorder characterized by a progressive decline in insulin action (insulin resistance), followed by the inability of β-cells to compensate for insulin resistance (pancreatic β-cell dysfunction) [13]. Insulin resistance is a characteristic metabolic defect that precedes overt β-cell dysfunction and is primarily associated with resistance to insulin-mediated glucose disposal at the periphery and compensatory hyperinsulinemia [14]. The β-cells normally compensate for insulin resistance by secreting larger amounts of insulin to maintain glucose homeostasis. In

the course of time, however, this β-cell function gets impaired, leading to deterioration in glucose homeostasis and subsequent development of impaired glucose tolerance and onset of frank diabetes [15, 16].

According to current WHO diagnostic criteria, diabetes mellitus can be diagnosed clinically in three ways, and each, in the absence of uneven hyperglycemia, must be confirmed on a subsequent day by any of the three methods, namely, the levels of fasting plasma glucose (FPG) less than 120 mg/dl, two-hour postprandial glucose less than 140 mg/dl, and random glucose less than 180 mg/dl [17, 18]. In recent times, the levels of glycosylated haemoglobin (HbA1c), a measure of the percentage of hemoglobin that has been bound to circulating blood glucose over the last two to three months, have been used as a reliable, non-manipulative, and reproducible biochemical index of both diagnostic and prognostic value in the field of early diagnosis and treatment of diabetes [19]. HbA1c is defined as a specific glycosylated haemoglobin formed by a non-enzymatic ketamine reaction between the carbonyl group of the glucose moiety and the N-terminal of the amino acid, valine, in one or both β-chains of haemoglobin [20]. In a single measure, it can be performed at any time of the day and does not necessitate any special preparation such as fasting. Recently, HbA1c percentages of less than 7 are regarded as good glycemic control in diabetic individuals [21, 22].

The primary objective of diabetic treatment is to save the life of the diabetic individual and alleviate the allied symptoms, and the secondary objective includes the prevention of long-term secondary complications that arise out of chronic hyperglycemia by eliminating the associated risk factors to increase life permanence. The non-pharmacological therapy or management of diabetes mellitus is recommended for the individuals who have impaired blood glucose levels or impaired glucose tolerance (IGT) and mainly includes diet control and physical exercise. Overweight or obese individuals are advised by the clinicians to restrict calorie intake by consuming food with low fat content. However, it is mandatory to follow the guidelines prescribed by the physicians and qualified dieticians before the commencement of the non-pharmacological strategy to avoid unpleasant incidents arising out of acute hypoglycemia or hyperglycemia.The chief microvascular complications include diabetic retinopathy, diabetic neuropathy, and diabetic nephropathy, and the macro vascular complications include cardiovascular complications and cardiac stroke [23, 24]. Nearly 60% of individuals with diabetes mellitus have more than one secondary complication caused by chronic hyperglycemia. The etiology of late-onset complications of diabetes mellitus is not

established yet but is probably multi-factorial and related to the quality of blood glucose control [25].

The impediments of diabetes mellitus are broadly in two types, namely acute and chronic complications as well as micro and macrovascular complications. Acute complications essentially include acute hypoglycemia, diabetic ketoacidosis, hyperglycemic hyperosmolar state, and malignant hyperthermia-like syndrome with rhabdomyolysis [26].The established manifestations include increased sweating, trembling, uncontrolled tears, confusion, loss of recognition, difficulty in speaking or slurred speech, loss of coordination, inability to drink or eat, drowsiness, staring at eye level, palpitation, tingling around the mouth, and severe hypoglycemia, which may lead to unconsciousness and rarely mortality [27]. Acute complications often occur in type 1 diabetes due to inappropriate doses of insulin administration in the form of injections managed independently by the patient, or they may be severe, thus threatening life and requiring intensive monitoring. Outcomes are improved with timely recognition of the patient at risk, their symptoms, and appropriate intervention [28]. The complications of acute hypoglycemia often differ from individual to individual. Diabetic hypoglycemia occurs if the blood glucose level of an individual falls below the physiological level of 70 mg/dl [26].

Pharmacologically, individuals with chronically elevated levels of fasting, postprandial, and random blood glucose and HbA1c levels are treated with drugs having diverse mechanism(s) of action, such as drugs capable of stimulating insulin secretion from the β-cells of the pancreas (sulfonylureas), improving insulin sensitivity (thiazolidinediones), regulating the process of gluconeogenesis, glycogenesis, and glycogenolysis (Biguanides), interfering with the absorption of glucose and other nutrients in the intestine (α-glucosidase inhibitors) to control the chronic hyperglycemia and its secondary complications [29, 30]. However, insulin forms the cornerstone for the successful treatment of both type 1 and type 2 diabetes.

Metformin has been used as a first-line pharmacotherapy in diabetes for more than 50 years. Metformin traces its origins to the traditional medicinal plant *Galegaofficinalis*, which was used to treat diabetes symptoms in Europe in the 18th century. It was rich in guanidines; later, several mono-guanidines and diguanidines were developed [31]. Though the derivatives possess significant hypoglycemic properties, the toxicity profile associated with them disallowed them from achieving extensive recognition as effective antidiabetic medications [32]. The three vital members of the biguanide family were phenformin, buphormin, and metformin [33]. In the 1950s all three biguanides were approved for diabetic

treatment in Europe [34]. Due to the risk of development of lactic acidosis, phenformin and buphormin were withdrawn nearly 20 years after their introduction. Since metformin showed a substantially reduced incidence of lactic acidosis, even in patients with compromised renal function, it was developed as an antidiabetic drug by Sterne in the year 1950 and was first introduced for therapeutic application in France in the year 1959 [35]. The American Diabetes Association approved metformin as an oral antidiabetic drug. Subsequently, the European Association for the Study of Diabetes (EASD) also approved metformin for the treatment of type 2 diabetes [36, 28, 37]. On the basis of data obtained through clinical trials of over 60,000 patients in Canada and other countries, metformin was approved for marketing as metformin hydrochloride in Canada in 1972 [38]. Metformin was added to the WHO's essential medicines list in the year 2011. [39, 40].

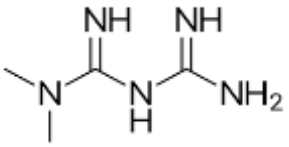


Figure 1: Structure of Metformin

Metformin is currently considered the first line of treatment for type 2 diabetes and the backbone for combination therapy [41]. Metformin (1, 1-dimethyl biguanide) is an important member of the biguanides, which occupies great significance in the clinical field for the treatment of type 2 diabetes [42]. Besides reducing the glucose level, it has an insulin-sensitizing effect with multiple actions on tissues such as the liver, skeletal muscle, endothelium, adipose tissue, and ovary [43]. Above all, metformin is relatively much cheaper than insulin and avoids possible risk factors associated with severe hypoglycemia and weight gain [44].

The pharmacokinetics and metabolic studies evidenced that metformin does not appear to be metabolized in humans. It takes 1.5 hours to start functioning after absorption. In the digestive tract, it is absorbed in the small intestine [45]. The mean plasma half-life following oral administration of metformin ranges between 4 and 8 hours, and the active duration is about 6-8 hours [46, 47, 48, 49]. However, plasma half-life is significantly prolonged in patients with renal impairment and a close relationship to creatinine clearance. A negative correlation has been described between oral dose and drug absorption [50]. It is rapidly distributed to all tissues and does not bind with plasma proteins [51, 52]. In contrast to phenformin, metformin does not undergo liver metabolism and is excreted unchanged by the kidneys. The typical metformin dose ranges from 250 to 2500 mg/day for the treatment of

T2DM [53, 54, 54, 55, 56]. In humans, the peak concentration is reached between 1 and 3 hours after the physiological oral dose of metformin [46, 47]. In most cases, metformin concentration reaches a maximum concentration within 2 hours of oral administration. However, the bioavailability of metformin at therapeutic doses reaches 50 to 60% but may drop as the dose is increased [57,58] Metformin is also excreted by the salivary glands at a markedly lower concentration when compared to plasma, with a half-time of about 9 hours. In fact, the unpleasant taste that diabetic patients experience is attributed to the presence of metformin in their saliva [46].

Metformin is excreted swiftly by the kidneys without any metabolic conversion, and the clearance rate is about four times greater than the clearance rate of creatinine in diabetic patients with normal renal function [47]. However, the clearance rate of metformin depends on the half-time of metformin present in the plasma. Functional impairment of the kidneys significantly reduces the excretion of metformin, which ultimately results in an excessive transfer of metformin into the tissues. The clearance of metformin from the tissues is normally 10 times slower than its elimination by the kidneys [59]. Metformin has no clinically relevant interactions with other drugs because it is not metabolized and does not inhibit the metabolism of other drugs [48, 60].

Experimental and clinical data evidenced that metformin administration tends to inhibit hepatic gluconeogenesis, leading to curtailment in endogenous glucose production by the liver without a concomitant increase in plasma insulin levels [61, 62]. Gluconeogenesis is considered as a crucial process in chronic hyperglycemia, since enhanced and unregulated hepatic glucose production is a prominent pathophysiological mechanism, and controlling it can improve the prognosis of the disease to a better extent [63]. In fact, gluconeogenesis is responsible for about 28-97% of total hepatic glucose output, which in turn further depends on the feeding status in the case of normal individuals and can be higher in patients with chronic T2D [64]. Metformin’s antihyperglycemic impact via the reduction of hepatic gluconeogenesis was first revealed by Zhou *et al*., (2001), and they observed that it is activated AMPK, a cellular basis for the blood glucose lowering action of metformin [65].

Metformin acts through the inhibition of Krebs cycle and/or oxidative phosphorylation by activation of AMP kinase, reduction of intestinal carbohydrate absorption leading to reduced post-prandial hyperglycemia, enhanced insulin-stimulated glucose transport in skeletal muscle through the increased recruitment and activity of GLUT4 glucose transporters, enhances the activity of IRS-2 (“Insulin Receptor Substrate 2”) to boost glucose uptake by the cells and enhanced non-oxidative glucose disposal into the\ skeletal muscle,

increased free fatty acid esterification and inhibition of lipolysis in adipose tissue, protection of β-cells from glucose toxicity and lipotoxicity including the protection of β- secretory capacity, increased secretion of glucagon-like peptide-1- glucose uptake by peripheral tissues mitochondrial oxidative phosphorylation and mitochondrial respiration. This inhibition is primarily occurring in energy consumption reactions with the reduction of ATP formation and a negative shift of the NADH/NAD+ redox potential [66-71].ATP is known to allosterically block the activity of the enzyme pyruvate kinase, and hence a reduction in ATP concentration leads to enhanced pyruvate kinase activity and decreased glucose output [72, 73].

Metformin was also found to activate AMPK activity intracelluarly, which acts as a sensor of energy in the cell, and it is observed that on activation of AMPK, the catabolic processes of the cells get triggered and lead to generation of ATP, whereas the anabolic pathways are disabled using ATP for synthetic processes [65, 74,75]. The decline in energy levels by diminished synthesis of ATP and resultant AMP by increased activity of enzyme adenylate kinase is observed [76]. Evidence also shows that metformin can even elevate AMP levels by blocking the activity of an enzyme, namely AMP deaminase that breaks down AMP [77]. Metformin, being a hydrophilic molecule, has been observed to have metal-binding properties, primarily with copper [78]. Metformin's hydrophilic nature makes it arduous to cross the cell membrane, and hence it depends on the membrane transporters and Organic Cation Transporters (OCT), which are responsible for the transport of drugs and other molecules across the cell membranes. Metformin has been found to be a substrate of OCT3 present in the brush border membrane of the enterocytes [79].

Reduced appetite is a functional action of metformin contributing to weight loss, which is beneficial, given that the vast majority of T2DM patients are obese. Insulin-stimulated glucose transport in insulin-resistant human skeletal muscle is also augmented by metformin administration [80]. In adipose tissue, metformin facilitates free fatty acid esterification and inhibits lipolysis. The increase in peripheral tissue glucose disposal may result in a 20% to 30% reduction in blood glucose levels. Of note, reduction in blood glucose and of lipolysis protects β-cells from glucose toxicity and lipotoxicity, respectively, which in turn may protect the β-cell secretory functions [81, 82, 83]. It is now widely accepted that metformin has a beneficial effect on serum lipids, inflammation, and cardiovascular diseases [84, 85, 86]. Most of the beneficial and therapeutic efficacies of metformin are attributable to the amelioration of insulin resistance. Since metformin treatment does not promote insulin secretion from the β-cells of the pancreas, it is not associated with the risk of hypoglycemia.

In general, the assortment and the combined efficacy of the cellular functions of metformin justify its characterization as a “multitasking medication.” In addition, evidence also shows that there is a disruption in intestinal microbiota in the patients with T2DM, and metformin administration was found to modulate the microbiota texture by decreasing the count of Bacteroidesfragilis [87]. However, if the HbA1c levels are higher than 7.0% after a prolonged period of treatment with metformin, a second medication can be added. Unluckily, metformin has several side effects (from mild to serious) that cause lack of adherence, and therefore it is the antidiabetic oral therapy with the lowest compliance [88].

Resveratrol is a most convincing polyphenolic composite found in many fruits and vegetables, such as peanut sprouts, grapes, and peanuts. Initially, it was extracted from *Veratrumgrandiflorum* or white hellebore, plants [89]. It attracted wider attention only in 1992, when the cardioprotective effect of red wine was attributed to the presence of resveratrol. The word “resveratrol” is derived from a Latin word: “res” means “which comes from,”from the plant“veratrum and ol” indicates the presence of alcohol moiety in the structure. The epidemiological finding of an inverse relationship between consumption of red wine and incidence of cardiovascular disease has led to the “French paradox,” which is consistent with its known activity [90]. The resveratrol content in grapes varies from 0.16 to 3.54 mg/g; dry grape skin contains about 24 mg/g of resveratrol [91]. The amount found in grape skin also varies with grape cultivar, its geographic origin, and exposure to fungal infection. It also exists in other nuts and berries, such as cranberry juice, containing about 0.2 mg/l. It is also documented that red wine contains a much larger number of polyphenolic compounds than white wine. The resveratrol concentration ranges from 0.1 to 14.3 mg/l in different types of red wine. At the same time, white wine contains only 0.1–2.1 mg/l of resveratrol [92].

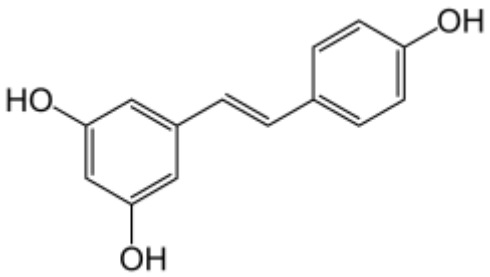


Figure 2: Structure of Resveratrol

Resveratrol is a stilbenoid, a derivative of stilbene, derived from the enzyme stilbene synthase using one molecule of 4-coumaroyl-Co-A and three molecules of malonyl-Co-A as substrate. It exists as two geometric isomers: *cis*- and *trans*-resveratrol. *Trans*-resveratrol in

the powder form is stable under 'accelerated stability' conditions of 75% humidity and 40°C in the presence of air. Resveratrol content also remains stable in the skin of grapes and pomace taken after fermentation and when stored for longer periods of time [93]. The molecular formula of resveratrol is $C_{14}H_{12}O_3$, and the molecular mass is 228.24 gm/mol. The melting point of resveratrol is around 256-257°C. Resveratrol is safe and did not cause any adverse effects [94]. Resveratrol is metabolized by hydroxylation, glucuronidation, sulfation, and hydrogenation. Urinary excretion of resveratrol and its metabolites was rapid, with 77% of all urinary agent-derived species excreted within 4 hours after the lowest dose. Excretion was mainly through urine (around 73%) and feces after 12 hours. The half-life of resveratrol and its metabolites was 9 9hours [95, 96]. In recent decades, it has attracted significant attention from medical chemists, diet nutritionists, and health professionals because of its numerous beneficial effects, including antiangiogenic, immunomodulatory, antimicrobial, neurological, anticancer, antidiabetic, and cardiovascular disease (CVD) prevention [97-104]. More recently, we have reported the beneficial and pharmacological properties of resveratrol [105].

Streptozotocin (STZ) is the most prominent diabetogenic chemical [106] that is widely used in experimental animals for creating animal models of type 1 and type 2 diabetes [107].Streptozotocin (also called Streptozocin) or 2-deoxy-2 ([methyl (nitroso) amino] carbonyl) amino)-(α and β)-D-glucopyranose [108], was discovered in 1959 as a natural antibiotic produced by *Streptomyces achromogenes;* its toxicity towards pancreatic β-cells (diabetogenic action) was reported in 1963 [109] byRakieten [110]. STZ molecule (molecular formula=$C_8H_{15}N_3O_7$, molecular weight≈265 [111] has two parts: (1) a glucopyranosyl group, which facilitates its uptake by pancreatic β-cells by glucose transporter 2 (GLUT2), and (2) a nitrosourea group, which destructs pancreatic β-cells [109]. More recently, we have reported the optimization of the protocol for the successful induction of streptozotocin-induced experimental type 2 diabetes in experimental rats [112].

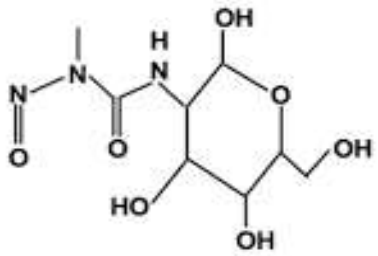


Figure 3: Structure of Streptozotocin

Having these beneficial as well as pharmacological aspects of metformin and resveratrol, in the present study, we have designed and synthesized a new metformin-resveratrol-aldehyde complex as an organic ligand and evaluated its toxicity as well as antidiabetic properties in a high-fat diet fed to low-dose STZ-induced experimental type 2 diabetes in rats.

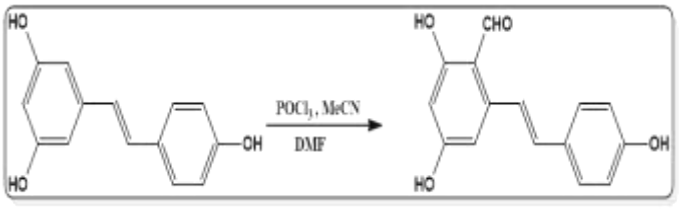
Materials and Methods

Chemicals

Resveratrol, metformin, streptozotocin, and insulin were obtained from Sigma Aldrich, USA. An ultra-sensitive ELISA kit for rat insulin assay was purchased from Crystal Chem Inc., Life Technologies, India. All the other chemicals and reagents used were of analytical grade and obtained from the Southern Indian Scientific Corporation, Chennai, India.

Synthesis of Resveratrol Aldehyde

Resveratrol aldehyde (RA) (2, 4-dihydroxy-6-((E)-2-(4-hydroxyphenyl)) benzaldehyde) was synthesized by the methods of [113, 114]. Resveratrol was treated with Vilsmeier reagent ($POCl_3$, DMF, and MeCN) to synthesize resveratrol aldehyde. Briefly, freshly distilled $POCl_3$ (0.6 ml, 6 mmol) was added dropwise to a solution of resveratrol (912 mg, 4 mmol) and DMF (464 ml, 6 mmol) in 20 ml of MeCN that was maintained in an ice water bath. The mixture was stirred continuously for 1 hr. at room temperature. Then, the solution was added to a mixture of ice and water, and the yellow mixture was stirred at 40°C in a water bath and extracted with EtOAc (3 × 10 ml) and evaporated. The yellow-colored crystals of RA were obtained with a yield of 78%. The overall scheme is represented as Scheme 1.

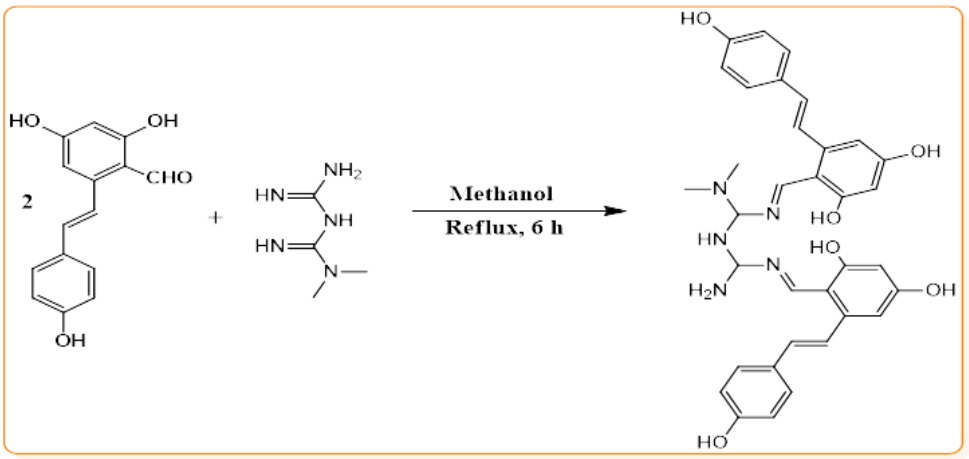


Scheme- 1: Synthesis of Resveratrol Aldehyde

Synthesis of a mixed ligand from Metformin hydrochloride and Resveratrol Aldehyde (M - RA).

Mixed ligand 4-(E)-((amino(E)-(2,4-dihydroxy -6-(4-Mixed ligand 4-(E)-hydroxyphenethyl)benzylidene)amino)(dimethylamine)methyl)amino)methyl)amino) methyl)-5-(4-hydroxyphenethyl)benzene-1,3-diol was prepared by refluxing the mixture of 1 mmol (0.2 g) of metformin with 2 mmol (0.5 g) of resveratrol aldehyde in 50 mL of methanol for about 6 hours.

The pale-yellow-colored compound was dried, and the yield was 92 mg (72%). The proposed scheme is represented in Scheme 2.



Scheme -2: Synthesis of Metformin- Resveratrol Aldehyde mixed ligand.

The physico-chemical parameters of resveratrol aldehyde and metformin-resveratrolaldehyde mixed ligand were recorded. The resveratrol aldehyde and the mixed ligand were subjected to spectral characterization by FT-IR, ^{1}H NMR, ^{13}C NMR, and mass spectral analysis, as we have reported earlier [115].

Experimental Animals

Male albino rats of the Wistar strain weighing around 160 to 180 g were procured from the Tamil Nadu Veterinary and Animal Sciences University, Chennai, and were housed in the Biomedical Research Unit and Lab Animal Centre, Saveetha Dental College and Hospitals, Chennai, under standard husbandry conditions (12 ± 1 h light and dark cycle, relative humidity 55% ± 10%). The animals were fed with a balanced diet (Hindustan Lever Ltd., Bangalore, India) and water *ad libitum*. The rat pellet diet is composed of 55% nitrogen-free extract, 21% protein, 5% fat, and 4% fiber (w/w) with sufficient levels of vitamins and minerals. The experimental design was strictly conducted according to the ethical norms approved by the Ministry of Social Justice and Empowerment, Government of India, and Institutional Animal Ethics Committee Guidelines (Approval number-BRULAC/SDCH/SIMATS/IAEC/08-2022/137) for the examination of experimental pain in conscious animals and to fix the effective dose of the Met-Res-Aldehyde complex for treatment.

Acute Toxicity and Dosage Fixation Studies

Acute toxicity studies were performed as per OECD guidelines for testing of chemicals in normal rats. Graded doses (2.5 mg, 5 mg, 7.5 mg, and 10 mg/kg b.w./rat/day) of Met-Res-Aldehyde complex dissolved in 5% DMSO were orally administered to rats using

gavages. All observations were systematically recorded, with individual records being maintained for each animal. Cage side observations included the evaluation of skin and fur, eyes, respiratory effects, lethargy, autonomic effects such as salivation, diarrhea, and urination, and central nervous system effects including tremors and convulsions, changes in the level of activity, gait, and posture. The changes in food consumption, fluid intake, and body weight were continuously monitored for a period of 30 days. Macroscopic examinations were also performed on vital organs. The suitable optimum dosage of the drug was assessed by administering graded dosages of the complex for different periods (15, 30, and 45 days) to determine the dose-dependent hypoglycemic effect in HFD fed-low-dose-STZ-induced diabetic rats by monitoring the fasting blood glucose levels periodically.

Experimental Design

The rats were allocated into two dietary regimens by feeding either a normal pellet diet (NPD) or a high-fat diet (HFD) for 2 weeks of dietary manipulation. HFD contains powdered NPD, 365 g/kg; lard, 310 g/kg; casein, 250 g/kg; cholesterol, 10 g/kg; vitamin and mineral mix, 60 g/kg; DL-methionine, 3 g/kg; yeast powder, 1 g/kg; and NaCl, 1 g/kg. After 2 weeks of HFD supplementation, Group II, Group III, and Group IV rats were injected with a single dose of STZ (35 mg/kg b.w./rat); control rats (Group I) fed with NPD were injected intraperitoneally with the same volume of freshly prepared cold citrate buffer (pH 4.5, 0.1 mol/L) only [116]. After one week of STZ injection, rats having fasting blood glucose levels ≥ 300 mg/dL were considered diabetic rats and chosen for further studies.

The animals were divided into four groups, each comprising six rats, as follows:

Group 1 : Control.
Group 2 : HFD+STZ induced experimental type 2 diabetic rats.
Group 3 : HFD+STZ-induced diabetic rats treated with Met-Res-Aldehyde complex (5 mg/kg b.w./rat/day) for 30 days.
Group 4 : HFD+STZ-induced diabetic rats treated with metformin 500 mg/kg b.w./rat/day for 30 days.

During the experimental period, body weight, blood glucose, food and water consumption, and physical examinations were determined at regular intervals. The dosage was adjusted every week according to any change in body weight to maintain a similar dose per kg of body weight of rats over the entire period of study for each group. At the end of the treatment period, the rats were fasted overnight, anesthetized (ketamine, 80 mg b.w. i.p.), and sacrificed by cervical decapitation. The blood was collected with and without anticoagulant for plasma or serum separation, respectively. The liver and kidney tissues were selectively dissected out and washed in ice-cold saline and used for further experimental studies.

A known amount of the selected tissues was homogenized in Tris-HCl buffer (100 mM, pH 7.4) at 4°C in a Potter-Elvehjem homogenizer with a Teflon pestle at 600 rpm for 3 minutes. The homogenate was centrifuged at 12,000xg for 30 minutes at 4°C. The supernatant was collected as tissue homogenate, which was used to assay various parameters. The protein content in the tissue homogenate was estimated by the method of Lowry *et al*., (1951) [117]. A portion of the wet liver tissue was used for the estimation of glycogen content.

Oral Glucose Tolerance Test (OGTT)

On the day prior to sacrifice, an oral glucose tolerance test (OGTT) was performed in all the groups of rats. Blood samples were collected through retro-orbital bleeding from all groups of rats deprived of food overnight. Successive blood samples were taken at 0, 30, 60, 90, and 120 minutes following the oral administration of 2 mg/kg b.w. of glucose solution [118]. All the blood samples were collected with EDTA for the estimation of glucose by the glucose oxidase/peroxidase diagnostic enzyme kit (Span Diagnostic Chemicals, Surat, India), and the analysis was performed according to the manufacturer's instructions.

Homeostasis Model Assessment of Insulin Resistance (HOMA-IR)

As the insulin abnormality cannot be accurately detected by a single determination of insulin or glucose levels, the insulin resistance was evaluated by homeostasis model assessment of insulin resistance (HOMA-IR) as follows [119].

HOMA-IR = Fasting insulin level × Fasting blood glucose/405

Biochemical Parameters

At the end of the experimental period, overnight fasted rats were anesthetized using ketamine (80 mg/kg b.w./rat.) and sacrificed by cervical decapitation. Blood samples were collected with and without anticoagulant for separation of plasma and serum, respectively. For the estimation of glycogen [120], liver tissue was excised, washed with ice-cold saline, stored at −70°C, and used. The basic biochemical parameters, such as fasting blood glucose [121], glycosylated hemoglobin [122], plasma protein [117] blood urea [123] and serum creatinine [124] levels, were estimated. Urine strips were used to detect the presence of glucose in urine. The levels of plasma insulin and C-peptide were assayed by ELISA using a rat insulin assay kit (Linco Research, St. Charles, MO, USA). The supernatant obtained from the centrifugation of the liver homogenate was used as an enzyme source for the assay of hexokinase, pyruvate kinase, lactate dehydrogenase, glucose-6-phosphatase, fructose-1, 6-bisphosphatase, glucose-6-phosphate dehydrogenase, glycogen synthase, and glycogen phosphorylase.

Statistical Analysis

The values are expressed as mean values of six rats in each group ± SEM. Data analysis was done with SPSS software. The hypothesis testing method included one-way analysis of variance (ANOVA) followed by post hoc testing performed with least significance difference (LSD). The value of $P < 0.05$ was considered to indicate statistical significance.

Results and Discussion

Effects of the Met-Res-Aldehyde complex on changes in body weight, food, and water intake. The changes in body weight, food intake, and water intake of control and experimental rats are represented in Table 1 as well as Figures 4a, 4b, and 4c. Body weight was significantly decreased, whereas food intake and water intake levels were significantly increased in experimental diabetic rats compared with the normal control group of rats in the present study. No significant changes were observed in normal control group of rats. All these changes observed in diabetic rats were significantly improved on the oral administration of the Met-Res-Aldehyde complex, evidencing that the newly synthesized complex treatment causes improvement in metabolic activity of the system and maintains glucose homeostasis. The data obtained also provides evidence for the non-toxic as well as beneficial effect of the newly synthesized complex.

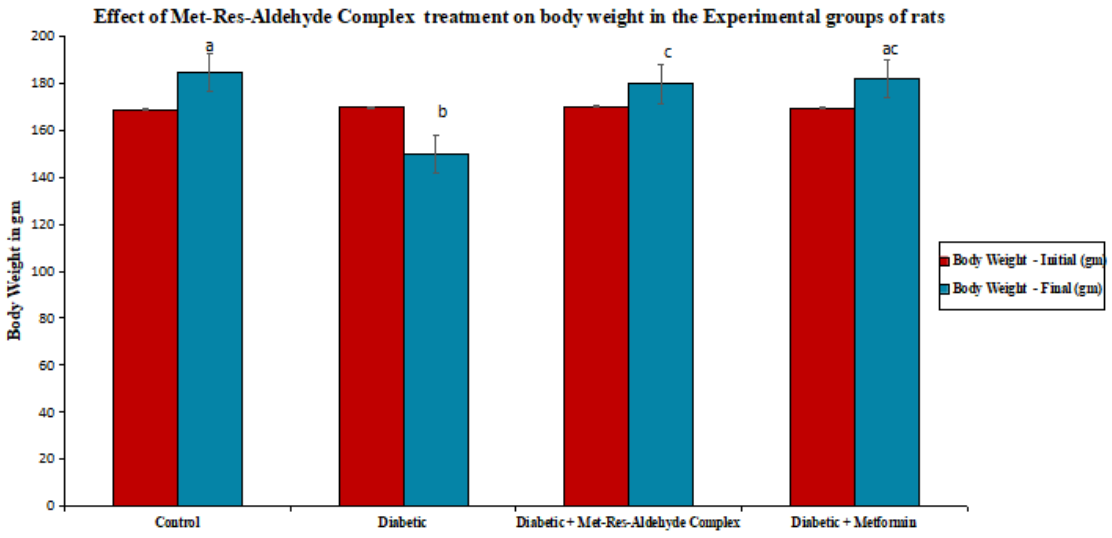
The aim of the present study was to investigate the anti-diabetic potential of the newly synthesized Met-Res-Aldehyde complex in high fat diet fed- low dose STZ-induced experimental diabetes in rats. Diabetes mellitus due to impaired glucose homeostasis is the frequently arising endocrine disorder that leads to the dysfunctional characteristics of multisystem in the body. Systemic and metabolic disturbances due to diabetes ultimately result in chronic hyperglycemia. In the diabetic control group, the characteristic loss of body weight is caused by an increase in muscle wasting and loss of tissue proteins [125]. The difference in the body weight observed during the period of treatment with the Met-Res-Aldehyde complex was less as compared to the diabetic control group, which may be due to its tissue protective effect in controlling muscle wasting which in turn may be due to the reversal of gluconeogenesis and may also be due to proper glycemic control. Based upon these results, it can be hypothesized that the Met-Res-Aldehyde complex probably acts by releasing more insulin from the residual pancreatic β-cells of pancreas. Treatment with the Met-Res-Aldehyde complex as well as metformin resulted in increased body weight and decreased food and water intake in diabetic rats. An increase in the body weight of HFD fed-low dose STZ-induced diabetic rats treated with the Met-Res-Aldehyde complex might be due to an enhancement in glycemic control and increased synthesis of structural proteins [126]. Earlier, we reported that oral administration of resveratrol at a concentration of 5 mg/kg b.w./rat/day for 30 days significantly improved the body weight gain in streptozotocin-nicotinamide-induced experimental type 2 diabetes in rats [97].

The diabetic group of rats showed a significant increase in food and water intake when compared to the control group of rats. Subsequent to the oral administration of the complex as well as metformin to the diabetic group of rats, the levels were found to be similar to that of the control group of rats. The classic symptoms of diabetes, such as polyphagia and polydipsia, were exhibited in high fat diet fed- low-dose-STZ-induced experimental diabetic rats, and this may be attributed to the non-availability of glucose to the cells due to insulin deficiency and/or insulin resistance. Increased food and water intake was observed in the diabetic group of rats, indicating the improvement in the polyphagic and polydipsic condition, followed by weight loss due to excessive breakdown of tissue proteins. However, oral treatment with the newly synthesized complex to the diabetic group of rats decreased the food and water consumption, which could be due to an improved control of hyperglycemia.

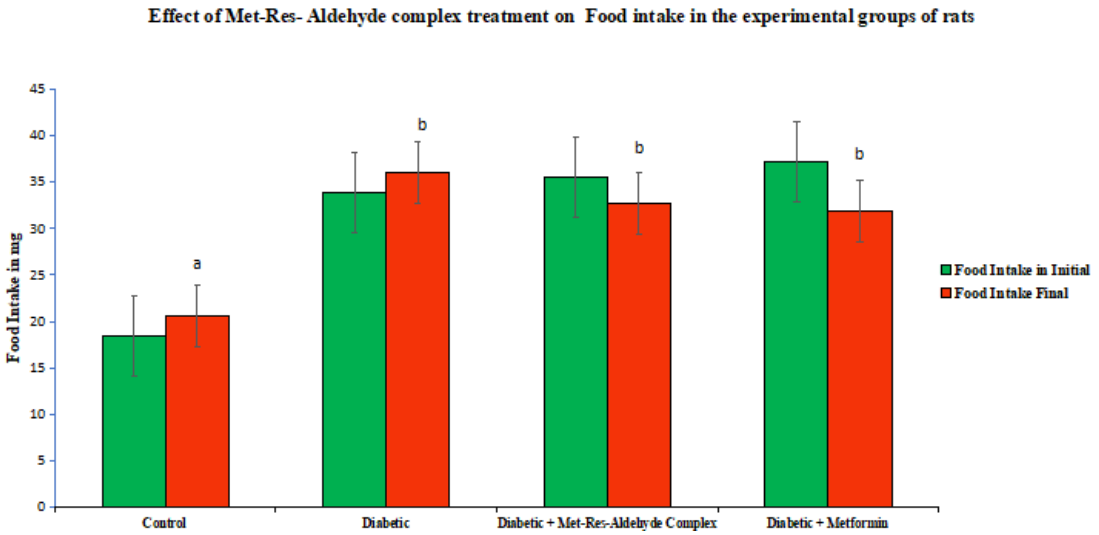
Table -1 Effect of Met-Res - Aldehyde complex treatment on body weight, food and water intake in the experimental groups of rats after 30 days.

Groups	Body Weight - Initial (g)	Body Weight - final (g)	Food intake (mg/rat/day)Initial	Food intake (mg/rat/day)Final	Water intake (ml/rat/day) Initial	Water intake (ml/rat/day) Final
Control	168.66± 0.71	184.66±1.11 [a]	18.5 ± 5.46	20.66± 4.25[a]	28.6 ± 7.85	32.5± 1.80[a]
Diabetic	169.83± 0.60	150.00±0.85 [b]	33.83 ±1.07	36 ± 1.46[b]	60.2 ± 4.01	72.83± 13.01[b]
Diabetic+ Met-Res-Aldehyde complex	170.16± 0.70	179.66±0.76 0[c]	35.5 ±1.52	32.66± 1.40[b]	59.8 ± 2.72	34.66 ± 1.58[a]
Diabetic + Metformin	169.33 ±1.25	182.00 ±0.96[ac]	37.16± 0.60	31.83± 0.60[b]	57 ± 4.09	32.16 ± 1.51[a]

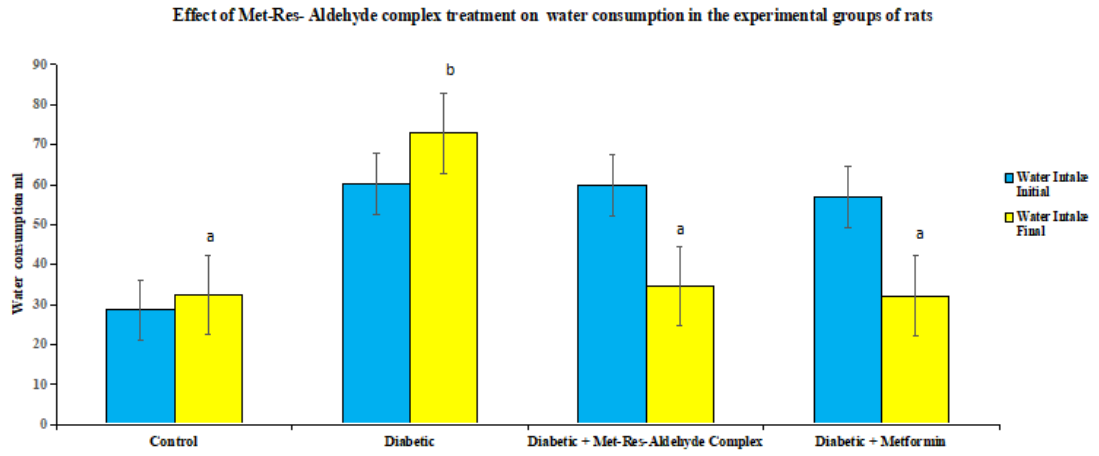
Values are presented as mean ± standard error of the mean (n=6). One-way Anova followed by post hoc test LSD was done. Results were compared with [a]control rats, [b]dibetic rats. Values are statistically significant at $p<0.05$.



Values are presented as mean ± standard error of the mean (n=6). One-way Anova followed by post hoc test LSD was done. Results were compared with [a]control rats, [b]dibetic rats. Values are statistically significant at $p<0.05$.



Values are presented as mean ± standard error of the mean (n=6). One-way Anova followed by post hoc test LSD was done. Results were compared with [a]control rats, [b]dibetic rats. Values are statistically significant at $p<0.05$



Values are presented as mean ± standard error of the mean (n=6). One-way Anova followed by post hoc test LSD was done. Results were compared with [a]control rats, [b]dibetic rats. Values are statistically significant at $p<0.05$

Effect of oral administration of the Met-Res-Aldehyde complex on glucose tolerance in diabetic rats after oral glucose load.

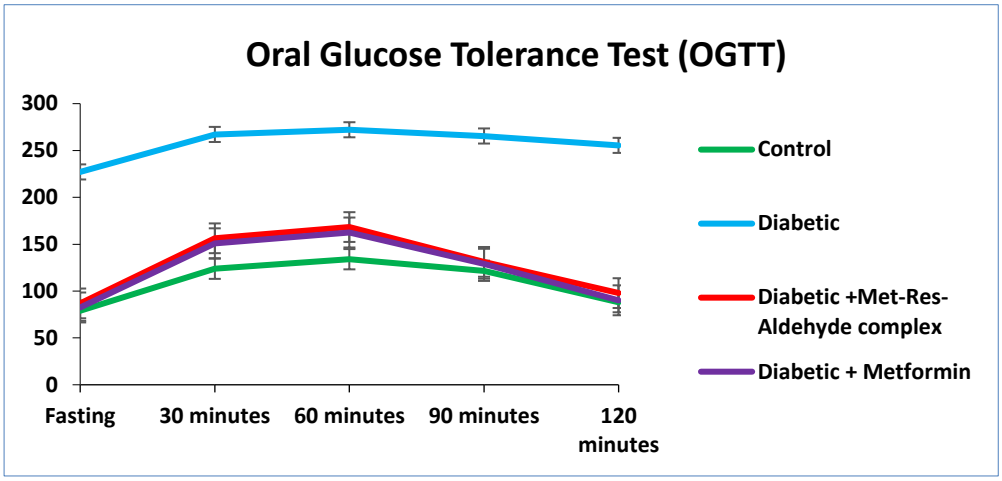
Figure-5shows the blood glucose levels of control and experimental groups of rats after an oral glucose load. After the oral glucose load, blood glucose levels peaked at 60 min and then gradually declined to near physiological levels at 120 min, indicating the maintenance of normoglycemia in the control group of rats, whereas, in the case of the high fat diet fed -low-dose-streptozotocin-induced experimental type 2 diabetic group of rats, the blood glucose concentration before the oral glucose load was significantly higher than the

glucose levels observed in the control group of rats, and the peak increase in blood glucose concentration was observed after 60 min and remained elevated over the next 60 min [127,128].

A high-fat diet fed with a low dose of STZ causes selective as well as irreversible damage to the insulin secretion of β-cells of the pancreas, which results in increased levels of blood glucose, which in turn may be due to increased production and/or decreased utilization. Oral administration of the Met-Res-Aldehyde complex (metformin-resveratrol aldehyde complex) to HFD-STZ-induced diabetic rats at a concentration of 5mg/Kg b.w./rat/day for 30 days showed a statistically significant decrease in blood glucose concentration at 60 and 120 min., indicating its blood glucose-lowering efficiency, which in turn may be due to the insulin secretory and/or insulin mimetic properties of the newly synthesized complex, and the hypoglycemic efficacy was comparable with metformin. This effect may occur due to a reduction in intestinal glucose absorption or induction of a glycogenic process along with a reduction in glycogenolysis and gluconeogenesis. The observed levels of blood glucose in untreated diabetic rats were due to the selective toxic effect of STZ, which causes tissue damage in the pancreas that destroys β-cells and results in insulin deficiency. Insulin deficiency ultimately causes increased blood glucose [129]. The reduction in blood glucose levels brought about by the Met-Res-Aldehyde complex was quite comparable with the reduction brought about by metformin, a standard reference drug that has been widely prescribed for the treatment of type 2 diabetes [130].

The oral glucose tolerance test (OGTT) is an established measure of effective glucose utilization by the system that generally aids in the premature diagnosis of diabetes [131,132]. OGTT is widely used to evaluate obvious insulin release and insulin resistance in various clinical conditions. Impaired glucose tolerance (IGT) due to pancreatic dysfunction results in the defective utilization of glucose by the tissues and increased hepatic gluconeogenesis [133]. OGTT has been the basis for diagnosing diabetes for several decades. It efficiently detects prediabetes as well as individuals with impaired glucose tolerance. It has the effectiveness for evaluating insulin sensitivity and β-cell function during glucose administration via a physiological transmission [134]. OGTT is widely used to evaluate the disease progression and outcome of therapeutic treatment and to assess the physiological and pathophysiological conditions of diabetes mellitus. It provides significant and valuable information for predicting the ensuing incidence of diabetes. The improved glycemic control in oral glucose tolerance tests by the Met-Res-Aldehyde complex shows that the complex treatment is capable of lowering the blood glucose levels during oral glucose load [135]. Further, our results are

inconsistent with a previous report that oral administration of resveratrol at a concentration of 5 mg/kg b.w./rat/day for 30 days in streptozotocin-nicotinamide-induced experimental type 2 diabetes [97].



Values are presented as mean ± standard error of the mean (n=6). One-way Anova followed by post hoc test LSD was done. Results were compared with [a]control rats, [b]dibeticrats.Values are statistically significant at $p<0.05$.

The homeostatic model assessment is a mathematical method used to quantify insulin resistance (HOMA-IR) and pancreatic β-cell function. In the present study, diabetic rats showed a significant increase in HOMA-IR when compared with the control rats. Met-Res-Aldehyde complex administration significantly decreased the HOMA-IR index in diabetic rats. Diabetic rats showed a significant elevation of HOMA-IR, and it was decreased significantly upon administration of the Met-Res-Aldehyde complex and metformin. The altered level of HOMA-IR was reverted to near normal in diabetic rats treated with the newly synthesized complex. Insulin resistance is a characteristic metabolic defect that precedes overt β-cell dysfunction and is primarily associated with resistance to insulin-mediated glucose disposal at the periphery and compensatory hyperinsulinemia. Pancreatic β-cell dysfunction with progressive loss of insulin secretion, consequent to the development of insulin resistance, is the established key defect associated with characteristics of type 2 diabetes. High fat diet feeding results in insulin resistance through the Randle or glucose-fatty acid cycle. This results in inhibition insulin-stimulated glucose transport, thereby diminishing the β-cell function through chronic elevation of free fatty acids [136]. The observed decrease in HOMA-IR evidenced that the complex treatment significantly controlled the insulin resistance and improved the pancreatic β-cell function via ameliorating the glucolipotoxicity (Figure -6).

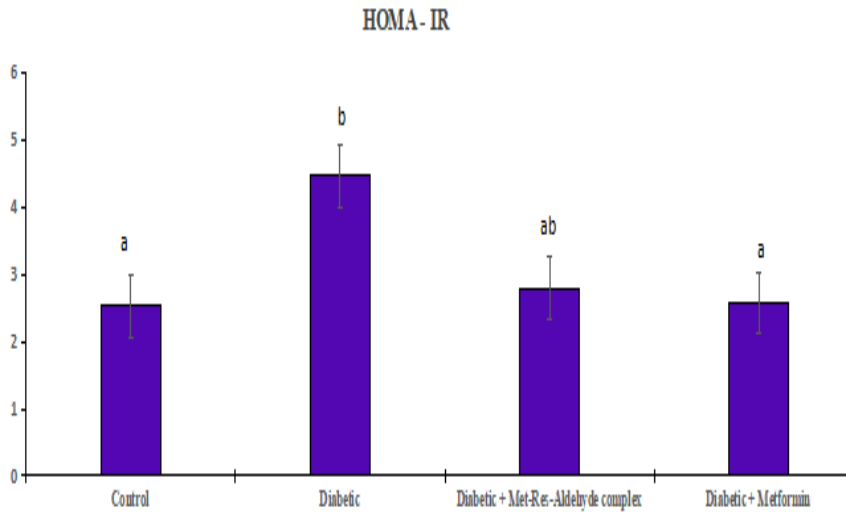


Figure 6: Effect of Met-Res-Aldehyde complex treatment on HOMA-IR .Values are given as means ± SEM for six rats in each group. One – way ANOVA followed by post hoc test LSD was done. Values are statistically significant at *p

The homeostasis model assessment of insulin resistance (HOMA-IR) developed by [137] Matthews *et al.,* (1985) has been extensively used for the assessment of insulin resistance. Insulin resistance is the primary metabolic defect associated with obesity and appears to be the primary mediator of metabolic syndrome [138]. The majority of genes associated with the initiation and onset of diabetes mellitus have been linked to the β-cell dysfunction and impairment of β-cell mass. If the β-cell mass is reduced by 50%, the secretory burden for the remaining β-cells increases by 100%, thereby leading to chronic β-cell stress [139]. The altered level of HOMA-IR was reverted back to near normal level in diabetic rats treated with the Met-Res-Aldehyde complex, evidencing the insulin sensitivity reduction efficacy of the newly synthesized complex.

Effect of oral administration of Met-Res-Aldehyde complex on blood glucose, plasma insulin, C-peptide, hemoglobin, glycosylated hemoglobin and urine sugar levels in experimental groups of rats.

Table-2 shows the levels of blood glucose, plasma insulin, C-peptide, haemoglobin, glycosylated haemoglobin, and urine sugar in control and experimental groups of rats. There was a significant elevation in the levels of blood glucose, glycosylated haemoglobin of diabetic rats as compared with the control group of rats. Upon treatment with the Met-Res-Aldehyde complex, the diabetic rats showed a significant decrease in the levels of blood

glucose and glycosylated haemoglobin, and an increase in haemoglobin content, which were comparable with the control group of rats. Moreover, the significantly diminished plasma insulin level of diabetic rats was improved to near physiological level by the administration of the Met-Res-Aldehyde complex. The urine sugar found in the diabetic rats was drastically controlled by the oral administration of the newly synthesized complex as well as metformin.

Table -2 showsthe levels of blood glucose, plasma insulin, C-peptide, haemoglobin, glycosylated haemoglobin and urine sugar in control and experimental groups of rats.

Groups	**Control**	**Diabetic**	**Diabetic+Met-Res- Aldehyde complex**	**Diabetic+ Metformin**
Blood Glucose	78.16 ± 2.31^{a}	257.16 ± 4.86^{b}	88.5 ± 1.38^{a}	86.16 ± 2.02^{a}
Plasma Insulin	13.5 ± 0.76^{a}	7.16 ± 1.60^{b}	11.83 ± 1.04^{a}	12.83 ± 0.60^{a}
C-Peptide	2.06 ± 0.20^{a}	0.075 ± 0.03^{b}	1.31 ± 0.25^{a}	1.55 ± 0.25^{a}
Hemoglobin	15.66 ± 1.45^{a}	10.5 ± 0.76^{b}	12.83 ± 0.47^{ab}	14.33 ± 0.66^{a}
Glycosylated haemoglobin	5.21 ± 0.25a	8.81 ± 0.28^{b}	6.9 ± 0.35^{c}	6.58 ± 0.57^{ac}
Urine Sugar	Nil	+++	Nil	Nil

Units: mg/dl for blood glucose, µU/ml for plasma insulin, pmol/ml for plasma C-peptide, g/dl for hemoglobin, % hemoglobin for HbA1c,+++ indicates more than 2% sugar.

Blood glucose is a reliable biochemical index for the diagnosis of diabetic conditions and is regulated by insulin that aids in the regulation of uptake, storage, and utilization of blood glucose. STZ administration causes irreversible damage to the pancreatic β-cell through the generation of excessive oxidative stress, which resulted in increased levels of blood glucose. The elevated levels of fasting blood glucose level observed in the diabetic group of rats may be primarily due to the excessive release of glucose from the liver by the processes of gluconeogenesis and glycogenolysis. Based on the results obtained from this methodical study, it can be concluded that oral administration of the Met-Res-Aldehyde complex at a concentration of 5 mg/kg body weight/rat/day for 30 days significantly reduces the blood glucose levels in high-fat-diet-fed, low-dose-streptozotocin-induced experimental type 2 diabetic rats, which could be due to improvement in insulin secretion and/or reduction of insulin resistance. Likewise, the observed increase in fasting blood glucose level in high

fat diet fed - low-dose STZ-induced experimental diabetic rats may be due to increased production and/or diminished utilization.

All vertebrate life forms on the earth are fuelled by glucose, which is derived from the foods consumed. However, the living body can derive glucose from proteins and fats. The glucose is absorbed in the small intestine by the intestinal brush border villi and enters the bloodstream and is transported to every living cell in the body, wherein it is burned or oxidized in the process known as cellular respiration to generate energy in the form of ATP. Fasting glucose levels are decisive for early diagnosis and management of diabetes and provide a baseline for treatment effectiveness. Maintenance of fasting blood levels within the physiological level can reduce the development of secondary complications of diabetes such as retinopathy, neuropathy, nephropathy, and cardiovascular complications[140,141,142]. Additionally, FBG level is often used to exclude diabetes mellitus in those suspected of diabetes insipidus. Thus, fasting blood glucose level is the "standard" test for the diagnosis of diabetes. The liver plays an essential role in the maintenance of normal blood glucose levels and is the primary organ responsible for endogenous glucose production. In fasting conditions, the liver produces glucose, whereas in the postprandial state, it stores excess glucose in the form of glycogen [143]. Oral administration of the Met-Res-Aldehyde complex treatment to a diabetic group of rats significantly reduced the fasting blood glucose level, evidencing its anti-hyperglycemic nature, which in turn may be due to its insulin secretory and/or insulin mimetic properties. Therefore, it is concluded that the Met-Res-Aldehyde complex possesses notable antidiabetic activity.

The decreased plasma insulin level in experimental diabetic rats was restored to near physiological levels in the Met-Res-Aldehyde complex as well as in metformin-treated diabetic rats. The observed decrease in insulin level in the experimental diabetic rats is mainly due to the specific cytotoxic effect of STZ on the pancreatic β-cell. Additionally, the insulin resistance induced by the high fat diet feeding is also responsible for the observed decrease in the levels of insulin. The observed increase in insulin level in newly synthesized complex-treated diabetic rats evidenced that the treatment with the complex improved the insulin secretion as well as the tissue-protective nature of the complex. Further, treatment with the complex results in the stimulation of insulin secretion from the remnant cells of pancreatic β-cells. Dysregulation of carbohydrate metabolizing enzymes in diabetes due to insulin deficiency and/or its resistance increases the hepatic glucose production and decreases the storage of glucose as glycogen [144,145,146]. Insulin essentially controls the hepatic

glucose production by regulating the activities of key enzymes involved in gluconeogenesis and glycogen metabolism [147].

C-peptide is a cleavage product of insulin biosynthesis and is subsequently released along with insulin in equimolar amounts to the blood circulation [148]. It consists of 31 amino acids and has a half-life of 30 minutes. C-peptide has been considered to be a biologically inert substance [149]. It is considered to be a reliable maker of residual β-cell function and plays a vital role in the maintenance of vascular homeostasis and exerts physiological effects of importance for the prevention and treatment of T1DM [150, 151]. The prime task of C-peptide is to facilitate proper folding of proinsulin by aiding disulfide bond formation within A- and B-chains [152, 153]. C-peptide possesses insulin-mimetic property, thereby activating insulin receptors and increasing the rate of glycogenesis and diminishing glucose-stimulated insulin secretion [154]. The nanomolar concentration of C-peptide binds exclusively to G protein-coupled receptors with successive activation of Ca^{2+}-dependent intracellular signaling pathways and stimulation of Na^{+}/K^{+}-ATPase activities. Physiological concentrations of C-peptide activate extracellular signal-regulated kinases, phosphatidylinositol 3-kinase (PI3K), and protein kinases C (PKC), elevate intracellular calcium, and stimulate peroxisome proliferator-activated receptor-gamma (PPAR-gamma). C-peptide is reported to alleviate incipient diabetic nephropathy by reducing glomerular hyperfiltration, improving functional reserve, reducing urinary albumin excretion, and preventing glomerular and renal hypertrophy in diabetic rats [155]. C-peptide is a potential marker of pancreatic β-cell destruction and is an index of endogenous insulin secretory capacity [156]. The oral treatment with the newly synthesized complex to diabetic rats elevated the C-peptide level through the activation of remnant pancreatic β-cells. The antidiabetic activity of the complex is associated with an increase in plasma insulin and C-peptide levels, suggesting an insulin mimetic activity of the complex. Thus, the increased levels of insulin and C-peptide observed in the complex-treated rats evidenced that the complex is capable of stimulating insulin secretion from the remnant pancreatic β-cell.

The deficiency and/or inefficiency of insulin may be responsible for the decreased levels of haemoglobin in experimental diabetic rats [157]. The observed decrease in the level of haemoglobin in experimental diabetic rats may be due to the formation of glycosylated hemoglobin, and the restoration of haemoglobin content was observed in diabetic rats treated with the complex as well as metformin for 30 days, evidencing the antihyperglycemic efficacy of the newly synthesized complex.

The marked increase in glycosylated haemoglobin (HbA1c) levels observed in diabetic animals is in accordance with a previous report [158,159]. However, treatment with the Met-Res-Aldehyde complex as well as metformin decreased the elevation of HbA1C, thereby increasing the level of total haemoglobin in diabetic rats. The decrease in HbA1c levels and increase in total hemoglobin levels might be due to improved glycemic control by the Met-Res-Aldehyde complex. Analysis of glycatedhaemoglobin (HbA1c) in blood provides evidence about an individual's average blood glucose levels during the previous two or three months, which is the predicted half-life of red blood cells (RBCs). In the normal 120-day life span of the red blood cell, due to chronic hyperglycemia, a glucose molecule binds with haemoglobin through a non-enzymatic pathway, forming glycosylated hemoglobin. This phenomenon was first identified by Koenig*et al*., (1976) [160]. The HbA1c is now recommended as a standard of care for testing and monitoring diabetes, specifically type 2 diabetes [161]. Extensive studies on HbA1c bring out its clinical significance as a reliable, reproducible, convenient, and non-manipulative biochemical index in assessing the degree of protein glycation and metabolic control as compared to a one-point blood glucose assessment. HbA1c is likely to be a more physiological assessment of glucose intolerance than the artificial conditions of the oral glucose tolerance test, and hence it is considered as a preferential tool for the early diagnosis and prognosis of diabetes mellitus [162].

During chronic hyperglycemia, the excessive blood glucose irreversibly binds to the N-terminal valine of the β-chain of haemoglobin. The process of glycation at other positions, such as lysine on the β-chain or at sites on the α- chain, may be imperative at higher levels of glycation. The percent glycation of haemoglobin also depends on the average age of the erythrocytes in the blood samples, and the percent HbA1c values are higher in older cells [163]. HbA1c determination may also be used as a significant tool to stratify the risk of development of both micro- and macrovascular complications due to persistent hyperglycemia. Each one percent reduction in glycatedhaemoglobin was associated with a significant reduction in diabetes-related secondary complications [164].

Glycosylated haemoglobin is defined as a function of glucose glycation at one or more sites on the haemoglobin molecule and the International Diabetes Federation recommended the diagnosis of diabetes mellitus by assessing HbA1c levels. Extensive studies on HbA1c bring out the importance of HbA1c as a non-manipulative and reliable biochemical parameter in assessing the metabolic control as compared to one-point blood glucose estimation [165]. During persistent hyperglycemia, the excessive glucose present in

the blood irreversibly binds to the N-terminal valine of the β-chain of haemoglobin [166]. However, the process of glycation at other positions, such as lysine on the β-chain or at sites on the α-chain, may be imperative at higher levels of glycation. The formation of HbA1c is a two-step process in which glucose initially binds with the N-terminal valine of the β-chain to form an unstable aldimine, which subsequently undergoes an Amadori rearrangement to form a stable ketoamine. The first reaction is rapid and reversible, while the second step is irreversible. However, there is rapid formation and dissociation of the aldimine dependent upon the ambient glucose concentration and a slower rate of ketoamine formation. The percent glycation of haemoglobin depends on the average age of the erythrocytes in the blood sample, and the percent HbA1c is higher in older cells. HbA1c level represents average glycemia over the entire 120-day lifespan of the red blood cells [167,168].

HbA1c remains in the blood circulation for the rest of the lifetime of erythrocytes. The non-enzymatic, irreversible covalent bonding of excessive glucose present in the circulation with haemoglobin in the blood results in the generation of glycosylated haemoglobin which is a critical parameter for assessing the long-term glycemic control and predicting the incidence of secondary complications of diabetes [169]. HbA1c was introduced into clinical practice in the 1980s and subsequently has become a cornerstone of clinical therapy [170]. The International Diabetes Federation and the American College of Endocrinology recommend HbA1c values below 6.5% as the normal range, while the range recommended by the American Diabetes Association extends to 7%. However, higher than expected values can be observed in people with a lower RBC turnover, such as those with vitamin B12 or folate deficiency, and lower than expected. Since HbA1c reflects the average plasma glucose level maintained over the previous 8 to 12 weeks, it is often considered a vital index in the diagnosis and prognosis of diabetes. Oral administration of the newly synthesized complex significantly decreased the levels of glycosylated haemoglobin, suggesting that it may prevent oxidative damage caused by the glycation reaction in chronic hyperglycemic conditions. These results on the levels of fasting blood glucose and glycosylated haemoglobin indicate the glucose lowering as well as beneficial effects of the complex in preventing the pathogenesis of diabetic complications caused by impaired glucose metabolism.

Urine sugar present in diabetic rats was absent in complex-treated diabetic rats, evidencing the hypoglycemic as well as renoprotective efficacy of the newly synthesized complex. Monitoring glucose in the body is a key component of managing and detecting metabolic conditions, especially diabetes. Sugar (glucose) in urine is medically termed

glucosuria. The consequence of chronic hyperglycemia on the sugar concentrations present in urine has been known since ancient times. Normally, kidneys filter glucose from the blood and reabsorb it back into the bloodstream through renal tubules. However, when blood sugar levels are too high, exceeding the renal threshold (around 180 mg/dL), the kidneys can no longer reabsorb all the glucose, and the excess spills into the urine. Below the renal threshold level of blood glucose, sugar is absent in the urine, while above this level, appreciable amounts of sugar appear in the urine and gradually increase both in total amount and in percentage concentration as the blood sugar levels become progressively higher. Thus, the appearance of glucose in urine is reflected in the concept of renal threshold for glucose excretion. In general, urine sugar concentration defines the level of plasma glucose.

While blood sugar testing is common, checking normal sugar levels in urine also provides valuable insights into your health. Urine glucose testing is a non-invasive and cost-effective method often used as an initial screening tool or to monitor diabetic patients. Glucose in urine can be detected through routine urine tests and is often a sign of uncontrolled blood sugar levels, potentially indicating diabetes or other underlying health issues. The ease of collection and testing makes assessment of urine sugar concentration more acceptable for the physicians than the measurement of the blood glucose concentration. One limitation of urine sugar concentration as an indicator of plasma glucose concentration is the wide range of glucose levels found at each concentration of urine sugar. Hence, multiple urine sugar determinations may be useful for defining the average plasma glucose concentration and thereby be helpful for the general management of diabetes. Glucosuria not only indicates uncontrolled diabetes but also potentially gestational diabetes, kidney diseases (renal glycosuria), severe stress, or certain medications, and it requires prompt medical attention to manage underlying causes like diabetes mellitus, as excess sugar in urine can feed bacteria, thereby causing the risk of urinary tract infections and serious renal disorders.

The effect of oral administration of the newly synthesized metformin-resveratrol aldehyde complex on the activities of pathophysiological enzymes such as aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) in the serumof control and experimental groups of rats are represented in Table 3. The activities of these enzymes in the diabetic group of rats were significantly increased as compared with the control group of rats. However, oral treatment with the complex significantly normalized the altered levels in comparison with the diabetic group of rats. The efficacy of the complex was comparable with diabetic rats treated with metformin.Table 3. The activities of serum transaminases and alkaline phosphates in the control and experimental groups of rats.

Table -3 The activities of serum transaminases and alkaline phosphatase in the control and experimental groups of rats.

GROUPS	AST	ALT	ALP
Control	38.83 ± 1.77[a]	18.18±1.44[a]	84.68±0.86[a]
Diabetic	51.33 ± 2.62[b]	47.52±2.38[b]	155.85±5.66[b]
Diabetic+Met-Res-Aldehyde complex	34.16 ± 1.01[a]	23.53±1.75[a]	99.865±4.58[c]
Diabetic + Metformin	32.16±1. 07[a]	21.71±1.17[a]	101.88±1.00[c]

Enzyme activities are expressed as:AST and ALT: µ moles of pyruvate liberated/h/mgof protein; ALP: µ moles of phenol liberated/min/mg of protein. Results are expressed as mean ± SEM [n = 6]. One-way ANOVA followed by post-hoc test LSD. The results were compared with [a] control rats, [b] diabetic rats. Values arestatistically significant at *P < 0.05.

Aminotransferases such as AST and ALT are cytosolic enzymes that have leaked into the blood circulation and serve as a marker of tissue injury, more specifically hepatocytes, as well as renal injury. The observed decrease in the activities of AST and ALT in the liver and kidney tissues indicates the extensive tissue damage caused by STZ, which is further evidenced by the increased activities of these enzymes in the serum of the diabetic group of rats [171-173]. Further, the elevated levels of these enzymes whose plasma concentration is above the homeostatic limit could be associated with various forms of disorders that affect the functional integrity of the liver and other vital organs [174]. Alkaline phosphatase is a membrane-bound enzyme, and it acts as a marker of biliary function and cholestasis. It is hypothesized that elevations in the activities of enzymes in the serum are considered as predictors of diabetes [175]. Further, elevation in the activity of these enzymes, whose gene transcription is suppressed by insulin, could indicate impairment in the insulin signaling pathway rather than purely tissue injury [176]. Other potential explanations for the observed elevation in the activity of the enzymes in the insulin-resistant state include oxidative stress from reactive lipid peroxidation, peroxisomal β-oxidation, and recruited inflammatory cells. The insulin-resistant state is also characterized by an increase in proinflammatory cytokines such as TNF - α, which may also contribute to hepatocellular injury [177]. However, the diabetic group of rats treated with the newly synthesized complex at a concentration of 5 mg/kg b.w./day for 30 days significantly diminished the activity of these enzymes to their basal levels, suggesting the tissue-protective nature of the complex. Earlier, we reported the tissue-protective nature of resveratrol in streptozotocin-nicotinamide-induced experimental type 2 diabetes in rats [97].

Effect of Met-Res-Aldehyde complex on levels of plasma protein, urea, creatinine and uric acid.

The levels of plasma protein, urea, creatinine, and uric acid are illustrated in Table 4. The induced diabetic rats also showed a significant increase in the urea, uric acid, and creatinine and decreased levels of plasma protein as compared with control rats. It is well known that the absolute or relative lack of insulin secretion leads to defective amino acid/protein metabolism, which may be a more important factor than chronic hyperglycemia in the etiology of diabetic complications [178]. Moreover, the total muscle protein catabolism is due to a net increase in protein breakdown rather than a decline in protein synthesis [179]. The alterations in protein metabolism could indeed be responsible for many of the chronic complications of diabetes, since they may involve both structural and functional proteins. Imbalance between synthesis and catabolism of proteins can have dramatic consequences in the metabolism of many tissues such as gut, skeletal muscle, and heart [180].

Experimentally induced diabetes in a rat model indicates several alterations of amino acid metabolism, which may be attributed to increased muscle proteolysis, reduced protein synthesis, an energy-dependent process in the liver, and stimulated hepatic gluconeogenesis utilizing gluconeogenic amino acids [181]. Dighe*et al.,* (1984) reported that accelerated proteolysis of uncontrolled diabetes occurs as a result of deranged glucagon-mediated regulation of cAMP formation during insulin deficiency [182]. This readily accounts for the observed decreased protein content in the experimental diabetic rats. The observed reduction of the level of total proteins in experimental diabetic rats was attributed to localized damage in the endoplasmic reticulum, which results in the loss of p450, leading to its functional failure with a decrease in protein synthesis. The rise in protein levels in the treated groups suggests the stabilization of endoplasmic reticulum leading to protein synthesis [183]. Administration of the Met-Res-Aldehyde complex may enhance the protein synthesis by stabilizing the structure and functions of the endoplasmic reticulum.

Table-4 Effect of Met-Res- Aldehyde complex on plasma protein, blood urea and serum creatinine and Uric acid in control and experimental groups of rats.

Groups	**Plasma Protein (g/dl)**	**Urea (mg/dl)**	**Serum Creatinine(mg/dl)**	**Uric acid(mg/dl)**
Control	8.16 ± 0.54^{a}	25.5 ± 1.17^{a}	0.89 ± 0.02^{a}	4.36 ± 0.31^{a}
Diabetic	5.33 ± 0.33^{b}	49.66 ± 7.10^{b}	2.11 ± 0.43^{b}	7.61 ± 1.20^{b}
Diabetic + Met-Res -	6.16 ± 0.47^{b}	29.33 ± 0.71^{a}	1.27 ± 0.01^{ab}	3.60 ± 0.41^{a}

Aldehyde complex				
Diabetic + Metformin	7.16 ± 0.54[ab]	32.83 ± 1.40[a]	1.22 ± 0.03[a]	3.55 ± 0.33[a]

Values are presented as mean±standard error of the mean (n=6). One-way Anova followed by post hoc test LSD was done. Results were compared with [a]control rats, [b]diabetic rats. Values are statistically significant at $p<0.05$.

The levels of urea, uric acid, and creatinine were significantly increased in the diabetic group, and treatment with the Met-Res-Aldehyde complex significantly reversed these values to physiological values. A similar effect was observed in the metformin-treated group. Levels of urea, uric acid, and creatinine acid are the key indices in the hyperglycemia-mediated renal dysfunction [184]. Urea and creatinine are nitrogenous end products of metabolism. Urea is the primary metabolite derived from dietary protein and tissue protein turnover. Creatinine is a byproduct of the breakdown of creatine and phosphocreatine, which are considered energy storage compounds in muscles. It is produced in the muscles by the non-enzymatic changes of creatine and phosphocreatinine. The serum creatinine level may vary based on a number of factors, including the diet composition, muscle mass, and gender. An increase in creatinine level usually occurs simultaneously with an increase in blood urea nitrogen [185]. Serum creatinine level concentration is often used as a variable not only to assess the impairment of kidney function but also as a clinical endpoint to detect treatment related to toxic effects of compounds on the kidney in experimental animals [186,187]. The liver has a crucial role in the assembly of creatinine through methylation of guanidine aminoacetic acid [188].

Urea is an organic compound known to play a vital role in the metabolism of nitrogen-containing compounds [189]. Oral treatment with the Met-Res-Aldehyde complex reversed the levels to near normalcy, denoting that it antagonizes metabolic complications like diabetic nephropathy. Uric acid, one of the major endogenous water-soluble antioxidants of the body, has been thought to be a metabolically inert end product of purine metabolism [190]. Elevated levels of serum uric acid are due to either an increase in uric acid production or a decrease in its excretion [191]. Accumulation of purines is the main source for the production of uric acid by the activity of xanthine oxidase. This accumulated purine evidence the increased oxidative stress, which is closely related to diabetes and its vascular complications. Thus, the elevated levels of circulating uric acid level may be an indicator that the body is trying to protect itself from the deleterious effects of free radicals by increasing the products of endogenous antioxidants such as uric acid [192]. The decreased levels of urea, uric acid, and creatinine and increased level of plasma protein are comparable with metformin-treated rats.

Effect of Met-Res-Aldehyde complex supplementation on the activity of carbohydrate metabolizing enzymes in the liver tissue of control and experimental groups of rats.

The activities of (E.C.No - 2.7.1.1), pyruvate kinase (E.C.No - 2.7.1.40), lactate dehydrogenase (E.C.No - 1.1.1.27), glucose-6-phosphatase (E.C.No - 3.1.3.9), fructose-1, 6-bisphosphatase (E.C.No - 3.1.3.11) and glucose-6-phosphate dehydrogenase(E.C.No - 1.1.149) in liver tissue of control and experimental groups of rats were presented in Tables 5 and 6, respectively. A significant alteration in the activity of these enzymes was observed in the liver tissues of experimental diabetic rats when compared to the control group of rats. However, upon treatment with the Met-Res-Aldehyde complex in diabetic rats, the activities of these enzymes were reversed to normal, and the efficacy was similar to that of metformin-treated rats (Table 5).

Table -5 Effect of Met-Res-Aldehyde complexon the activities of Hexokinase, Pyruvate kinase and Lactatedehydrogenase in the liver tissue of control and experimental groups of rats.

Groups	Hexokinase	Pyruvate Kinase	Lactate Dehydrogenase
Control	255.83 ± 2.08[a]	208.16 ±6.96[a]	236.5 ± 14.12[a]
Diabetic	139.33 ± 2.96[b]	139.83 ±24.40[b]	417.83 ± 77.83[b]
Diabetic+Met-Res - Aldehyde complex	205.33 ±1.25[a]	180.33 ±5.94[ab]	310.5 ± 3.54[ab]
Diabetic + Metformin	216.83 ±5.74[a]	187 ± 2.29[ab]	292.83 ± 4.23[ab]

Units are expressed as: µmoles of glucose-6-phosphate formed/h/mg of protein for hexokinase, mU/mg of protein for pyruvate kinase. Values are presented as mean ± standard error of the mean (n=6). One-way Anova followed by post hoc test LSD was done. Results were compared with [a]control rats, [b]dibeticrats.Values are statistically significant at $p<0.05$.

Diabetes mellitus due to insufficient secretion and/or resistance to insulin action causes disturbances in the uptake of glucose and its utilization in a number of ways. The glycolytic pathway is the initiation step of glucose oxidation to generate energy in the form of ATP, and the key enzymes involved in the glucose metabolism are hexokinase, pyruvate kinase, lactate dehydrogenase, glucose-6-phosphatase, glucose-6-phosphate dehydrogenase, and fructose-1, 6-bisphosphatase. In diabetes, impairment of function occurs at various stages of glucose metabolism, which includes intestinal absorption, intracellular transport, decreased efficiency of the glycolytic pathway, the tricarboxylic acid cycle, the hexose monophosphate (HMP) shunt, and the glycogen synthetic and degradation pathways [193]. Also, glucose synthesis from gluconeogenesis enhances the level of blood glucose in diabetic patients. The

activities of hexokinase, pyruvate kinase, and glucose-6-phosphate dehydrogenase were significantly diminished, and the activities of lactate dehydrogenase, glucose-6-phosphatase, and fructose-1.6-bisphosphatase were significantly increased in the liver and kidney tissues of HFD fed- low-dose STZ-induced diabetic rats. Treatment with the newly synthesized complex to a diabetic group of rats, similar to metformin, significantly regulated the altered activities of these enzymes to near normalcy in liver and kidney tissues.

The liver is the primary site of endogenous glucose production [194] with a minor contribution from the kidney [195] and produces glucose by gluconeogenesis as well as glycogenolysis. Elevated levels of endogenous glucose production are a common abnormality associated with diabetes that, in concurrence with deprived pancreatic function and reduced glucose clearance; contribute to chronic hyperglycemia, a characteristic feature of diabetes. Insulin regulates the metabolism by modulating the uptake and utilization of blood glucose in target tissues such as liver, skeletal muscle, and adipose tissue by modulating the activities of several metabolic enzymes. Deng *et al*., (2008) reported that resveratrol improves muscular glucose uptake via both insulin-dependent and non-insulin-dependent pathways [196].

Glycolysis, the major metabolic pathway, utilizes glucose as an energy source in cells. The first step in the sequence of reactions of glycolysis is the phosphorylation of glucose to prepare it for further breakdown in order to play a crucial function in tissue intermediary metabolism. The vital reason for the immediate phosphorylation of glucose is to prevent diffusion out of the cell. This conversion is catalyzed by the enzyme hexokinase. This enzyme catalyzed the addition of a charged phosphate group (phosphorylation) to glucose to form glucose-6-phosphate in the presence of Mg^{2+} and ATP. Hexokinases have been found in every organism tested, ranging from bacteria, yeast, plants, and vertebrates, including human beings. There are four important mammalian hexokinase isoenzymes designated as hexokinase I, II, III, and IV, or hexokinases A, B, C, and D, that vary in their sub-cellular locations and substrate specificity. The molecular weight of the hexokinases was found to be approximately 100 KD [197].

Each isoenzyme has a common ATP binding site core surrounded by variable sequences that determine substrate specificity/affinity and other properties. The mammalian hexokinase IV, also referred to as glucokinase, has unique characteristics and functions compared to other hexokinases. It plays an important regulatory role in carbohydrate metabolism in the liver, pancreas, brain, and intestine. In the pancreatic β-cell, the enzyme glucokinase serves as a glucose sensor to control the release of insulin and glucagon. Reports on animal models and isolated hepatocytes established that hepatic hexokinase exerts a strong

impact on glucose utilization and controls the process of glycogenesis [198] and their levels are very low in both human and rodent diabetes. Insulin administration rapidly reinstates hexokinase activity in the hepatocytes. Because of these observations, restoration of hepatic hexokinase activity provides a possible therapeutic strategy for diabetes treatment. Hexokinases I, II, and III have a high affinity for glucose and are feedback-inhibited by physiologic concentrations of glucose-6-phosphate, whereas glucokinase (hexokinase IV or glucokinase), the major glucose phosphorylating enzyme, has a lower affinity for glucose, and its abundance is regulated transcriptionally by insulin and glucagon and post-translationally by the glucokinase regulatory protein [199]. Hexokinase has high affinity for glucose even at low concentrations (below 1 mM) and glycogen synthesis, whereas hexokinase IV, or glucokinase, can only phosphorylate glucose, if the concentration of this substrate is high enough; its Km for glucose is 100 times higher than hexokinase I, II and III [200].

The modulation of hexokinase activity, the prime enzyme for maintaining normoglycemia, occurs due to the changes in the amount of enzyme present within the cell. This may be the reason for diminished utilization of glucose in the system and increased amount of glucose in the circulation. Primary cultures of rat hepatocytes have exhibited the induction of hexokinase activity by insulin, which is the result of an increase of synthesis of the enzyme [201]. In STZ-induced diabetic rats, the low level of hexokinase synthesis and its activity were correlated with low levels of mRNA coding for hexokinase, and administration of insulin increases translatable hexokinase mRNA, the rate of synthesis, and the activity of enzymes [202]. Hence, a possible explanation of observed enhanced activity of hexokinase is that the newly synthesized complex treatment may activate the mRNA coding for hexokinase in diabetic rats. The markedly decreased level of insulin in the experimentally induced diabetic group of rats ultimately leads to impairment in the activity of hexokinase, since insulin deficiency is a hallmark of diabetes [203]. However, the modest increase in the activity of hexokinase as observed in the diabetic rats treated with the newly synthesized complex suggests the protection of the hepatic and extrahepatic tissues against STZ-induced selective pancreatic β-cell toxicity by stimulating insulin secretion from the remnant β-cells. The data obtained also provide evidence that a modest augmentation of hexokinase activity in the liver and kidney tissues enhances glucose metabolism and promotes overall glucose homeostasis in complex treated diabetic rats and the efficacy was comparable with metformin treatment.

Pyruvate kinase (PK) is a ubiquitously expressed, rate-controlling, key glycolytic enzyme that catalyzes the final irreversible conversion of phosphoenol pyruvate to pyruvate

with the release of ATP. The product, pyruvate, feeds into a number of metabolic pathways, such as the TCA cycle and gluconeogenesis, which place pyruvate kinase at a primary intersection. Johnson *et al.,* (1979) first reported the crystalline structure of PK, isolated from cat muscle [204]. Four distinct isoenzymes are distinguished in vertebrate tissues: M1 (in skeletal muscle), M2 (in kidney, adipose tissue, and lungs), L (in liver), and R (in red blood cells). Each of these isoenzymes has different kinetic properties reflecting the different metabolic requirements of the tissues [205]. Several physiological activators control the activity of PK, which essentially includes H^+, K^+, Mn^{2+}, and Mg^{2+} [206].

PK is regulated by its own substrate, phosphoenol pyruvate, and fructose-1, 6-bisphosphate, and an intermediate in the glycolytic pathway, which both up-regulate pyruvate kinase. In doing so, it drives glycolysis to operate faster when more substrate is available [207]. It has also been reported that nutrients themselves play an important role in the regulation of PK activity. PK and G6PDH deficiencies are approximately equally common and together constitute most of the cases of the human hereditary disorders, including non-spherocytichaemolytic anemia due to erythroenzymopathies [208]. High fat diet feeding decreased the activity of PK, while a high-sucrose diet increased it *in vivo* and *in vitro* through independent acute hormone actions [209,210]. PK isoenzymes in body fluids can be quantitatively assayed [211]. In all eukaryotes, PK is cytosolic, and almost all organisms have at least one PK gene and one enzyme [212]. The decrease in the activity of PK in diabetic conditions alters the glucose metabolism and ATP production, which might be promptly responsible for the reduced glycolysis and amplified gluconeogenesis [213]. Oral administration of the Met-Res-Aldehyde complex to the diabetic rats showed a notable increase in the PK activity suggesting the regulatory role of the complex in ameliorating chronic hyperglycemia.

The observed decrease in the activity of PK in the liver and kidney tissues of experimental diabetic rats readily accounts for the decreased utilization of glucose (glycolysis) and increased production of glucose (gluconeogenesis) by the liver and kidney, indicating that these two pathways are altered in diabetes. Gluconeogenesis and glycolysis are coordinated so that one pathway is relatively active, while the other is highly inactive [214]. Likewise, when gluconeogenesis is stimulated, as in starvation, glycolysis must be suppressed in liver and kidney tissues [215]. The existence of multiple isoenzymes of PK facilitates these demands [216]. The effect of insulin on the activity of PK has been investigated in several studies. The total liver PK activity was found to be decreased by 40% when rats were rendered diabetic and increased back to normal values within 1 or 2 days of

insulin therapy [217]. An increase in rat liver PK activity was observed within 10 minutes after the administration of insulin via the portal vein [218].

The observed increase in the activity of PK in the liver and kidney tissues of newly synthesized complex-treated diabetic rats is probably the result of an altered endocrine status in addition to any possible direct effect of the complex on the PK activity in these cells. However, on the basis of previous reports, it is assumed that the PK activity is accelerated by the complex at least partially by facilitating the glycolytic pathway and suppression of gluconeogenesis via its insulin mimetic actions [98].

Lactate dehydrogenase is a terminal glycolytic enzyme that facilitates the interconversion of pyruvate to lactate to yield energy under anaerobic conditions [219] and the interconversion reaction occurs in both cytosolic and mitochondrial compartments [220]. This enzyme is a tetramer composed of two different kinds of subunits: M-muscle type and H-heart type. The biosynthesis of each of these subunits is apparently controlled by separate genes. LDH activity is altered by glucose, insulin, and NADH, as well as increases in mitochondrial membrane potential, cytosolic free ATP, and cytosolic free calcium ions [221]. The decreased activity of LDH in tissues could be important to ensure that a high proportion of both pyruvate and NADH supplied by the glycolytic pathway is subsequently oxidized by mitochondria. Indeed, the elevated levels of LDH are observed in experimental diabetic animals, which are attributed to impaired glucose-stimulated insulin secretion [222]. Thus, the normal glucose metabolism and insulin secretion in T cells are perturbed with the increased activity of LDH, and it therefore may be directly responsible for insulin secretory defects in diabetes. Oral administration of the Met-Res-Aldehyde complex as well as metformin to diabetic groups of rats showed a significant reduction in the LDH activity, probably due to the regulation of the NAD+/NADH ratio by the oxidation of glucose.

The activities of glucose-6-phosphatase and fructose 1, 6-bis phosphatase were significantly increased in diabetic rats when compared to control rats. The activity of glucose-6-phosphate dehydrogenase was significantly decreased. However, upon treatment with the Met-Res-Aldehyde complex in diabetic rats, the activities of these hepatic enzymes reverted to normalcy and the effect was comparable to metformin-treated rats (Table -6).

Table – 6 Effect of Met-Res- Aldehyde complex on the levels of glu-6-phosphatase, fructose-1,6-bisphosphatase and glucose- 6-phosphate dehydrogenase in the control and experimental groups of rats.

Groups	**Glucose-6-phosphatase**	**Fructose - 1, 6 - bisphosphatase**	**Glucose-6-phosphate dehydrogenase**
Control	1053.33 ± 20.50[a]	446.16 ± 25.93[a]	512.83 ± 43.80[a]

Diabetic	1841.83 ± 236.96[b]	809.16± 123.44[b]	244.83 ± 48.93[b]
Diabetic+Met-Res-Aldehyde complex	1210 ± 21.64[a]	513.66 ± 3.41[a]	395.5 ± 46.83[ab]
Diabetic + Metformin	1126.66 ± 6.85[a]	523.33 ± 8.53[a]	425 ± 7.30[a]

Units are expressed as: µmol of pi liberated/h/mg of protein for glucose-6-phosphatase and fructose-1,6-bisphosphatase and µmol of NADPH/min/mg of protein for glucose-6-phosphate dehydrogenase. Values are presented as mean ± standard error of the mean (n=6). One-way Anova followed by post hoc test LSD was done. Results were compared with [a]control rats, [b]dibetic rats. Values are statistically significant at $p<0.05$.

Glucose-6-phosphatase is a crucial gluconeogenic enzyme in the homeostatic regulation of blood glucose and is critical in providing glucose to other organs during diabetes, prolonged fasting, or starvation [223,224]. It catalyzes the dephosphorylation of glucose-6-phosphate to free glucose as the terminal step in gluconeogenesis and glycogenolysis. This reaction occurs in the lumen of the endoplasmic reticulum, and the enzyme complex is composed of a glucose-6-phosphate catalytic subunit that hydrolyzes the glucose-6-phosphate to glucose and phosphate [225]. The activity of glucose-6-phosphatase is stimulated by cAMP and repressed by insulin, and its diminished activity leads to severe metabolic disorders mainly characterized by hypoglycemia. Van Schaftingen and Gerin (2002) demonstrated that glucose-6-phosphatase activity impairs hepatic glucose utilization while simultaneously enhancing hepatic glucose production [226]. Insulin deficiency achieved in experimental diabetic rats enhances glucose-6-phosphatase activity in both liver and kidney tissues, and the activity is reverted back to normal level after treatment with the synthesized complex.

Fructose-1, 6-bisphosphatase is a highly regulated, rate-limiting enzyme that catalyzes the dephosphorylation of fructose-1, 6-biphosphate to fructose-6-phosphate, the second to last step in the gluconeogenic pathway [224]. Its activity is abundant in the liver and kidney tissues but is scantily expressed in the pancreatic β-cells under normal conditions and appears as a target for efficient and safe glycaemic control in diabetes [227]. The increased activity of fructose-1,6-bisphosphatase in the hepatic and renal tissues of HFD-STZ-induced diabetic rats was suggested as a possible mechanism for the production of increased endogenous glucose after it was shown that diabetics have an increase in gluconeogenesis from glycerol [228,229].Activation of gluconeogenic enzymes is due to the state of insulin deficiency and availability of a surplus of gluconeogenic substrates [140] [Chen *et al*., 2000] In our study, oral administration of the metformin-resveratrol aldehyde complex diminishes the activities of

fructose-1, 6-bisphosphatase in HFD-STZ-induced diabetic rats. The reduction in the activities of glucose-6-phosphatase and fructose-1, 6-bisphosphatase can lead to decreased gluconeogenesis, thereby reducing the endogenous production of glucose.

Glucose-6-phosphate dehydrogenase (G6PDH) an archetypical X-linked "housekeeping" enzyme, catalyzes the first and rate-limiting step in the pentose phosphate pathway, which results in the production of ribose-5-phosphate and NADPH, which are needed for the maintenance of reduced glutathione and reductive biosynthesis [230]. The activity of glucose-6-phosphate dehydrogenase is found to be decreased in diabetic conditions [231]. Oral administration of the Met-Res-Aldehyde complex to HFD fed- low dose STZ-induced diabetic rats significantly increased the activity of glucose-6-phosphate dehydrogenase. Earlier reports have demonstrated that NADPH produced by G6PDH is essential for both the generation of reactive oxygen species such as superoxide anions and nitric oxide and the elimination of these radicals via glutathione peroxidase and catalase in hepatic as well as extrahepatic tissues [232]. The activity of G6PDH is also regulated through alternative splicing [233] in response to hormonal and nutritional cues such as glucose and lipids [234]. Increased expression of G6PDH has been associated with increasing glutathione levels and resistance to oxidative stress [235]. Notably, it has also been shown that modest changes in G6PDH activity itself have significant effects on cell growth and death in a variety of cell types [236]. However, the observed decrease in the activity of G6PDH in experimental diabetic rats might also suggest a decrease in metabolism via a phosphogluconate oxidation pathway [237].This is consistent with previously published data in which G6PDH activity has also been observed in liver and kidney tissues of diabetic rats [238,239]. Further, the present study suggests that decreased G6PDH activity is of significance in the pathogenesis of diabetic complications, and the activity of G6PDH is regulated to near normalcy by the treatment with the newly synthesized complex.

Table -7 represents' the effect of Met-Res-Aldehyde complex treatment on the level of glycogen and the activities of glycogen synthase and glycogen phosphorylase in the liver of control and experimental groups of rats. A significant decrease in the glycogen content as well as in the glycogen synthase activity and a concomitant increase in the activity of glycogen phosphorylase were observed in the liver of HFD-STZ-induced diabetic rats as compared with control rats. Treatment with reinstated the glycogen level and the activities of glycogen synthase and glycogen phosphorylase were significantly improved to near physiological levels when compared to the control group of rats. The effect was comparable to metformin treatment.

Table – 7 Effect of Met- Res - Aldehyde complex on the levels of Glycogen, Glycogen synthase and Glycogen phosphorylase in the control and experimental groups of rats.

Groups	Glycogen	Glycogen synthase	Glycogen phosphorylase
Control	59.5 ± 7.91[a]	791.16 ± 79.58[a]	601.16 ± 10.89[a]
Diabetic	27.5 ± 6.17[b]	525.83 ± 43.97[b]	837 ± 68.61[b]
Diabetic+Met-Res-Aldehyde complex	47.83 ± 3.00[ab]	714.5 ± 21.39[a]	668.66 ± 16.16[a]
Diabetic + Metformin	49.5 ± 1.17[a]	735.33 ± 16.57[a]	681 ± 7.97[a]

Units are expressed as: µmol of UDP formed/h/mg protein for glycogen synthase and µmol pi liberated/h/mg protein for glycogen phosphorylase. Values are presented as mean ± standard error of the mean (n=6). One-way Anova followed by post hoc test LSD was done. Results were compared with [a]control rats, [b]dibetic rats. Values are statistically significant at $p<0.05$.

Glycogen is the primary intracellular storage form of glucose for virtually every organism from yeast to primates, and its levels in various tissues are a direct reflection of insulin activity, as insulin promotes intracellular glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase [240]. Glycogen forms an energy reserve that can be quickly mobilized to meet an instant need for glucose but one that is less compact than the energy reserves of triglycerides. Under classical definitions, the functions of glycogen seem obvious: muscle glycogen is degraded to generate ATP during increased energy demand, whereas hepatic glycogen is broken down for the release of glucose into the bloodstream to supply other tissues [241]. The liver plays a central role in the maintenance of blood glucose homeostasis. Glycogen metabolism in hepatic tissue is one of the major metabolic processes involved in glucose homeostasis. It has been well established that the balance between the levels of insulin and glucagon is vital for this regulation. Glycogen synthase, a crucial and rate-limiting enzyme in tissues with non-oxidative glucose disposal, catalyzes the conversion of glucose-1-phosphate to glycogen in animal cells [242]. There are two mammalian isoforms of glycogen synthase. One appears to be expressed only in the liver tissue, while the second is expressed in skeletal and cardiac muscles as well as adipose tissue, kidney, and brain. The activity of glycogen synthase is regulated by decreased cellular glycogen content, hormone signaling, sub-cellular localization, targeting of phosphatase, and allosteric activation by glucose-6-phosphate [243].

Glycogen phosphorylase, a rate-limiting enzyme of glycogenolysis, cleaves alpha (1 to 4) linkages to remove glucose molecules from the glycogen. This enzyme exists as a dimer with each subunit linked to the essential cofactor pyridoxal phosphate, which donates the phosphate as

an electron donor for the release of glucose-1-phosphate [241]. Its activity is regulated by phosphorylation and by allosteric binding of AMP, ATP, glucose-6-phosphate, and glucose [244]. Since streptozotocin causes selective destruction of pancreatic β-cells, resulting in an apparent decline in insulin levels, it is responsible for the decreased glycogen levels in major storage tissues such as liver, kidney, and skeletal muscle, as they depend on insulin for the entry of glucose into the cells for energy generation. During diabetic conditions, the glycogen content, glycogen synthase activity, and responsiveness to insulin signaling are diminished, and glycogen phosphorylase activity is significantly increased. The observed significant increase of liver glycogen level in the Met-Res-Aldehyde complex-treated diabetic groups may be due to reactivation of the glycogen synthase system. Similarly, the decreased activity of glycogen phosphorylase showed that the Met-Res-Aldehyde complex and metformin treatment produce more insulin from the remnant pancreatic β-cells and increase glycogen content in the liver. The efficacy was comparable with diabetic rats treated with metformin alone. Earlier, we reported that the oral administration of resveratrol at a concentration of 5 mg/kg b.w./rat/day for a period of 30 days to streptozotocin-nicotinamide-induced experimental type 2 diabetic rats modulated the activity of carbohydrate and glycogen metabolizing enzymes by stimulating the remnants of β-cells to secrete more insulin, thereby normalizing the altered glycogen content in the liver [97,98].The experimental results obtained indicate that the Met-Res-Aldehyde complex has considerable antidiabetic activity and is capable of maintaining the liver glycogen level.

Conclusion

Based on the results obtained from this methodical study, it can be concluded that oral administration of the metformin-resveratrol aldehyde complex at a concentration of 5 mg/kg body weight/rat/day for 30 days significantly reduces the blood glucose levels in high fat diet fed-low-dose-streptozotocin-induced experimental type 2 diabetic rats, which could be due to improvement in insulin secretion and/or insulin resistance. Oral treatment with the newly synthesized complex restored the activities of key enzymes involved in the metabolism of glucose and glycogen. Therefore, it is concluded that the Met-Res-Aldehyde complex is non-toxic and has complementary potency to develop as an antihyperglycemic agent for the treatment of diabetes mellitus and its secondary complications.

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REFEENCES

1. King LS. What is disease? Philosophy of science. 1954 Jul;21(3):193-203.
2. Alwan A, MacLean DR. A review of non-communicable disease in low-and middle-income countries. International health. 2009 Sep 1; 1(1):3-9.
3. Asogwa OA, Boateng D, Marzà-Florensa A, Peters S, Levitt N, van Olmen J, Klipstein-Grobusch K. Multimorbidity of non-communicable diseases in low-income and middle-income countries: a systematic review and meta-analysis. BMJ open. 2022 Jan 1; 12(1):e049133.
4. Bennett JE, Kontis V, Mathers CD, Guillot M, Rehm J, Chalkidou K, Kengne AP, Carrillo-Larco RM, Bawah AA, Dain K, Varghese C. NCD Countdown 2030: pathways to achieving Sustainable Development Goal target 3.4. The Lancet. 2020 Sep 26; 396(10255):918-34.
5. Bates G, Newberry P, McClatchey R, Newman J, Ayres S. How can health be further integrated in urban development policymaking in the United Kingdom? A systems mapping approach. Health Research Policy and Systems. 2025 Jul 29; 23(1):96.
6. Sobry A, Kizito W, Van den Bergh R, Tayler-Smith K, Isaakidis P, Cheti E, Kosgei RJ, Vandenbulcke A, Ndegwa Z, Reid T. Caseload, management and treatment outcomes of patients with hypertension and/or diabetes mellitus in a primary health care programme in an informal setting. Tropical Medicine & International Health. 2014 Jan; 19(1):47-57.
7. Bhattacharya S, Heidler P, Varshney S. Incorporating neglected non-communicable diseases into the national health program—A review. Frontiers in public health. 2023 Jan 10; 10:1093170.
8. Chen H, Chen G, Zheng X, Guo Y. Contribution of specific diseases and injuries to changes in health adjusted life expectancy in 187 countries from 1990 to 2013: retrospective observational study. bmj. 2019 Mar 27; 364.
9. Xi JY, Zhang WJ, Chen Z, Zhang YT, Chen LC, Zhang YQ, Lin X, Hao YT. Potential gains in health-adjusted life expectancy by reducing burden of noncommunicable diseases in 188 countries: a population-based study. Value in Health. 2023 Jun 1; 26(6):802-9.
10. Goswami N. A dual burden dilemma: Navigating the global impact of communicable and non-communicable diseases and the way forward. International Journal of Medical Research. 2024 Jul 1; 12(3):65-77.
11. Irandoust K, Daroudi R, Tajvar M, Yaseri M. Global and regional impact of health determinants on life expectancy and health-adjusted life expectancy, 2000–2018: an econometric analysis based on the Global Burden of Disease study 2019. Frontiers in Public Health. 2025 Apr 17; 13:1566469.
12. Chakraborty A, Bhattacharyya M. Diabetes, hypertension and cardiovascular disease-an unsolved enigma. Phytotherapy in the Management of Diabetes and Hypertension. 2012 Nov 15:97-130.
13. Mauricio D, Gratacòs M, Franch-Nadal J. Managing diabetes across female reproductive stages. Trends in Endocrinology & Metabolism. 2025 Mar 14

14. Reaven GM. Insulin resistance and compensatory hyperinsulinemia: role in hypertension, dyslipidemia, and coronary heart disease. American heart journal. 1991 Apr 1; 121(4):1283-8.
15. Zhao X, An X, Yang C, Sun W, Ji H, Lian F. The crucial role and mechanism of insulin resistance in metabolic disease. Frontiers in endocrinology. 2023 Mar 28; 14:1149239.
16. Mader JK, Wong JC, Freckmann G, Garcia-Tirado J, Hirsch IB, Johnson SB, Kerr D, Kim SH, Lal R, Montaser E, O'Donnell H. The use of continuous glucose monitoring to diagnose stage 2 type 1 diabetes. Journal of Diabetes Science and Technology. 2025 Jul; 19(4):1109-27.
17. Harris MI. Classification, diagnostic criteria, and screening for diabetes. Diabetes in America. 1995; 2:15-36.
18. McCance DR, Hanson RL, Pettitt DJ, Bennett PH, Hadden DR, Knowler WC. Diagnosing diabetes mellitus–do we need new criteria?. Diabetologia. 1997 Feb; 40:247-55.
19. Xue X, Li J, Zheng W, Zhang B, Wang S, Zhang J, Yang Z. Contributions of 2-h post-load glucose, fasting blood glucose and glycosylated haemoglobin elevations to the prevalence of diabetes and pre-diabetes in adults: A systematic analysis of global data. Diabetes, Obesity and Metabolism. 2025 Dec;27(12):7285-98
20. Al-hashimi H, Merie G, Mustafa M. The Relationship between HbA1c and Diabetes. Pharaonic Journal of Science. 2025 Jun 23;1(1):46-60
21. Kaiafa G, Veneti S, Polychronopoulos G, Pilalas D, Daios S, Kanellos I, Didangelos T, Pagoni S, Savopoulos C. Is HbA1c an ideal biomarker of well-controlled diabetes?. Postgraduate Medical Journal. 2021 Jun; 97(1148):380-3.
22. Deng L, Jia L, Wu XL, Cheng M. Association Between Body Mass Index and Glycemic Control in Type 2 Diabetes Mellitus: A Cross-Sectional Study. Diabetes, Metabolic Syndrome and Obesity. 2025 Dec 31:555-63.
23. Donnelly R, Emslie-Smith AM, Gardner ID, Morris AD. Vascular complications of diabetes. Bmj. 2000 Apr 15; 320(7241):1062-6.
24. Zakir M, Ahuja N, Surksha MA, Sachdev R, Kalariya Y, Nasir M, Kashif M, Shahzeen F, Tayyab A, moazzam Khan MS, Junejo M. Cardiovascular complications of diabetes: from microvascular to macrovascular pathways. Cureus. 2023 Sep 24; 15(9).
25. Ralapanawa DM, Jayawickreme KP, Ekanayake EM. Fibrocalculous pancreatic diabetes: a case report. BMC Research Notes. 2015 Dec; 8:1-4.
26. Nakhleh A, Shehadeh N. Hypoglycemia in diabetes: An update on pathophysiology, treatment, and prevention. World journal of diabetes. 2021 Dec 15; 12(12):2036.
27. Greene DA. Acute and chronic complications of diabetes mellitus in older patients. The American journal of medicine. 1986 May 16;80(5):39-53.
28. Nathan DM. Long-term complications of diabetes mellitus. New England journal of medicine. 1993 Jun 10; 328(23):1676-85.
29. Babu PV, Liu D, Gilbert ER. Recent advances in understanding the anti-diabetic actions of dietary flavonoids. The Journal of nutritional biochemistry. 2013 Nov 1; 24(11):1777-89.
30. Martiniakova M, Sarocka A, Penzes N, Biro R, Kovacova V, Mondockova V, Sevcikova A, Ciernikova S, Omelka R. Protective Role of Dietary Polyphenols in the Management and Treatment of Type 2 Diabetes Mellitus. Nutrients. 2025 Jan 13; 17(2):275.
31. Drzewoski J, Hanefeld M. The current and potential therapeutic use of metformin—the good old drug. Pharmaceuticals. 2021 Feb 5; 14(2):122.

32. Chikhale HU, Rishipathak DD. In Silico Prediction, Molecular Docking Study for Identification of Novel Nitrogen Substituted Benzoxazole Derivative for Their Potential Biological Activity. Chemistry Africa. 2025 Feb 13:1-3.
33. Di Magno L, Di Pastena F, Bordone R, Coni S, Canettieri G. The mechanism of action of biguanides: New answers to a complex question. Cancers. 2022 Jun 30; 14(13):3220.
34. Ramadori GP. Synthalin, Buformin, Phenformin, and Metformin: A Century of Intestinal "Glucose Excretion" as Oral Antidiabetic Strategy in Overweight/Obese Patients. Livers. 2025 Jul 31; 5(3):35.
35. Sterne J. The present state of knowledge on the mode of action of the antidiabetic diguanides. Metabolism. 1964 Sep 1; 13(9):791-8.
36. Winer N, Sowers JR. Epidemiology of diabetes. J Clin Pharmacol. 2004; 44:397–405.
37. Davies MJ, Aroda VR, Collins BS, Gabbay RA, Green J, Maruthur NM, Rosas SE, Del Prato S, Mathieu C, Mingrone G, Rossing P. Management of hyperglycaemia in type 2 diabetes, 2022. A consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Diabetologia. 2022 Dec; 65(12):1925-66.
38. Lucis OJ. The status of metformin in Canada. Canadian Medical Association Journal. 1983 Jan 1; 128(1):24.
39. Chow CK, Ramasundarahettige C, Hu W, AlHabib KF, Avezum A, Cheng X, Chifamba J, Dagenais G, Dans A, Egbujie BA, Gupta R. Availability and affordability of essential medicines for diabetes across high-income, middle-income, and low-income countries: a prospective epidemiological study. The lancet Diabetes & endocrinology. 2018 Oct 1; 6(10):798-808.
40. Millard C, Kadam AB, Mahajan R, Pollock AM, Brhlikova P. Availability of brands of six essential medicines in 124 pharmacies in Maharashtra. Journal of global health. 2018 Jan 23; 8(1):010402.
41. Foretz M, Viollet B. New hopes for metformin-Towards a better understanding of its mechanisms of action. Médecine/sciences. 2014 Oct 1; 30(1):82-92.
42. Fryer LG, Parbu-Patel A, Carling D. The anti-diabetic drugs rosiglitazone and metformin stimulate AMP-activated protein kinase through distinct signaling pathways. Journal of Biological Chemistry. 2002 Jul 12; 277(28):25226-32.
43. Riachi R, Khalife E, Kędzia A, Niechciał E. Understanding Insulin Actions Beyond Glycemic Control: A Narrative Review. Journal of Clinical Medicine. 2025 Jul 16; 14(14):5039.
44. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes research and clinical practice. 2010 Jan 1; 87(1):4-14.
45. Vidon N, Chaussade S, Noel M, Metformin in the digestive tract. Diabetes Res Clin Pract. 1988;4:223–. Scheen AJ. Clinical pharmacokinetics of metformin. Clin Pharmacokinet.
46. Pentikäinen PJ, Neuvonen PJ, Penttilä A. Pharmacokinetics of metformin after intravenous and oral administration to man. Eur J Clin Pharmacol. 1979; 16:195–202.
47. Tucker GT, Casey C, Phillips PJ, Metformin kinetics in healthy subjects and patients with diabetes mellitus. Br J Clin Pharmacol. 1981; 12:235–46.
48. Scheen AJ. Clinical pharmacokinetics of metformin. Clin Pharmacokinet.1996;30:359–71
49. Çevik UA, Işik A, Karakaya A. ADMET and Physicochemical Assessments in Drug Design. Computational Methods for Rational Drug Design. 2025 Jan 20:123-51.

50. Padrini R. Clinical pharmacokinetics and pharmacodynamics of direct oral anticoagulants in patients with renal failure. European journal of drug metabolism and pharmacokinetics. 2019 Feb 11; 44(1):1-2.
51. Roberts JA, Pea F, Lipman J. The clinical relevance of plasma protein binding changes. Clinical pharmacokinetics. 2013 Jan; 52(1):1-8.
52. Çevik UA, Işik A, Karakaya A. ADMET and Physicochemical Assessments in Drug Design. Computational Methods for Rational Drug Design. 2025 Jan 20:123-51.
53. Wildasin EM, Skaar DJ, Kirchain WR, Hulse M. Metformin, a promising oral antihyperglycemic for the treatment of noninsulin-dependent diabetes mellitus. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy. 1997 Jan 2; 17(1):62-73..
54. Papanas N, Maltezos E. Metformin: a review of its use in the treatment of type 2 diabetes. Clinical Medicine. Therapeutics. 2009 Jan; 1:CMT-S1085.
55. Kinaan M, Ding H, Triggle CR. Metformin: an old drug for the treatment of diabetes but a new drug for the protection of the endothelium. Medical principles and practice. 2015 May 28; 24(5):401-15.
56. Bailey CJ. Metformin: Therapeutic profile in the treatment of type 2 diabetes. Diabetes, Obesity and Metabolism. 2024 Aug; 26:3-19.
57. McCreight LJ, Bailey CJ, Pearson ER. Metformin and the gastrointestinal tract. Diabetologia. 2016 Mar; 59(3):426-35.
58. Henry RR, Frias JP, Walsh B, Skare S, Hemming J, Burns C, Bicsak TA, Baron A, Fineman M. Improved glycemic control with minimal systemic metformin exposure: Effects of Metformin Delayed-Release (Metformin DR) targeting the lower bowel over 16 weeks in a randomized trial in subjects with type 2 diabetes. PloS one. 2018 Sep 25; 13(9):e0203946.
59. Szymczak-Pajor I, Wenclewska S, Śliwińska A. Metabolic action of metformin. Pharmaceuticals. 2022 Jun 30; 15(7):810.
60. Chen B, Li T, Wu Y, Song L, Wang Y, Bian Y, Qiu Y, Yang Z. Lipotoxicity: A New Perspective in Type 2 Diabetes Mellitus. Diabetes, Metabolic Syndrome and Obesity. 2025 Dec 31:1223-37.
61. Hundal RS, Krssak M, Dufour S, Laurent D, Lebon V, Chandramouli V, Inzucchi SE, Schumann WC, Petersen KF, Landau BR, Shulman GI. Mechanism by which metformin reduces glucose production in type 2 diabetes. Diabetes. 2000 Dec 1; 49(12):2063-9.
62. Johnson AB, Webster JM, Sum CF, The impact of metformin therapy on hepatic glucose production and skeletal muscle glycogen synthase activity in overweight type II diabetic patients. Metabolism. 1993;42(9):1217–1222. Doi: 10.1016/0026-0495(93)90284-u.
63. LaMoia TE, Shulman GI. Cellular and molecular mechanisms of metformin action. Endocr Rev. 2021; 42(1):77–96. doi:10.1210/endrev/ bnaa023.
64. Consoli A, Nurjhan N. Contribution of gluconeogenesis to overall glucose output in diabetic and nondiabetic men. Ann Med. 1990; 22 (3):191–195. Doi: 10.3109/07853899009147268 28.
65. Zhou G, Myers R, Li Y, Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Invest. 2001; 108(8):1167–1174. Doi: 10.1172/jci1350535.
66. Bailey CJ, Mynett KJ, Page T. Importance of the intestine as a site of metformin-stimulated glucose utilization. Br J Pharmacol. 1994; 112:671–5.
67. Bailey CJ, Turner RC. Metformin. New Engl J Med. 1996; 334:574–9.

68. Musi N, Hirshman MF, Nygren J, Metformin increases AMP-activated protein kinase activity in skeletal muscle of subjects with type 2 diabetes. Diabetes. 2002; 51:2074–81.
69. Bailey CJ, Wilcock C, Scarpello JHB. Metformin and the intestine. Diabetologia. 2008; 51:1552–3.
70. White MF. Metformin and insulin meet in a most atypical way. Cell Metab. 2009; 9:485–7.
71. El-Mir MY, Nogueira V, Fontaine E, Avéret N, Rigoulet M, Leverve X. Dimethylbiguanide inhibits cell respiration via an indirect effect targeted on the respiratory chain complex I. Journal of Biological Chemistry. 2000 Jan 7; 275(1):223-8.
72. Argaud D, Roth H, Wiernsperger N, Leverve XM. Metformin decreases gluconeogenesis by enhancing the pyruvate kinase flux in isolated rat hepatocytes. Eur J Biochem. 1993; 213(3):1341–1348. doi:10.1111/j.1432-1033.1993.tb17886.x38.
73. Hundal RS, Inzucchi SE. Metformin: new understandings, new uses. Drugs. 2003; 63(18):1879–1894. doi:10.2165/00003495-200363180-00001
74. Miller RA, Birnbaum MJ. An energetic tale of AMPK-independent effects of metformin. J Clin Invest. 2010; 120(7):2267–2270. doi:10.1172/ jci43661
75. Pernicova I, Korbonits M. Metformin—mode of action and clinical implications for diabetes and cancer. Nat Rev Endocrinol. 2014; 10 (3):143–156. doi:10.1038/nrendo.2013.256.
76. Ouyang J, Parakhia RA, Ochs RS. Metformin activates AMP kinase through inhibition of AMP deaminase. J Biol Chem. 2011; 286(1):1–11. doi:10.1074/jbc.M110.121806.
77. Owen MR, Doran E, Halestrap AP. Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain. Biochem J. 2000;3:607–614.34
78. Logie L, Harthill J, Patel K, Cellular responses to the metal-binding properties of metformin. Diabetes. 2012; 61(6):1423–1433. Doi:10.2337/db11-096119.
79. Shirasaka Y, Lee N, Zha W, Wagner D, Wang J. Involvement of organic cation transporter 3 (Oct3/Slc22a3) in the bioavailability and pharmacokinetics of antidiabetic metformin in mice. Drug Metab Pharmacokinet. 2016; 31(5):385–388. doi:10.1016/j.dmpk.2016.04.00525.
80. He L, Sabet A, Djedjos S, Metformin and insulin suppress hepatic gluconeogenesis through phosphorylation of CREB binding protein. Cell.2009; 137:635–46.
81. Rooyackers O, Myrenfors P, Nygren J, Thorell A, Ljungqvist O. Insulin stimulated glucose disposal in peripheral tissues studied with microdialysis and stable isotope tracers. Clinical Nutrition. 2004 Aug 1; 23(4):743-52.
82. Jazet IM, Schaart G, Gastaldelli A, Ferrannini E, Hesselink MK, Schrauwen P, Romijn JA, Maassen JA, Pijl H, Ouwens DM, Meinders AE. Loss of 50% of excess weight using a very low energy diet improves insulin-stimulated glucose disposal and skeletal muscle insulin signalling in obese insulin-treated type 2 diabetic patients. Diabetologia. 2008 Feb; 51(2):309-19.
83. Grubelnik V, Zmazek J, Završnik M, Marhl M. Lipotoxicity in a vicious cycle of pancreatic β cell exhaustion. Biomedicines. 2022 Jul 7; 10(7):1627.
84. Molavi B, Rassouli N, Bagwe S, Rasouli N. A review of thiazolidinediones and metformin in the treatment of type 2 diabetes with focus on cardiovascular complications. Vasc Health Risk Manag. 2007; 3:967–73.
85. Scarpello JHB, Howlett HCS. Metformin monotherapy and clinical uses. Diabetes Vasc Dis Res. 2008; 5:157–67.

86. Bailey CJ. Metformin: effects on micro- and macrovascular complications in type 2 diabetes. Cardiovasc Drugs Ther. 2008; 22:215–24.
87. Wu H, Esteve E, Tremaroli V, Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug. Nat Med. 2017; 23(7):850–858. doi:10.1038/nm.4345.
88. McGovern A, Tippu Z, Hinton W, Munro N, Whyte M, de Lusignan S. Comparison of medication adherence and persistence in type 2 diabetes: A systematic review and meta-analysis. Diabetes, Obesity and Metabolism. 2018 Apr; 20(4):1040-3.
89. Kaur A, Tiwari R, Tiwari G, Ramachandran V. Resveratrol: A vital therapeutic agent with multiple health benefits. Drug research. 2022 Jan; 72(01):5-17.
90. Renaud SC, Guéguen R, Schenker J, d'Houtaud A. Alcohol and mortality in middle-aged men from eastern France. Epidemiology. 1998 Mar 1;9(2):184-8.
91. Waffo-Téguo P, Krisa S, Pawlus AD, Richard T, Monti JP, Mérillon JM. Grapevine stilbenoids: Bioavailability and neuroprotection. InNatural Products: Phytochemistry, Botany, Metabolism of Alkaloids, Phenolics and Terpenes 2025 Nov 8 (pp. 1-37). Berlin, Heidelberg: Springer Berlin Heidelberg.
92. Lim CG, AG Koffas M. Bioavailability and recent advances in the bioactivity of flavonoid and stilbene compounds. Current Organic Chemistry. 2010 Oct 1; 14(16):1727-51.
93. Prokop J, Abrman P, Seligson AL, Sovak M. Resveratrol and its glycon piceid are stable polyphenols. Journal of medicinal food. 2006 Mar 1; 9(1):11-4.
94. Costa CE, Romaní A, Domingues L. Overview of resveratrol properties, applications, and advances in microbial precision fermentation. Critical Reviews in Biotechnology. 2025 May 19; 45(4):788-804.
95. Hemdan DE, El-Shiekh RA. Resveratrol: A promising candidate for treating a range of diseases. Bulletin of Faculty of Pharmacy Cairo University. 2025; 63(1):4.
96. Goel H, Nagarajan K, Nainwal LM, Bhardwaj S. Analytical Techniques for the Quantification and Validation of Resveratrol: A Review. Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistry. 2025.
97. Palsamy P, Subramanian S. Resveratrol, a natural phytoalexin, normalizes hyperglycemia in streptozotocin-nicotinamide induced experimental diabetic rats, Biomed. Pharmacother, 62(2008)9.
98. Palsamy P, Subramanian S. Modulatory effects of resveratrol on attenuating the key enzymes activities of carbohydrate metabolism in streptozotocin–nicotinamide-induced diabetic rats, Chem. Biol. Interact,179(2009)2.
99. Palsamy P, Subramanian S. Ameliorative potential of resveratrol on proinflammatory cytokines, hyperglycemia mediated oxidative stress, and pancreatic β-cell dysfunction in streptozotocinnicotinamideinduced diabetic rats, J. Cell. Physiol, 224(2010)2.
100. Palsamy P, Sivakumar S, Subramanian S. resveratrol attenuates hyperglycemia-mediated oxidative stress, proinflammatory cytokines and protects hepatocytes ultrastructure in streptozotocin–nicotinamide-induced experimental diabetic rats, Chem. Biol. Interact ,2(2010)186.
101. Palsamy P, Subramanian S. Resveratrol protects diabetic kidney by attenuating hyperglycemia-mediated oxidative stress and renal inflammatory cytokines via Nrf2–Keap1 signaling,BBA-Mol. Basis Dis ,1812(2011)7.
102. Alvarez MV, Moreira MR, Ponce A. Antiquorum sensing and antimicrobial activity of natural agents with potential use in food. Journal of Food Safety. 2012 Aug; 32(3):379-87.
103. Oliveira AR, Domingues FC, Ferreira S. The influence of resveratrol adaptation on resistance to antibiotics, benzalkonium chloride, heat and acid stresses of

Staphylococcus aureus and Listeria monocytogenes. Food Control. 2017 Mar 1; 73:1420-5.

104. Prokop A, Magiera A, Olszewska MA. Proanthocyanidins as Therapeutic Agents in Inflammation-Related Skin Disorders. International Journal of Molecular Sciences. 2025 Oct 17; 26(20):10116.
105. Rajitha R, Iyyam Pillai. S and Subramanian. S. Resveratrol: An incredible Naturaceutical with numerous Health Benefits. Journal of Dalian University of Technology. Volume 32, Issue 7, 2025, Page No-248. https://doi.org/10.5281/Zenodo.16760149
106. Ghasemi A, Khalifi S, Jedi S. Streptozotocin-nicotinamide-induced rat model of type 2 diabetes. Acta Physiologica Hungarica. 2014 Dec 1; 101(4):408-20.
107. King AJ. The use of animal models in diabetes research. British journal of pharmacology. 2012 Jun; 166(3):877-94.
108. Kawada J, Toide K, Nishida M, Yoshimura Y, Tsujihara K. New Diabetogenic Streptozocin Analogue, 3-O-Methyl-2-{[(methylnitrosoamino) carbonyl] amino}-D-glucopyranose: Evidence for a Glucose Recognition Site on Pancreatic B-Cells. Diabetes. 1986 Jan 1; 35(1):74-7.
109. Capdevila J, Ducreux M, García Carbonero R, Grande E, Halfdanarson T, Pavel M, Tafuto S, Welin S, Valentí V, Salazar R. Streptozotocin, 1982–2022: Forty years from theFDA's approval to treat pancreatic neuroendocrine tumors. Neuroendocrinology. 2022 May 10; 112(12):1155-67.
110. Lenzen S. The mechanisms of alloxan-and streptozotocin-induced diabetes. Diabetologia. 2008 Feb; 51(2):216-26.
111. Junod AL, Lambert AE, Orci L, Pictet R, Gonet AE, Renold AE. Studies of thediabetogenic action of streptozotocin. Proceedings of the Society for Experimental Biology and Medicine. 1967 Oct; 126(1):201-5.
112. Sorimuthu Pillai Subramanian, Rajitha Rajendran, Subramanian Iyyam Pillai. Optimization Of Protocol For The Successful Induction Of Streptozotocin-Induced, YMER, Volume 24: Issue 11 (Nov)-2025. ISSN: 0044-0477.
113. Huang XF, Li HQ, Shi L, Xue JY, Ruan BF, Zhu HL. Synthesis of resveratrol analogues, and evaluation of their cytotoxic and xanthine oxidase inhibitory activities. Chemistry & Biodiversity. 2008 Apr; 5(4):636-42.
114. Basheer L, Schultz K, Fichman M, Kerem Z. Use of in vitro and predictive in silico models to study the inhibition of cytochrome P4503A by stilbenes. PloS one. 2015 Oct 20; 10(10):e0141061.
115. Iyyam Pillai Subramanian,, Rajitha Rajendran, Sorimuthu Pillai Subramanian,2021.Synthesis and characterization of a new metformin - resveratrol aldehyde ligand and evaluation of its glucose uptake efficacy using rat l6 skeletal muscle cell lines. Journal of medical pharmaceutical and allied sciences, Volume 10 - Issue 6, 1497, November - December 2021, Page – 4008-4017.
116. Wang HJ, Shen W, Neng J, Wu T, Li YJ. Low dose streptozotocin (STZ) combined with high energy intake can effectively induce type 2 diabetes through altering the related gene expression. Asia Pacific journal of clinical nutrition. 2007; 16:412.
117. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J biol Chem. 1951 Nov 1; 193(1):265-75.
118. Lee CW, Lee HS, Cha YJ, Joo WH, Kang DO, Moon JY. In vivo investigation of anti-diabetic properties of ripe onion juice in normal and streptozotocin-induced diabetic rats. Preventive nutrition and food science. 2013 Sep; 18(3):169.
119. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, Quon MJ. Quantitative insulin sensitivity check index: a simple, accurate method for assessing

insulin sensitivity in humans. The Journal of Clinical Endocrinology & Metabolism. 2000 Jul 1; 85(7):2402-10.

120. Hassid WZ, Abraham S. Chemical procedures for analysis of polysaccharides. in Methods in Enzymology, S. Colowick and N. Kaplan, Eds., vol. 3, pp. 34–36, Academic Press, New York, NY, USA, 1957.
121. Trinder P. Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. Journal of clinical pathology. 1969 Mar 1; 22(2):158-61.
122. Nayak SS, Pattabiraman TN. A new colorimetric method for the estimation of glycosylated hemoglobin. Clinica Chimica Acta. 1981 Feb 5; 109(3):267-74.
123. Natelson S, Scott ML, Beffa C. A rapid method for the estimation of urea in biologic fluids: by means of the reaction between diacetyl and urea. American Journal of Clinical Pathology. 1951 Mar 1; 21(3_ts):275-81.
124. Brod J, Sirota JH. The renal clearance of endogenous "creatinine" in man. The Journal of Clinical Investigation. 1948 Sep 1; 27(5):645-54.
125. Rao BK, Sudarshan PR, Rajasekhar MD, Nagaraju N, Rao CA. Antidiabetic activity of Terminalia pallida fruit in alloxan induced diabetic rats. Journal of Ethnopharmacology. 2003 Mar 1; 85(1):169-72Kang MH, Choi MK. Antidiabetic Activities Analysis by Oral Glucose Tolerance Test in Rats. Reproductive & Developmental Biology 2010; 34(4): 305-308
126. Sathibabu Uddandrao VV, Brahmanaidu P, Ravindarnaik R, Suresh P, Vadivukkarasi S, Saravanan G. Restorative potentiality of S-allylcysteine against diabetic nephropathy through attenuation of oxidative stress and inflammation in streptozotocin-nicotinamide-induced diabetic rats. European journal of nutrition. 2019 Sep 1; 58:2425-37.
127. Nagy C, Einwallner E. Study of in vivo glucose metabolism in high-fat diet-fed mice using oral glucose tolerance test (OGTT) and insulin tolerance test (ITT). Journal of visualized experiments: JoVE. 2018 Jan 7(131):56672.
128. Sato VH, Chewchinda S, Goli AS, Sato H, Nontakham J, Vongsak B. Oral glucose tolerance test (OGTT) evidence for the postprandial anti-hyperglycemic property of Salacca zalacca (Gaertn.) voss seed extract. Molecules. 2023 Sep 23; 28(19):6775.
129. Amniattalab A, Malekinejad H, Rezabakhsh A, Rokhsartalab-Azar S, Alizade-Fanalou S. Silymarin: a novel natural agent to restore defective pancreatic β cells in streptozotocin (STZ)-induced diabetic rats. Iranian journal of pharmaceutical research: IJPR. 2016;15(3):493
130. Mahmoud MF, El Ashry FE, El Maraghy NN, Fahmy A. Studies on the antidiabetic activities of Momordica charantia fruit juice in streptozotocin-induced diabetic rats. Pharmaceutical biology. 2017 Jan 1; 55(1):758-65.
131. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. Diabetic medicine. 1998 Jul; 15(7):539-53.
132. Kuo FY, Cheng KC, Li Y, Cheng JT. Oral glucose tolerance test in diabetes, the old method revisited. World journal of diabetes. 2021 Jun 15; 12(6):786.
133. Abdul-Ghani MA, Sabbah M, Kher J, Minuchin O, Vardi P, Raz I. Different contributions of insulin resistance and beta-cell dysfunction in overweight Israeli Arabs with IFG and IGT. Diabetes/metabolism research and reviews. 2006 Mar; 22(2):126-30.
134. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes care. 1999 Sep 1; 22(9):1462-70.

135. Ziel FH, Venkatesan N, Davidson MB. Glucose transport is rate limiting for skeletal muscle glucose metabolism in normal and STZ-induced diabetic rats. Diabetes. 1988 Jul 1; 37(7):885-90.
136. Randle PJ. Endocrine control of metabolism. Annual Review of Physiology. 1963 Mar; 25(1):291-324.
137. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner R. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. diabetologia. 1985 Jul;28(7):412-
138. Eizirik DL, Pasquali L, Cnop M. Pancreatic β-cells in type 1 and type 2 diabetes mellitus: different pathways to failure. Nature Reviews Endocrinology. 2020 Jul; 16(7):349-62.
139. Ward WK, Bolgiano DC, McKnight B, Halter JB, Porte D. Diminished B cell secretory capacity in patients with noninsulin-dependent diabetes mellitus. The Journal of clinical investigation. 1984 Oct 1; 74(4):1318-28.).
140. Chen R, Meseck M, McEvoy RC, Woo SL. Glucose-stimulated and self-limiting insulin production by glucose 6-phosphatase promoter driven insulin expression in hepatoma cells. Gene Therapy. 2000 Nov; 7(21):1802-9.
141. Gromova LV, Fetissov SO, Gruzdkov AA. Mechanisms of glucose absorption in the small intestine in health and metabolic diseases and their role in appetite regulation. Nutrients. 2021 Jul 20; 13(7):2474.
142. Younes S. The Relationship in Renal disease in individuals with diabetes. Clinical Endocrinology and Metabolism. 2024; 3(2).
143. Sheehan JP. Fasting hyperglycemia: etiology, diagnosis, and treatment. Diabetes technology & therapeutics. 2004 Aug 1; 6(4):525-33.
144. Girard J. The inhibitory effects of insulin on hepatic glucose production are both direct and indirect. Diabetes. 2006 Dec 1; 55(Supplement_2):S65-9.
145. Roden M. Hepatic glucose production and insulin resistance. Wiener Medizinische Wochenschrift. 2008 Oct;158(19):558-61
146. Jiang S, Young JL, Wang K, Qian Y, Cai L. Diabetic-induced alterations in hepatic glucose and lipid metabolism: the role of type 1 and type 2 diabetes mellitus. Molecular medicine reports. 2020 Aug; 22(2):603-11.
147. Weiskorn J, Saboo B, Danne T. Current and future strategies in insulin development and treatment. Hormone Research in Paediatrics. 2025 Aug 24; 98(4):396-404.
148. Wahren J, Kallas Å, Sima AA. The clinical potential of C-peptide replacement in type 1 diabetes. Diabetes. 2012 Apr 1; 61(4):761-72.
149. Steiner DF. Insulin-induced liver hyperplasia: evidence for a negative liver-size-correcting process. Hepatotrophic Factors. 1978:229-46.
150. Silva J, Steiner D. Assessment of metabolic syndrome in patients diagnosed with melasma in a dermatology service. Portuguese Journal of Dermatology & Venereology. 2025 Apr 1; 83(2).
151. Thevis M, Thomas A, Schänzer W. Insulin. Doping in sports: Biochemical principles, effects and analysis. 2009 Sep 17:209-26.
152. Jeffrey PD, Milthorpe BK, Nichol LW. Polymerization pattern of insulin at pH 7.0. Biochemistry. 1976 Oct 1; 15(21):4660-5.
153. Wojtaszewski JF, Hansen BF, Gade, Kiens B, Markuns JF, Goodyear LJ, Richter EA. Insulin signaling and insulin sensitivity after exercise in human skeletal muscle. Diabetes. 2000 Mar 1; 49(3):325-31.
154. Wahren J, Ekberg K, Johansson J, Henriksson M, Pramanik A, Johansson BL, Rigler R, Jörnvall H. Role of C-peptide in human physiology. American Journal of Physiology-Endocrinology and Metabolism. 2000 May 1;278(5):E759-68.

155. Zhong ZH, Pramanik A, Ekberg K, Jansson OT, Jörnvall H, Wahren J, Rigler R. Insulin binding monitored by fluorescence correlation spectroscopy. Diabetologia. 2001 Sep 1; 44(9).
156. Ahn SH, Montero M, Odell D, Roundy S, Wright PK. Anisotropic material properties of fused deposition modeling ABS. Rapid prototyping journal. 2002 Oct 1; 8(4):248-57.
157. Esfandiari E, Dorali A, Ghanadian M, Rashidi B, Mostafavi FS. Protective and therapeutic effects of Phoenix dactylifera leaf extract on pancreatic β-cells and diabetic parameters in streptozotocin-induced diabetic rats. Comparative Clinical Pathology. 2020 Aug; 29(4):847-54.
158. Pari L, Sankaranarayanan C. Beneficial effects of thymoquinone on hepatic key enzymes in streptozotocin–nicotinamide induced diabetic rats. Life sciences. 2009 Dec 16; 85(23-26):830-4.
159. Sundaram R, Shanthi P, Sachdanandam P. Effect of iridoid glucoside on plasma lipid profile, tissue fatty acid changes, inflammatory cytokines, and GLUT4 expression in skeletal muscle of streptozotocin-induced diabetic rats. Molecular and cellular biochemistry. 2013 Aug; 380:43-55.
160. Koenig LR, Murray FW. Ice-bearing cumulus cloud evolution: Numerical simulation and general comparison against observations. Journal of Applied Meteorology and Climatology. 1976 Jul; 15(7):747-62.
161. Chang AT, Noble J. Estimation of HbA1c like glycosylated proteins in kidneys of streptozotocin diabetes and controlled rats. Diabetes. 1979; 28:408-15.
162. Davidson MB, Schriger DL, Peters AL, Lorber B. Relationship between fasting plasma glucose and glycosylated hemoglobin: potential for false-positive diagnoses of type 2 diabetes using new diagnostic criteria. Jama. 1999 Apr 7; 281(13):1203-10.
163. Abdel-Wahab YH, O'Harte FP, Ratcliff H, McClenaghan NH, Barnett CR, Flatt PR. Glycation of insulin in the islets of Langerhans of normal and diabetic animals. Diabetes. 1996 Nov 1;45(11):1489-96
164. Stratton I, Gough S, Cramb R, Smith J, Sikaris K, Nightingale P, Manley S. Diagnosis of diabetes: HbA1c versus WHO criteria. Diabetes and Primary Care. 2010 Apr 16; 12(2):87-96.
165. Chandalia HB, Krishnaswamy PR. Glycated hemoglobin. Current Science. 2002 Dec 25:1522-32.
166. Yabe-Nishimura C. Aldose reductase in glucose toxicity: a potential target for the prevention of diabetic complications. Pharmacological reviews. 1998 Mar 1; 50(1):21-33.
167. Monnier VM, Cerami A. 7 Non-enzymatic glycosylation and browning of proteins in diabetes. Clinics in Endocrinology and Metabolism. 1982 Jul 1; 11(2):431-52.
168. Little RR. Glycated hemoglobin standardization--National Glycohemoglobin Standardization Program (NGSP) perspective. Clinical Chemistry & Laboratory Medicine. 2003 Sep 1; 41(9).
169. Edelman D, Olsen MK, Dudley TK, Harris AC, Oddone EZ. Utility of hemoglobin A1c in predicting diabetes risk. Journal of general internal medicine. 2004 Dec; 19(12):1175-80.
170. Massi-Benedetti M. Changing targets in the treatment of type 2 diabetes. Current medical research and opinion. 2006 Aug 1; 22(sup2):S5-13.
171. Ong KC, Khoo HE. Effects of myricetin on glycemia and glycogen metabolism in diabetic rats. Life sciences. 2000 Aug 25; 67(14):1695-705.

172. Mansour HA, Newairy AS, Yousef MI, Sheweita SA. Biochemical study on the effects of some Egyptian herbs in alloxan-induced diabetic rats. Toxicology. 2002 Jan 25; 170(3):221-8.
173. Férard G, Imbert-Bismut F, Messous D, Piton A, Ueda S, Poynard T, Lessinger JM. A reference material for traceability of aspartate aminotransferase (AST) results. Clinical Chemistry & Laboratory Medicine. 2005 May 1; 43(5).
174. Giannini EG, Testa R, Savarino V. Liver enzyme alteration: a guide for clinicians. Cmaj. 2005 Feb 1;172(3):367-79.
175. Harris EH. Elevated liver function tests in type 2 diabetes. Clinical diabetes. 2005 Jul 1; 23(3):115-9.
176. O'Brien RM, Granner DK. Regulation of gene expression by insulin. Biochemical Journal. 1991 Sep 15; 278(Pt 3):609.
177. Grove J, Daly AK, Bassendine MF, Day CP. Association of a tumor necrosis factor promoter polymorphism with susceptibility to alcoholic steatohepatitis. Hepatology. 1997 Jul; 26(1):143-6.
178. Tomás E, LIN YS, Dagher Z, Saha A, Luo Z, Ido Y, Ruderman NB. Hyperglycemia and insulin resistance: possible mechanisms. Annals of the New York Academy of Sciences. 2002 Jun; 967(1):43-51.
179. Møller N, Nair KS. Diabetes and protein metabolism. Diabetes. 2008 Jan 1; 57(1):3-4.
180. Fuller SJ, Mynett JR, Sugden PH. Stimulation of cardiac protein synthesis by insulin-like growth factors. Biochemical Journal. 1992 Feb 15;282(1):85-90.Sugden PH,
181. Fando JL, Jolin T, Salinas M, Dominguez F, Herrera E. The effect of streptozotocin diabetes on brain protein synthesis in the rat. Diabete & Metabolisme. 1985 Apr 1; 11(2):92-7.
182. Dighe RR, Rojas FJ, Birnbaumer L, Garber AJ. Glucagon-stimulable adenylyl cyclase in rat liver. The impact of streptozotocin-induced diabetes mellitus. The Journal of clinical investigation. 1984 Apr 1; 73(4):1013-23.
183. Naskar S, Islam A, Mazumder UK, Saha P, Haldar PK, Gupta M. In vitro and in vivo antioxidant potential of hydromethanolic extracts of Phoenix dactylifera fruits. Journal of Scientific Research. 2010; 2(1):144-57.
184. Almdal TP, Vilstrup H. Effects of streptozotocin-induced diabetes and diet on nitrogen loss from organs and on the capacity of urea synthesis in rats. Diabetologia. 1987 Dec;30(12):952-6.
185. Higgins C. Urea and creatinine concentration, the urea: creatinine ratio. Acute Care Test Hand. 2016 Oct: 1-8.
186. Travlos GS, Morris RW, Elwell MR, Duke A, Rosenblum S, Thompson MB. Frequency and relationships of clinical chemistry and liver and kidney histopathology findings in 13-week toxicity studies in rats. Toxicology. 1996 Jan 22; 107(1):17-29.
187. Sandilands EA, Dhaun N, Dear JW, Webb DJ. Measurement of renal function in patients with chronic kidney disease. British journal of clinical pharmacology. 2013 Oct; 76(4):504-15.
188. Hamilton RW, Gardner LB, PENN AS, GOLDBERG M. Acute tubular necrosis caused by exercise-induced myoglobinuria. Annals of internal medicine. 1972 Jul 1; 77(1):77-82.
189. Kurzer F, Sanderson PM. Urea in the history of organic chemistry: Isolation from natural sources. Journal of Chemical Education. 1956 Sep; 33(9):452.
190. Facchini F, Chen YD, Hollenbeck CB, Reaven GM. Relationship between resistance to insulin-mediated glucose uptake, urinary uric acid clearance, and plasma uric acid concentration. Jama. 1991 Dec 4; 266(21):3008-11.

191. Modan M, Halkin H, Karasik A, Lusky A. Elevated serum uric acid—a facet of hyperinsulinaemia. Diabetologia. 1987 Sep; 30(9):713-8.
192. Becker BF, Massoudy P, Permanetter B, Raschke P, Zahler S. Possible significance of free oxygen radicals for reperfusion injury. Zeitschrift fur Kardiologie. 1993 Jan 1; 82:49-58.
193. Jung S. Variation in antioxidant metabolism of young and mature leaves of Arabidopsis thaliana subjected to drought. Plant Science. 2004 Feb 1; 166(2):459-66.
194. Roden M, Bernroider E. Hepatic glucose metabolism in humans—its role in health and disease. Best practice & research Clinical endocrinology & metabolism. 2003 Sep 1; 17(3):365-83.
195. Cersosimo E, Molina PE, Abumrad NN. Renal glucose production during insulin-induced hypoglycemia. Diabetes. 1997 Apr 1; 46(4):643-6.
196. Deng JY, Hsieh PS, Huang JP, Lu LS, Hung LM. Activation of estrogen receptor is crucial for resveratrol-stimulating muscular glucose uptake via both insulin-dependent and-independent pathways. Diabetes. 2008 Jul 1; 57(7):1814-23.
197. Wilson JE. Isozymes of mammalian hexokinase: structure, subcellular localization and metabolic function. Journal of Experimental Biology. 2003 Jun 15; 206(12):2049-57.
198. Postic C, Decaux JF, Girard J. Regulation of hepatic glucokinase gene expression. FRONTIERS IN DIABETES. 2004 Jun 3; 16:180-92.
199. Collier JJ, Scott DK. Sweet changes: glucose homeostasis can be altered by manipulating genes controlling hepatic glucose metabolism. Molecular Endocrinology. 2004 May 1; 18(5):1051-63.
200. Ferre T, Riu E, Bosch F, Valera A. Evidence from transgenic mice that glucokinase is rate limiting for glucose utilization in the liver. The FASEB Journal. 1996 Aug; 10(10):1213-8.
201. Katz NR, Nauck MA, Wilson PT. Induction of glucokinase by insulin under the permissive action of dexamethasone in primary rat hepatocyte cultures. Biochemical and Biophysical Research Communications. 1979 May 14; 88(1):23-9.
202. Spence JT. Levels of translatable mRNA coding for rat liver glucokinase. Journal of Biological Chemistry. 1983 Aug 10; 258(15):9143-6.
203. Gupta D, Raju J, Baquer NZ. Change in the lipid profile, lipogenic and related enzymes in the livers of experimental diabetic rats: effect of insulin and vanadate. Diabetes Research and Clinical Practice. 1999 Oct 1; 46(1):1-7.
204. Johnson SC, Bailey T, Becker RR, Cardenas JM. Isolation and sequence determination of a peptide located in or near the active site of bovine muscle pyruvate kinase. Biochemical and Biophysical Research Communications. 1979 Sep 27; 90(2):525-30.
205. Staal GE, Jansen G, Roos D. Pyruvate kinase and the. The Journal of clinical investigation. 1984 Jul 1; 74(1):231-5.
206. Mesecar AD, Nowak T. Metal-ion-mediated allosteric triggering of yeast pyruvate kinase. 1. A multidimensional kinetic linked-function analysis. Biochemistry. 1997 Jun 3; 36(22):6792-802.
207. Tani K, Fujii H, Takahashi K, Ogura H, Kanno H, Hayasaka K, Narisawa K, Nakahata T, Akabane T, Morisaki T, Tsutsumi H. Two homozygous cases of erythrocyte pyruvate kinase (PK) deficiency in Japan: PK Sendai and PK Shinshu. American journal of hematology. 1988 Jul; 28(3):186-90.
208. Baronciani L, Beutler E. Analysis of pyruvate kinase-deficiency mutations that produce nonspherocytic hemolytic anemia. Proceedings of the National Academy of Sciences. 1993 May 1; 90(9):4324-7.

209. Bizeau ME, Short C, Thresher JS, Commerford SR, Willis WT, Pagliassotti MJ. Increased pyruvate flux capacities account for diet-induced increases in gluconeogenesis in vitro. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2001 Aug 1; 281(2):R427-33.
210. Kawaguchi T, Takenoshita M, Kabashima T, Uyeda K. Glucose and cAMP regulate the L-type pyruvate kinase gene by phosphorylation/dephosphorylation of the carbohydrate response element binding protein. Proceedings of the National Academy of Sciences. 2001 Nov 20; 98(24):13710-5.
211. Muñoz ME, Ponce E. Pyruvate kinase: current status of regulatory and functional properties. Comparative biochemistry and physiology Part B: biochemistry and molecular biology. 2003 Jun 1; 135(2):197-218.
212. Negm FB, Cornel FA, Plaxton WC. Suborganellar localization and molecular characterization of nonproteolytic degraded leukoplast pyruvate kinase from developing castor oil seeds. Plant physiology. 1995 Dec; 109(4):1461.
213. Taylor R, Agius L. The biochemistry of diabetes. Biochemical Journal. 1988 Mar 15; 250(3):625.
214. Berg JM, Tymoczko JL, Stryer L. Gluconeogenesis and glycolysis are reciprocally regulated. Biochemistry. 2002.
215. YAMADA K, NOGUCHI T. Nutrient and hormonal regulation of pyruvate kinase gene expression. Biochemical Journal. 1999 Jan 1; 337(1):1-1.
216. Boles E, Schulte F, Miosga T, Freidel K, Schlüter E, Zimmermann FK, Hollenberg CP, Heinisch JJ. Characterization of a glucose-repressed pyruvate kinase (Pyk2p) in Saccharomyces cerevisiae that is catalytically insensitive to fructose-1, 6-bisphosphate. Journal of bacteriology. 1997 May; 179(9):2987-93.
217. Weber JB, Perry PW, Upchurch RP. The influence of temperature and time on the adsorption of paraquat, diquat, 2, 4-D and prometone by clays, charcoal, and an anion-exchange resin. Soil Science Society of America Journal. 1965 Nov; 29(6):678-88.
218. Taunton OD, Stifel FB, Greene HL, Herman RH. Rapid reciprocal changes in rat hepatic glycolytic enzyme and fructose diphosphatase activities following insulin and glucagon injection. Journal of Biological Chemistry. 1974 Nov 25; 249(22):7228-39.
219. Kavanagh DJ, Andrade J, May J. Beating the urge: Implications of research into substance-related desires. Addictive Behaviors. 2004 Sep 1; 29(7):1359-72.
220. Boucher JI, Jacobowitz JR, Beckett BC, Classen S, Theobald DL. An atomic-resolution view of neofunctionalization in the evolution of apicomplexan lactate dehydrogenases. Elife. 2014 Jun 25; 3:e02304.
221. Ainscow EK, Brand MD. Internal regulation of ATP turnover, glycolysis and oxidative phosphorylation in rat hepatocytes. European journal of biochemistry. 1999 Dec 15; 266(3):737-49.
222. .Kurosaki E, Nakano R, Momose K, Shimaya A, Suzuki T, Shibasaki M, Shikama H. Hypoglycemic agent YM440 suppresses hepatic glucose output via gluconeogenesis by reducing glucose-6-phosphatase activity in obese Zucker rats. European journal of pharmacology. 2003 May 9;468(2):151-8
223. Bouche C, Serdy S, Kahn CR, Goldfine AB. The cellular fate of glucose and its relevance in type 2 diabetes. Endocrine reviews. 2004 Oct 1;25(5):807-30
224. Pilkis SJ, El-Maghrabi MR, Claus TH. Fructose-2, 6-bisphosphate in control of hepatic gluconeogenesis: from metabolites to molecular genetics. Diabetes Care. 1990 Jun 1; 13(6):582-99.
225. Chou KM, Cheng YC. An exonucleolytic activity of human apurinic/apyrimidinic endonuclease on 3′ mispaired DNA. Nature. 2002 Feb 7; 415(6872):655-9.

226. Van Schaftingen E, Gerin I. The glucose-6-phosphatase system. Biochemical Journal. 2002 Mar 15; 362(3):513-32.
227. Massillon D, Barzilai N, Chen W, Hu M, Rossetti L. Glucose Regulates in Vivo Glucose-6-phosphatase Gene Expression in the Liver of Diabetic Rats. Journal of Biological Chemistry. 1996 Apr 26; 271(17):9871-4.
228. Nurjhan NU, Consoli A, Gerich J. Increased lipolysis and its consequences on gluconeogenesis in non-insulin-dependent diabetes mellitus. The Journal of clinical investigation. 1992 Jan 1; 89(1):169-75.
229. Andrikopoulos S, Rosella G, Gaskin E, Thorburn A, Kaczmarczyk S, Zajac JD, Proietto J. Impaired regulation of hepatic fructose-1, 6-bisphosphatase in the New Zealand obese mouse model of NIDDM. Diabetes. 1993 Dec 1; 42(12):1731-6.
230. Hauschild R, von Schaewen A. Differential regulation of glucose-6-phosphate dehydrogenase isoenzyme activities in potato. Plant physiology. 2003 Sep 1; 133(1):47-62.
231. Hagg SA, Taylor SI, Ruberman NB. Glucose metabolism in perfused skeletal muscle. Pyruvate dehydrogenase activity in starvation, diabetes and exercise. Biochemical Journal. 1976 Aug 15;158(2):203-10
232. Guru A, Ranjan D, Jaiswal D, Hidangmayum A, Dwivedi P. Role of Osmolytes in ROS-Scavenging and Maintaining Homeostasis Under Abiotic Stress. InPlant Signaling Molecules in Regulation of ROS-Scavenging System: Adapting to Climate Change 2025 Nov 6 (pp. 61-86). Singapore: Springer Nature Singapore.
233. Tao Y, Farsetta DL, Nibert ML, Harrison SC. RNA synthesis in a cage—structural studies of reovirus polymerase λ3. Cell. 2002 Nov 27; 111(5):733-45.
234. Salati LM, Szeszel-Fedorowicz W, Tao H, Gibson MA, Amir-Ahmady B, Stabile LP, Hodge DL. Nutritional regulation of mRNA processing. The Journal of nutrition. 2004 Sep 1; 134(9):2437S-43S.
235. Salvemini D, Wang ZQ, Zweier JL, Samouilov A, Macarthur H, Misko TP, Currie MG, Cuzzocrea S, Sikorski JA, Riley DP. A nonpeptidyl mimic of superoxide dismutase with therapeutic activity in rats. Science. 1999 Oct 8; 286(5438):304-6.
236. Tian WN, Braunstein LD, Apse K, Pang J, Rose M, Tian X, Stanton RC. Importance of glucose-6-phosphate dehydrogenase activity in cell death. American Journal of Physiology-Cell Physiology. 1999 May 1; 276(5):C1121-31.
237. Yaish HM, Niazi GA, Al Shaalan M, Khan S, Ahmed GS. Increased incidence of hyperbilirubinaemia in ‘unchallenged’glucose-6-phosphate dehydrogenase deficiency in term Saudi newborns. Annals of tropical paediatrics. 1991 Jan 1; 11(3):259-66.
238. McDermott BM, Flatt PR, Strain JJ. Effects of copper deficiency and experimental diabetes on tissue antioxidant enzyme levels in rats. Annals of nutrition and metabolism. 1994 Nov 20; 38(5):263-9.
239. Xu Y, Osborne BW, Stanton RC. Diabetes causes inhibition of glucose-6-phosphate dehydrogenase via activation of PKA, which contributes to oxidative stress in rat kidney cortex. American journal of physiology-renal physiology. 2005 Nov; 289(5):F1040-7.
240. Malini P, Kanchana G, Rajadurai MU. Antibiabetic efficacy of ellagic acid in streptozotocin-induced diabetes mellitus in albino wistar rats. Asian J Pharm Clin Res. 2011; 4(3):124-8.
241. Greenberg CC, Danos AM, Brady MJ. Central role for protein targeting to glycogen in the maintenance of cellular glycogen stores in 3T3-L1 adipocytes. Molecular and cellular biology. 2006 Jan 1; 26(1):334-42.

242. Youn JH, Youn MS, Bergman RN. Synergism of glucose and fructose in net glycogen synthesis in perfused rat livers. Journal of Biological Chemistry. 1986 Dec 5; 261(34):15960-9.
243. Lawrence Jr JC, Roach PJ. New insights into the role and mechanism of glycogen synthase activation by insulin. Diabetes. 1997 Apr 1; 46(4):541-7.
244. Bollen M, Vandenheede JR, Goris J, Stalmans W. Characterization of glycogen-synthase phosphatase and phosphorylase phosphatase in subcellular liver fractions. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research. 1988 Apr 2; 969(1):66-77.