FORMULATION AND CHARACTERIZATION OF HERBAL NANOPARTICLES FOR ANTIDIABETIC THERAPY

Email Id-mayuritaware911103@gmail.com

Ms. Mayuri Taware (B pharm)
Dr. Uttekar .P.S (Ph.D)
Mr.Harshwardhan Kole
Ms.Ankita Balshire(M pharm)

NAME OF THE COLLEGE-
Dnyan, Kala, Krida, & Krishi Pratishthan's
LATE LAXMIBAI PHADTARE COLLEGE OF PHARMACY
At Post: Kalamb Walchandnagar, Tal.Indapur, Dist. Pune - 413114.

Abstract

Gymnemic acid from _Gymnema sylvestre_ has poor oral bioavailability. Researchers formulated chitosan-TPP nanoparticles by ionic gelation. DLS showed 180±15 nm size, PDI 0.21, zeta +28mV. FTIR confirmed no chemical interaction. EE% was 78.3%. In-vitro release was sustained over 24h at pH 7.4. α-glucosidase inhibition IC50 improved from 42.5 µg/mL for free extract to 18.2 µg/mL for NPs. STZ-diabetic rats showed better glucose reduction vs free extract.Green synthesized ZnO nanoparticles using Momordica charantia

Bitter melon extract was used as reducing/capping agent for ZnO nanoparticles. UV-Vis showed SPR peak at 365 nm. TEM confirmed hexagonal 25-40 nm particles. XRD + FTIR verified crystalline ZnO structure. NPs showed 85% α-amylase inhibition at 100 µg/mL vs 54% for extract. In diabetic mice, 10 mg/kg ZnO NPs reduced fasting blood glucose by 48% in 21 days with improved insulin levels. Characterization proved stable, biocompatible NPs with synergistic herb + nano effect.

Berberine from _Berberis aristata_ suffers from low solubility. Nanoprecipitation with PLGA-PEG gave 145 nm particles, PDI 0.18, zeta -22mV. DSC/XRD confirmed amorphous conversion → higher solubility. EE% 82.1%. Release followed Higuchi model for 72h. In diabetic rats, oral NPs lowered HbA1c 1.8% vs 0.9% with free berberine at same dose. TEM + SEM confirmed spherical morphology for better cellular uptake.

KEYWORDS

- Herbal Nanoparticles
- Antidiabetic Therapy
- Diabetes Mellitus
- Nanotechnology
- Drug Delivery System
- Phytochemicals
- Herbal Extracts
- Nanoformulation
- Bioavailability Enhancement
- Controlled Drug Release
- Chitosan Nanoparticles
- Antidiabetic Activity
- Nanomedicine
- Characterization Studies
- Phytotherapy

1. INTRODUCTION-

Diabetes mellitus is a group of metabolic diseases characterized by an increase in blood glucose level associated with atypical metabolism of basic foods like carbohydrates, proteins and fats due to inadequate or lack of insulin secretion by beta cells of islets of Langerhans of the pancreas and peripheral tissues like adipose, skeletal muscle and liver tissue. It is also associated with hyper amino acid anaemia and hyperlipidaemia [1, 2]. Long-term suffering from diabetes mellitus may lead to critical complications like retinopathy leading to blindness, neuropathy, including dysfunctions of endocrine organs and nephropathy, which may cause renal failure. Patients suffering from diabetes mellitus are also at high risk of vascular, cardiovascular, peripheral and cerebrovascular diseases [3]. The world prevalence rate of diabetes mellitus may increase from 8.3% (366 million) in 2011 to 9.9% (522 million) approaching the year 2030 as estimated from recent studies and surveys. The highest increase may occur in developing countries like India and China. The United States of America also has a huge number of patients suffering from diabetes mellitus. Newly developed drugs are frequently tested for the prevention and treatment of diabetes and its complications. New approaches of current therapies for the treatment of diabetes mainly include maintaining diet, exercise and use of carbohydrate digestive enzyme inhibitors, which is responsible for inhibition of glucose absorption in intestine and reduction of cellular glucose uptake. Diabetes mellitus can be treated with allopathic oral hypoglycaemic agents, which are associated with mild-to-severe side effects related to hypoglycaemia, skin reactions, gastrointestinal problems, nausea and haematological disorders [4].

Diabetes mellitus is a serious and pervasive metabolic syndrome characterized by high glucose levels in blood. It can be classified into two major types: type 1 and type 2 diabetes mellitus. Over the past few decades, the prevalence of diabetes has climbed exponentially. In 2014, approximately 422 million people constituting 8.5% of the total global population were diagnosed diabetic [5,6]. Among them, 7% of people with diabetes were categorized as type 2 diabetic patients [6]. It has been predicted that approximately 642 million people will suffer from diabetes mellitus by 2040 [7]. Diabetes is a silent killer which can provoke a number of slowly or rapidly developing pathogenesis, such as nephropathy, retinopathy, neuropathy, cardiomyopathy, peripheral arterial disease, coronary artery disease, and stroke. Glycaemic control has been regarded to be the principal therapeutic intervention in diabetes mellitus. However, multiple risk factors in diabetes, lethal complications, and establishment of vasculopathy before diagnosis necessitate developing

novel therapeutic strategies for the effective management of diabetes. Insulin, a polypeptide hormone, is secreted from β cells of islets of Langerhans in the pancreas and regulates the uptake and utilization of glucose by the tissues to produce energy. For type 1 diabetic patients, typical treatment includes injections of long-acting insulin to maintain a basal level of insulin, in combination with bolus injections of fast-acting insulin at mealtimes. Several hypoglycaemic agents singly or in combination with insulin are clinically used to treat type 2 diabetes mellitus [6]. However, the clinically available antidiabetic agents disappoint both clinicians and patients owing to their untoward effects, which increasingly shifted the focus toward the discovery of novel antidiabetic agents.

The elucidation of the incretin effect has catalyzed the development of cutting-edge therapies that have markedly transformed the management of type 2 diabetes mellitus (T2DM). Mediated predominantly by the incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), this effect enhances insulin secretion in response to oral glucose intake, playing a vital role in maintaining glucose homeostasis. The enzyme dipeptidyl peptidase-4 (DPP-4) regulates the duration of incretin hormone activity, thereby influencing its metabolic effects [8]. Incretin-based therapies currently include DPP-4 inhibitors and GLP-1 receptor agonists (GLP-1 RAs), with the recent introduction of the dual GIP/GLP-1 agonist tirzepatide [9,10]. While GLP-1 RAs and tirzepatide have demonstrated essential cardiovascular (CV) benefits, concerns regarding their associations with thyroid and pancreatic cancers have emerged, leading to cautionary guidelines. Nonetheless, given their impact on weight reduction, these therapies may, under certain conditions, offer protective effects against malignancy.

Diabetes mellitus is a complex and a fast growing medical problem throughout the globe, in both developed and developing countries. As per WHO report, diabetes is a multifarious group of disorders that disturbs the metabolism of carbohydrates, fat and protein and results in a shortage or lack of insulin secretion and/or reduced sensitivity of the tissue to insulin. Despite advances in understanding and management of this metabolic disorder, the rate of morbidity and mortality due to this disorder is increasing every year. Approximately 285 million people have been diagnosed with diabetes mellitus worldwide and this gure is expected to double by the year 2030 [11]. The number of diabetes mellitus (DM) cases is rapidly increasing worldwide and its complications are a major cause of disability and hospitalization, posing a signicantnancial burden. Various antidiabetic drugs such as biguanides, sulfonylureas, meglitinides, thiazolidinediones, -glucosidase inhibitors, incretin mimetics, dipeptidyl peptidase-IV (DPP-IV) inhibitors and insulin are currently available to reduce, control and manage diabetes mellitus. Most classes of these pharmaceutical drugs have serious side/adverse effects. For instance, sulfonylurea results in hypo glycaemia, which though usually mild to moderate, can cause mild headache, fatal complication, weight gain [12,13], increase food intake, gastrointestinal disturbances and cardiovascular mortality. Metformin (under class biguanides) leads to transient nausea, anorexia or diarrhoea, abdominal discomfort, lactic acidosis with severe renal impairment and renal hypoperfusion [14,15]. Thiazolidinediones group of drugs also causes gastrointestinal disturbances, weight gain, anaemia, headache, visual disturbances, dizziness, haematuria, impotence, less commonly fatigue insomnia, vertigo, hypoglycaemia and proteinuria. These scenarios drive researchers in search of a new class of therapeutic antidiabetic compounds that is essential to overcome diabetic problems including various secondary complications.

Mechanism

Diabetes mellitus (DM) is a set of related diseases in which the body cannot regulate the amount of sugar (specifically, glucose) in the blood. The blood delivers glucose to provide the body with energy to perform all of a person's daily activities. The liver converts the food a person eats into glucose. The glucose is then released into the bloodstream. In a healthy person, the blood glucose level is regulated by several hormones, primarily insulin. Insulin is produced by the pancreas, a small organ between the stomach and liver. The

pancreas also makes other important enzymes released directly into the gut that helps digest food. Insulin allows glucose to move out of the blood into cells throughout the body where it is used for fuel. People suffered diabetes either do not produce enough insulin (type 1 diabetes) or cannot use insulin properly (type 2 diabetes), or both (which occurs with several forms of diabetes). In diabetes, glucose in the blood cannot move efficiently into cells, so blood glucose levels remain high. This not only starves all the cells that need the glucose for fuel, but also harms certain organs and tissues exposed to the high glucose levels.

TYPES OF DIABETES

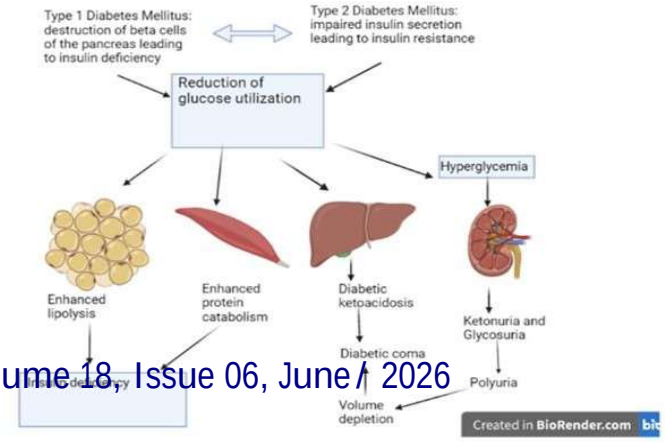
There are two main types of diabetes type 1 and type 2 with their clinical relevance shown in Table 1 and another type of diabetes is gestational diabetes mellitus.

Type 1 diabetes

Type 1 diabetes is an autoimmune disease in which the β-cells of the pancreas do not produce sufficient insulin, a hormone which helps use blood sugar (glucose) for energy. The cells become starved of energy and there will be excess of glucose in the blood. This is then followed by life threatening conditions of hypoglycemia, low blood sugar, and hyperglycemia, high blood sugar. When hypoglycemia develops, cells do not get enough glucose and patients suffer of confusion, loss of consciousness, and coma. Even death can results when the brain is deprived of glucose for too long. Hyperglycemia and prolonged absence of insulin may lead to ketoacidosis, which is accumulation of ketones in the blood when the body uses fat for energy instead of glucose. This is because fatty acids cannot be converted into glucose at steady state. Ketones make the blood acidic and slow down all body functions. This also leads to a coma and eventually death [16-21].

T1DM is one of the most common endocrine and metabolic conditions occurring in childhood. In the vast majority of patients (70–90%), the loss of βcells is the consequence T1DMrelated autoimmunity (concomitant with the formation of T1DMassociated autoantibodies); these patients have autoimmune T1DM (also known as type 1a diabetes mellitus). In a smaller subset of patients, no immune responses or autoantibodies are detected, and the cause of βcell destruction is unknown (idiopathic T1DM or type 1b diabetes mellitus); this type has a strong genetic component5. Unless otherwise specified, the term T1DM refers to autoimmune T1DM in this Primer. T1DM is associated with the appearance of auto antibodies many months or years before symptom onset. These autoantibodies are not thought to be pathogenetic but serve as biomarkers of the development of auto immunity. Characteristic autoantibodies associated with T1DM are those that target insulin, 65 kDa glutamic acid decarboxylase (GAD65; also known as glutamate decarboxyl ase 2), insulinomaassociated protein 2 (IA2) or zinc transporter 8 (ZNT8)6–8. Individuals with specific HLA genotypes (which encode MHC proteins) — that is, HLADR and HLADQ genotypes (HLA DRDQ) — have an increased risk of developing two or more autoantibodies and T1DM. The first βcell targeting autoantibody to appear during early childhood usually targets insulin or GAD65 (that is, anti insulin or antiGAD65 autoantibodies), but these auto antibodies can both be present, whereas it is rare to observe IA2 autoantibody or ZNT8 autoantibody first. What triggers the appearance of a first appearing βcell targeting autoantibody is unclear but is under scrutiny in several studies of children who are being followed up since birth.

Type 2 diabetes



Type 2 diabetes mellitus is a complex endocrine and metabolic disorder. The interaction between several genetic and environmental factors results in a heterogeneous and progressive disorder with variable degrees of insulin resistance and pancreatic β-cell dysfunction. Overweight and obesity are major contributors to the development of insulin resistance and impaired glucose tolerance. When β cells have not longer able to secrete sufficient insulin to overcome insulin resistance, impaired glucose tolerance progresses to type-2 diabetes. When insulin secretion is no longer sufficient to overcome insulin resistance, glucose intolerance progresses to type 2 diabetes. The decline in β-cell function seems to involve chronic hyperglycaemia (glucotoxicity), chronic exposure to non-esterifies fatty acids (lipotoxicity), oxidative stress, inflammation, and amyloid formation [22] Patients with type 2 diabetes usually have pancreatic α-cell dysfunction that results in increased (or non suppressed) glucagon secretion in the presence of hyperglycaemia and probably reduced prandial GLP 1 secretion.

It is estimated that 366 million people had DM in 2011; by 2030 this would have risen to 552 million.8 The number of people with type 2 DM is increasing in every country with 80% of people with DM living in low- and middle-income countries. DM caused 4.6 million deaths in 2011. It is estimated that 439 million people would have type 2 DM by the year 2030. The incidence of type 2 DM varies substantially from one geographical region to the other as a result of environmental and lifestyle risk factors. Literature search has shown that there are few data available on the prevalence of type 2 DM in Africa as a whole. Studies examining data trends within Africa point to evidence of a dramatic increase in prevalence in both rural and urban setting, and affecting both gender equally. The majority of the DM burden in Africa appears to be type 2 DM, with less than 10% of DM cases being type 1 DM. A 2011 Centre for Disease Control and Prevention (CDC) report estimates that DM affects about 25.8 million people in the US (7.8% of the population) in 2010 with 90% to 95% of them being type 2 DM. It is predicted that the prevalence of DM in adults of which type 2 DM is becoming prominent will increase in the next two decades and much of the increase will occur in developing countries where the majority of patients are aged between 45 and 64 years.

Gestational diabetes mellitus (GDM)

Gestational diabetes mellitus (GDM) is a type of DM determined in the second or third trimester of pregnancy that is not clearly overt diabetes. GDM is a provisional disorder that happens in pregnancy and brings enduring danger of type II diabetes2, Women with slightly elevated blood glucose levels are diagnosed as having gestational diabetes, whilst women with substantially elevated blood glucose levels are classified as having diabetes mellitus in pregnancy. GDM tends to arise from the 24th week of pregnancy. Screening by means of an oral glucose tolerance test is therefore recommended and must be conducted early in pregnancy for high risk women, and between the 24th and 28th week of pregnancy in all other women. Women with hyperglycemia diagnosed during pregnancy are at greater risk of adverse pregnancy outcomes such as: very high blood pressure and foetal macrosomia, with the vaginal birth being difficult and risky. In some cases, clinicians prescribe insulin or oral medication in order to control the blood glucose levels. Notwithstanding, gestational diabetes normally disappears after delivery but women who have been previously diagnosed are in danger of presenting GDM in subsequent pregnancies and type IIDM later in their life. In addition, infants beared by mothers with GDM also have a higher risk of developing type II diabetes during adolescence or early adulthood [23,24].

Clinical detection is important, since therapy will reduce perinatal morbidity and mortality. Dysglycemia risk in GDM is a continuum, and risk assessment for GDM should occur at the first prenatal visit. Two groups, the International Association of Diabetes and Pregnancy Study Groups(IADPSG) and the National Institutes of Health (NIH) Consensus Group recommend different testing methods for the diagnosis of

GDM. A large-scale (~25,000 pregnant women) multinational epidemiologic study demonstrated that risk of adverse maternal and neonatal outcomes continuously increased as a function of maternal glycemia at 24-28 weeks, even within ranges previously considered normal for pregnancy. These observations led to a revision in the diagnostic criteria recommended by IADPSG for GDM using a “one-step” 75-gram OGTT. A NIH Consensus Development Conference, using the same data, continues to recommend the “two-step” approach to diagnosis. The stated reason was the lack of interventional trials to prove the new criteria could decrease poor outcomes, as it was observational.

PATHOPHYSIOLOGY

The pathophysiology of diabetes is largely based on insulin resistance, and many research studies have examined the factors such as environmental and genetic factors that promotetype 2 diabetes mellitus (Fig.01) [25]. It is dependent on thebody’s insulin level and its utilization. Insulin is absent in type 1 diabetes mellitus, while in type 2 diabetes mellitus, the effect of insulin is resisted by the peripheral tissues. The brain, an organ greatly dependent on the blood glucose concentration for proper func tioning, is served by the pancreatic beta cells’ release of insulin in response to elevated blood glucose concentrations. Oral anti hyperglycemic and insulin are drugs that reverse hyperglycemia [26,27].

Also, the plasma concentration of glucose signals the central nervous system to release energy fuel for utilization. An increase in autonomic activity is caused by low blood glucose concentration. Hypoglycemia is evidence of low blood glucose levels, which aids in the diagnosis of diabetes. A chain of events occurs as a response to hypoglycemia: reduced insulin secretion, increased secretion of glucagon and epineph rine (counter-regulatory hormones of glucose), heightened sympathoa drenal symptoms, and in the worst case, intellectual disability, convulsions, stroke, syncope, or coma. The administration of glucose intravenously or orally is a rapid treatment to reverse hypoglycemia and return the blood glucose concentration to normal levels [27]. In type 1 diabetes mellitus, the resulting progression of this disease is dependent on the rate of the autoimmune destruction of the pancreatic β-cells. Diabetic ketoacidosis (DKA) is a grave syndrome present in diabetic patients. The body breaks down fat at a faster rate than normal, causing the liver to process the fat into ketones and making the blood acidic. Diabetic ketoacidosis (DKA) can likely occur in children and young adults because of β-cell destruction, it is usually described as the first manifestation of the illness. The disease progresses at a very slow rate, coupled with a gradual increase in fasting plasma glucose levels. Nevertheless, based on increasing insulin deficiency, patients become insulin-dependent together with the presence of severe hyperglycemia and ketoacidosis. Furthermore, due to the severity and progression of type 1 diabetes mellitus, the patient becomes fully dependent on insulin therapy for his or her survival. The pathophysiology of type 2 diabetes mellitus is distinguished by insulin deficiency and insulin resistance, which have been linked to inflammatory cytokines in the plasma and high levels of fatty acids, leading to deficient glucose transport into target cells, elevated breakdown of fat, and increase dhepatic glucose production. Consequent hyperglycemia is caused by the over secretion of glucagon and a deficiency of insulin by the glucagon-secreting alpha cell and the insulin-secreting beta cell, respectively. In the case of type 2 diabetes mellitus, the disease is diagnosed because patients cannot increase

Type 2 diabetes mellitus may go undiagnosed at the early stage because it progresses rather slowly and asymptomatically, sometimes with mild hyperglycemia. The appearance of other symptoms like polydipsia, weight loss, blurry vision and growth impairment comes at the late or advanced stage of the disease. The etiology of this form of diabetes involves a combination of genetic and environmental factors. The disease is often related to various lifestyle factors such as poor diet, age, lack of exercise, family history of diabetes, obesity, earlier gestational diabetes mellitus in women, and pathophysiological conditions like atherosclerosis, dyslipidemia, and hypertension. Gestational diabetes mellitus is the measure of diabetes or glucose intolerance confirmed at the second or third trimester of pregnancy and gestation. At the onset of

pregnancy, it is noted that fasting and random blood are lower than the normal blood concentration value, and an exponential increase in blood glucose levels in the third trimester confirms gestational diabetes mellitus.

Treatment of Diabetes Mellitus

Many of the drugs available in the market are associated with one or more side effects and hence treatment for DM makes this disease a major health problem around the world. The search for effective drugs for the treatment of DM with less or no side effects is continuing. The major components of the treatment of diabetes are: Diet (combined with exercise if possible)

Diabetics should focus on:

Weight control

Nutritional requirements allowing good glycaemic control with blood glucose levels as close to normal as possible

Correcting any associated blood lipid abnormalitiesAntihyperglycaemic drug

Drawback in Conventional Delivery of Anti Diabetic Drugs:

Most of the oral hypoglycemic drugs are available either in the form of tablets and capsules. However, these conventional dosage forms offer various limitations such as short duration of action due to their short half-lives, frequent dosing, a risk of hypoglycemia, lowbioavailability, high protein binding, gastric irritation, diarrhea, insolubility in water and do not comply with the safety and efficacy of the patients [28]. Subcutaneous administrations of insulin associated with various limitations like multiple insulin injections, self-injection, difficulties in using the vial and syringe technique, local tissue necrosis, infection, nerve damage, high cost, potential dosing errors and fear of painful injections are among the barriers to compliance for patients. These limitations revealed the limited accessibility of conventional dosage forms at desired site of action, higher systemic toxicity, narrow therapeutic window, complex dosing schedule for long-term treatment and lead to poor patient compliance [29]. Several research studies are going on in the area of nanotechnology with the aid of nano size particles in order to overcome such limitations in the delivery of anti-diabetic drugs. There are various types of nanotechnology-based drug delivery systems including polymer-drug conjugates, micellar formulations, liposomes, nano sized drug particles and protein-bound drugs [30]. This manuscript throws more light on the need for nanoparticulate drug delivery systems, their advantages, limitations and recent advances in the application of such drug delivery systems in the delivery of anti-diabetic drugs.

Role of Herbal Medicines in Diabetes Management [31-32]

Herbal medicines have long been used as complementary or alternative therapies in the management of diabetes mellitus due to their potential to modulate glucose metabolism, improve insulin function, and reduce complications associated with chronic hyperglycemia.

Mechanisms of ActionHerbal remedies exhibit diverse mechanisms that contribute to anti-diabetic effects:

Improving insulin secretion and sensitivity:Some herbal compounds can stimulate pancreatic β-cells to release insulin and enhance peripheral tissue responsiveness to insulin.

Inhibition of carbohydrate-metabolizing enzymes:

Certain phytochemicals block enzymes like α-glucosidase and α-amylase, slowing carbohydrate breakdown and postprandial glucose rise.

Antioxidant and anti-inflammatory effects:

Phytochemicals such as flavonoids and phenolics reduce oxidative stress and inflammation, which are major contributors to insulin resistance and diabetic complications.

Enhanced glucose uptake:

Some herbs increase GLUT4 expression in muscle and adipose tissues, improving cellular glucose uptake and utilization.These multi-targeted actions make herbal medicines promising adjuncts to conventional therapy, especially in type 2 diabetes.Advantages of Herbal Medicine

Reduced side effects:

Compared to some synthetic hypoglycemic drugs, herbal medicines are often associated with lower adverse effects, which enhances patient compliance.

Cost-effectiveness and accessibility:Many herbal therapies are inexpensive and widely available, particularly in low-resource settings. Potential to manage complications:

Due to their antioxidant, anti-inflammatory, and lipid-modulating properties, herbs may also help mitigate diabetic complications such as neuropathy and cardiovascular dysfunction

PLANTS WITH ANTIDIABETIC POTENTIAL

Many traditional plant treatments for diabetes exist, a hidden wealth of potentially useful natural products for diabetes control. Nonetheless, few traditional antidiabetic plants have received scientific or medical scrutiny, despite recommendations by the World Health Organization in 1980 that this should be undertaken. Medicinal plants that are the most effective and the most commonly studied in relation to diabetes and its complications are: Gentiana olivieri griseb (Gentianaceae), Bauhinia forficata koeingii (Leguminosae), Eugenia jambolana L. (Myrtaceae), Lactuca indica L. (Compositae), Mucuna pruriens Bak. (Leguminosae), Tinospora cordifolia W. (Menispermaceae), Momordica charantia L. (Cucurbitaceae), Aporosa lindleyana Baill (Euphorbiaceae), Cogent db, Myrtus communis L. (Myrtaceae), Rhizoma Polygonati Odorati (Liliaceae), and Terminalia pallida Brand. (Combretaceae). Most of these plants have shown varying degrees of hypoglycemic and anti- hyperglycemic activity. Among active medicinal herbs, Momordica charantia L. (Cucurbitaceae), Pterocarpus marsupium Roxb. (Leguminoceae), and Trigonella foenum greacum L. (Leguminosae) have been reported to be beneficial for treatment of type 2 diabetes. The following traditional medicinal plants or natural products are commonly used in the control or/and treatment of diabetes and its complications. Aporosa lindleyana Baill

The aqueous and alcoholic extracts (100 mg/kg) of Aporosa lindleyana Baill (Euphorbiaceae) reduced blood glucose in normal rats from 80.49/2.7 to 69.89/2.0 mg% and from 82.69/1.9 to 70.89/3.2 mg%, respectively, 3 h after oral administration of the extract (PB/0.001), and also significantly lowered blood glucose level in alloxan-induced diabetic rats from 3069/3.37 to 1609/2.46 and 3289/4.15 to 1529/3.86 mg%, respectively, 3 h after oral administration of the extract (PB/0.001). The antihyperglycemic activity of Aporosa lindleyana Baill (Euphorbiaceae) was compared with that of tolbutamide, an oral hypoglycemic agent [33]. Cogent db Oral administration of 0.15, 0.30, or 0.45 g/kg body wt. of the aqueous solution of Cogent db herbs: Azardirachta indica A. juss (Meliaceae), Phyllanthus emblica L. (Euphorbiaceae), Terminalia belerica Roxb. (Combretaceae), Terminalia chebula Retz. (Combretaceae), Tribulus terrestris L. (Zygophyllaceae), Trigonella foenum graecum L. (Leguminosae), Cuecuma longa L. (Zingiberaceae), Syzigium cumini L. (Myrtaceae), Rotula aquatica Lour. (Lythraceae)] for 40 days exhibited a significant reduction in blood glucose and glycosylated hemoglobin and increased plasma insulin and total hemoglobin along with having antihyperlipidemic effects in diabetic rats. The effective dose was found to be 0.45g/kg body weight (bw). The antidiabetic effect of Cogent db was greater than that observed with glibenclamide [34]

Myrtus Communis L. (Myrtaceae)

Myrtus communis L. (Myrtaceae) leaves and the volatile oil (Myrtii Oleum; MO) obtained from the leaves are used to lower the blood glucose level in type-2 diabetic patients in Turkish folk medicine. MO significantly lowered blood glucose by 51% in alloxan-diabetic rabbits by 4 h through the following days at a dose of 50 mg/kg ($P<0.001$) The hypoglycemic dose (50 mg/kg) was also determined by performing the

oral glucose tolerance test in normal rabbits. The hypoglycemic effect of the MO was 21% higher in rabbits, which received the glucose load orally, when compared with the control group. However, MO did not affect serum insulin concentrations in normal and alloxan diabetic rabbits but reduced the serum triglyceride concentrations by 14% in alloxan-diabetic rabbits. The above observations show that MO exerts hypoglycemic as well as mild hypotriglyceridemic activity in diabetic animals [35].

Allium sativum (Garlic)

Allium sativum, or garlic, is a member of the family Liliaceae17. There is evidence that a daily dose of garlic ethanolic extract (10 ml/kg/day) will reduce blood sugar levels. The anti-diabetic medication glibenclamide was compared to garlic extract and found to be less effective. It was discovered that ethyl acetate, ethanol, and petroleum ether extract all had anti-diabetic effects in rats with Streptozotocin (STZ)-induced diabetes. Anti-platelet, antibacterial, blood pressure lowering, and cholesterol lowering are just few of the medicinal effects of garlic [36].

Aloe barbadensis (Aloe vera)

The common houseplant aloe vera has been used for centuries as a home remedy for a wide range of medical problems. Gel and latex are the two main components of the plant. The aloe vera gel comes from the leaf's mucilage or pulp, while the aloe latex, sometimes called "aloe juice," is a bitter yellow secretion from the pericyclic tubules just under the leaf's outer epidermis. Extracts of aloe gum enhance glucose tolerance in both diabetic and non-diabetic rats. The exudates from Aloe barbadensis leaves had a hypoglycemic effect on alloxanized diabetic rats when given in chronic dosages, but not when given in single doses. Bitter principle extracted from the same plant exhibited a hypoglycemic effect when administered in both single and chronic doses to diabetic rats. In order for aloe vera and its bitter component to have any effect, the beta cells in the pancreas need to have their insulin production or release stimulated. This herb promotes faster healing of wound in diabetic mice and has an anti- inflammatory impact that is dose-reliant, according to the research. Blood glucose levels were significantly lowered after administering extract of aloe vera leaf in water orally. Anti-diabetic, antioxidant, and a four-fold increase in glutathione levels in diabetic rats are only some of the therapeutic benefits of aloe vera gel [37-40]

Acacia arabica (Babul)

It occurs naturally over the entire country of India. The plant extracts combat diabetes by stimulating the body's natural insulin production. While alloxanized rats do not experience hypoglycemia, control rats do. Normal rabbits were fed Acacia arabica seed powder (2, 3, or 4 g/kg body weight) and experienced hypoglycemia due to the resulting liberation of insulin from pancreatic beta cells [41].

Azadirachta indica (Neem)

Extracts of Azadirachta indica in water and alcohol displayed anti-hyperglycemic action in rats treated with streptozotocin, with the augmented glucose absorption and glycogen accumulation in separated rat hemidiaphragm being the likely mechanism(s) at work. In addition to its anti- diabetic characteristics, this plant possesses anti-bacterial, anti-malarial, anti-fertility, hepato- protective, and antioxidant activities [42,43].

Ocimum sanctum (Tulsi)

Tulsi is the name most people use to refer to it. This plant has a long history of therapeutic usage. An aqueous extract of Ocimum sanctum leaves was found to significantly lower blood sugar levels in both normal and alloxan induced diabetic mice. Fasting blood sugar, uronic acid, total amino acid, total cholesterol, triglyceride, and total lipid were all significantly decreased in diabetic rats, demonstrating the hypoglycemic and hypolipidemic actions of Tulsi. By the end of the trial's on 15th and 30th days, participants who were given plant extract orally (200 mg/kg) had reduced their plasma glucose concentrations by 9.06% and 26.4%

respectively. Renal glycogen content in diabetic rats was increased by a factor of ten, while glycogen concentrations in skeletal muscle and the liver were reduced by 68% and 75% respectively [44-46].

Challenges with herbal drugs

There are two entirely different approaches in the future research on TCM/TIM. The “sharp shooter” approach is to select a particular plant with a specific activity or a biological target. By using bioactivity-guided isolation and structural elucidation, one can discover a new chemical entity for a specific disease target. Many Western drugs, such as chemotherapeutic agent paclitaxel, were discovered this way, while some others such as metformin were developed upon further structural modification. Obviously, this is a validated approach for drug diseases.

This approach is termed the “shotgun” approach. While it is not necessary to identify the exact active ingredient(s), it is still necessary to have good quality control of the preparations to ensure reproducibility. Certain chemical markers in the preparations can be selected as markers to standardize the raw materials and processing procedures. Once are producible preparation is obtained and its biological activity is established, it can be used to treat certain given disease in the general patient population. Because the extract contains multiple components which may interact with multiple disease targets, it might be advantageous to a single chemical entity, especially in the area of disease prevention. However, pharmaceutical scientists are facing unique challenges in developing herbal products as anti-DM agents.

Patentability.

Because herbal medicines derive from natural plants, their active components cannot be patented as novel materials. It is also difficult to patent their usage since much information is already in the public domain. But it is possible to patent a unique combination and/or the extraction process. Product standardization

This should be achieved via proper control on raw material, extract pro cess and final formulation. Without effective quality control, consistency of the herbal product may be compromised. Improved methods for quality control of herbal products, such as bioactivity- guided pharmacokinetic methods and genomic fingerprinting techniques are promising.

Placebo-controlled, randomized clinical trials

TCM/ TIM physician’s philosophy to individualize formula for different patients has significantly hindered the systematic scientific investigation according to West ern medicine standards. In comparis onto their West erncounter parts, the anti-DM efficacies of TCM/TIM herbs have not been well studied using randomized, double-blinded clinical trials, although many animal studies have been carried out. However, the implementation of placebo- controlled, randomized clinical trials is a prerequisite for the evidence-based practice of using TCM/TIM preparations for DM prevention.

Toxicity and herb-drug interaction

TCM herbs are generally thought to be relatively safe and with milder side effects. However, their activities are usually not as potent as Western medications. Thus high doses (sometimes as high as 10g per day) are usually required to achieve optimal therapeutic efficacy. In addition, the toxicity of herbal products cannot be ignored. Most common complaints of herbal supplements ingestion are gastrointestinal related, including stomach upset, diarrhea, constipation, nausea, and vomiting. In addition, more serious adverse effects may also occur. For example, ginseng abuse syndrome is a result of chronic ingestion of excessive amounts of ginseng.

This is characterized by hypertension and CNS stimulation, insomnia, and nervousness is also an increased awareness of herb-drug inter action in pharmacokinetics and pharmacodynamics. When used in combination with established anti-DM medications, herbal supplementation may predispose patients at an increased risk of hypoglycemia. For example, Ginkgo biloba extract interacts with selective serotonin reuptake inhibitors

used in the treatment of depression, resulting in the serotonin syndrome, and with thiazide diuretics resulting in decreased efficacy [47].

NEED FOR NANOTECHNOLOGY IN HERBAL DRUG DELIVERY

Phytotherapeutics needs a scientific approach to deliver the herbal drugs in a sustained manner to increase patient compliance and avoid repeated administration, which can be achieved by designing novel drug delivery system. Nanotechnology is one such approach that helps to increase the therapeutic value by reducing toxicity and increasing the bio-availability besides reducing repeated doses. It is a part of drug delivery systems where the drug is uniformly distributed in the body through the blood stream and is delivered at only specific sites thereby improving the therapeutic index. Nanostructured-based drug delivery system offers many advantages, some of which include: (i) they can pass through the smallest and narrow capillary vessels due to their ultra-tiny volume; (ii) they can penetrate cells and tissue gap to arrive at target organs such as liver, spleen, lungs, spinal cord and lymph [98]; (iii) they can provide controlled release for prolong period. These unique properties make nanostructured-based drug delivery system a better choice to deliver drug compared to conventional drug delivery system. The animal experiments demonstrated that the nanoparticles (NPs) enter the bloodstream and end up in organs such as liver and kidney. NPs also are able to protect the drug from degradation in the gastrointestinal tract, release the incorporated drug in a controlled manner thereby minimising side effects [48].

Nanotechnology is a novel and cutting-edge strategy for delivering nano-therapeutics in drug delivery to deal with diabetes. Nanotechnology-driven nanocarriers have brought about a revolutionary impact within the realm of pharmaceutical research and development, facilitating precise manipulation of the shape, particle size, surface characteristics, and release of pharmacologically active ingredients at the specific target sites, ensuring a rationalized rate and dosage [49]. Nanocarriers have been prepared using various types of natural, organic, and inorganic materials that belong to polymers, metals, ceramic, and lipids. Pharmacologically active moieties are incorporated into nanocarriers through physical interactions, including entrapment or encapsulation and surface attachment. These variations could certainly be introduced by the unique properties of several nanocarriers that resolve the drawbacks related with conventional drug delivery systems. The treatment of diabetes typically involves the use of insulin, oral hypoglycemic agents, or incretin-based therapies. However, conventional delivery methods face challenges, such as enzymatic breakdown, suboptimal pharmacokinetics, and invasive administration techniques [50]. Nanocarriers offer an innovative approach by providing benefits, such as improved drug stability and absorption, targeted delivery to specific tissues or cells, controlled or stimuli-responsive drug release, enhanced bioavailability, reduced side effects, and better patient adherence (figure 1 A). For instance, liposomal delivery systems excel in enhancing the permeability and cellular uptake of antidiabetic drugs, enabling targeted release and improving therapeutic efficiency. In contrast, non-liposomal carriers often struggle to cross lipid bilayers, limiting their effectiveness in delivering drugs to the target cells (figure 2 B) [51]. Additionally, by facilitating non-invasive insulin administration modalities such as oral or nasal delivery, nanocarriers assist in alleviating injection-related consumer compliance concerns.

In recent years, a budding interest has been generated in nano pharmaceuticals due to increased number of advancements with an aim to focus on engineering novel applications. The active phytoconstituents and standardized extracts are the main sources from which nano phytomedicines are prepared. By lowering the side effects and toxicity associated with the drugs, the herbal treatment not only helps to increase the bioavailability but also increases the therapeutic value at the same time [52]. In this regard, nanotechnology has a vital role to play in herbal medicines. Further, drug delivery systems incorporating nanotechnology is all set to spread extensively. The problems associated with synthetic drugs can be overcome by nanotechnology based herbal drug delivery systems and subsequently enhancing the potency of medicinal

plants in the near future [53]. Due to the insufficient processing difficulties and logistic justification since long time, herbal medicines were not taken into consideration so as to develop and design novel formulations. However, these shortcomings have been resolved as scientific needs (such as pharmacological mechanistic pathway, pharmacokinetics determination, accurate dose calculation, site of action, etc.) of herbal drugs are solved by modern phytopharmaceutical research such that it can be incorporated in novel drug delivery systems such as solid dispersions, nanoparticles, matrix systems, microemulsions, solid lipid nanoparticles and liposomes. Thus, herbal drug incorporated modern dosage forms with enhanced efficacy and potency can be utilized in a way better for designing and developing novel drug delivery systems [52]

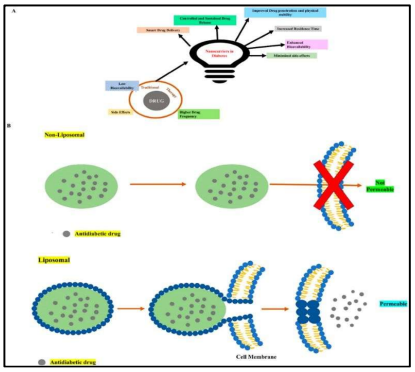


Figure 2: Nanotechnology-based approaches to address challenges associated with traditional therapy

Herbal remedies were selected as feasible drug candidate for delivery through a nano delivery system because of the following properties:

1.Effective chloroform, petrol, acetone, and methanolic extracts are available which may not be suitable for delivery as such.

2These are the bulk drugs so dose reduction is intended.

3.Currently marketed formulations lack target specificity for various chronic diseases.

4.Some other side effects are associated with currently marketed formulations.

5.Patient non-compliance due to large doses and less effectiveness with the available formulations

NANO DRUG DELIVERY SYSTEMS FOR HERBAL EXTRACTS

Phytotherapeutics need a scientific approach to deliver the components in a sustained manner to increase patient compliance and avoid repeated administration. This can be achieved by designing NDDSs for herbal constituents NDDSs not only reduce the repeated administration to overcome non compliance but also help to increase the therapeutic value by reducing toxicity and increasing the bioavailability [54]. The novel carriers should ideally fulfill two prerequisites. First, it should deliver the drug at a rate directed by the needs of the body over the period of treatment. Second, it should channel the active entity of herbal drug to the site of action. Conventional dosage forms including prolonged release dosage forms are unable to meet none of these.

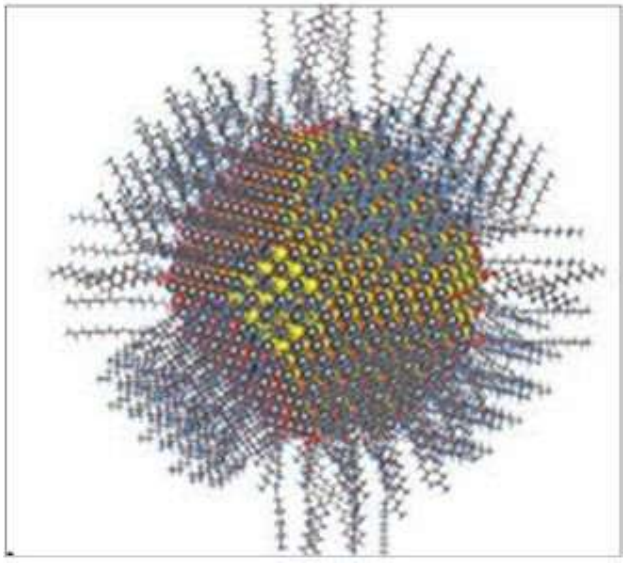
It has a number of advantages for herbal drugs including enhancement of solubility and bioavailability, protection from toxicity, enhancement of pharmacological activity, enhancement of stability, improving tissue macrophages distribution, sustained delivery, and protection from physical and chemical degradation. Thus, the nano-sized NDDSs of herbal drugs have a potential future for enhancing the activity and overcoming problems associated with plant medicines. Nanocarriers applying to herbal remedies will carry the optimum amount of the drug to their site of action bypassing all the barriers such as acidic pH of stomach, liver metabolism, and increase the prolonged circulation of the drug in the blood due to their small size.Hence, use of herbal remedies in an NDDS

will enhance the improvement in the use of herbal remedies that will come forth to treat the various chronological diseases.[55]

Nanoparticles

Nanoparticles are particles between 1 and 100 nanometers in size. In nanotechnology, a particle is defined as a small object that behaves as a whole unit with respect to its transport and properties. Particles are further classified according to diameter [56]

Figure 3: Nanoparticle



Nanoparticles are composed of synthetic or semisynthetic polymers having nano or sub nano- sized structures. In nanotechnology, a small object that used as a whole unit with respect to its transport is defined as particle [57]. Nanoparticles can easily reach the effective site as the formulation is encapsulated in it easily. Microencapsulation of herbal extract in nanoparticulate is an effective way used to protect food ingredients or drug from volatile losses, deterioration, or interaction with other ingredients. Nanoparticles show several advantages like solubility enhancement, efficacy enhancement, bioavailability enhancement, dose reduction and improved absorption of herbal medicines. Nanoparticles were developed with biocompatible and biodegradable polymers with uniform in size, spherical in shape and smooth surface to counter the poor solubility problem and toxicity of triptolide [58].

Nanoparticles have better efficacy as it enhances the herbal drug solubility and helps to deliver the drug to a specific site. Drug delivery in small particle size has quicker dissolution in the blood as it increases the total surface area of the drugs [59].

Nanoparticle systems with mean particle size well above the 100nm standard have also been reported in literature, including nano sized curcuminoids [60], paclitaxel [61] and praziquantel [62] which have a mean particle size of 450,147.7, and even higher than200nm, respectively.

In addition, nanoparticles could also be defined as being submicronic (b1lm) colloidal systems [63]. Then a no spheres have a matrix type structure in which the active ingredient is dispersed throughout (the particles), whereas then a no capsules have a polymeric membrane and an active ingredient core. Nanonization possesses many advantages, such as increasing compound solubility, reducing medicinal doses, and improving the absorbency of herbal medicines compared with there spective crude drugs preparations.

Properties of nanoparticles [64-66

They are effectively a bridge between bulk materials and atomic or molecular structure
The high surface area to volume ratio of nanoparticles provides a tremendous driving force for diffusion, especially at elevated temperatures. Sintering can take place at lower temperatures, over shorter time scales than for larger particles
Suspensions of nanoparticles are possible since the interaction of the particle surface with the solvent is strong enough to overcome density differences, which otherwise usually result in a material either sinking or floating in a liquid
Nanoparticles also often possess unexpected optical properties as they are small enough to confine their electrons and produce quantum effects. For example, gold nanoparticles appear deep red to black in solution

Nanoparticles with one-half hydrophilic and the other half hydrophobic are termed Janus particles and are particularly effective for stabilizing emulsions. They can self- assemble at water/oil interfaces and act as solid surfactants
The photocatalytic activity of the nanoparticles must not lead to a self-destruction of the composite system, and it is essential to check this point before fixing a combination of polymer matrix and nanoparticles.Role of nanoparticles [67]
To deliver the drug in the small particle size that enhances the entire surface area of the drugs
allocating quicker dissolution in the blood
Drug delivery system is targeted in a specific manner
Permeation of the drugs across epithelial and endothelial barriers
To deliver the drugs at sites of action
Combined therapy of the two different modalities or drugs.
TYPES OF NANOPARTICLES
There are two types of nanoparticles, and they are inorganic and organic.
Inorganic nanoparticles
The various types of inorganic particles, namely, magnetic, metallic, ceramic and nanoshells, their description, size, advantages, disadvantages, and applications are produced in Table 1.
Organic nanoparticles
The various types of organic nanoparticles, namely, carbon nanotubes, quantum dots, dendrimers, liposome and polymers, their description and size are described in Table 2
Table 1: Inorganic nanoparticles [68]
Table 2: Organic nanoparticles [68]
HERBAL NANOPARTICLES IN ANTIDIABETIC THERAPY
In recent years, a budding interest has been generated in nanopharmaceuticals due to increased number of advancements with an aim to focus on engineering novel applications. The active phytoconstituents and standardized extracts are the main sources from which nanophytomedicines are prepared. By lowering the side effects and toxicity associated with the drugs, the herbal treatment not only helps to increase the bioavailability but also increases the therapeutic value at the same time [69].
In this regard, nanotechnology has a vital role to play in herbal medicines. Further, drug delivery systems incorporating nanotechnology is all set to spread extensively. The problems associated with synthetic drugs can be overcome by nanotechnology based herbal drug delivery systems and subsequently enhancing the potency of medicinal plants in the near future [70]. Due to the insufficient processing difficulties and logistic justification since long time, herbal medicines were not taken into consideration so as to develop and design novel formulations. However, these shortcomings have been resolved as scientific needs (such as pharmacological mechanistic pathway, pharmacokinetics determination, accurate dose calculation, site of

action, etc.) of herbal drugs are solved by modern phytopharmaceutical research such that it can be incorporated in novel drug delivery systems such as solid dispersions, nanoparticles, matrix systems, microemulsions, solid lipid nanoparticles and liposomes. Thus, herbal drug incorporated modern dosage forms with enhanced efficacy and potency can be utilized in a way better for designing and developing novel drug delivery systems [69].

Phyto therapeutics needs a scientific approach to deliver the herbal drugs in a sustained manner to increase patient compliance and avoid repeated administration, which can be achieved by designing novel drug delivery system. Nanotechnology is one such approach that helps to increase the therapeutic value by reducing toxicity and increasing the bio-availability besides reducing repeated doses. It isa part of drug delivery systems where the drug is uniformly distributed in the body through the blood stream and is delivered at only specific sites thereby improving the therapeutic index. Nanostructured-based drug delivery system offers many advantages, some of which include: (i) they can pass through the smallest and narrow capillary vessels due to their ultra-tiny volume; (ii) they can penetrate cells and tissue gap to arrive at target organs such as liver, spleen, lungs, spinal cord and lymph [71]; (iii) they can provide controlled release for prolong period. These unique properties make nanostructured-based drug delivery system a better choice to deliver drug compared to conventional drug delivery system.

The animal experiments demonstrated that the nanoparticles (NPs) enter the bloodstream and end up in organs such as liver and kidney. NPs also are able to protect the drug from degradation in the gastrointestinal tract, release the incorporated drug in a controlled manner thereby minimising side effects [72]

Complementary and alternative drug formulations need to be developed using several existing phytochemicals already in traditional use to treat several symptoms of diabetes and its complications. However, orthodox medicines were proven to be superior to herbal drugs in the onset of action in spite of substant ial amounts of phyto-antioxidants in herbs. The reason behind this is that their efficacy is seen only in long-term treatment and hence research is needed to enhance their action and bioavailability to targeted organs/organ systems. NPs and nano scaled materials have many physical, chemical and biological properties that render the mattr active for biomedical applications [73]. They are usually in the range of 100–1000 nm, however, some of the minute NPs, which are smaller than 100 nm across, attract water on the inside and are water repelling on the outside [74]. When they reach the bloodstream, they break down in response to the pH of blood and then release the drug. NPs are used to deliver both small-molecule and large macromolecular (i.e. DNA, RNA and proteins) therapeutics, as well as to diagnose and monitor the progression of disease [75]. Biosynthesis of NPs using plant extracts is the favourite method of green, exploited to a vast extent because the plants are widely distributed, easily available, advancement over physical and chemical methods, safe to handle and with a range of metabolites and compatibility for pharmaceutical and biomedical applications as they do not use toxic chemicals in the synthesis protocols [76,77].

NPs can be used as diagnostic tools for the early detection and prevention of diseases owing to their unique biological, physical, optical, chemical, and magnetic properties. For example, NPs can be used as an imaging probe in diseased tissues or organs, due to their nanoscale size or by using specific binding ligands on NP's surfaces. In addition, due to their longer circulation time and being more targetable in the body by changing their size, surface charge, and other properties, NPs are widely explored as contrasting agents for different types of biomedical imaging modalities, including magnetic resonance imaging (MRI) which can be used in the early diagnosis of DM. Moreover, NPs have a large surface-to-volume ratio, which can offer a better matrix for the immobilization of enzymes and improve enzyme–substrate interactions by circumventing free enzyme aggregation, which in turn enhances enzymatic activity. Several researchers have assessed different

materials that can be used as matrices for enzyme immobilization, including magnetite NPs, gold NPs (AuNPs), and carbon nanotubes [78,79].

Overall, the synthesis of highly sensitive biosensors (such as nanosensors), as well as NPs that improve glucose sensor function, will ultimately improve the lives of patients living with DM. A biosensor is an analytical device and powerful diagnostic tool to detect the presence or concentration of a biological analyte [80].

It consists of three main parts:

Components that recognize biological elements (such as enzymes, receptors, antibodies, nucleic acids, aptamer, microorganisms, and lectins) and produce signals

Signal transducer (such as electrochemical, optical, thermometric, piezoelectric, and magnetic) that converts the biorecognition event into a measurable signal, and

A reader device that converts the signal into a readable form [81,82].

At present, glucose oxidase (GO) is the standard enzyme in glucose biosensors and it is widely used to measure glucose levels in biological samples based on the enzyme-catalyzed, oxidation mechanism. In glucose biosensors, immobilized GO catalyzes the oxidation of β-D-glucose to gluconic acid in the presence of flavin adenine dinucleotide (FAD) as co-factors that are reduced to FADH2. The reduced FADH2 is regenerated by reacting with molecular oxygen, leading to the formation of hydrogen peroxides (H2O2) which are oxidized at a catalytic— classically platinum (Pt)—anode. Finally, the electrode recognizes the number of electron transfers, and this electron flow is proportional to the number of glucose molecules present in the sample, such as blood.

Although glucose biosensors based on GO are widely used in clinical diagnosis due to their low cost, availability, good sensitivity, relatively good selectivity to glucose, and fast and real- time detection, it still has certain limitations including the poor electron transfer efficiency between the active center of GO and electrode materials. Recently, several researchers have synthesized nanomaterials with excellent conductivity and biocompatibility (such as graphene, AuNPs, and conducting polymers) as electron transport media to improve the performance of enzyme-based biosensors [83,84]. In addition, the incorporation of NPs into the sensors provides a variety of advantages, including increased surface area, more efficient electron transfer from enzyme to electrode due to the excellent conductivity and small bandgap, enhanced stability, and the ability to include additional catalytic steps [85,86,87]. The most common application of nanotechnology for sensors in diabetes is the use of NPs to assist standard enzymatic electrochemical detection of glucose or direct detection of glucose oxidation at an electrode; i.e., nonenzymatic glucose sensors.

Application of Nanoparticles as Therapeutic/Anti-Diabetic Agents

Macro and micro minerals play a key role in the proper functioning of proteins, many enzymes, and transcriptional factors. For instance, Zn, Mg, and Mn are cofactors for several enzymes. Zn and Cr are involved in the synthesis and secretion of insulin from the pancreatic beta-cells and increase the insulin receptor activity on target tissues, respectively. The insulin molecule forms polymers with zinc in β-cell granules and zinc plays a major role in the secretion of insulin from pancreatic β cells. The antidiabetic and insulin-like effect of zinc has been reported in several in vitro and in vivo studies [88]. Zn enhances the phosphorylation of the insulin receptor β-subunit and successively stimulates the phosphatidyl inositol 3-kinase and protein kinase [89,90]. As a result, of the several kinds the literature showed, Zn oxide NPs (novel agent to deliver Zn) have great implication for DM treatment [91-94].

El-Gharbawy et al., have synthesized ZnONPs with a median size of ~20 nm via the sol-gel method using zinc acetate and methanol as precursors to revive the function and structure of β- cells. ZnONPs alone or together with vildagliptin (DPP-IV inhibitor) significantly decreased microRNA-103 and microRNA-143

expression (potential biomarkers for type 2 DM) compared to the control diabetic group, indicating its antidiabetic effects. In addition, ZnONPs improved many indices of diabetic dysfunction (such as glucose tolerance, weight loss, insulin levels, fructosamine levels, pancreatic SOD activity, and pancreas histology) and have a significant antidiabetic effect. The anti-diabetic properties of ZnONPs were briefly discussed by Kim San Tang in his review paper entitled "The current and future perspectives of zinc oxide NPs in the treatment of DM" [95].

In addition, several researchers reported that inorganic NPs have a promising potential in treating DM due to their anti-oxidative activity against reactive oxygen species (ROS) [96]. ROS (e.g., superoxide anion (O2•−), hydroxyl radical (OH•), hydrogen peroxide (H2O2), and hypochlorous acid (HOCl)) are derivatives of molecular oxygen or partially reduced metabolites of oxygen that own strong oxidizing capabilities [97]. At physiological concentrations, ROS play a great role in cell metabolism and as a signaling molecule that regulates cell growth and differentiation. However, at high concentrations ROS oxidizes and damages cellular macromolecules (such as protein, lipid, and DNA) that ultimately affect their biological function, leading to cell apoptosis and necrosis. Moreover, when a greater imbalance occurs in favor of the ROS, oxidative stress results, which is identified as the causal factor for cardiovascular disease, diabetes, rheumatoid arthritis, cancer, and neurodegenerative disorders. It is, therefore, advantageous to employ exogenous antioxidants or agents which can control the level of ROS through different chemical reactions.

Currently, several NPs with catalytic activity toward ROS are designed and used to protect against oxidative stress-related toxicity. For example, Weaver et al., have synthesized enzyme- mimetic auto-catalytic, antioxidant, self-renewing cerium oxide nanoparticle (CONP)- composite hydrogel encapsulated β-cells. Although cytotoxicity was observed after phagocytosis of low concentrations (~1 mM) of CONPs by β-cells, the authors reported that co-incubation of CONPs with β-cells has potent cytoprotection from superoxide exposure. In addition, after CONPs-composite hydrogel was embedded within alginate hydrogels, an excellent cytoprotection effect from the free radical attack was observed without cytotoxicity, to encapsulated β-cells at up to 10 mM concentration of CONPs.

Similarly, BarathManiKanth et al., have synthesized gold nanoparticles (AuNPs) to control the hyperglycemic conditions in streptozotocin-induced diabetic mice [98]. The author reported that AuNPs tend to inhibit lipid peroxidation and ROS generation during hyperglycemia. In addition, the AuNPs exhibited an insistent control over the blood glucose level, lipids, and serum biochemical profiles in diabetic mice near the control mice provoking their effective role in controlling and increasing organ functions for better utilization of blood glucose.

In addition, manganese-based NPs have an antioxidant effect and could aid in the survival and continued function of the embedded cells by reducing free radical load and improving oxygenation. MnO2 NPs display numerous useful properties including high reactivity with H2O2 and the capability to fully recover the oxygen, which makes them the best choice in glucose-responsive materials. Wu et al., used manganese oxide NPs to catalytically remove H2O2 produced through the oxidation of glucose in their self-regulating insulin release device. Similarly, Tootoonchi et al., have synthesized and characterized four manganese oxide nanoparticles and evaluated their catalytic ability to remove ROS and produce oxygen, acting as in vitro enzyme mimetics [99]. The synthesized manganese NPs have minimal cytotoxicity to murine insulinoma cells and protect cells from H2O2 exposure.

On the contrary, different studies showed that the minerals imbalance can be a cause of DM, primarily correlated with ROS generation which finally ends up in oxidative stress that will decrease the insulin gene promoter activity and mRNA expression in pancreatic islet cells. Compared with the liver, β-cells have low antioxidant (i.e., 1% catalase, 2% glutathione peroxidase, and 29% superoxide dismutase (SOD) activities and as a result are more susceptible to oxidative stress. Like free metals, their derivative NPs have multifaced

effects on DM. The foremost common negative outcome associated with the therapeutic application of NPs is the excessive generation of ROS which is considered a key factor in metal NP- induced toxicity. This shows that metal oxide NPs have a dual effect on DM treatments, and hence their antioxidant or pro-oxidant properties must be assessed before being used to treat DM.

Natural nanocarriers in the treatment of T2DM and its complications

Finding effective diabetes therapies is the next step after a diagnosis has been made. Given that around 80–90% of diabetes cases are represented by T2DM and just 5–10% are T1DM, recent research has investigated the potential use of nanomaterials in both T1DM and T2DM therapies, with a focus on the latter one. Antidiabetic drugs can be used efficiently to promote insulin secretion or reduce glucose production. Contrarily, in T1DM, where the pancreas produces very little or no insulin, treatment with insulin is necessary. Despite this, nanomaterials containing natural (e.g., Momordica charantia, Panax ginseng, Allium cepa, Euphorbia hirta) and synthetic (e.g., insulin, glibenclamide, metformin) antidiabetic drugs improved clinical outcomes in both T1DM and T2DM, due to the innovative properties of NPs, such as increased drug targeting, sustained hypo glycemic effect, and reduced risk of adverse reactions [100].

Several studies have indicated that natural polysaccharides exhibit noteworthy hypoglycemic effects through various mechanisms. These include the repair of pancreatic islet cells, enhancement of insulin sensitivity, regulation of related digestive enzyme activity, and modulation of key enzymes involved in the metabolism of liver glucose. In the last twenty years, there has been a significant surge in employing nanotechnology for the development of medications targeting diabetes, with several normoglyce mic nanocarriers being developed. The appropriate term for normoglycemic nanocarriers is hypoglycemic nanocarriers which are designed to deliver drugs that lower blood glucose levels in patients with diabetes.

Systems using nanoencapsulation have many benefits and are frequently used in differ ent industries. Both hydrophobic and hydrophilic molecules can be encapsulated, thus improving stability, decreasing toxicity, and protecting against degradation. Drugs can be attached to the surface of nanocarriers or enclosed within their matrix [101]. Apart from possessing the appropriate particle size, variable surface charge, and drug-loading capac ity, nanocarriers also have various other advantages for drug delivery. Polysaccharide-based nanocarriers, for instance, demonstrate reduced toxicity, increased safety, enhanced encapsulation efficiency, targeted drug delivery, controlled release, and accelerated intestinal absorption [102].

Rationale of Herbal Nanotechnology in Anti-Diabetic Therapy [103]

Diabetes mellitus is a chronic metabolic disease that requires long-term management. Many plant-derived compounds (like flavonoids, alkaloids, polyphenols) show antidiabetic activity, but their clinical use is limited by:

Poor water solubility

Low gastrointestinal stability

Rapid metabolism and low bioavailability

Inadequate tissue targeting

Nanotechnology (like nanoparticles, nanoemulsions, liposomes, solid lipid nanoparticles) is applied to enhance the efficacy of herbal compounds in diabetes therapy because it can:

1Improve solubility and absorption of poorly soluble herbal bioactive molecules.

2..Protect compounds from degradation in the digestive tract.

3.Enable sustained or controlled release, improving therapeutic effects over longer periods.

4.Enhance targeting to tissues involved in glucose metabolism (e.g., pancreas, liver, muscle), potentially increasing insulin sensitivity and reducing glucose levels.

5.Reduce dose and side effects, since nanoformulations can require smaller effective doses with less systemic exposure.

LITERATURE REVIEW

1. Ateeb M et.al (2026)

Diabetes and cancer are two severe, multifaceted, and long-lasting diseases. Each year, diabetes mellitus and cancer claim the lives of about 11 million individuals. Recently, metal-based nanoparticles (MNPs), which were produced utilizing plant extracts in an ecologically benign way, have appeared as a superior choice for the management of these deadly illnesses. The current study investigated the antioxidant, anti-diabetic, and anticancer properties of greenly generated silver nanoparticles (AgNPs) and Grewia asiatica Linn (G. asiatica), a leaf extract from the Malvaceae family. The results indicate the color transition of the DPPH solution from purple to yellow, with the crude extracts exhibiting robust antioxidant activity (78.68% ± 0.02) and the nanoparticles demonstrating even higher activity (81.62% ± 0.02) against DPPH. Furthermore, with IC50 values of 177.3 µg/ml, 99.8 µg/ml, and 73 µg/ml, respectively, AgNPs efficiently prevent the development of human breast (MCF-7), cervical (HeLa), and liver (HepG2) cancer cells at exceptionally low doses. Additionally, the AgNPs exhibited superior anti-diabetic activity, as demonstrated by alpha-glucosidase and alpha-amylase inhibition assays (85.03% ± 0.02 and 75.15± 0.01 accordingly). AgNPs were therefore shown to be a potent α-glucosidase inhibitor, a free radical scavenger, and an efficient medicinal substance that targets human breast cancer cell lines (MCF-7). To determine the nanoparticles' anticancer and antidiabetic actions, further research is necessary [104].

2. Parui RK et.al (2026)

In the Quantum Year 2025, the importance of the quantum tunneling discovery has been remembered through this article. Although the influence of quantum tunneling spreads across almost all fields of science, its effect as quantum medicine in the safeguard of human life, particularly type 2 Diabetes Mellitus (T2DM), has been discussed. Nanotechnology offers scientists the ability to describe the manipulation of matter on an atomic, molecular, and nano- level scale, where unique quantum mechanical effects take place, i.e., reduction of at least one dimension at the nanoscale particles can be useful in the field of health care and medicine, particularly in diabetic treatment. At present, diabetes has turned into a chronic disease, and day by day, healthcare expenses are increasing more and more. Neem synthesis nanoparticles from herbs and plants turn into a substitute medicine that is less cost effective compare to chemical medicine. Natural environment, which can be considered as an open mine of herbs

and plants like aloe vera, neem, tulsi, turmeric, etc., that are easily available. These have potential for treating as green synthesis materials which are capable of providing gold (Au), silver (Ag), and other nanoparticles. Fresh neem leaf extract (i.e., neem extract) exhibit magical role for quick support during sudden sugar spikes in the human bloodstream. “Neem extract” shows promise for T2DM by potentially improving insulin sensitivity during glucose uptake as well as lowering blood sugar. The realistic scenario is “neem juice/extract” has special characteristic like (a) increase in glucose uptake which can help the cells for taking more amount glucose, (b) anti-inflammatory property that acts as an anti-diabetic, (c) quick support during sugar spikes and (d) promising potential that can be used in clinical trials for blood sugar management for diabetes [105].

3. Sharma S et.al (2026)

Curcuma caesia, a lesser known therapeutically potential herb, rich in bioactive compounds but still limitedly used for pharmaceutical applications due to poor solubility, stability and bioavailability of active constituents. To overcome these limitations, the present study aims to synthesis unipot copper oxide nanoparticles through eco-friendly green synthesis approach using *Curcuma caesia* extract as stabilizing and reducing agent. The synthesised nanoparticles were initially optimized using one variable at a time approach followed by statistical validation through Response Surface Methodology framework based on Box-Behnken Design. Synthesised nanoparticles were further characterized using UV-Vis spectroscopy, FTIR, SEM, TEM and XRD which confirmed the crystalline spherical nanoparticles of 50-70 nm size with distinct Surface Plasmon Resonance peak at 430 nm. FTIR analysis revealed the synthesis mechanism including reduction and capping of nanoparticles with bioactive compound. Further, biological assays demonstrated that synthesised nanoparticles exhibited strong antioxidant activity with IC50 value of 13.037 nM through DPPH assay followed by strong antibacterial activity, membrane lipid peroxidation (75.2-16.4%), anti-inflammatory (62-72%) and anti-diabetic activity (36.62-59.79%). These findings highlight the potential of *Curcuma caesia* mediated copper oxide nanoparticles as strong therapeutic agent and directing it towards biomedical applications [106].

4. Datta K et.al (2025)

Diabetes is a chronic metabolic disorder that is characterized by high postprandial blood sugar levels and increased fasting, which disrupts physiological balance and causes organ damage. Owing to the global health risk of type 2 diabetes, natural remedies have shown promise as

viable alternatives because of their outstanding antidiabetic properties. Nevertheless, the therapeutic use of these compounds is rather restricted due to their inadequate solubility, instability in the gastrointestinal tract, low absorption, and other related factors. Currently, the development of nanoscale systems is a notable approach to enhancing the delivery of phytochemicals. This study aims to investigate the advancements in drug delivery techniques using nanoparticles, with a particular focus on enhancing the effectiveness of herbal remedies in the treatment of diabetes. This study aims to enrich our understanding of nanotechnology's potential in enlightening drug delivery systems by employing database repositories like PubMed, Scopus, Google Scholar, and Web of Science. Based on their categorization and structure, nano-systems are classified into liposomes, nanostructured lipid carriers, phytosomes, niosomes, solid lipid nanoparticles, self-nano emulsifying drug delivery systems, and inorganic nano-carriers. This study intricately describes the formulation process, selection criteria, and mechanism of herb-loaded nanoparticles using an example of the pharmacokinetic and pharmacodynamic properties of antidiabetic herbal drugs. Researchers have proven that nano-formulations of herb-loaded antidiabetic drugs improve compliance and therapeutic efficacy by resolving pharmacokinetic and biopharmaceutical issues. We could expect the creation of nano-formulations to be a viable method for optimizing the therapeutic effectiveness of plant-produced antidiabetic compounds [107].

5. Afshin N et.al (2025)

Diabetes mellitus (DM) is a non-communicable, life-threatening syndrome prevalent worldwide. One effective treatment for DM is the medicinal use of green synthesized silver nanoparticles (AgNPs), which are eco-friendly and cost-effective. This study investigates the antidiabetic potential of greensynthesizedAgNPs derived from a polyherbal formulation (PHF). Characterization of PHF-AgNPs included UV–Vis spectroscopy, FTIR, XRD, SEM, and EDX. Diabetes was induced in albino Wistar rats (N = 30, n = 6/group, 150–200 g, 8 weeks old) via intraperitoneal alloxan injection (150 mg). Groups are as follows: 1) untreated control, 2) diabetic control (150 mg/kg b.w. alloxan), 3) glibenclamide (0.5 mg/kg), 4) PHF-AgNPs (10 mg/kg), and 5) PHF-AgNPs (20 mg/kg). Blood glucose levels (BGL) were monitored on days 0, 7, 14, and 21. Blood samples were collected for the liver, kidney, and lipid profile analysis before euthanization. The results showed that PHF AgNPs had an average size of 20 nm and exhibited significant reductions in BGL, with PHF-AgNPs at both 10 mg/kg and 20 mg/kg demonstrating superior effects compared to glibenclamide. Histopathological analysis revealed tissue regeneration in the liver, kidney, and pancreas, indicating healing of

alloxan-induced damage. Additionally, treatment improved liver and kidney function markers, and lipid profiles, with reductions in cholesterol, triglycerides, ALT, AST, and creatinine levels compared to the diabetic control group. These findings suggest that green synthesized PHF- AgNPs effectively improved blood glucose control, body weight, and organ health, positioning them as a promising antidiabetic agent with potential for further clinical development [108].

6. Abbigeri MB et.al (2025)

The weed Martynia annua traditionally known as Kakanasika is annual herbaceous plant known for its multiple medicinal properties such as anthelmintic, analgesic, antipyretic, antibacterial, anti-convulsant, anti-fertility, anti nociceptive, antioxidant, CNS depressant and wound healing activity. The aqueous root extract of M. annua was subjected to qualitative analysis, revealing the presence of terpeniods, indicative of its rich phytochemicals composition. Utilizing a green synthesis approach, silver nanoparticles (AgNPs) were successfully synthesized from the plant extract. Characterization through UV-Visible spectroscopy, FTIR, DLS, and SEM/EDX confirmed the formation of AgNPs with polygonal morphology and an average size of 64 nm, with the PDI of 0.385. Additionally, the AgNPs demonstrated moderate stability, evidenced by a zeta potential of −21.6 mV. Evaluation of the synthesized AgNPs focused on their anti-diabetic potential. The green synthesized R-AgNPs were potent antioxidant agents. They exhibited significant inhibition of alpha amylase, a pivotal enzyme in carbohydrate metabolism, suggesting their efficacy as anti-diabetic agents. Moreover, the AgNPs enhanced glucose uptake by yeast cells, indicating their promising therapeutic role in managing diabetes mellitus. This study highlights the pharmacological importance of M.annua, particularly its aqueous root extract, in the eco-friendly synthesis of AgNPs with potential therapeutic implications. Further investigation into the mechanism of action and clinical efficacy of these AgNPs in diabetes management is warranted [109].

7. Upadhyay P et.al (2025)

Herbal medicines have served humanity for numerous generations all across the world. Current methods in phytochemical and phytopharmacological sciences base the clinical applicability of numerous medicinal plants on the composition of active compounds and how much of these compounds are present in samples. Numerous therapeutic compounds such as flavonoids, tannins along terpenoids exist as watersoluble substances yet they have limited potential for absorption. Multiple barriers prevent these compounds from penetrating cell membranes or taking absorption or crossing cell membrane barriers because of their large molecular size and

poor absorption and inability to cross cell membranes. This causes them to have low bioavailability and reduced efficacy. Plant extracts fail to enter clinical practice due to these circumscribing factors. Researchers have extensively recommended using nanotechnology to overcome the obstacles related to herbal medicine delivery. Nanoscale technology increases the efficacy of plant extracts by reducing the amount of administration required while reducing side effects and producing therapeutic advantages. Nanocarriers maintain active components at their best concentrations during therapy while guiding them to specific destinations. Treatment methods that exist in the conservative healthcare system typically do not achieve these standards. This section evaluates both nanotechnology principles and their use in herbal drug delivery systems. The drug delivery system using herbal nanotechnology remains essential for diabetes management because polymeric and lipid nanoparticles, liposomes, dendrimers, and niosomes show superior performance than traditional oral hypoglycaemic agent treatments [110].

8. Sher A et.al (2025)

Diabetes mellitus (DM), a serious health risk, can result in several problems and fatalities. This study investigates greensynthesized silver nanoparticles from Asparagus officinalis (AO- AgNPs) for their anti-diabetic properties. AO-AgNPs were analyzed using UV-Vis FTIR XRD, EDX, and SEM. UV-Vis spectroscopy with an extract peak of 383nm and AO-AgNPs was 420nm. The extract FTIR peaks were from 528 cm-1 to 2730 cm−1, whereas of AO-AgNPs peaks were from 632 cm−1 to 3352 cm−1. XRD diffraction peak patterns were from 111 to 331 in the 2θ range. The SEM revealed that the AO-AgNPs were uniformly sized and smooth structure. In yeast glucose uptake assay, AO-AgNPs had significant effects of (78.6 ± 0.78%) and alpha-amylase inhibitory activity (88.8 ± 0.89%) compared to crude extract and standard. Alpha-glucosidase inhibition by crude extract, AO-AgNPs and metformin at 35 µg/ml were highest at 78.7 ± 1.05%, 85.3 ± 0.65 %, and 89.6±0.88%, respectively. In the glucose adsorption assay, AO-AgNPs (9.7±0.2%) and the crude extract has (8.4±0.3%). In an in vivo study Alloxan-induced diabetic mice showed that AO-AgNPs (10, 20, and 30 mg/kg b.w.) effectively lowered blood sugar levels and supported pancreatic and liver cell regeneration. Quercetin and beta-sitosterol were identified as promising compounds through docking studies. Overall, AO-AgNPs demonstrate significant potential as anti-diabetic agents [111].

9. Meng L et.al (2025)

This work introduces the design and preparation of a new type of crosslinked polymers template based on chitosan-pectin polysaccharides (CHT-PEC) to encapsulate silver nanoparticles as a novel biological agent by using *Anethum graveolens* leaf extract as a natural reducing reagent. CHT-PEC hydrogel was formed by hydrogen bonds and crosslinked by glutaraldehyde to have a natural reducing/capping and stabilizer template for immobilizing nanoparticles (CHT-PCT/Ag NPs). The prepared CHT-PCT/Ag nanocomposite was confirmed by various advanced techniques like FT-IR, UV-vis, FE-SEM, TEM, elemental mapping, EDX, and XRD techniques. TEM images showed the particles shape was spherical and monodispersed with a size of 10–15 nm. The CHT-PCT/Ag NPs pure crystalline nature was recorded by the related XRD pattern. In the *in vivo* study, gestational diabetes mellitus was induced in rats *via* intraperitoneal administration of streptozotocin. The subjects were subsequently divided into five distinct groups: the CHT-PCT/Ag nanocomposite-100 μg/kg group, the CHT-PCT/Ag nanocomposite-400 μg/kg group, the glibenclamide group, the untreated rats, and the normal pregnancy rats (n = 10). The CHT-PCT/Ag nanocomposite was administered intragastrically over 25 days. On the concluding day, the concentrations of ALT, AST, ALP, and blood glucose were evaluated. After processing the tissues, the volumes of the liver and its sub-compartments were estimated. The CHT-PCT/Ag nanocomposite demonstrates the capability to lower the increased levels of AST and ALP enzymes. In the untreated group, the introduction of CHT-PCT/Ag nanocomposite led to a reduction in blood glucose levels. The streptozotocin administration caused a significant increase in the volume of hepatocytes and sinusoids. Nevertheless, following treatment with CHT-PCT/Ag nanocomposite-400, a marked decrease in their volume was observed. Conversely, the volumes of the portal vein and bile ducts remained consistent across the experimental groups. The presence of CHT-PCT/Ag nanocomposite resulted in alterations in the volume of the hepatic arteries and central vein. This study highlights the antidiabetic and hepatoprotective properties of CHT-PCT/Ag nanocomposite, suggesting its potential as a supplementary measure to protect liver while simultaneously prevent the gestational diabetes [112].

10. Parvez MM et.al (2025)

The study was carried out to evaluate the efficacy of bitter gourd extract (BGE) and zinc oxide nanoparticles (ZnONPs) against streptozotocin (STZ) induced diabetes nephropathy (DN) in male Swiss Albino mice. Multiple intraperitoneal (IP) injection of STZ was used to induce

diabetes in experimental mice and were divided into five groups viz., T0 = control (no diabetes and no treatment), T1 = STZ-induced DN without treatment, T2 = STZ-induced DN treated with BGE, T3 = STZ induced DN treated with ZnONPs and T4 = STZ-induced DN treated with both BGE and ZnONPs. The respective groups received single oral daily dose of BGE @ 5g kg-1 body weight and ZnONPs @ 8 mg kg-1 body weight, where control and T1 groups were given normal distilled water orally. Data were recorded on blood glucose level, blood urea nitrogen levels and serum creatinine at 5 days after STZ administration and thereafter 7, 14 and 21 days after administration of bitter gourd extract and ZnO nanoparticles. Bitter gourd extract and ZnONPs were found operative to reduce the blood glucose level, creatine level and blood urea nitrogen level in nephropathic mice where combined effect was more effective than the individual effects against diabetic nephropathy. The findings provide considerable evidence about the efficacy of BGE and ZnONPs which could help to design the clinical application of BGE and ZnONPs on a standardized formulation to protest the development of diabetic nephropathy. However, BGE and ZnONPs possess a potential anti-diabetic as well as anti nephropathic activity, which could be validated by further mechanistic investigations [113].

11. Thirugnanasambandan T et.al (2024)

Since conventionally prescribed drugs lead the side-effects, complementary and alternative medicines are more effective for autoimmune diseases, including cancer, diabetes, and psoriasis. On the other hand, green synthesis using plant extracts results in nanoparticles of very small size in homogenous solution and they can be eliminated from the body. The extracts of leaf, seed, flower, and root can reduce the precursor salts and the bio-active components are adsorbed on the surface of nanoparticles. Plants having antidiabetic effects have been used to prepare nanoparticles; thus, obtained nano formulations are analyzed for their antidiabetic activities. In addition, various natural nanoparticles like curcumin and quercetin are used for the treatment of diabetes. Polymers, liposomes, and solid lipid nanoparticles encapsulated with phytochemicals having antidiabetic activity are also revealed. This review gleaned the potentials of plant-based biosynthesized nanoparticles to be antidiabetic agents [114].

12. Archana TM et.al (2024)

Natural products have emerged as promising candidates for the development of nano- antidiabetic drugs, presenting a novel approach to combat the rising global burden of diabetes. These bioactive compounds with intricate chemical entities and multifaceted pharmacological effects are mainly of plant origin. In the context of diabetes, natural products exhibit a wide

range of modes of action that include modulation of insulin secretion, expediting insulin signalling cascades, improving insulin sensitivity, regulating enzymes involved in glucose metabolism, and attenuating oxidative stress and inflammations. These intricate mechanisms of actions, combined with the potential synergistic interactions among the phytocompounds, contribute to their profound anti-diabetic effects. Despite the promising pharmacokinetic properties exhibited by the phytoconstituents, poor solubility and bioavailability are the main challenges faced by this system of treating diabetes. The utilization of nanotechnology in formulating natural products as nano-antidiabetic drugs offers numerous advantages over conventional approaches. Nanoencapsulation techniques enable precise control over the physicochemical properties of drug, such as solubility, stability, and bioavailability, thereby enhancing their therapeutic efficacy. Furthermore, nanoscale drug delivery systems facilitate targeted delivery and sustained release of active compounds, optimizing their pharmacokinetics and minimizing potential side effects. Beyond their therapeutic benefits, nano-antidiabetic drugs offer sustainable and environmentally friendly approaches to diabetes management [115].

13. Rachana R et.al (2024)

Diabetes is a severe chronic disorder with hyperglycemia, either due to dysfunctional pancreas (type I) or due to malfunctioned glucose utilizing entities like: liver, straight muscle or adipocytes (type II). type 1 or insulin-dependent/Juvenile diabetes and type 2 diabetes mellitus (DM) or non-insulin dependent/adult-onset diabetes are the two broad classifications of diabetes. In case of type 1 diabetes injectable insulin is the major form of medication given and in case of type 2 DM, α-glucosidase inhibitors, insulin sensitizers, SGLT2, insulin secretagogues, biguanides, amylin antagonists, incretin mimetics, and inhibitors are majorly used. Monotherapy and combination therapies are the preferred therapies to manage type 2 diabetes nowadays. Conventional antidiabetic drugs majorly lack cost effectiveness and have side effects and so the world is now again shifting towards using traditional medications based on natural products. Traditional drugs also have many complexities such as: low bioavailability, small shelf life and non-targetability. To address such issues in both the cases of modern-day medicine or natural products-based medicines: nanotechnology-based drug delivery systems are being devised. The present review introduces the basics of diabetes and discusses nanotechnology-based drug delivery for diabetic patients, majorly for natural products-based antidiabetic [116].

14. Sarkhel S et.al (2024)

Diabetes is a widespread metabolic illness. Mismanagement of diabetes can lead to severe complications that tremendously impact patients' quality of life. The assimilation of nanotechnology in diabetes care holds the potential to revolutionize treatment paradigms, improve patient outcomes, and reduce the economic burden associated with this pervasive disease. This manuscript explores the multifaceted utilization of nanomaterials in diabetes care, emphasizing the unique features of nano-based medication delivery methods and smart drug delivery mechanisms. Additionally, this paper talks about research on nanocarrier-integrated oral, transdermal, and inhalable insulin delivery; dendrimer- and nanocarrier-coupled antisense oligonucleotide-driven gene therapy; the implementation of gold nanoparticles and quantum dots for glucose surveillance; and nucleic acid therapies. There are certain restrictions when using medication delivery methods that are commonly available to handle diabetes. In order to increase efficacy and safety, the rapidly developing science of nanotechnology is also being explored and employed in medical biology. Nanomaterials like liposomes, dendrimers, niosomes, polymeric and metallic nanocarriers, and solid lipid nanoparticles are among the nanocarriers that have been developed for better delivery of various oral hypoglycemic agents in comparison to conventional therapies. These nanocarriers provide great control over elevated blood glucose levels, making them one of the most intriguing and promising technologies available today. Furthermore, adding additional ligands to nanocarriers allows for more focused distribution while protecting the encapsulated hypoglycemic drugs [117].

15. Sivalingam AM et.al (2024)

Type-2 Diabetes Mellitus (T2DM), one of the most common metabolic illnesses, is characterized by elevated blood sugar levels caused by impaired insulin secretion, insulin action, or both. Plant-mediated green nanomaterial production is becoming increasingly popular due to its low cost and environmental friendliness. The study's goal is to manufacture Cos tus spicatus with AgNPs, characterize them by different analytical methods like UV- visible, FT-IR, SEM, TEM, XRD, and EDX spectroscopy, and conduct additional in vitro and in vivo experiments, different biomarkers SGOT, SGPT, and ALP were used;. In results UV– visible analysis confirmed the successful bio-reduction of silver nanoparticles as Cs-AgNPs, its confirmed at 448 nm, indicated that SPR phenomenon, inFT-IR analysis peaks noticed at 619 cm^{-1} and 453 cm^{-1}, it indicates C–Br and C–Cl bonding vibrations. In SEM analysis confirmed that the nanoparticles AgNPs were crystalline, spherical, and clustered, with sizes

ranged 10 to 25 nm. In TEM analysis showed that homogeneous polycrystalline shapes 0.295 in size, XRD confirmed crystalline nature of AgNPs; at (311), (220), (200), and (111). In EDX the dominance of AgNPs observed (66.75%), followed by carbon (22.02%) and oxygen (11.23%). In antidiabbetic the animals treated with C. spicatus rhizome ethanol extract exhibited no damage in kidney its recovery observed at 0, 7, and 21 days. In 21 days observation of kidney body weight significantly increased and 21 days noticed that it returned to near-normal levels. Furthermore, in 0 days to 14 days observation the acute hyperlipidemia of TC, TG, LDL and HDL level of C. spicatus effects in STZ-induced levels decreased (SGOT:
108.39 ± 3.73# to 85 ± 1.92#, SGPT: 106 ± 2.19** to 73.67 ± 2.37#, ALP: 124.59 ± 2.37# to

80.17 ± 0.85**), indicating improved kidney function and anti-diabetic activity. These results, with **± 0.0001 indicating the significance of the control and silver nanoparticles as Cs-AgNPs STZ groups, treatment demonstrates significant kidney tissue restoration and promising anti- diabetic activity, offering potential in diabetes management strat egies. silver nanoparticles as Cs-AgNPs together show promising in restoring kidney tissue and managing diabetes, its concluded that, the research has shown that the rhizome extract, when combined with nanoparticles, demonstrates potent antioxidant and enzyme inhibitory effects, especially targeting the key enzymes implicated in Type 2 Diabetes Mellitus (T2DM) [118].

16. Mishra SB et.al (2024)

Raphanus sativus L., which belongs to the Brassicaceae family and is commonly known as Radish, is a vegetable grown and consumed around the world as a part of the human diet. All parts, including the seeds, of this plant are intended for diabetes treatment. However, due to the presence of poor water-soluble phytoconstituents and the low bioavailability of seeds, their nanoparticles were prepared by nanoprecipitation followed by the probe sonication method. They were also characterized by FTIR, FESEM, and zeta sizer. In addition, the plant extract was subjected to quantitative estimation of total flavonoid and phenolic compounds. The particle size and polydispersity index of prepared nanoparticles were found to be 203.76±2.24 nm and 0.89, respectively. The Zeta potential of the prepared nanoparticles was -6.29 mV. The in vitro antidiabetic effect of the prepared plant extract and its nanoparticles were estimated using the alpha-amylase and alpha-glucosidase inhibition methods and compared with the standard drug Acarbose, which shows significant inhibition of both amylolytic enzymes in a dose-dependent manner [119].

17. Elias A et.al (2024)

Diabetes mellitus, a metabolic disorder, elevates blood sugar levels, resulting in complications affecting various bodily systems such as the nerves, eyes, kidneys, and other organs. The administration of oral antihyperglycemic medications can potentially induce adverse effects, especially when combined, leading to drug–drug interactions. To address these challenges, there is potential in introducing innovative antioxidant compounds targeted at specific organs for therapeutic action. Currently, metal nanoparticles have gained prominence in healthcare due to their exceptional biocompatibility, stability, cost-effectiveness, and environmentally friendly attributes. In this study, green synthesis methods were employed to produce gold nanoparticles (AuNPs) using the *Amaranthus gangeticus* plant, known for its antidiabetic properties. The leaf extract of *A. gangeticus* was utilized to synthesize these green AuNPs using a 1 mM gold chloride solution. Various characterization techniques, including UV spectroscopy, FTIR analysis, X-ray diffraction analysis (XRD), transmission electron microscopy (TEM), and scanning electron microscopy (SEM), were employed to analyse the synthesized herbal-mediated AuNPs. Additionally, the in vivo antidiabetic efficacy of the produced AuNPs was evaluated. The induction of diabetes using STZ resulted in elevated levels of blood glucose, cholesterol, triglycerides, LDL, and VLDL and a significant loss in body weight. However, these detrimental effects were mitigated after treating diabetic rats for 28 days, with significant improvements observed at a dosage range of 1 mg/kg of AuNPs compared to the group treated solely with the plant extract. The findings suggest that the plant- mediated AuNPs demonstrate significant potential as antidiabetic agents compared to the crude extract alone [120].

18. Revathi G et.al (2023)

Diabetes is a highly complex disease that has an adverse impact on the lives of individuals, and the current medicines used to manage diabetes have obvious side effects. Medicinal plants, on the other hand, may serve as an alternate source of anti-diabetic drugs. A polyherbal combination has a higher and more extensive therapeutic potential than a single herb. Yet, due to deterioration during the absorption process, the usage of this drug still yields inadequate results. Encapsulation of polyherbal drug with chitosan nanoparticles is one of the key ways to solve this problem due to its biocombatibilty, slow and targeted drug delivery characteristics. In the present study, the chitosan was derived from prawn shell and the chitosan nanoparticles had been prepared by ionic-gelation method. The anti-diabetic polyherbal drug

(Andrographis paniculata, Andrographis alata, Adhatoda zeylanica, Gymnema sylvestre, Syzygium cumini, and Justicia glabra) was encapsulated with a bio-derived chitosan biopolymer. The drug loading efficiency was about 85 %. The chemical and physical properties of the chitosan and drug-loaded chitosan nanoparticles had been analyzed by FT-IR absorption, XRD, SEM, TEM and EDAX analysis. The antidiabetic efficiency, hepatoprotective activity and antihyperlipedimic activity of the chitosan nanoparticles, polyherbal drug and polyherbal drug encapsulated with chitosan nanoparticles were assessed in a group of rats. The polyherbal drug reduced the serum glucose level from 306.4 mg/dL to 134.47 mg/dL, while the polyherbal drug encapsulated with chitosan nanoparticles reduced to 127.017 mg/dL. This was very close to the serum glucose level of non-diabetic rat (124.65 mg/dL). Further, it considerably increased the insulin level close to that of non-diabetic rat. Thus, the polyherbal drug encapsulated with chitosan nanoparticles showed superior efficiency in antidiabetic and also diabetic complications [121].

19. Khan HA et.al (2023)

One of the most widespread metabolic diseases, Type-2 Diabetes Mellitus (T2DM) is defined by high blood sugar levels brought on by decreased insulin secretion, reduced insulin action, or both. Due to its cost-effectiveness and eco-friendliness, plant-mediated green synthesis of nanomaterials has become more and more popular. The aim of the study is to synthesize AgNPs, their characterizations and further *in-vitro* and *in-vivo* studies. Several methods were used to morphologically characterise the AgNPs. The AgNPs were crystalline, spherical, and clustered, with sizes ranging from 20 to 50 nm. AgNPs were found to contain various functional groups using Fourier transform infrared spectroscopy. This study focuses on the green- synthesis of AgNPs from *Fagonia cretica* (*F. cretica*) leaves extract to evaluate their synthesized AgNPs for *in-vitro* and *in-vivo* anti-diabetic function. For the *in-vivo* tests, 20 male Balb/C albino-mice were split up into four different groups. Anti-diabetic *in-vivo* studies showed significant weight gain and a decrease in all biochemical markers (pancreas panel, liver function panel, renal function panel, and lipid profile) in Streptozotocin (STZ)-induced diabetic mice. *In vitro* anti-diabetic investigations were also conducted on AgNPs, comprising α- amylase, α-glucosidase inhibitions, and antioxidant assays. AgNPs showed antioxidant activity in both the DPPH and ABTS assays. The research showed that the isolated nanoparticles have powerful antioxidant and enzyme inhibitory properties, especially against the main enzymes involved in T2DM [122].

20. Kumari S et.al (2023)

Nowadays rapidly and continuously increasing population is creating a significant problem with adverse social, economic, personal, and health outcomes and environmental damage. There are many options available in the market for birth control. Due to the side effects of allopathic or synthetic medicine, herbal medicines are becoming popular day by day since herbal drugs are found safe, economical, and readily available for therapeutic uses. Medicinal plants are regarded as rich resources of traditional medicines; many modern medicines are produced from these plants. The usefulness of herbal medicines can be increased with the help of nanoparticles. A nanoparticle can be defined as a particle of matter that is between 1- 100 nanometers in diameter. Nanoparticles have several properties that distinguish them from bulk materials simply by size, chemical reactivity, absorption, and biological mobility. Herbal nanoparticles are also used in anticancer, anti-diabetic, antimicrobial and antioxidant drugs. Plants have various metabolites that can cause potent antifertility and fertility. This is high time when active components of herbal plants should be further investigated for their antifertility activity. This review aims to provide glimpses and emphasize the importance of herbal nanoparticles in fertility control [123].

21. Khan HA et.al (2023)

Therapeutic moieties derived from medicinal plants as well as plants-based ecofriendly processes for producing selenium nanoparticles have shown great promise in the management of type 2 diabetes mellitus (T2DM). The current study was aimed to assess the anti-diabetic potentials of Fagonia cretica mediated biogenic selenium nanoparticles (FcSeNPs) using in- vitro and in-vivo approaches. The bio-synthesized FcSeNPs were characterized using various techniques including UV–VIS spectrophotometry and FTIR analysis. The in-vitro efficacy of FcSeNPs were assessed against α-glucosidase, α-amylase enzymes as well as the anti-radical studies were performed using DPPH and ABTS free radicals scavenging assays. For in-vivo studies, 20 Male Balb/C albino-mice were randomly divided into 4 groups (n =5) including normal group, disease group (Diabetic group with no treatment), control group and treatment group (Diabetic group treated with FcSeNPs). Further, biochemistry markers including pancreas, liver, kidney and lipid profile were assessed for all treatment groups. The FcSeNPs exhibited a dose- dependent inhibition against α-amylase and α-glucosidase at 62–1000 µg mL

1 concentration with IC50 values of 92 and 100 µg mL 1 respectively. In antioxidant experiments, the FcSeNPs demonstrated significant radicals scavenging effect against DPPH

and ABTS radicals. In STZ-induced diabetic mice, a considerable decline in blood glucose level was observed after treatment with FcSeNPs. Anti-hyperglycemic effect of FcSeNPs treated animals were high (105 ±3.22**) as compared to standard drug (128.6 ±2.73** mg dL 1). Biochemical investigations revealed that all biochemical parameters for pancreas, liver function, renal function panel and lipid profile were significantly lowered in FcSeNPs treated animals. Our findings indicate a preliminary multi-target efficacy for FcSeNPs against type-2 diabetes and thus warrant further detailed studies [124].

22. Naik J et.al (2023)

Phytochemicals capacity to reduce metal into nanoparticles has sparked a new eco-friendly method to build green nanotechnology. Synthesis of nanoparticles using a biological approach with valuable medicinal applications is an essential feature of modern nanotechnology. The present study attempts to synthesize silver (PE-AgNPs) and zinc oxide (PE-ZnONPs) nanoparticles using *Phyllanthus emblica* fruit ethanolic extract and evaluates their therapeutic potential. In our present study, the secondary metabolites of the extract were determined by Gas Chromatography-Mass Spectra (GC-MS.) Nanoparticles were characterized using analytical tools. The PE-AgNPs and PE-ZnONPs nanoparticles have shown potential inhibition activity against α-amylase ($IC50$ 170.60 ± 0.94 and 160.82 ± 0.88 µg/mL), α-glucosidase
(IC_{50} 80.24 ± 1.38 and 70.02 ± 0.59 µg/mL) and increased glucose uptake (180.42 ± 0.78 and 110.52 ± 0.84 µg/mL). *In-vitro* MTT assay of PE-AgNPs and PE-ZnONPs nanoparticles revealed the effective anticancer potential against the A549 cancer cell line with an IC_{50} value of 27.52 ± 1.12 and 70.64 ± 1.4 µg/mL. The synthesized bioactive compounds can be the alternative eco-friendly potential for an antidiabetic and anticancer substitute which can be further recommended for pharmacological applications [125].

23. Bakshi, Jyoti, et.al (2022)

In the present study, the anti-diabetic and antimicrobial properties of berberine were improved using non-ionic guar gum and ionic acacia gum as nanocarriers. Berberine loaded guar-acacia gum nanocomplexes were synthesized by employing ionic complexation method. The formulation was characterized by dynamic light scattering (DLS), Fourier-transform infrared spectroscopy (FTIR), Transmission electron microscopy (TEM), Scanning electron microscopy (SEM) and evaluated for in vitro dissolution study, anti-diabetic activity and antimicrobial activity. The optimized berberine loaded guar-acacia gum nanocomplexes had a particle size of 290.2 nm as indicated by DLS and drug entrapment efficiency of 96.5%.

Morphological analysis revealed that berberine nanocomplexes were spherical-shaped with a smooth surface and size in the range of 100–250 nm. Moreover, berberine loaded guar-acacia nanocomplexes showed good stability and controlled released property in vitro. Antimicrobial activity against bacterial strains and fungal strains demonstrated the higher antimicrobial potential of berberine loaded gum nanocomplexes than gum nanocomplexes (blank) and pure berberine as indicated by the greater zone of inhibition diameter. In vitro anti-diabetic assessment showed higher percentage inhibition of the α-amylase enzyme by berberine loaded gum nanocomplexes as compared to pure berberine and blank nanocomplexes. In conclusion, the improved biological potency of berberine upon encapsulation into gum nanocomplexes indicates that berberine loaded guar-acacia gum nanocomplexes can be used as a promising candidate against diabetes and pathogenic microorganisms in the near future [126].

24. Vijayaraj Surendran et.al (2022)

The rutin loaded chitosan-alginate nanoparticles (RCANP) were prepared using an ion gelation method. The optimized RCANP4 formulation composed of rutin: alginate: chitosan with the ratio of 1.24:5:2. The particle size, zeta potential, and entrapment efficiency of RCANP4 formulation were found to be 168.4 ± 11.23 nm, -24.7 ± 1.5 mV, and 91.23 ± 1.1%, respectively. The *in vitro* drug release of RCANP4 formulation was found to be 88.89 ± 2.9% within 24 h. The Fourier transform infrared spectroscopy (FT-IR) of RCANP4 revealed all characteristic groups of rutin, confirming the successful loading of rutin into the nanoparticles. Methods: Due to rutin entrapment in the chitosan sodium alginate matrix, a broad curve was observed in the Differential Scanning Calorimetry (DSC) study of RCANP4. The RCANP4 was found to be uniform and spherical revealed from Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). RCANP4 showed 3.54 times more bioavailability than free rutin, resulting in more internalization of rutin in systemic circulation. The results of plasma glucose levels of diabetic rats administered with RCANP4 and rutin were evident that RCANP4 showed effective antidiabetic activity compared to rutin. Results: The results obtained for glucose uptake in HepG2 cells, the RCANP4 caused a significant ($P < 0.05$) increase in glucose uptake in contrast to rutin. *In vitro* cytotoxicity results explained that RCANP4 could significantly ($P < 0.05$) reduce the cells viability rate compared with rutin. It may be due to the internalization of RCANP4 formulations in systemic circulation. Conclusion: The results also showed that RCANP4 could significantly reduce cell viability over 24 h and 48 h compared to free rutin [127].

25. Sreeharsha Nagaraja et.al (2022)

Diabetes mellitus (DM) and its complications are a severe public health concern due to the high incidence, morbidity, and mortality rates. The present study aims to synthesize and characterize silver nanoparticles (AgNPs) using the aqueous leaf extract of *Psidium guajava* (PGE) for investigating its antidiabetic activity. *Psidium guajava* silver nanoparticles (PGAg NPs) were prepared and characterized by various parameters. The in vivo study was conducted using PGE and PGAg NPs in Streptozotocin (STZ)-induced diabetic rats to assess their antidiabetic properties. STZ of 55 mg/kg was injected to induce diabetes. The PGE, PGAg NPs at a dose of 200 and 400 mg/kg and standard drug Metformin (100 mg/kg) were administered daily to diabetic rats for 21 days through the oral route. Blood glucose level, body weight changes, lipid profiles, and histopathology of the rats' liver and pancreas were examined. In the diabetic rats, PGE and PGAg NPs produced a drastic decrease in the blood glucose level, preventing subsequent weight loss and ameliorating lipid profile parameters. The histopathological findings revealed the improvements in pancreas and liver cells due to the repercussion of PGE and PGAg NPs. A compelling effect was observed in all doses of PGE and PGAg NPs; however, PGAg NPs exhibited a more promising result. Thus, from the results, it is concluded that the synthesized PGAg NPs has potent antidiabetic activity due to its enhanced surface area and smaller particle size of nanoparticles [128].

26. Debele TA et.al (2022)

Diabetes mellitus (DM) is a chronic metabolic disorder of carbohydrates, lipids, and proteins due to a deficiency of insulin secretion or failure to respond to insulin secreted from pancreatic cells, which leads to high blood glucose levels. DM is one of the top four noncommunicable diseases and causes of death worldwide. Even though great achievements were made in the management and treatment of DM, there are still certain limitations, mainly related to the early diagnosis, and lack of appropriate delivery of insulin and other anti-diabetic agents. Nanotechnology is an emerging field in the area of nanomedicine and NP based anti-diabetic agent delivery is reported to enhance efficacy by increasing bioavailability and target site accumulation. Moreover, theranostic NPs can be used as diagnostic tools for the early detection and prevention of diseases owing to their unique biological, physiochemical, and magnetic properties. NPs have been synthesized from a variety of organic and inorganic materials including polysaccharides, dendrimers, proteins, lipids, DNA, carbon nanotubes, quantum

dots, and mesoporous materials within the nanoscale size. This review focuses on the role of NPs, derived from organic and inorganic materials, in the diagnosis and treatment of DM [129].

27. Sengani M et.al (2022)

The current study demonstrated a green, friendly, low-cost biosynthesis of silver nanoparticles (AgNPs) from Kigelia africana leaves (Lam.) Benth. extract (KAE) as both a major capping and reducing agent. The produced AgNPs were characterized using a variety of analytical methods, like the X-ray powder diffraction (XRD), HRTEM, Fourier transforms infrared (FTIR), and UV–Vis spectrophotometer. The formation of AgNPs with maximum absorbance at max = 435 nm was endorsed by surface plasmon resonance. FTIR analysis revealed that biological macromolecules of KAE were involved in the stabilization and synthesis of AgNPs. At the same time, HRTEM images revealed that the average particle size of the spherical AgNPs ranged from about 25 nm to 35 nm. Further, cytotoxicity assessment of AgNPs was done using the RINm5F insulinoma cell line with an MTT assay. Followed by, the RINm5F insulinoma cells treated with AgNPs and KAE, the expression of the Peroxisome proliferator-activated receptor gamma (PPARγ) gene was accessed. The results showed gene expression was upregulated in the RINm5F insulinoma cell line thus confirming AgNPs and KAE anti-diabetic efficacy. Furthermore, the findings show that nanotechnology has enhanced the effectiveness of current methodologies in gene expression and regulation which has contributed to the emergence of different forms of advanced regulatory systems [130].

28. Perumalsamy R et.al (2022)

Myristica fragrans, also known as nutmeg, is a spice that cures various diseases. This study aimed to synthesize silver nanoparticles from a hydroethanolic extract of *Myristica fragrans* seeds (MFHE) and evaluate their anti-diabetic properties. To MFHE, $AgNO_3$ solution was added and exposed to sunlight to produce silver nanoparticles from hydroethanolic seed extract of *Myristica fragrans* (MFHENP). The MFHENP was characterized by numerous techniques. UV-visible spectroscopy confirmed the formation of silver nanoparticles by the absorption peak at 430nm. Scanning electron microscopy (SEM) studies revealed the shape and size of the particles at the range of 50–60nm. Energy-dispersive X-ray spectroscopy (EDX) disclosed the presence of silver ions. X-ray diffraction spectrum confirmed the crystalline nature of silver nanoparticles by the peak at 39°. FTIR analysis revealed the functional groups present in MFHE as well as in MFHENP and zeta potential analysis was found to be 14mV.

Furthermore, in vitro anti-diabetic activity was investigated. MFHENP showed significant efficiency against the inhibition of alpha-amylase and alpha-glucosidase enzymes and also MFHENP retarded the glucose transport across the membrane which is analyzed by glucose diffusion and glucose uptake assays. Acarbose is used as a standard for all these methods and MFHENP efficiency proves their therapeutic potential for the treatment of diabetes mellitus [131].

29. Faisal S et.al (2022)

In this work, an ecofriendly approach for biogenic production of copper oxide nanoparticles (CuO-NPs) was proposed by utilizing the *Bacopa monnieri* leaf extract as a reducing and stabilizing agent. The synthesis of CuO-NPs was instantly confirmed by a shift in the color of the copper solution from blue to dark gray. The use of UV–visible spectroscopy revealed a strong narrow peak at 535 nm, confirming the existence of monoclinic-shaped nanoparticles. The average size of CuO-NPs was 34.4 nm, according to scanning electron microscopy and transmission electron microscopy studies. The pristine crystalline nature of CuO-NPs was confirmed by X-ray diffraction. The monoclinic form of CuO-NPs with a crystallite size of 22 nm was determined by the sharp narrow peaks corresponding to 273, 541, 698, 684, and 366 Bragg's planes at different 2θ values. The presence of different reducing metabolites on the surface of CuO was shown by Fourier transform infrared analysis. The biological efficacy of CuO-NPs was tested against *Helicobacter felis*, *Helicobacter suis*, *Helicobacter salomonis.* and *Helicobacter bizzozeronii*. *H. suis* was the most susceptible strain with an inhibition zone of 15.84 ± 0.89 mm at 5 mg/mL of NPs, while the most tolerant strain was *H. bizzozeronii* with a 13.11 ± 0.83 mm of inhibition zone. In in vivo analgesic activity, CuO-NPs showed superior efficiency compared to controls. The maximum latency time observed was
7.14 ± 0.12 s at a dose level of 400 mg/kg after 90 min, followed by 5.21 ± 0.29 s at 400 mg/kg after 60 min, demonstrating 65 and 61% of analgesia, respectively. Diclofenac sodium was used as a standard with a latency time of 8.6 ± 0.23 s. The results observed in the rat paw edema assays showed a significant inhibitory activity of the plant-mediated CuO-NPs. The percentage inhibition of edema was 74% after 48 h for the group treated with CuO-NPs compared to the control group treated with diclofenac (100 mg/kg) with 24% edema inhibition. The solution of CuO-NPs produced 82% inhibition of edema after 21 days when compared with that of the standard drug diclofenac (73%). CuO-NPs vividly lowered glucose levels in STZ-induced diabetic mice, according to our findings. Blood glucose levels were reduced by about 33.66 and 32.19% in CuO-NP and (CuO-NP + insulin) groups of mice, respectively. From the

abovementioned calculations, we can easily conclude that *B. monnieri*-synthesized CuO-NPs will be a potential antibacterial, anti-diabetic, and anti-inflammatory agent on in vivo and in vitro basis [132].

30. Zolkepli H et.al (2022)

Diabetes mellitus is a prevalent metabolic syndrome that is associated with high blood glucose levels. The number of diabetic patients is increasing every year and the total number of cases is expected to reach more than 600 million worldwide by 2045. Modern antidiabetic drugs alleviate hyperglycaemia and complications that are caused by high blood glucose levels. However, due to the side effects of these drugs, plant extracts and bioactive compounds with antidiabetic properties have been gaining attention as alternative treatments for diabetes. Natural products are biocompatible, cheaper and expected to cause fewer side effects than the current antidiabetic drugs. In this review, various nanocarrier systems are discussed, such as liposomes, niosomes, polymeric nanoparticles, nanoemulsions, solid lipid nanoparticles and metallic nanoparticles. These systems have been applied to overcome the limitations of the current drugs and simultaneously improve the efficacy of plant-based antidiabetic drugs. The main challenges in the formulation of plant-based nanocarriers are the loading capacity of the plant extracts and the stability of the carriers. A brief review of lipid nanocarriers and the amphipathic properties of phospholipids and liposomes that encapsulate hydrophilic, hydrophobic and amphiphilic drugs is also described. A special emphasis is placed on metallic nanoparticles, with their advantages and associated complications being reported to highlight their effectiveness for treating hyperglycaemia. The present review could be an interesting paper for researchers who are working in the field of using plant extract-loaded nanoparticles as antidiabetic therapies [133].

31. Jeyabharathi S et.al (2021)

Zinc deficiency is one of the important nutritional problems, particularly in developing countries. In humans, zinc plays a significant role in the synthesis, secretion and storage of insulin in beta cells. The low level of zinc in the pancreas is associated with increased risks of hyperglycemic conditions. As a novel attempt, herbal-based zinc oxide nanoparticles (ZnONPs) were synthesized from anti-diabetic medicinal plant Costus *igneus.* The synthesized ZnONPs were characterized by UV–vis Spectroscopy, Fourier Transformer Infra-Red spectroscopy (FTIR), X-ray diffraction (XRD), and Scanning Electron Microscopy (SEM) coupled with Energy Dispersive X-ray analysis (EDX). The

zebrafish embryos in the early blastula stage (~48 hpf) were exposed to different concentrations of ZnONPs and its toxic effects were evaluated. Furthermore, the hyperglycemic condition was induced in adult zebrafish using alloxan. The hyperglycemic condition induced zebrafishes were exposed to various concentrations of ZnONPs. Results revealed the promising effects of ZnONPs in lowering the blood glucose level. The ZnONPs also exhibited excellent anti-bacterial activity against Staphylococcus epidermidis and Enterobacter *aeurogens*. In the present study, ZnONPs exhibited synergistic effects in terms of reducing the blood glucose level in hyperglycemic zebrafish and also showed high anti-bacterial activity [134].

32. Suchithra MR et.al (2021)

Diabetes mellitus is an endocrine disorder that affects 85% of the people because of higher sugar level in blood. Due to this metabolic disorder and improper function of organs especially kidney results in urinary tract infection causing struvite urinary stone. So, in the present study was carried out to study the green synthesized silver nanoparticles using *Hybanthus enneaspermus* stem bark extracts on antioxidant, antidiabetic and antiurolithiatic activity under *in vitro* conditions. By the treatment of aqueous solution of 5 mM silver nitrate (AgNO3) with stem bark extracts, silver nanoparticles could be quickly synthesized within 1 h. These silver nanoparticles were characterized using UV–Visible spectroscopy, Fourier Transform Infrared Spectroscopy (FT-IR), Transmission Electron Microscope (TEM), X-ray diffraction (XRD) and Dynamic Light Scattering (DLS) for further confirmation. TEM analysis found that the silver nanoparticles are spherical in shape. X-ray diffraction confirmed that silver nanostructure exhibit a face centered cubic crystal structure. DLS showed that the nanoparticles size is 644.2 nm. By increasing the concentration of silver nanoparticles, weight of the formed crystals reduced from 0.94 g to 0.13 g in struvite crystals and analysed by FTIR analyses. This multidisciplinary approach showed a better percentage of inhibition such as antioxidant, antidiabetic and antiurolithiatic activity of silver nanoparticles [135].

33. Das, Aparoop, et al.(2020)

Diabetes is one amongst the chronic metabolic diseases affecting millions of people across the world. Apart from proper selection of drugs and doses, conventional drugs pose unwanted side effects to the diabetics. Due to the limited side effects, cost and easy accessibility, there is a rising interest in the field of research incorporating compounds from natural backgrounds. However, most of the biologically active constituents have low absorption capability despite

their property of high solubility in water. Due to their low absorption capability, most of them are unable to cross the lipid bilayers of the cell owing to their large molecular sizes that produce failure in achieving bioavailability followed by the loss of efficacy. In recent years, nanotechnology-based formulations have given new lease of life to such problems with their myriad of formulations that include nanospheres, nanocapsules, liposomes, proliposomes, solid lipid nanoparticles [SLNs] and nano-emulsion. Combining herbal drugs with nanotechnology may potentiate the action of the plant extracts or active constituent by increasing their solubility, bioavailability and efficacy as well as by reducing the required dose and side effects. Therefore, the objective behind presenting this chapter is to outline works on the nanotechnology-based anti-diabetic herbal formulations reported till date [136].

34. Subhajit Maity et.al (2020)

The insolubility of bioflavonoids in water and low bioavailability restrict their uses to a great extent, which can be overcome by encapsulated polymer nanoparticles. We have prepared naringenin (a bioflavonoid) bearing polymer PLGA (N-PLGA) nanoparticles by emulsion- diffusion-evaporation method. The encapsulation efficiency was nearly 70%. N-PLGA nanoparticles were about 129 nm in diameters, as determined by DLS and approximately 90 nm diameters determined by AFM and TEM. The nanoparticles exhibited higher melting temperature (350 °C) than free naringenin (253 °C). FT-IR spectroscopic study confirmed the formulation of N-PLGA nanoparticles. Free naringenin and N-PLGA nanoparticles were tested in streptozotocin-induced diabetic rats. Diabetic rats were treated (i.p) with 10 mg free naringenin or N-PLGA nanoparticles containing 10 mg naringenin/kg body weight. After 10 days, second dose was administered to both group of rats, as the blood glucose level was still higher than normal. After second dose, blood glucose level became normal in nanoparticle-treated rats, but not in free naringenin-treated rats. Significant reduction in glycated hemoglobin level, increase in insulin level and improvement of dyslipidemia and oxidative stress parameters were found in N-PLGA treated-group, in comparison with the respective parameters in free naringenin-treated group. The findings thus suggest better antidiabetic potential of N-PLGA nanoparticles in comparison with the free flavonoid [137].

35. Das, Aparoop, et al (2020)

Diabetes is one amongst the chronic metabolic diseases affecting millions of people across the world. Apart from proper selection of drugs and doses, conventional drugs pose unwanted side effects to the diabetics. Due to the limited side effects, cost and easy accessibility, there is a

rising interest in the field of research incorporating compounds from natural backgrounds. However, most of the biologically active constituents have low absorption capability despite their property of high solubility in water. Due to their low absorption capability, most of them are unable to cross the lipid bilayers of the cell owing to their large molecular sizes that produce failure in achieving bioavailability followed by the loss of efficacy. In recent years, nanotechnology-based formulations have given new lease of life to such problems with their myriad of formulations that include nanospheres, nanocapsules, liposomes, proliposomes, solid lipid nanoparticles [SLNs] and nano-emulsion. Combining herbal drugs with nanotechnology may potentiate the action of the plant extracts or active constituent by increasing their solubility, bioavailability and efficacy as well as by reducing the required dose and side effects. Therefore, the objective behind presenting this chapter is to outline works on the nanotechnology-based anti-diabetic herbal formulations reported till date [138].

36. Dewanjee, Saikat, et al (2020)

Diabetes mellitus is a life-threatening metabolic syndrome. Over the past few decades, the incidence of diabetes has climbed exponentially. Several therapeutic approaches have been undertaken, but the occurrence and risk still remain unabated. Several plant-derived small molecules have been proposed to be effective against diabetes and associated vascular complications via acting on several therapeutic targets. In addition, the biocompatibility of these phytochemicals increasingly enhances the interest of exploiting them as therapeutic negotiators. However, poor pharmacokinetic and biopharmaceutical attributes of these phytochemicals largely restrict their clinical usefulness as therapeutic agents. Several pharmaceutical attempts have been undertaken to enhance their compliance and therapeutic efficacy. In this regard, the application of nanotechnology has been proven to be the best approach to improve the compliance and clinical efficacy by overturning the pharmacokinetic and biopharmaceutical obstacles associated with the plant-derived antidiabetic agents. This review gives a comprehensive and up-to-date overview of the nanoformulations of phytochemicals in the management of diabetes and associated complications. The effects of nanosizing on pharmacokinetic, biopharmaceutical and therapeutic profiles of plant-derived small molecules, such as curcumin, resveratrol, naringenin, quercetin, apigenin, baicalin, luteolin, rosmarinic acid, berberine, gymnemic acid, emodin, scutellarin, catechins, thymoquinone, ferulic acid, stevioside, and others have been discussed comprehensively in this review [139].

37. Sati, Satish C., et al (2020)

Metal nanoparticles (AgNPs and ZnONPs) were synthesized using a green methodology with the green leaves extract of the Bedu (*Ficus palmata*) tree as a reducing agent and the support of natural fibers. The synthesized AgNPs and ZnONPs were characterized by several techniques, including ultraviolet–visible spectral analysis, powder X-ray diffraction crystal analysis, scanning electron microscopy, EDAX, transmission electron microscopy, and Fourier transform infrared spectroscopy, which confirmed that the synthesized particles are in the nano range (1–100 nm), i.e., 30 nm for AgNPs with polydispersity and a spherical shape, whereas the average size of synthesized ZnONPs is 34 nm and they seem to exhibit a distorted spherical shape. The results of thermogravimetric analysis confirmed a weight loss of 18.02% for AgNPs under exothermic conditions due to the desorption of water, and ZnONPs show weight loss between 265 and 500 °C. Both synthesized MNPs are highly thermally stable. Anti- inflammatory and anti-diabetic studies of metal NPs have been evaluated. The AgNPs and ZnONPs of *F. palmata* leaves showed remarkably highly potent activity for respective strains. In vitro anti-diabetic activity was found for inhibition of α-amylases and α-glucosidases by synthesized silver nanoparticles [140].

38. Deng, Wenji, et al (2019)

Diabetes mellitus (DM) remains a great challenge in treatment due to pathological complexity. It has been proven that phytomedicines and natural medicines have prominent antidiabetic effects. This work aimed to develop selenium-layered nanoparticles (SeNPs) for oral delivery of mulberry leaf and Pueraria Lobata extracts (MPE), a group of phytomedicines with significant hypoglycemic activities, to achieve a synergic antidiabetic effect. MPE-loaded SeNPs (MPE-SeNPs) were prepared through a solvent diffusion/in situ reduction technique and characterized by particle size, ζ potential, morphology, entrapment efficiency (EE) and drug loading (DL). The resulting MPE-SeNPs were 120nm around in particle size with EE of 89.38% for rutin and 90.59% for puerarin, two marker components in MPE. MPE SeNPs exhibited a slow drug release and good physiological stability in the simulated digestive fluid. After oral administration, MPE-SeNPs produced significant hypoglycemic effects both in the normal and diabetic rats. Ex vivo intestinal imaging and cellular examinations demonstrated that MPE-SeNPs were provided with outstanding intestinal permeability and transepithelial transport aptness. It was also revealed that MPE-SeNPs could alleviate the oxidative stress, improve the pancreatic function, and promote the glucose utilization by adipocytes. Our study

provides new insight into the use of integrative nanomedicine containing phytomedicines and selenium for DM treatment [141].

39. Marella, Saritha et.al (2018)

Diabetes mellitus (DM) is a chronic illness that requires continuing medical care and patient self-management education to prevent acute complications and to reduce the risk of long-term complications. The number of people with diabetes is increasing due to population growth, ageing, urbanisation and increasing prevalence of obesity and physical inactivity. Apart from currently available therapeutic options, many herbal medicines have been recommended for the treatment of diabetes. Herbal drugs are prescribed widely because of their effectiveness, less side effects and relatively low cost. Several pharmacopoeias have provided parameters to maintain quality and standardise procedures in identification/authentication of herbal inputs and their products. Available literature related to folklore medicine used in the treatment of diabetes extended to nanoformulation of herbal drugs up to date was cited. The use of bioactive compounds leads to new hope to improve the life expectancy and health status of the population for the formulation of novel drugs. Recently, many studies have shown that nanotechnology has the potential to be used in different biological and medical applications, mainly as targeted drug delivery systems to minimise and delay the chronic effects of diabetes. Herein, the authors presented a thorough review of the available herbal medicines and the possibilities of developing their nanoformulations in the treatment of DM [142].

40. Arulanandraj, N., et al (2018)

Murva (Maerua oblongifolia) contains numerous bioactive compounds that may provide multiple health benefits, including antimicrobial, anti-fungal, anti-pyretic and anti-diabetic. Most of the therapeutic effects of murva have been attributed due to the presence of triterpenoids and alkaloids, in their composition. Although these compounds have been shown promising therapeutic effects under in-vitro conditions, they met with limited efficacy in clinical settings due to various reasons such as poor oral absorption and bioavailability. Different techniques have been proposed to improve the stability and bioavailability of the herbal drugs. Among such strategies, nanoparticulate based drug delivery systems are novel and promising tools. In this study, chitosan nanoparticles containing Murva (CNP1-CNP3) were synthesized by ionic gelation technique, which resulting in particles size smaller than 650nm. The encapsulation efficiency of nanoformulations was over 41.5%. The nanoformulations exhibited slow and sustained in vitro release over 99% of drug from the

Murva encapsulated chitosan nanoparticles after 24 hours. The synthesized nanoformulations were found to be a promising system for oral sustained administration of murva and also enhance its stability and bioavailability [143].

41. Shanker, Kalakotla, et.al (2017)

Diabetes is a metabolic disorder characterized by hyperglycemia, altered carbohydrate, lipid and protein metabolism. In recent studies, Nanoscience and nanotechnology are blazing fields for researchers; for researchers; of late there has been a prodigious excitement in the field of nanopharmacology to study silver nanoparticle (SNP) synthesis using natural products. Biological methods have been used to synthesize SNPs using medicinally active plants having an antidiabetic role, and this made us to assess the biologically synthesized SNPs from the seed extract of *Psoralea corylifolia* using 1 mM silver nitrate solution. The synthesized herbal- mediated SNPs (HMSNPs) were subjected to various characterization techniques such as X- ray diffraction analysis (XRD), energy dispersive X-ray (EDX) analysis, transmission electron microscope (TEM), and differential light scattering (DLS), respectively. In the current study the HMSNPs were tested to observe the *in vitro* antidiabetic activity and possible toxic effects in healthy female albino mice by following OECD guidelines-425. Huge data from biochemical, cellular, mouse, and chemical inhibitor studies have recognized protein tyrosine phosphatase 1B (PTP1B) as a major negative regulator of insulin signaling. In addition, corroboration suggests that insulin action can be enhanced by the inhibition of PTP1B. Keeping in view of the above fact, the PTP1B assay was done to determine the PTP1 B inhibitory effect of HMSNPs. It can be concluded that medicinal plants can be a good source for the synthe sis of HMSNPs. This study can be used for the development of valuable nanomedicines to treat various ailments, and it also highlights the safety and biocompatibility of SNPs within a biological cell; *in vivo* parameters need to be considered for further discoveries .[144]

.AIM AND OBJECTIVE

Aim: Formulation and characterization of herbal nanoparticles for antidiabetic therapy

Objectives:

To extract bioactive phytoconstituents from the selected herbal source using suitable extraction methods.

To formulate nanoparticles incorporating the herbal extract using an appropriate nanoparticle preparation technique (e.g., nanoprecipitation, ionic gelation, or emulsification).

To evaluate the physicochemical stability of the herbal nanoparticle formulation.

To assess the in vitro antidiabetic activity of the formulated nanoparticles (e.g., α-amylase or α-glucosidase

inhibition studies).

PLAN OF WORK

- Literature review
- Selection and Authentication of Plant Material
- Preparation of Herbal Extract
- Preliminary Phytochemical Screening
- Formulation of extract loaded nanoparticles
- Selection of Experimental Animals
- Assessment of Antidiabetic Activity
- Data Analysis
- Interpretation and Conclusion
- Report writing

5.1. Plant profile: Tulsi [145-147]

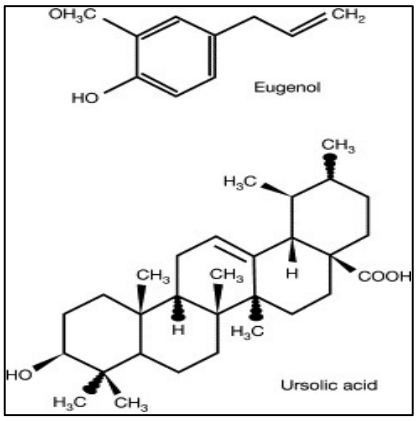


Figure 4: Chemical structure of active constituents of tulsi (Eugenol & Ursolic acid)

1.Botanical Identification

Scientific Name: *Ocimum sanctum*

Synonyms: Ocimum tenuiflorum, Ocimum basilicum var. sanctum

Family: Lamiaceae

Common Names: Tulsi (India), Holy Basil (English), Sacred Basil, Ocimum

Plant Type: Aromatic perennial shrub

2.Morphological Description

Height: 0–60 cm (can reach up to 1 m)

Stem: Erect, quadrangular, hairy, sometimes purplish

Leaves: Opposite, simple, ovate or elliptic, serrated margins, aromatic

Flowers: Small, purplish, white, or pink, arranged in terminal spikes

Fruit: 4–6 nutlets, brownish-black, small

Roots: Taproot system, brownish, aromatic

Geographical Distribution

Native to the Indian subcontinent

Cultivated widely across tropical and subtropical regions

Found in India, Nepal, Sri Lanka, Thailand, and Southeast Asia

Habitat and Cultivation

Thrives in warm, humid climates

Soil: Well-drained loamy or sandy soil

Requires moderate sunlight

Propagation: By seeds or stem cuttingsHarvesting: Leaves are harvested before flowering for maximum phytoconstituents

Phytochemical Constituents

Tulsi is rich in bioactive compounds with pharmacological properties.

Category	**Active Compounds**
Volatile Oils	Eugenol, Methyl eugenol, Caryophyllene, Linalool, Camphor, Ursolic acid
Phenolic Compounds	Rosmarinic acid, Flavonoids (Orientin, Vicenin, Apigenin, Luteolin)
Alkaloids	Ocimum alkaloids (Tulipin)
Terpenoids	Ursolic acid, Oleanolic acid
Polysaccharides	Mucilage and pectins
Other	Tannins, Saponins, Vitamin C, Beta-carotene

1. Pharmacological Properties

Tulsi exhibits a wide range of therapeutic effects:

Activity	**Mechanism**
Antipyretic	Reduces fever by modulating prostaglandins and cytokines

Anti-inflammatory	Inhibits COX-2 enzyme, reduces inflammation
Antioxidant	Scavenges free radicals, prevents oxidative stress
Antimicrobial	Effective against bacteria (Staphylococcus, E. coli), fungi (Candida) and viruses
Adaptogenic	Enhances resistance to stress via HPA axis modulation
Immunomodulatory	Stimulates antibody production, macrophage activity
Hepatoprotective	Protects liver cells against toxins
Cardioprotective	Reduces blood lipid levels, protects heart tissue
Anti-diabetic	Improves insulin sensitivity, lowers blood glucose
Neuroprotective	Protects neurons, improves memory and cognitive functions
Anti-cancer	Inhibits tumor growth, induces apoptosis in certain cancer cell lines

Traditional Uses (Ethnobotanical)

Ayurveda: Used as Rasayana (rejuvenator) and for respiratory ailments, fever, and stress

Unani Medicine: Treatment for cold, cough, and influenza

Folk Remedies: Tulsi leaves chewed for sore throat, crushed leaves applied on insect bites, decoction for digestion

Religious Significance: Sacred in Hindu households; considered purifying and protective

Dosage and Forms

Leaves: Fresh or dried, infusion, decoction, or powder

Juice: Leaf juice taken orally for cough, cold, and fever

Essential Oil: Used in aromatherapy and topical applications

Extracts: Alcoholic, aqueous, or hydroalcoholic extracts used for research

Toxicity and Safety

Generally regarded as safe when used orally in moderate amounts

Mild hypoglycemia may occur in diabetic patients if combined with medication

Rare allergic reactions reported with topical application

Excipients profile

5.1.1. Chitosan [148-150]

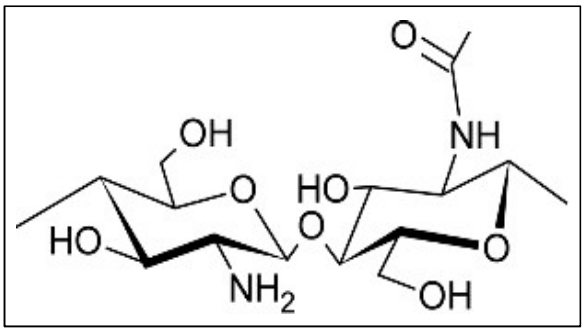


Figure 5: Chemical structure of Chitosan

1.General Information

Chemical Name: β-(1→4)-2-amino-2-deoxy-D-glucose polymer**Synonyms:** Chitin deacetylated, poly(D-glucosamine), deacetylated chitin

Molecular Formula: ($C_6H_{11}NO$)

Molecular Weight: 50,000–2,000,000 Da (depending on degree of polymerization)

Appearance: White to off-white fibrous powder

Odor: Mild, characteristic

Solubility: Soluble in dilute acids (acetic acid, citric acid), insoluble in water and organic solvents

pH of 1% solution: 4.0–6.5

2.Source and Preparation

Derived from chitin, which is extracted from crustacean shells (crabs, shrimps, lobsters)

Deacetylation process: Chitin is treated with concentrated alkali (NaOH) to remove acetyl groups, yielding chitosan

Degree of deacetylation (DD): 70–95%

DD influences solubility, viscosity, and bioadhesive properties

3.Physical and Chemical Properties

Property	Characteristic
Physical form	Powder, flakes, or granules
Hygroscopicity	Moderate; absorbs moisture from air
Viscosity	20–2000 cP depending on molecular weight and concentration
Charge	Positively charged at acidic pH (cationic polymer)
Biodegradability	Biodegradable by lysozyme and microbial enzymes
Thermal stability	Stable up to 220–250°C; decomposition above 280°C

4.Pharmaceutical Classification

Function: Polymer / excipient / film-former / mucoadhesive agent / controlled-release carrier

Category: Natural cationic polysaccharide

4.Mechanism of Action in Formulations

Mucoadhesion: Positively charged amino groups interact with negatively charged mucosal surfaces, enhancing retention time**Controlled Release:** Forms hydrogels and matrices that modulate drug release

Permeation Enhancement: Temporarily opens tight junctions in epithelial layers**Film-forming:** Can form flexible, biodegradable films for transdermal, ocular, or oral drug delivery.Common Pharmaceutical Applications

Oral Drug Delivery

1.Tablets, capsules, hydrogels

Sustained-release and colon-targeted formulations

2..Transdermal / Topical

Films, patches, wound dressings

3.Ocular / Nasal

In-situ gels for residence time

4.Nanoparticles / Microparticles

Encapsulation of drugs, peptides, proteins, and vaccines

5.Gene Delivery

Non-viral cationic polymer for DNA/RNA complexation

Advantages

Biocompatible and biodegradable

Non-toxic and non-immunogenic

Enhances bioavailability of poorly absorbed drugs

Good film-forming and gel-forming propertie

Natural and renewable polymer source

Limitations / Considerations

Solubility restricted to acidic media

Batch-to-batch variability depending on source and degree of deacetylation

Hygroscopic nature requires careful storage

May interact with anionic drugs, affecting release profile

Stability and Storage

Store in airtight containers

Keep in a cool, dry place (<25°C)

Protect from light and moisture to prevent degradationRegulatory Status

FDA / GRAS: Generally Recognized As Safe (GRAS) for food and pharmaceutical applications

Widely approved for use as excipient in oral, topical, and parenteral formulations

Sodium Tripolyphosphate [151,152]

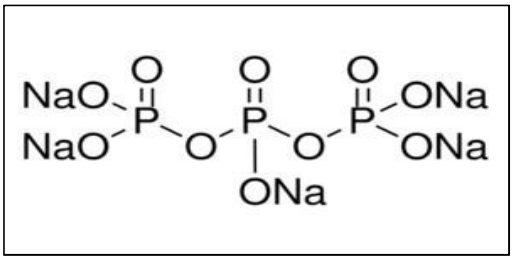


Figure 6: Chemical structure of Sodium tripolyphosphate

1.General Information

Chemical Name: Sodium tripolyphosphate

Chemical Formula: $Na_5P_3O_{10}$

Molecular Weight: 367.86 g/mol

CAS Number: 7758-29-4

Appearance: White crystalline powder

Odor: Odorless

Solubility: Soluble in water, insoluble in alcohol and organic solvents

pH of 1% solution: 9–10 (alkaline)

1.Source and Preparation

Produced by condensation of sodium phosphate salts: sodium dihydrogen phosphate and sodium carbonate or sodium hydroxide

Commercially available as food-grade, pharmaceutical-grade, and technical-grade Physical and Chemical Properties

Property	**Characteristic**
Physical form	Crystalline powder or granules
Hygroscopicity	Moderate; absorbs moisture from air
Thermal Stability	Stable up to 400°C; decomposes to sodium phosphate
Charge	Anionic polyelectrolyte
Solubility	Highly soluble in water, forming alkaline solutions
Density	2.53 g/cm³

Pharmaceutical Classification

Function: Crosslinking agent, sequestrant, buffering agent, stabilizer

Category: Inorganic excipient / polymer crosslinker

Mechanism of Action in Formulations

Crosslinking: Reacts with polymers like chitosan or gelatin to form ionic crosslinked networks
Sequestration / Chelation: Binds metal ions (Ca^{2+}, Mg^{2+}) to prevent precipitation or hardening
pH Stabilization: Maintains alkaline environment in aqueous systems

Viscosity Enhancement: Helps in forming stable gels and hydrogels

Common Pharmaceutical Applications

Oral and Topical Formulations

Stabilizer in suspensions and emulsions

Prevents caking in powders

Controlled Release Systems

Ionic crosslinker for chitosan nanoparticles, microspheres, and hydrogels

Enhances mechanical strength and drug encapsulation efficiency.Food and Cosmetic Applications

Emulsifier, sequestrant, and water-softening agent

Industrial Uses

Detergent builder (non-pharmaceutical)

Advantages

Non-toxic and water-soluble

Effective ionic crosslinker for cationic polymers like chitosan

Enhances mechanical stability of hydrogel and nanoparticle formulations

Improves shelf-life by chelating metal ions

Limitations / Considerations

Highly alkaline; may require pH adjustment in formulations

Excess amounts may lead to brittleness in gels or tablets

Hygroscopic nature; must be stored in airtight containers

Stability and Storage

Store in cool, dry, airtight containers

Protect from moisture and direct sunlight

Regulatory Status

GRAS / FDA Approved: Widely recognized as safe for food and pharmaceutical applications

Acceptable in oral, topical, and controlled-release formulations

5.1.2. Poloxamer 188 [153,154]

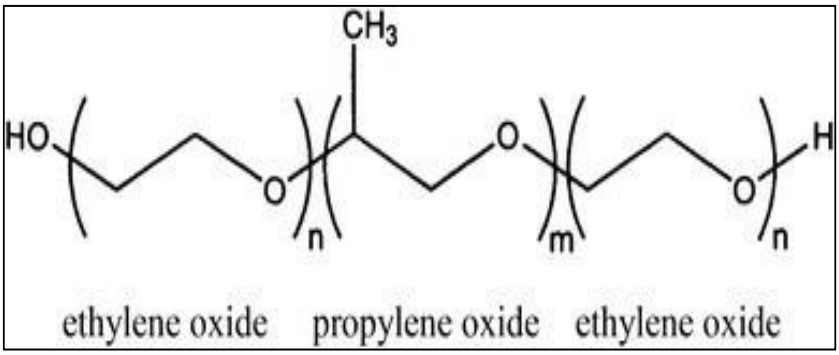


Figure 7: Chemical structure of Poloxamer 188

1. General Information

Parameter	**Details**
Generic Name	Poloxamer 188
Other Names	Pluronic® F68, Lutrol® F68
Chemical Name	Poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) block copolymer
Chemical Structure	HO–(C_2H_4O)a–(C_3H_6O)b–(C_2H_4O)a–H
Molecular Formula	Approx. $C_5H_{10}O_2$ repeated units
Average Molecular Weight	~7,680 – 9,510 g/mol
HLB Value	29 (Highly hydrophilic)
Category	Non-ionic surfactant
Pharmacopoeial Status	IP, USP, BP

2. Description

White to off-white waxy solid or powder

Odorless and tasteless

Freely soluble in water and alcohol

Practically insoluble in oils

Forms clear, colorless solutions in water

Functional Category

Poloxamer 188 is used as:

Surfactant

Emulsifying agent

Solubilizing agent

Wetting agent

Stabilizer

Dispersing agent

Mechanism of Action as Surfactant

Poloxamer 188 is a triblock copolymer consisting of hydrophobic polypropylene oxide (PPO) core and hydrophilic polyethylene oxide (PEO) chains.

Hydrophobic PPO portion interacts with nonpolar drug/oil

Hydrophilic PEO portion interacts with aqueous phase

Reduces interfacial tension

Forms micelles above Critical Micelle Concentration (CMC)

Enhances solubility of poorly water-soluble drugs

Applications in Pharmaceutical Formulations

Nanoparticles stabilizationLiposomes and micellesInjectable formulations

Ophthalmic preparations

Oral liquid formulationsTopical gels and creamsProtein and peptide stabilization

Advantages

Non-ionic → low irritation

High HLB → excellent for O/W emulsions

Biocompatible and low toxicity

Enhances drug solubility

Improves bioavailability

Approved for parenteral use

Protects proteins from aggregation

Incompatibilities

Strong oxidizing agents

May reduce preservative effectiveness

Incompatible with some phenolic compoundsStability and Storage
Stable under normal conditions

Protect from excessive heat

Store in well-closed container

Hygroscopic in nature

Safety Profile

Generally Recognized as Safe (GRAS)

Non-irritant at recommended concentrations

Safe for IV administration

Rare hypersensitivity reactions

5.2. Plant profile: Tulsi [145-147]

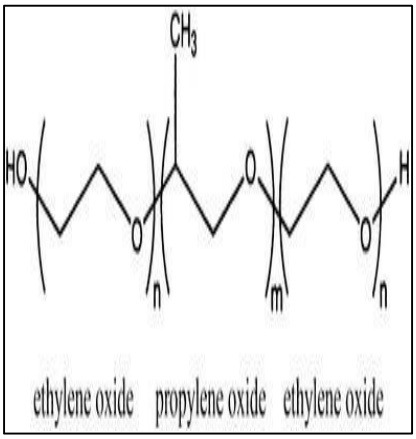


Figure 4: Chemical structure of active constituents of tulsi (Eugenol & Ursolic acid)

Botanical Identification

Scientific Name: *Ocimum sanctum*

Synonyms: Ocimum tenuiflorum, Ocimum basilicum var. sanctum

Family: Lamiacea

Common Names: Tulsi (India), Holy Basil (English), Sacred Basil, Ocimum

Plant Type: Aromatic perennial shrub

Morphological Description

Height: 30–60 cm (can reach up to 1 m)

Stem: Erect, quadrangular, hairy, sometimes purplish

Leaves: Opposite, simple, ovate or elliptic, serrated margins, aromatic

Flowers: Small, purplish, white, or pink, arranged in terminal spikes

Fruit: 4–6 nutlets, brownish-black, small

Roots: Taproot system, brownish, aromatic

Geographical Distribution

Native to the Indian subcontinent

Cultivated widely across tropical and subtropical regions

Found in India, Nepal, Sri Lanka, Thailand, and Southeast Asia

Habitat and Cultivation

Thrives in warm, humid climates

Soil: Well-drained loamy or sandy soil

Requires moderate sunlight

Propagation: By seeds or stem cuttings

Harvesting: Leaves are harvested before flowering for maximum phytoconstituents

Phytochemical ConstituentsTulsi is rich in bioactive compounds with pharmacological properties.

Category	Active Compounds
Volatile Oils	Eugenol, Methyl eugenol, Caryophyllene, Linalool, Camphor, Ursolic acid
Phenolic Compounds	Rosmarinic acid, Flavonoids (Orientin, Vicenin, Apigenin, Luteolin)
Alkaloids	Ocimum alkaloids (Tulipin)
Terpenoids	Ursolic acid, Oleanolic acid
Polysaccharides	Mucilage and pectins
Other	Tannins, Saponins, Vitamin C, Beta-carotene

2. Pharmacological Properties

Tulsi exhibits a wide range of therapeutic effects:

Activity	Mechanism
Antipyretic	Reduces fever by modulating prostaglandins and cytokines
Anti-inflammatory	Inhibits COX-2 enzyme, reduces inflammation
Antioxidant	Scavenges free radicals, prevents oxidative stress

Antimicrobial	Effective against bacteria (Staphylococcus, E. coli), fungi (Candida) and viruses
Adaptogenic	Enhances resistance to stress via HPA axis modulation
Immunomodulatory	Stimulates antibody production, macrophage activity
Hepatoprotective	Protects liver cells against toxins
Cardioprotective	Reduces blood lipid levels, protects heart tissue
Anti-diabetic	Improves insulin sensitivity, lowers blood glucose
Neuroprotective	Protects neurons, improves memory and cognitive functions
Anti-cancer	Inhibits tumor growth, induces apoptosis in certain cancer cell lines

Traditional Uses (Ethnobotanical)

Ayurveda: Used as Rasayana (rejuvenator) and for respiratory ailments, fever, and stress

Unani Medicine: Treatment for cold, cough, and influenza

Folk Remedies: Tulsi leaves chewed for sore throat, crushed leaves applied on insect bites, decoction for digestion

Religious Significance: Sacred in Hindu households; considered purifying and protective

Dosage and Forms

Leaves: Fresh or dried, infusion, decoction, or powder

Juice: Leaf juice taken orally for cough, cold, and fever

Essential Oil: Used in aromatherapy and topical applications

Extracts: Alcoholic, aqueous, or hydroalcoholic extracts used for research

3. Toxicity and Safety

- Generally regarded as safe when used orally in moderate amounts
- Mild hypoglycemia may occur in diabetic patients if combined with medication
- Rare allergic reactions reported with topical application

5.3. Excipients profile

5.3.1. Chitosan [148-150]

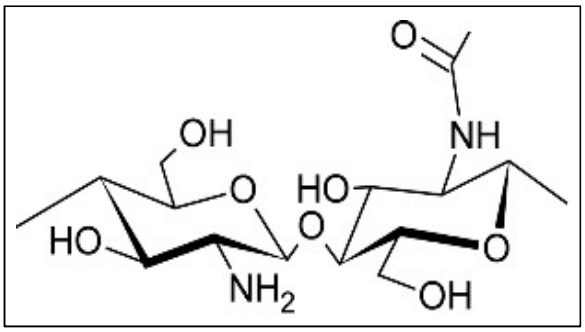


Figure 5: Chemical structure of Chitosan

1. General Information

- **Chemical Name:** β-(1→4)-2-amino-2-deoxy-D-glucose polymer
- **Synonyms:** Chitin deacetylated, poly(D-glucosamine), deacetylated chitin
- **Molecular Formula:** $(C_6H_{11}NO_4)n$
- **Molecular Weight:** 50,000–2,000,000 Da (depending on degree of polymerization)
- **Appearance:** White to off-white fibrous powder
- **Odor:** Mild, characteristic
- **Solubility:** Soluble in dilute acids (acetic acid, citric acid), insoluble in water and organic solvents
- **pH of 1% solution:** 4.0–6.5

2. Source and Preparation

- Derived from chitin, which is extracted from crustacean shells (crabs, shrimps, lobsters)
- Deacetylation process: Chitin is treated with concentrated alkali (NaOH) to remove acetyl groups, yielding chitosan
- Degree of deacetylation (DD): 70–95%
- DD influences solubility, viscosity, and bioadhesive properties

3. Physical and Chemical Properties

Property	Characteristic
Physical form	Powder, flakes, or granules
Hygroscopicity	Moderate; absorbs moisture from air
Viscosity	20–2000 cP depending on molecular weight and concentration
Charge	Positively charged at acidic pH (cationic polymer)
Biodegradability	Biodegradable by lysozyme and microbial enzymes
Thermal stability	Stable up to 220–250°C; decomposition above 280°C

Pharmaceutical Classification

Function: Polymer / excipient / film-former / mucoadhesive agent / controlled-release carrier

Category: Natural cationic polysaccharideMechanism of Action in Formulations

Mucoadhesion: Positively charged amino groups interact with negatively chargedmucosal surfaces, enhancing retention time

Controlled Release: Forms hydrogels and matrices that modulate drug release**Permeation Enhancement:** Temporarily opens tight junctions in epithelial layers**Film-forming:** Can form flexible, biodegradable films for transdermal, ocular, or oral drug delivery.

Common Pharmaceutical Applications

Oral Drug Delivery Tablets, capsules, hydrogels

Sustained-release and colon-targeted formulations

Transdermal / Topical

Films, patches, wound dressings

Ocular / Nasal

In-situ gels for prolonged residence time

Nanoparticles / Microparticles

Encapsulation of drugs, peptides, proteins, and vaccines

Gene Delivery

Non-viral cationic polymer for DNA/RNA complexation

Advantages

Biocompatible and biodegradableNon-toxic and non-immunogenic

Enhances bioavailability of poorly absorbed drugs

Good film-forming and gel-forming properties

Natural and renewable polymer source

4.Limitations / Considerations

Solubility restricted to acidic media

Batch-to-batch variability depending on source and degree of deacetylation

Hygroscopic nature requires careful storage

May interact with anionic drugs, affecting release profile

5.Stability and Storage

Store in airtight containers

Keep in a cool, dry place (<25°C)

Protect from light and moisture to prevent degradationRegulatory Status

FDA / GRAS: Generally Recognized As Safe (GRAS) for food and pharmaceutical applications

Widely approved for use as excipient in oral, topical, and parenteral formulations

5.3.2 Sodium Tripolyphosphate [151,152]

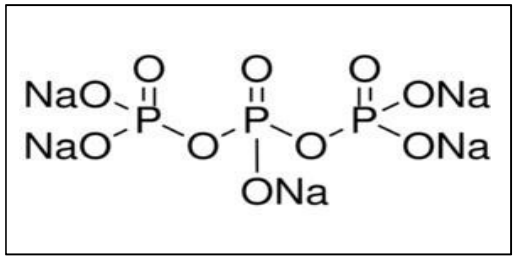


Figure 6: Chemical structure of Sodium tripolyphosphate

1.General Information

Chemical Name: Sodium tripolyphosphate

Chemical Formula: $Na_5P_3O_{10}$

Molecular Weight: 367.86 g/mol

CAS Number: 7758-29-4

Appearance: White crystalline powder

Odor: Odorless

Solubility: Soluble in water, insoluble in alcohol and organic solvents

pH of 1% solution: 9–10 (alkaline)

2. Source and Preparation

Produced by condensation of sodium phosphate salts: sodium dihydrogen phosphate sodium carbonate or sodium hydroxide

Commercially available as food-grade, pharmaceutical-grade, and technical-grade

3.Physicand Chemical Properties

Property	**Characteristic**
Physical form	Crystalline powder or granules
Hygroscopicity	Moderate; absorbs moisture from air
Thermal Stability	Stable up to 400°C; decomposes to sodium phosphate
Charge	Anionic polyelectrolyte
Solubility	Highly soluble in water, forming alkaline solutions
Density	2.53 g/cm³

4.Pharmaceutical Classification

Function: Crosslinking agent, sequestrant, buffering agent, stabilizer

Category: Inorganic excipient / polymer crosslinker

5.Mechanism of Action in Formulations

Crosslinking: Reacts with polymers like chitosan or gelatin to form ionic crosslinked networks

Sequestration / Chelation: Binds metal ions (Ca^{2+}, Mg^{2+}) to prevent precipitation or hardenig

pH Stabilization: Maintains alkaline environment in aqueous systems

Viscosity Enhancement: Helps in forming stable gels and hydrogels

6. Common Pharmaceutical Applications**Oral and Topical Formulations**

Stabilizer in suspensions and emulsions

Prevents caking in powders

Controlled Release Systems

Ionic crolinker for chitosan nanoparticles, microspheres, and hydrogels

Enhances mechanical strength and drug encapsulation efficiency.Food and Cosmetic Applications

Emulsifier, sequestrant, and water-softening agent

Industrial Uses

Detergent builder (non-pharmaceutical)

Advantages

Non-toxic and water-soluble

Effective ionic crosslinker for cationic polymers like chitosan

Enhances mechanical stability of hydrogel and nanoparticle formulations

Improves shelf-life by chelating metal ions

Limitations / Consideration

Highly alkaline; may require pH adjustment in formulations

Excess amounts may lead to brittleness in gels or tablets

Hygroscopic nature; must be stored in airtight containers

Stability and Storage

Store in cool, dry, airtight containers

Protect from moisture and direct sunlight

Regulatory Status

GRAS / FDA Approved: Widely recognized as safe for food and pharmaceutical applications

Acceptable in oral, topical, and controlled-release formulations

5.3.2. Poloxamer 188 [153,154]

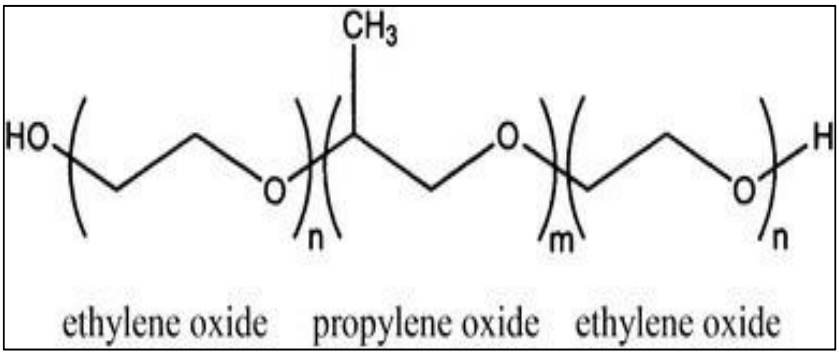


Figure 7: Chemical structure of Poloxamer 188

1.General Information

Parameter	**Details**
Generic Name	Poloxamer 188
Other Names	Pluronic® F68, Lutrol® F68
Chemical Name	Poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) block copolymer
Chemical Structure	HO–(C_2H_4O)a–(C_3H_6O)b–(C_2H_4O)a–H
Molecular Formula	Approx. $C_5H_{10}O_2$ repeated units
Average Molecular Weight	~7,680 – 9,510 g/mol
HLB Value	29 (Highly hydrophilic)
Category	Non-ionic surfactant
Pharmacopoeial Status	IP, USP, BP

2.Description

White to off-white waxy solid or powder

Odorless and tasteless

Freely soluble in water and alcohol

Practically insoluble in oils

Forms clear, colorless solutions in water

3. Functional Category

Poloxamer 188 is used as:

Surfactant

Emulsifying agent

Solubilizing agent

Wetting agent

Stabilizer

Dispersing agent

4. Mechanism of Action as Surfactant

Poloxamer 188 is a triblock copolymer consisting of hydrophobic polypropylene oxide (PPO) core and hydrophilic polyethylene oxide (PEO) chains.

Hydrophobic PPO portion interacts with nonpolar drug/oil

Hydrophilic PEO portion interacts with aqueous phase

Reduces interfacial tension

Forms micelles above Critical Micelle Concentration (CMC)

Enhances solubility of poorly water-soluble drugs

5. Applications in Pharmaceutical Formulations

Nanoparticles stabilization

Liposomes and micelles

Injectable formulations

Ophthalmic preparations

Oral liquid formulations

Topical gels and creams

Protein and peptide stabilization

7. Advantages

Non-ionic → low irritation

High HLB → excellent for O/W emulsions

Biocompatible and low toxicity

Enhances drug solubility

Improves bioavailability

Approved for parenteral use

Protects proteins from aggregation

8. Incompatibilities

Strong oxidizing agents

May reduce preservative effectiveness

Incompatible with some phenolic compounds

9. Stability and Storage

Stable under normal conditions

Protect from excessive heat

Store in well-closed container

Hygroscopic in nature

10. Safety Profile

Generally Recognized as Safe (GRAS)

Non-irritant at recommended concentrations

Safe for IV administration

Rare hypersensitivity reactions

RESULTS AND DISCUSSION

7.1. Collection and Drying of plant

The plant material of *Ocimum sanctum* was collected from Pune, a region located in Maharashtra, India. The collection was conducted following proper botanical practices and guidelines. It was done in the shade dry at room temperature. After drying, the obtained coarse powder was crushed in a grinder and then passed through a 40-mesh screen to remove the fine particles. The remaining coarse powder was then utilized for the next step.

7.2. Preliminary phytoconstituent tests for extract

Table 10: Preliminary phytoconstituent tests

Sr. No.	Test	Observation	Result
1.	Carbohydrates Test A) Fehling's test:	Brick red precipitate	Carbohydrates present
	B) Benedict's test	Orange colour	Carbohydrates present
2.	Alkaloid Test A) Dragendroff's test	No orange-brown precipitate	Alkaloids absent
	B) Hager's test	No yellowish precipitate	Alkaloids absent
	C) Wagner's test	No reddish brown precipitate	Alkaloids absent
3.	Glycosides Test A) Keller Killani test	A blue colored solution (in the layer)	Cardiac glycosides present
	B) Borntrager's test	No pink or red colour	Anthraquinone glycosides absent
4.	Flavonoid Test A) Ferric chloride test	Green precipitate	Flavonoid present
	B) Lead acetate test	Yellow colour	Flavonoid present
5.	Steroids Test A) Salkowski's test	No red colour (in lower layer)	Steroids absent

6.	Triterpenoids test	The violet tint	Triterpenoids present
7.	Tannins & Phenolic compounds	Deep blue-black colour	Tannins & Phenolic compounds present

Conclusion:

The preliminary phytochemical screening of the Tulsi extract (*Ocimum sanctum*) revealed the presence of several important bioactive constituents. The tests confirmed the presence of carbohydrates, cardiac glycosides, flavonoids, triterpenoids, and tannins & phenolic compounds, while alkaloids, anthraquinone glycosides, and steroids were absent in the extract. The presence of flavonoids, phenolic compounds, and triterpenoids indicates strong antioxidant potential of the plant extract. These phytoconstituents are known to contribute to various pharmacological activities, including antidiabetic effects. Flavonoids and phenolic compounds may help in reducing blood glucose levels and protecting pancreatic β-cells from oxidative stress. Therefore, the phytochemical profile suggests that Tulsi possesses promising antidiabetic properties and supports its traditional use in the management of diabetes.

7.3. TLC of active constituents

Table 11: TLC for Phytoconstituents

Phytoconstituents	**Mobile phase**	**RF value found**
Terpenoids	Toluene: Ethyl acetate 8:2	0.92
Flavonoids	Ethyl acetate: Methanol: Water 7:2:1	0.96

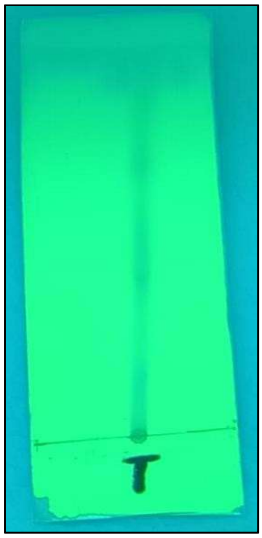


Figure 8: TLC for Phytoconstituents (Terpenoids)

Figure 9: TLC for Phytoconstituents (Flavonoids)

7.4. Optimization of ethanolic extract loaded nanoparticle

The ethanolic extract of *Ocimum sanctum* loaded nanoparticles was optimized using Box behnken design of 3 levels and 3 factors of total 13 experimental runs. The independent parameters includes Chitosan Concentration (%), Extract concentration (mg) and Stirring speed (rpm). The dependent parameters were Particle size (nm), Zeta potential (mV) and Entrapment efficiency (%).

7.5. Evaluations of nanoparticles

7.5.1Particle size, PDI and Zeta potential

Table 12: Particle size, PDI and Zeta potential of TF1-TF13

Formulation code	Particle size (nm)	PDI	Zeta potential (mV)
TF1	218.5	0.253	-19.56
TF2	231.9	0.286	-20.52
TF3	168.6	0.315	-18.85
TF4	135.4	0.184	-29.41
TF5	145.6	0.365	-18.85
TF6	178.9	0.275	-17.45
TF7	165.4	0.296	-18.96
TF8	215.7	0.348	-23.56
TF9	205.6	0.355	-20.75
TF10	186.7	0.248	-14.86
TF11	174.2	0.291	-26.54
TF12	196.4	0.198	-22.84
TF13	188.6	0.288	-25.26

Conclusion:

The Tulsi extract-loaded nanoparticles were successfully formulated with particle sizes ranging from 135.4 to 231.9 nm, indicating they are within the ideal nanoscale for improved bioavailability. The PDI values (0.184–0.365) suggest that most formulations have a moderate to good uniformity, with TF4 showing the narrowest distribution. The zeta potential values (- 14.86 to -29.41 mV) indicate that the nanoparticles possess adequate electrostatic stability, with

TF4 exhibiting the highest stability. Smaller particle sizes and appropriate surface charges are expected to enhance cellular uptake and therapeutic efficacy. The formulations with lower PDI and higher negative zeta potential are likely to maintain colloidal stability over time. Overall, the data confirm that the prepared nanoparticles are suitable for further in vitro and in vivo antidiabetic studies. The optimized formulations can provide sustained release and improved pharmacological activity of Tulsi extract.

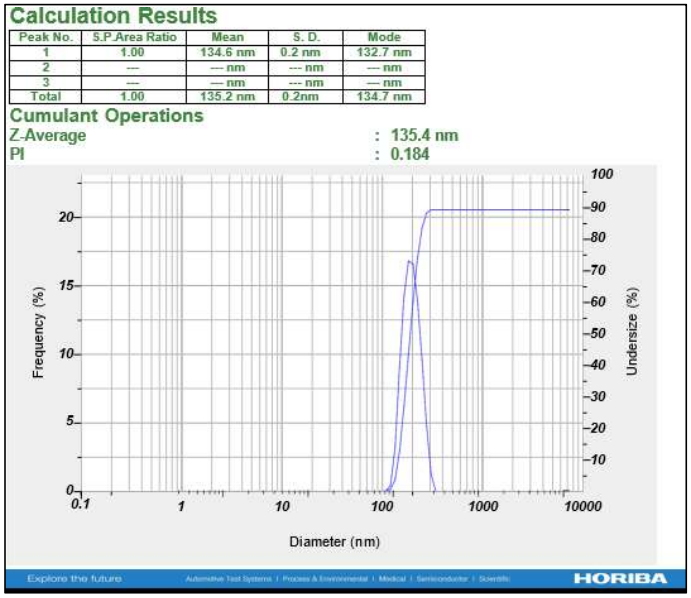


Figure 10: Particle size graph of TF4

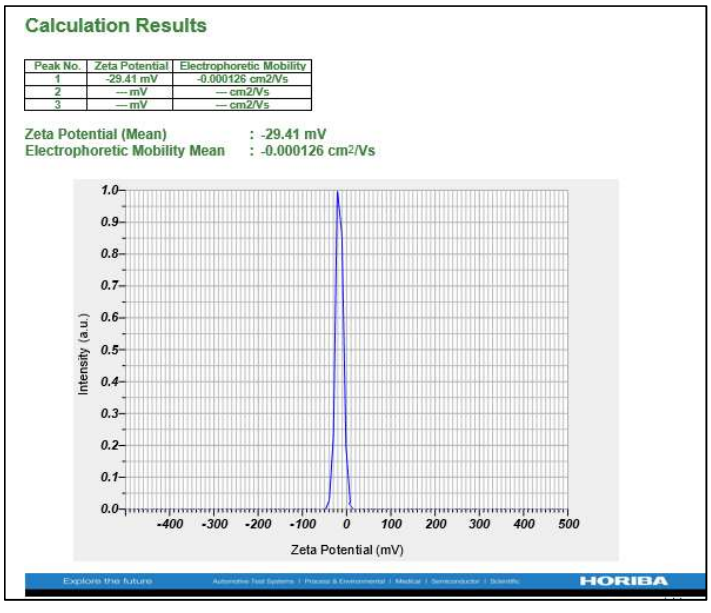


Figure 11: Zeta potential graph of TF4

ANOVA for 2FI model Response 1:

Particle size

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	8657.69	6	1442.95	8.72	0.0093	significant
A-Chitosan Conc	119.35	1	119.35	0.7211	0.4284	
B-Extract conc	282.03	1	282.03	1.70	0.2396	
C-Stirring speed	1529.05	1	1529.05	9.24	0.0228	
AB	4455.56	1	4455.56	26.92	0.0020	
AC	53.29	1	53.29	0.3220	0.5910	
BC	2218.41	1	2218.41	13.40	0.0106	
Residual	993.07	6	165.51			
Cor Total	9650.76	12				

Factor coding is **Coded**.

Sum of squares is **Type III - Partial**

The **Model F-value** of 8.72 implies the model is significant. There is only a 0.93% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case C, AB, BC are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Fit Statistics

Std. Dev.	12.87	**R^2**	0.8971
Mean	185.50	**Adjusted R^2**	0.7942
C.V. %	6.94	**Predicted R^2**	0.6295
		Adeq Precision	8.3431

Coefficients in Terms of Coded Factors

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	185.50	1	3.57	176.77	194.23	
A-Chitosan Conc	3.86	1	4.55	-7.27	14.99	1.0000
B-Extract conc	5.94	1	4.55	-5.19	17.07	1.0000
C-Stirring speed	13.83	1	4.55	2.70	24.95	1.0000
AB	-33.37	1	6.43	-49.11	-17.64	1.0000
AC	3.65	1	6.43	-12.09	19.39	1.0000
BC	-23.55	1	6.43	-39.29	-7.81	1.0000

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the VIFs are 1; VIFs greater than 1 indicate multi-colinearity, the higher the VIF the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable.

Final Equation in Terms of Coded Factors

Particle size	=
+185.50	
+3.86	A
+5.94	B
+13.83	C
-33.37	AB
+3.65	AC
-23.55	BC

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Final Equation in Terms of Actual Factors

Particle size	=
-128.47500	
+596.62500	Chitosan Conc
+28.66750	Extract conc
+0.214500	Stirring speed
-66.75000	Chitosan Conc * Extract conc
+0.146000	Chitosan Conc * Stirring speed
-0.018840	Extract conc * Stirring speed

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space.

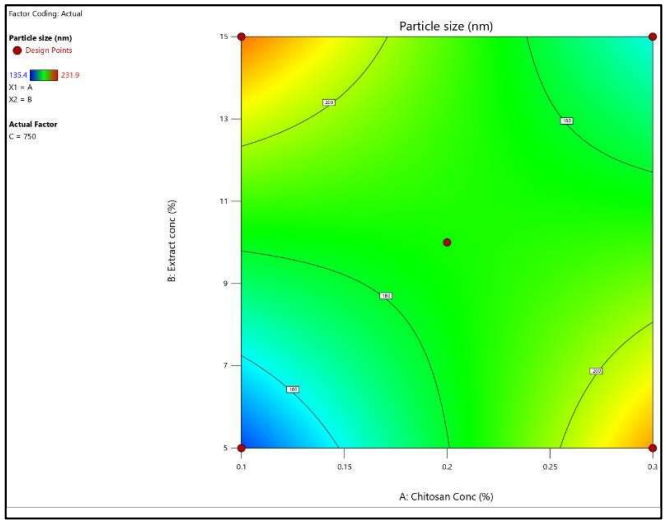


Figure 12: Contour plot for Particle size

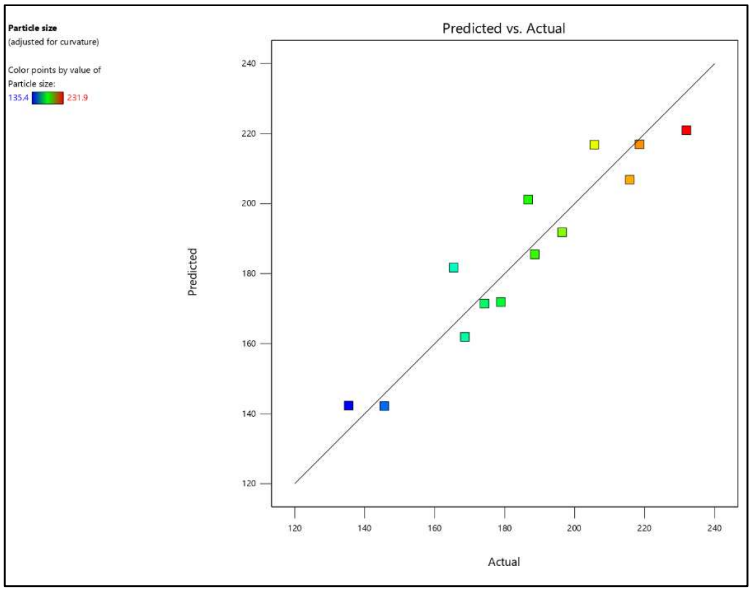


Figure 13: Predicted vs actual plot for Particle size

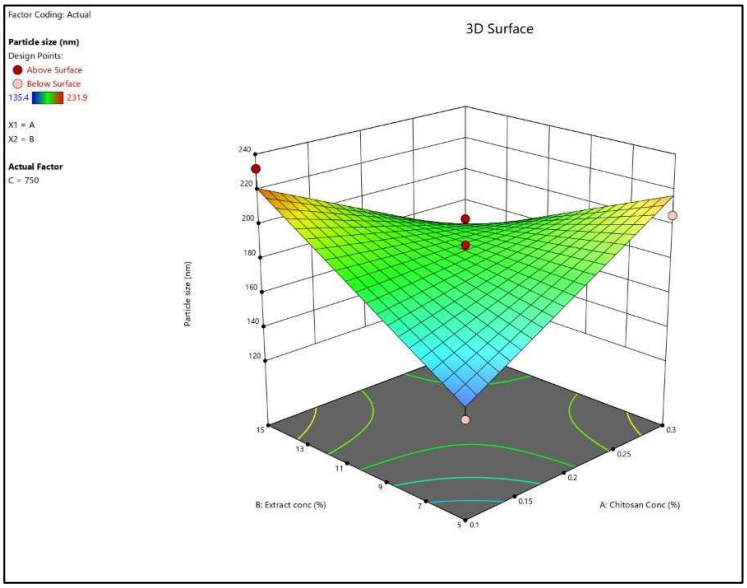


Figure 14: 3D Surface plot for Particle size

ANOVA for Quadratic model

Response 2: Zeta potential

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	188.08	9	20.90	10.44	0.0396	significant
A-Chitosan Conc	43.71	1	43.71	21.84	0.0185	
B-Extract conc	29.57	1	29.57	14.77	0.0311	
C-Stirring speed	6.52	1	6.52	3.26	0.1690	
AB	12.22	1	12.22	6.10	0.0900	
AC	24.06	1	24.06	12.02	0.0404	
BC	2.87	1	2.87	1.44	0.3169	
A^2	1.58	1	1.58	0.7890	0.4398	
B^2	31.44	1	31.44	15.71	0.0287	
C^2	27.90	1	27.90	13.94	0.0335	
Residual	6.01	3	2.00			
Cor Total	194.09	12				

Factor coding is **Coded**.

Sum of squares is **Type III - Partial**

The **Model F-value** of 10.44 implies the model is significant. There is only a 3.96% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, B, AC, B^2, C^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Fit Statistics

Std. Dev.	1.41	**R^2**	0.9691
Mean	-21.34	**Adjusted R^2**	0.8762
C.V. %	6.63	**Predicted R^2**	0.7648
		Adeq Precision	11.2863

Coefficients in Terms of Coded Factors

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	-25.26	1	1.41	-29.76	-20.76	
A-Chitosan Conc	2.34	1	0.5002	0.7456	3.93	1.0000
B-Extract conc	1.92	1	0.5002	0.3306	3.51	1.0000
C-Stirring speed	-0.9025	1	0.5002	-2.49	0.6894	1.0000
AB	-1.75	1	0.7074	-4.00	0.5038	1.0000
AC	-2.45	1	0.7074	-4.70	-0.2012	1.0000
BC	-0.8475	1	0.7074	-3.10	1.40	1.0000
A^2	-0.8313	1	0.9358	-3.81	2.15	1.35
B^2	3.71	1	0.9358	0.7306	6.69	1.35
C^2	3.49	1	0.9358	0.5156	6.47	1.35

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the VIFs are 1; VIFs greater than 1 indicate multi-colinearity, the higher the VIF the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable.

Final Equation in Terms of Coded Factors

Zeta potential	=
-25.26	
+2.34	A
+1.92	B
-0.9025	C
-1.75	AB
-2.45	AC
-0.8475	BC

-0.8313	A^2
+3.71	B^2
+3.49	C^2

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Final Equation in Terms of Actual Factors

Zeta potential	=
-14.90875	
+165.15000	Chitosan Conc
-1.37500	Extract conc
-0.061060	Stirring speed
-3.49500	Chitosan Conc * Extract conc
-0.098100	Chitosan Conc * Stirring speed
-0.000678	Extract conc * Stirring speed
-83.12500	Chitosan $Conc^2$
+0.148350	Extract $conc^2$
+0.000056	Stirring $speed^2$

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space.

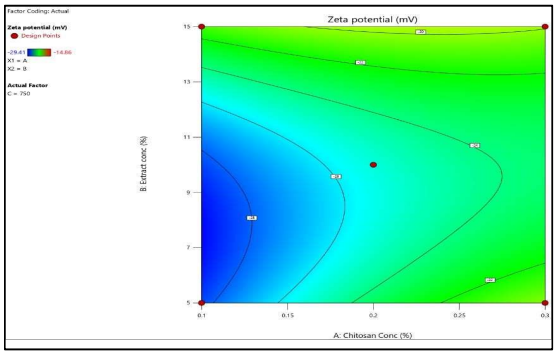
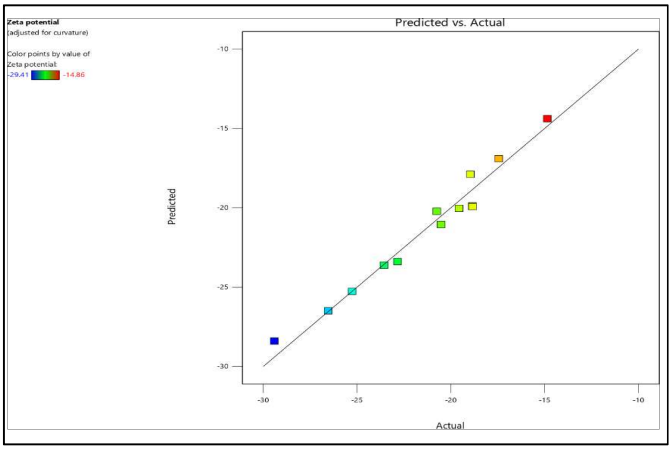


Figure 15: Contour plot for zeta potential

Figure 16: Predicted vs actual plot for zeta potential



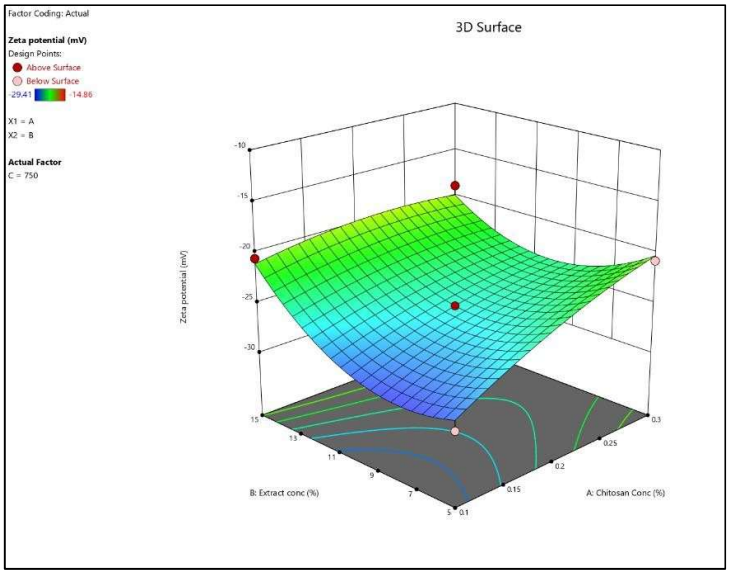


Figure 17: 3D Surface plot for zeta potential

7.5.2. Entrapment efficiency

Table 13: Entrapment efficiency of TF1-TF13

Formulation code	Entrapment efficiency (%)
TF1	78.96±0.002
TF2	79.96±0.003
TF3	83.37±0.002
TF4	87.25±0.001
TF5	80.42±0.001
TF6	82.75±0.002
TF7	76.86±0.004
TF8	81.52±0.001
TF9	78.45±0.003
TF10	78.65±0.003
TF11	84.33±0.004
TF12	83.25±0.001
F13	79.65±0.002

Conclusion: The entrapment efficiency of Tulsi extract-loaded nanoparticles ranged from 76.86% to 87.25%, indicating effective encapsulation of the extract in all formulations. TF4 showed the highest entrapment efficiency **(**87.25%**)**, suggesting optimal formulation parameters, while TF7 had the lowest **(**76.86%**)**. Most formulations achieved entrapment efficiency above 78%, demonstrating good consistency. The low standard deviations indicate reproducibility and precision in the nanoparticle preparation process. Overall, these results confirm that the nanoparticles are capable of efficiently retaining Tulsi extract, supporting their potential for enhanced antidiabetic efficacy.

ANOVA for Quadratic model Response 3:

Entrapment efficiency

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	99.35	9	11.04	35.51	0.0068	significant
A-Chitosan Conc	9.46	1	9.46	30.44	0.0117	
B-Extract conc	4.87	1	4.87	15.66	0.0288	
C-Stirring speed	3.86	1	3.86	12.43	0.0388	
AB	37.27	1	37.27	119.91	0.0016	
AC	0.0056	1	0.0056	0.0181	0.9015	
BC	0.0272	1	0.0272	0.0876	0.7866	
A^2	26.79	1	26.79	86.20	0.0026	
B^2	1.52	1	1.52	4.90	0.1137	
C^2	0.0283	1	0.0283	0.0910	0.7826	
Residual	0.9325	3	0.3108			
Cor Total	100.28	12				

Factor coding is **Coded**.

Sum of squares is **Type III - Partial**

The **Model F-value** of 35.51 implies the model is significant. There is only a 0.68% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, B, C, AB, A^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not

significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Fit Statistics

Std. Dev.	0.5575	**R²**	0.9907
Mean	81.19	**Adjusted R²**	0.9628
C.V. %	0.6867	**Predicted R²**	0.8652
		Adeq Precision	20.4762

Coefficients in Terms of Coded Factors

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	79.65	1	0.5575	77.88	81.42	
A-Chitosan Conc	-1.09	1	0.1971	-1.71	-0.4602	1.0000
B-Extract conc	-0.7800	1	0.1971	-1.41	-0.1527	1.0000
C-Stirring speed	-0.6950	1	0.1971	-1.32	-0.0677	1.0000
AB	3.05	1	0.2788	2.17	3.94	1.0000
AC	-0.0375	1	0.2788	-0.9246	0.8496	1.0000
BC	-0.0825	1	0.2788	-0.9696	0.8046	1.0000
A^2	3.42	1	0.3688	2.25	4.60	1.35
B^2	-0.8162	1	0.3688	-1.99	0.3573	1.35
C^2	-0.1113	1	0.3688	-1.28	1.06	1.35

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the VIFs are 1; VIFs greater than 1 indicate multi-colinearity, the higher the VIF the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable.

Final Equation in Terms of Coded Factors

Entrapment efficiency	=
+79.65	
-1.09	A
-0.7800	B
-0.6950	C
+3.05	AB
-0.0375	AC
-0.0825	BC
+3.42	A^2
-0.8162	B^2
-0.1113	C^2

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Final Equation in Terms of Actual Factors

Entrapment efficiency	=
+106.38875	
-207.75000	Chitosan Conc
-0.674500	Extract conc
+0.000850	Stirring speed
+6.10500	Chitosan Conc * Extract conc
-0.001500	Chitosan Conc * Stirring speed
-0.000066	Extract conc * Stirring speed
+342.37500	Chitosan $Conc^2$
-0.032650	Extract $conc^2$
-1.78000E-06	Stirring $speed^2$

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space.

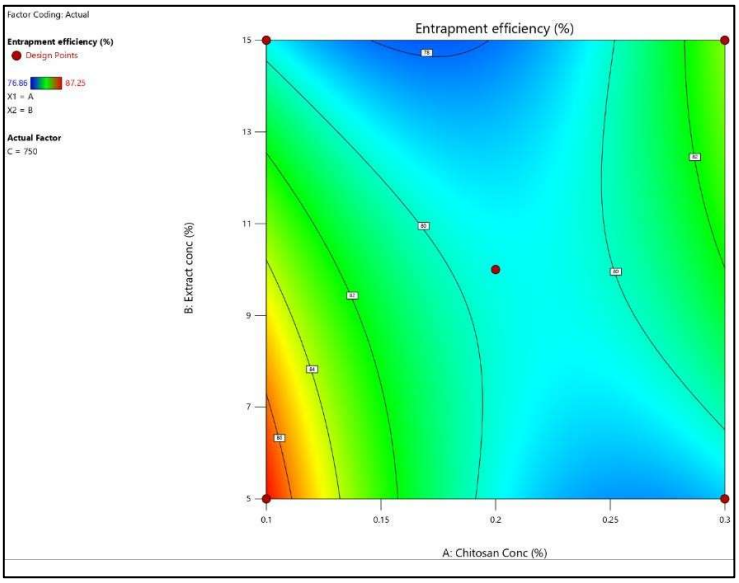


Figure 18: Contour plot for entrapment efficiency

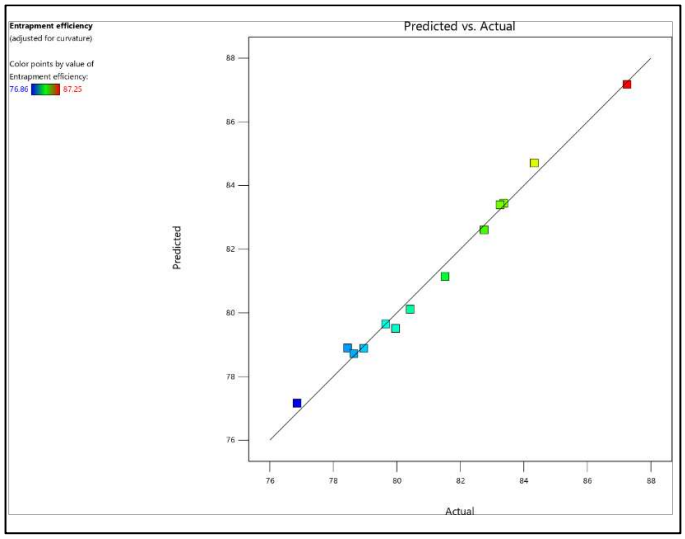


Figure 19: Predicted vs actual plot for entrapment efficiency

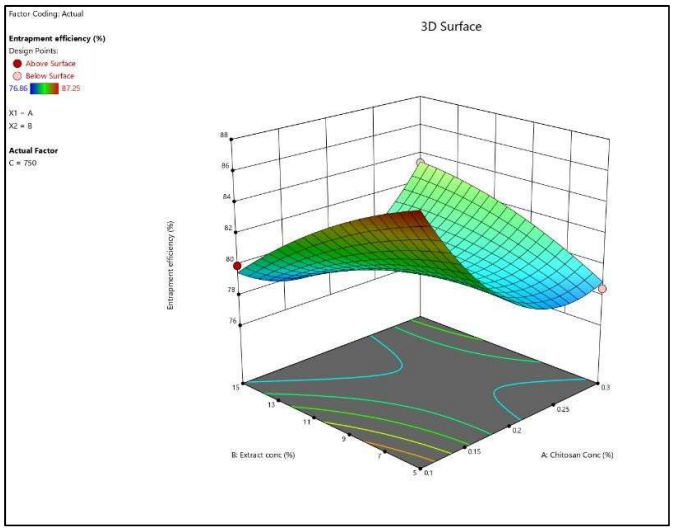


Figure 20: 3D Surface plot for entrapment efficiency

7.5.3. Drug content

Table 14: Drug content of TF1-TF13

Formulation code	Drug content (%)
TF1	92.45±0.003
TF2	87.54±0.004
TF3	83.26±0.004
TF4	95.26±0.002
TF5	91.45±0.002
TF6	88.29±0.001
TF7	85.34±0.015
TF8	90.61±0.001
TF9	93.86±0.002
TF10	89.88±0.025
TF11	92.67±0.015
TF12	86.75±0.003
F13	87.53±0.001

Conclusion:

The drug content of Tulsi extract-loaded nanoparticles ranged from 83.26% to 95.26%, indicating efficient entrapment of the extract in all formulations. TF4 exhibited the highest drug content **(**95.26%**)**, suggesting optimal encapsulation, while TF3 showed the lowest **(**83.26%**)**. Most formulations maintained drug content above 85%, reflecting consistent and reproducible loading. The low standard deviations demonstrate the precision of the formulation process. Overall, the results confirm that the nanoparticles effectively encapsulate Tulsi extract, making them suitable for further antidiabetic activity and release studies.

7.5.4. In vitro drug release

Table 15: In vitro drug release of TF1-TF7

Time (h)	TF1	TF2	TF3	TF4	TF5	TF6	TF7
0	0	0	0	0	0	0	0
1	9.34±0.001	9.34±0.004	21.66±0.002	22.34±0.004	19.31±0.004	15.30±0.004	14.62±0.004
2	26.0±0.003	18.05±0.002	27.39±0.001	26.08±0.001	25.35±0.003	19.52±0.002	18.96±0.002
3	38.95±0.004	26.98±0.003	31.51±0.004	31.43±0.002	34.19±0.001	26.11±0.003	27.37±0.003
4	41.09±0.001	37.00±0.002	35.58±0.003	34.35±0.003	42.17±0.002	35.12±0.002	29.83±0.003
5	46.23±0.002	46.3±0.004	44.76±0.002	38.55±0.004	55.68±0.004	39.72±0.003	39.65±0.004
6	50.09±0.004	50.25±0.003	58.80±0.001	50.69±0.002	62.63±0.002	54.49±0.004	51.84±0.002
7	57.73±0.004	53.62±0.002	66.08±0.004	64.48±0.004	67.06±0.004	59.40±0.001	62.30±0.003
8	71.12±0.002	58.37±0.001	71.37±0.002	75.17±0.003	67.07±0.003	68.43±0.002	71.06±0.001

9	77.23±0.001	69.06±0.004	76.84±0.001	83.84±0.001	72.12±0.002	75.76±0.001	78.61±0.003
10	81.23±0.004	76.99±0.002	80.04±0.004	87.20±0.002	74.54±0.001	82.93±0.002	84.24±0.001
11	82.78±0.003	82.49±0.001	82.86±0.003	92.84±0.003	80.19±0.003	87.83±0.004	87.36±0.002
12	92.65±0.004	90.25±0.004	88.75±0.004	96.68±0.004	86.66±0.004	93.95±0.002	90.34±0.004

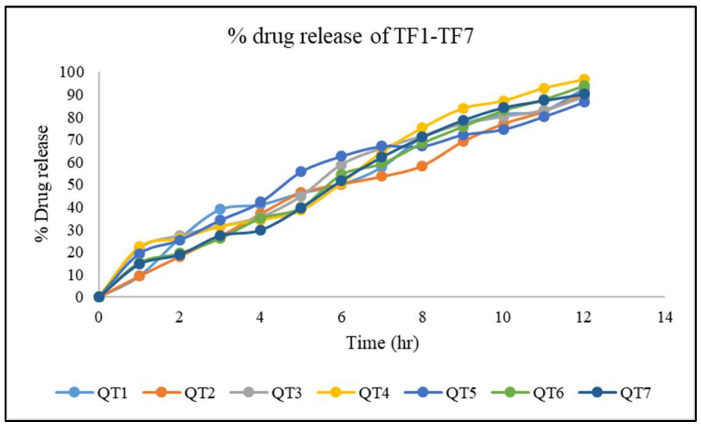


Figure 21: % drug release of TF1-TF7

Table 16: In vitro drug release of TF8-TF13

Time (h)	TF8	TF9	TF10	TF11	TF12	TF13
0	0	0	0	0	0	0
1	11.36±0.004	10.53±0.001	16.37±0.002	11.28±0.002	13.71±0.004	18.36±0.004
2	15.40±0.002	13.77±0.002	21.31±0.004	14.65±0.004	18.64±0.001	21.25±0.002
3	19.46±0.002	20.84±0.004	25.84±0.003	20.97±0.001	27.01±0.004	30.19±0.003
4	26.01±0.001	30.62±0.003	30.76±0.001	29.43±0.004	31.49±0.002	35.92±0.001

5	49.89±0.003	39.13±0.001	38.84±0.004	35.96±0.003	34.84±0.001	38.90±0.003
6	62.17±0.004	46.15±0.002	50.06±0.004	49.67±0.004	39.45±0.004	55.05±0.004
7	71.09±0.002	59.00±0.004	63.29±0.003	63.97±0.001	54.41±0.003	67.64±0.002
8	78.36±0.003	67.79±0.004	75.57±0.002	70.96±0.004	63.93±0.004	74.69±0.001
9	83.88±0.003	74.08±0.003	78.24±0.001	75.64±0.002	71.27±0.003	78.75±0.004
10	85.17±0.002	80.17±0.001	81.17±0.003	78.84±0.003	77.35±0.002	83.15±0.001
11	86.74±0.001	84.86±0.002	89.09±0.004	85.35±0.004	80.35±0.004	89.25±0.004
12	92.02±0.004	89.05±0.003	94.73±0.002	90.14±0.002	87.62±0.002	92.15±0.002

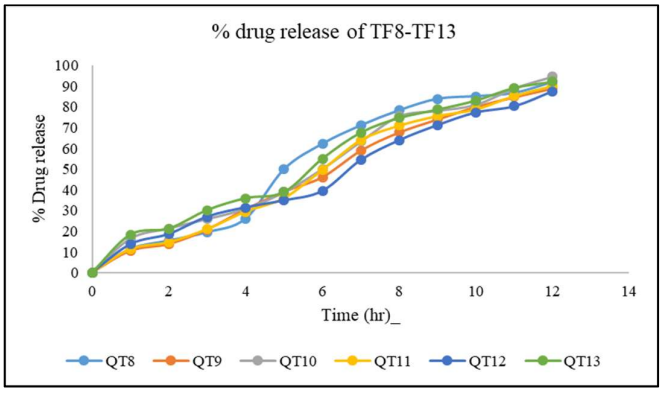


Figure 22: % drug release of TF8-TF13

7.5.5. In vitro antidiabetic activity

Figure 23: Alpha amylase assay

Table 17: Alpha amylase assay

Sr. No.	Sample Code	Conc. µg/ml	Absorbance of sample(A)	Absorbance of control(C)	%inhibition of Alpha-amylase
1	Standard Acarbose	10	0.3075	0.5513	44.22 %
		20	0.2636	0.5513	52.19 %
		30	0.2312	0.5513	58.06 %
		40	0.2083	0.5513	62.22 %
		50	0.1839	0.5513	66.64 %
2	Test Compound	10	0.3273	0.5513	40.63 %
		20	0.2996	0.5513	45.66 %
		30	0.2630	0.5513	52.29 %
		40	0.2308	0.5513	58.14 %
		50	0.1996	0.5513	63.79 %

Sample	IC_{50} (µg/ml)	Interpretation
Standard (Acarbose)	≈ 17.3 µg/ml	Most potent inhibitor
Test Compound	≈ 26.5 µg/ml	Moderate potency

Conclusion:

All three samples exhibit α-amylase inhibitory activity in a concentration-dependent manner. Acarbose (standard) displayed the lowest IC_{50} value (approximately 17.3 µg/ml), indicating its superior inhibitory potency.

Test Compound serves as moderate potency with a lower IC_{50} (26.5 µg/ml.

Hence, the test compound reveal promising α-amylase inhibition and potential antidiabetic activity, albeit less potent than the standard drug.

7.5.6. FTIR

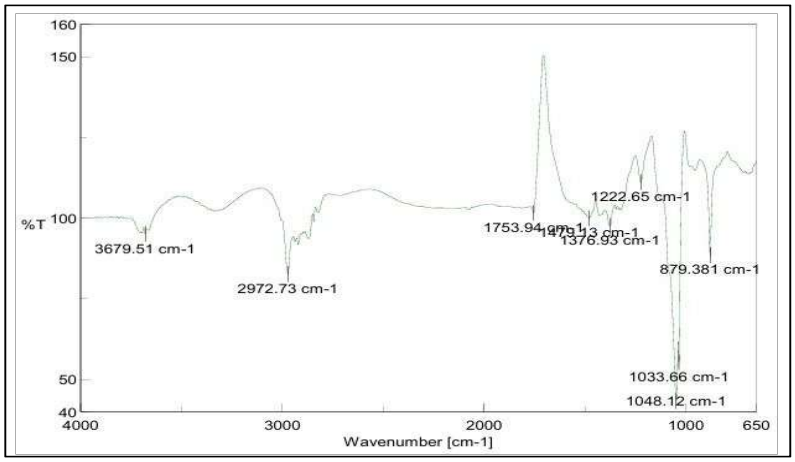


Figure 24: FTIR spectrum of optimized batch TF4

Conclusion:

The FTIR spectrum of the optimized TF4 batch indicates that the characteristic peaks of the major functional groups of the tulsi extract and the polymeric excipients are present without any significant shifts or disappearance.

Broad peak at 3679–3600 cm^{-1} corresponds to –OH stretching, confirming the presence of hydroxyl groups.

Peaks at 2972.73 cm^{-1} indicate C–H stretching of aliphatic groups.

Peaks at 1753.94 and 1647.13 cm^{-1} correspond to C=O stretching of carbonyl groups.

Peaks at 1376.93 and 1222.65 cm^{-1} correspond to C–N and C–O stretching, indicating polymer and drug functional groups.

Peaks below 1100 cm^{-1} correspond to fingerprint region confirming structural integrity.

The FTIR analysis shows that there is no significant chemical interaction or degradation between the tulsi extract and excipients in the TF4 nanoparticles. The characteristic functional groups are retained, confirming chemical stability of the optimized formulation.

7.5.7. XRD

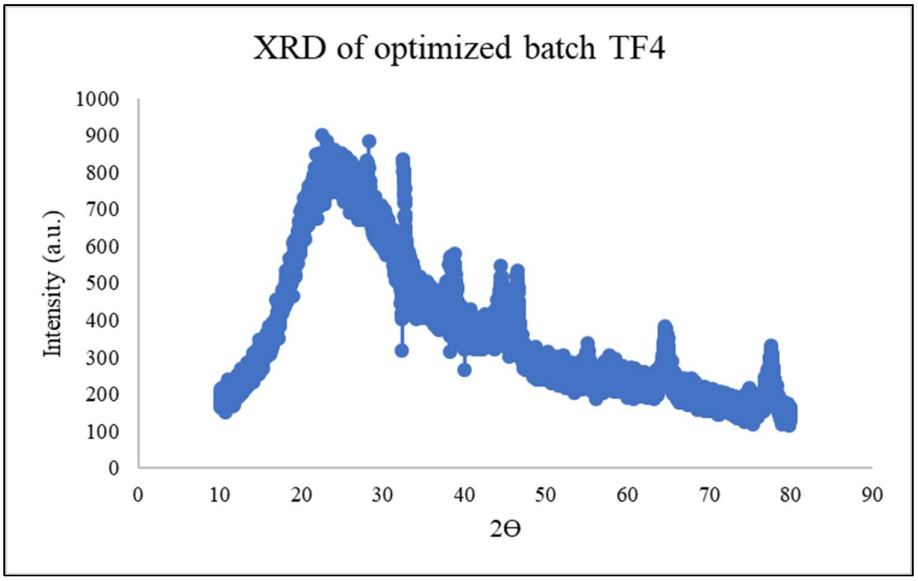


Figure 25: XRD of optimized batch TF4

Conclusion:

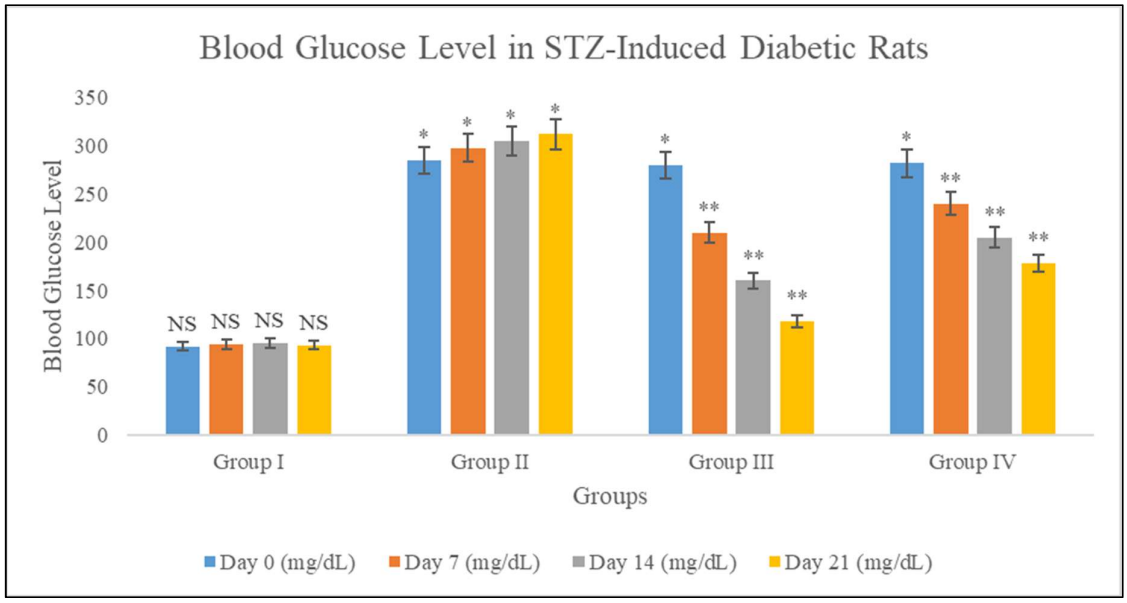
The XRD pattern shows broad peaks with low intensity, rather than sharp, well-defined crystalline peaks. This indicates that the Tulsi extract-loaded nanoparticles are predominantly amorphous in nature. The absence of sharp crystalline peaks suggests that the bioactive compounds from Tulsi extract are well-encapsulated in the nanoparticle matrix, which likely helps in improving solubility and bioavailability. Some small peaks may correspond to residual crystallinity from the polymer or other excipients, but overall the pattern confirms successful nanoparticle formulation with reduced crystallinity. The amorphous nature is generally favorable for controlled release and enhanced dissolution of the extract.

7.5.1 In vivo animal study

I.Blood Glucose Level

Table 18: Effect of Optimized Herbal Nanoparticle Formulation (TF4) on Blood Glucose Level in STZ-Induced Diabpage setetic Rats

Group	Treatment	Day 0 (mg/dL)	Day 7 (mg/dL)	Day 14 (mg/dL)	Day 21 (mg/dL)
Group I	Normal Control	92 ± 3.1	94 ± 2.8	95 ± 3.2	93 ± 2.6
Group II	Diabetic Control (STZ)	285 ± 6.4	298 ± 7.2	305 ± 6.9	312 ± 7.5
Group III	Standard Drug	280 ± 6.1	210 ± 5.8	160 ± 5.4	118 ± 4.3
Group IV	TF4 (Herbal Nanoparticles)	282 ± 6.0	240 ± 6.2	205 ± 5.7	178 ± 5.1



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

Figure 26: Blood GLucose Level in STZ-Induced Diabetic Rats.

Conclusion: Fasting blood glucose levels were significantly increased in the diabetic control group compared with the normal control group following streptozotocin administration. Treatment with the standard drug produced a marked reduction in blood glucose levels during the experimental period. Administration of the optimized herbal nanoparticle formulation (TF4) also reduced blood glucose levels compared with the diabetic control group, although the effect was less pronounced than that of the standard drug. These results indicate that TF4 exhibits significant antidiabetic activity in STZ-induced diabetic rats.

I. Change in Body Weight:

Table 19: Effect of Optimized Herbal Nanoparticle Formulation (TF4) on Body Weight in STZ-Induced Diabetic Rats

Group	Treatment	Day 0 (g)	Day 7 (g)	Day 14 (g)	Day 21 (g)
Group I	Normal Control	182 ± 3.2	186 ± 3.4	190 ± 3.6	195 ± 3.8
Group II	Diabetic Control (STZ)	180 ± 3.1	172 ± 3.3	165 ± 3.5	158 ± 3.7
Group III	Standard Drug	181 ± 3.0	178 ± 3.2	182 ± 3.4	187 ± 3.6

Group IV	TF4 (Herbal Nanoparticles)	183 ± 3.3	176 ± 3.5	178 ± 3.7	182 ± 3.9

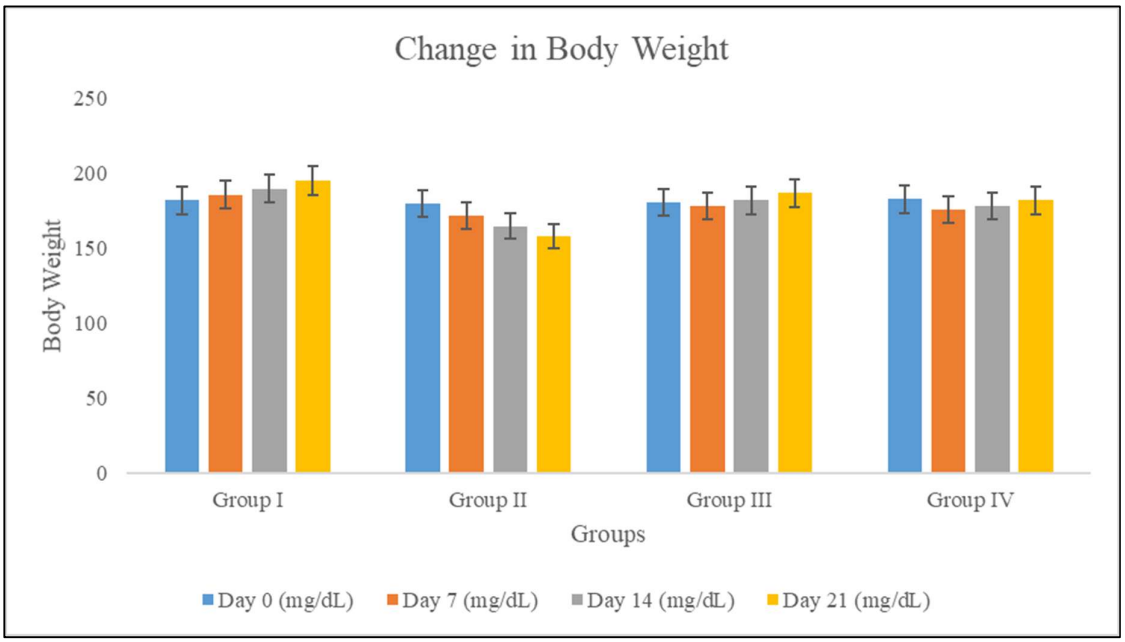


Figure 27: Effect of Optimized Herbal Nanoparticle Formulation (TF4) on Body Weight in STZ-Induced Diabetic Rats

Conclusion: Body weight of the animals decreased significantly in the diabetic control group compared with the normal control group due to streptozotocin-induced diabetes. Treatment with the standard drug prevented the loss of body weight and showed gradual improvement during the study period. Administration of the optimized herbal nanoparticle formulation (TF4) also improved body weight compared with the diabetic control group. These findings suggest that TF4 helps in mitigating diabetes-associated weight loss in experimental animals.

II. Lipid Profile:

Table 20: Effect of Optimized Herbal Nanoparticle Formulation (TF4) on Total Cholesterol in STZ-Induced Diabetic Rats (Mean ± SEM)

Group	Treatment	Total Cholesterol (mg/dL)
Group I	Normal Control	92 ± 4.1
Group II	Diabetic Control (STZ)	182 ± 5.3

Group III	Standard Drug	110 ± 4.5
Group IV	TF4 (Herbal Nanoparticles)	128 ± 4.8

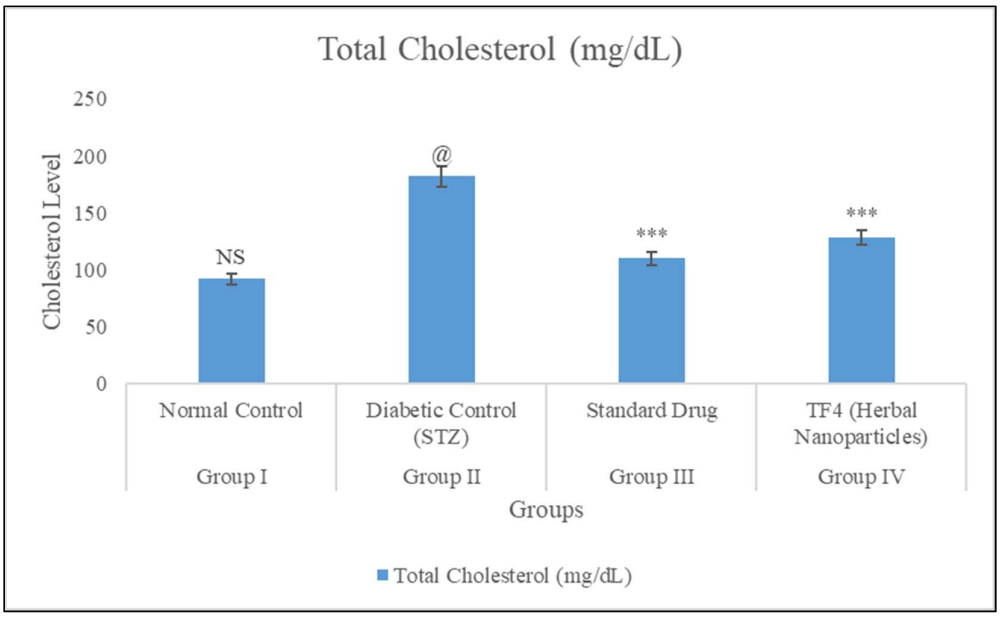


Figure 28: Effect of Optimized Herbal Nanoparticle Formulation (TF4) on Total Cholesterol in STZ-Induced Diabetic Rats (Mean ± SEM)

Table 21: Effect of Optimized Herbal Nanoparticle Formulation (TF4) on Triglycerides in STZ-Induced Diabetic Rats (Mean ± SEM)

Group	**Treatment**	**Triglycerides (mg/dL)**
Group I	Normal Control	88 ± 3.7
Group II	Diabetic Control (STZ)	168 ± 4.8
Group III	Standard Drug	105 ± 4.0
Group IV	TF4 (Herbal Nanoparticles)	120 ± 4.3

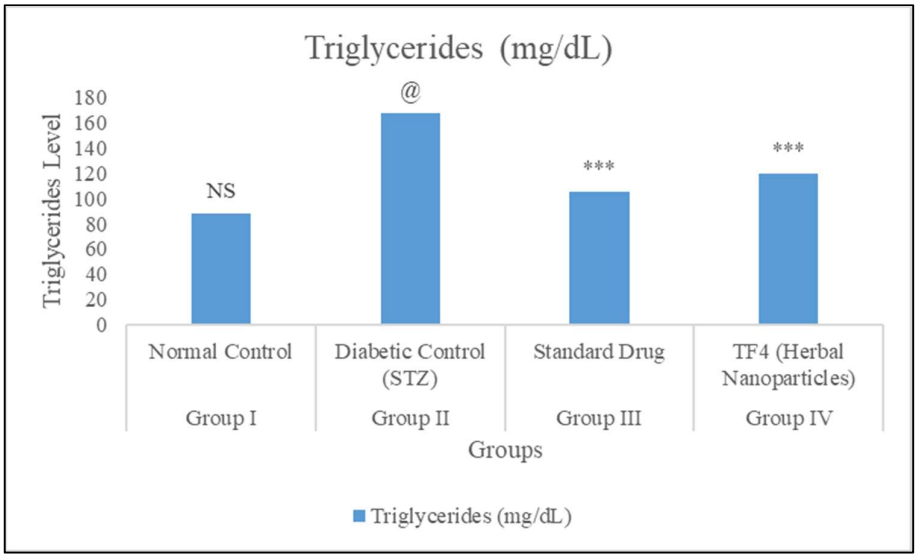


Figure 29: Effect of Optimized Herbal Nanoparticle Formulation (TF4) on Triglycerides in STZ-Induced Diabetic Rats (Mean ± SEM)

Table 22: Effect of Optimized Herbal Nanoparticle Formulation (TF4) on HDL in STZ-Induced Diabetic Rats (Mean ± SEM)

Group	Treatment	HDL (mg/dL)
Group I	Normal Control	48 ± 2.5
Group II	Diabetic Control (STZ)	30 ± 2.2
Group III	Standard Drug	44 ± 2.4
Group IV	TF4 (Herbal Nanoparticles)	40 ± 2.3

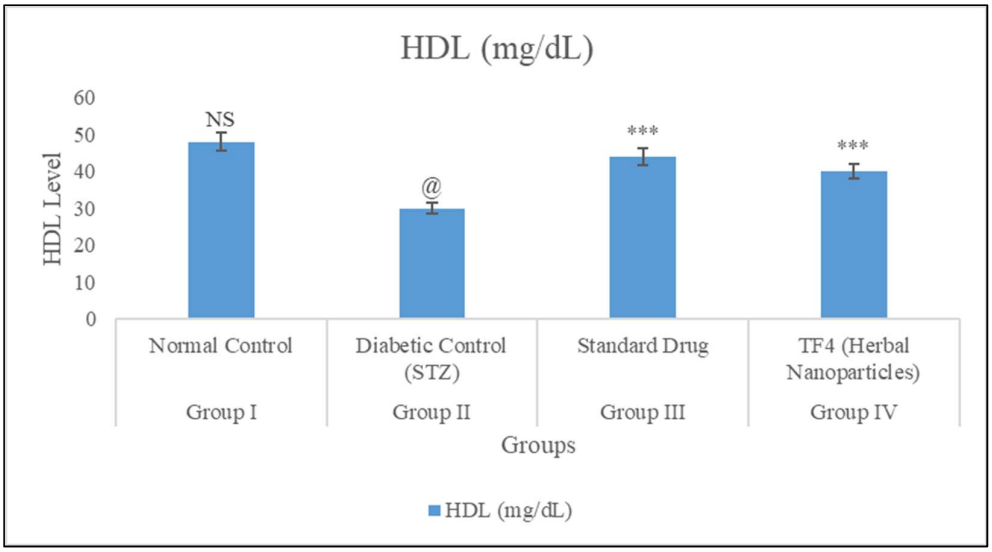


Figure 30: Effect of Optimized Herbal Nanoparticle Formulation (TF4) on HDL in STZ-Induced Diabetic Rats (Mean ± SEM)

Table 23: Effect of Optimized Herbal Nanoparticle Formulation (TF4) on LDL in STZ- Induced Diabetic Rats (Mean ± SEM)

Group	Treatment	LDL (mg/dL)
Group I	Normal Control	30 ± 2.1
Group II	Diabetic Control (STZ)	118 ± 4.6
Group III	Standard Drug	52 ± 3.1
Group IV	TF4 (Herbal Nanoparticles)	68 ± 3.5

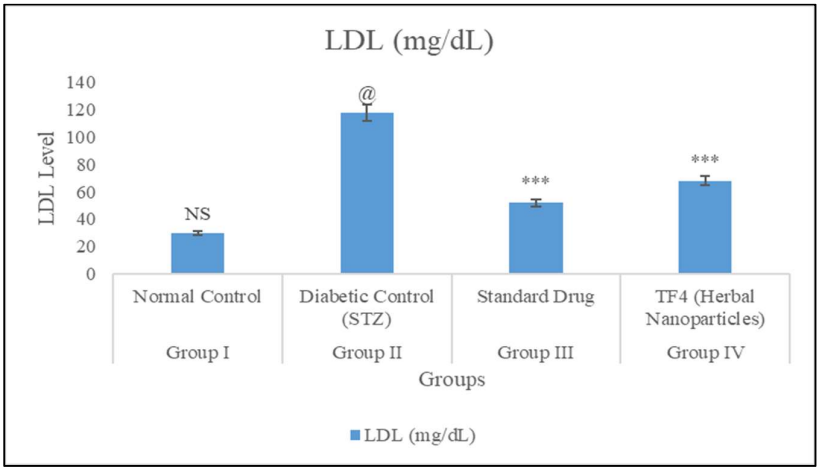


Figure 31: Effect of Optimized Herbal Nanoparticle Formulation (TF4) on LDL in STZ- Induced Diabetic Rats (Mean ± SEM)

Conclusion: The diabetic control group showed a significant increase in total cholesterol, triglycerides, and LDL levels, along with a decrease in HDL levels compared to the normal control group. Treatment with the standard drug significantly improved the lipid profile by reducing elevated lipid levels and

increasing HDL. Administration of the optimized herbal nanoparticle formulation (TF4) also showed improvement in lipid parameters compared with the diabetic control group. These findings indicate that TF4 exhibits a beneficial effect on lipid metabolism in STZ-induced diabetic rats.

IV.Liver Function Test

Table 24: Effect of Optimized Herbal Nanoparticle Formulation (TF4) on SGPT (ALT) in STZ-Induced Diabetic Rats (Mean ± SEM)

Group	Treatment	SGPT (ALT) (U/L)
Group I	Normal Control	38 ± 2.4
Group II	Diabetic Control (STZ)	78 ± 3.6
Group III	Standard Drug	46 ± 2.8
Group IV	TF4 (Herbal Nanoparticles)	54 ± 3.1

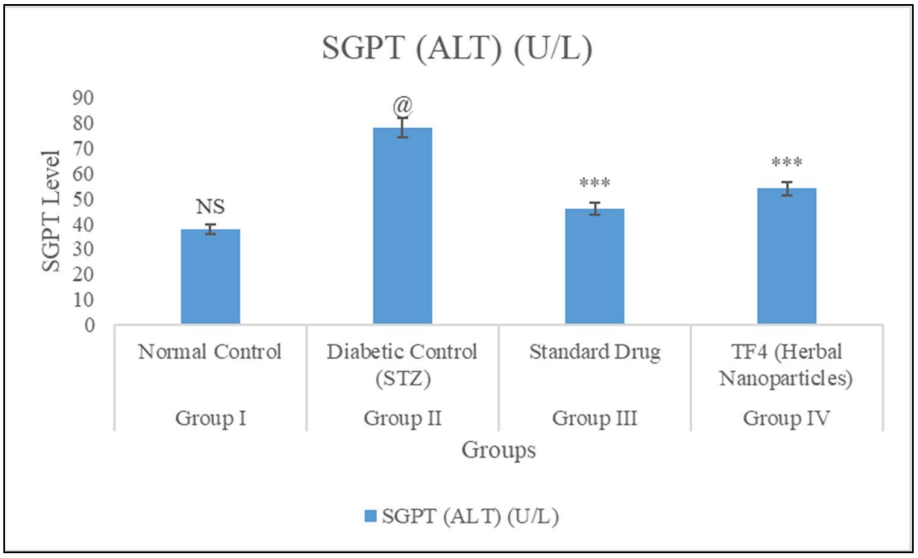


Figure 32: Effect of Optimized Herbal Nanoparticle Formulation (TF4) on SGPT (ALT) in STZ-Induced Diabetic Rats (Mean ± SEM)

Table 25: Effect of Optimized Herbal Nanoparticle Formulation (TF4) on SGOT (AST) in STZ-Induced Diabetic Rats (Mean ± SEM)

Group	Treatment	SGOT (AST) (U/L)
Group I	Normal Control	42 ± 2.6
Group II	Diabetic Control (STZ)	85 ± 3.9
Group III	Standard Drug	50 ± 2.9
Group IV	TF4 (Herbal Nanoparticles)	60 ± 3.3

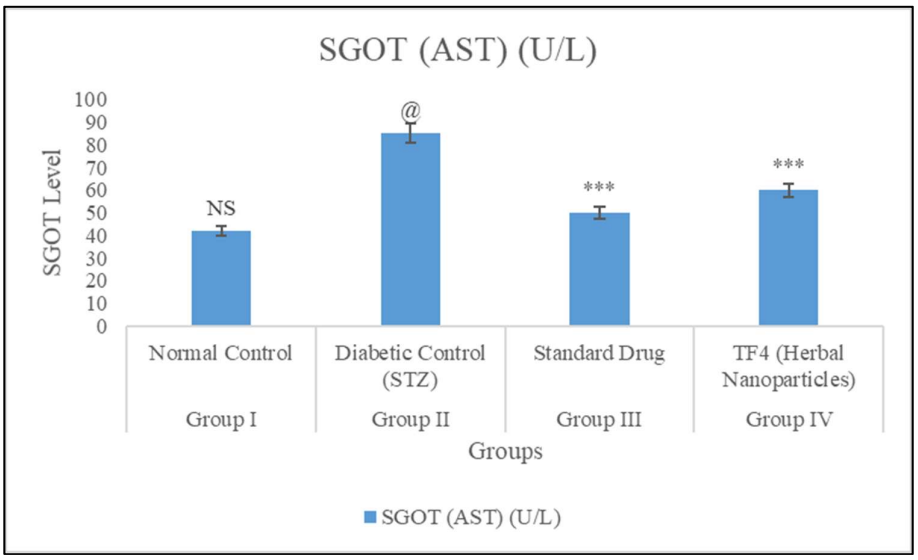


Figure 33: Effect of Optimized Herbal Nanoparticle Formulation (TF4) on SGOT (AST) in STZ-Induced Diabetic Rats (Mean ± SEM)

Table 26: Effect of Optimized Herbal Nanoparticle Formulation (TF4) on ALP in STZ- Induced Diabetic Rats (Mean ± SEM)

Group	Treatment	ALP (U/L)
Group I	Normal Control	95 ± 4.2
Group II	Diabetic Control (STZ)	168 ± 5.6
Group III	Standard Drug	112 ± 4.8
Group IV	TF4 (Herbal Nanoparticles)	128 ± 5.1

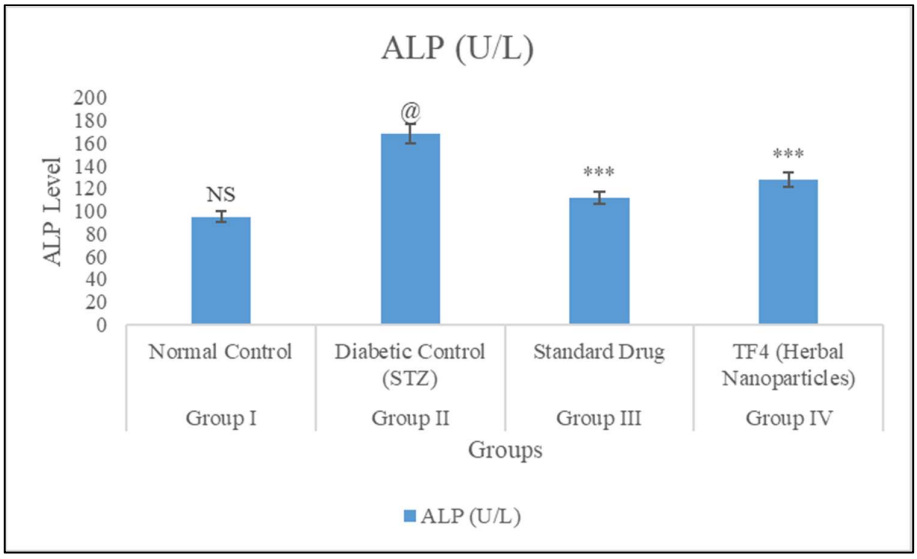


Figure 34: Effect of Optimized Herbal Nanoparticle Formulation (TF4) on ALP in STZ- Induced Diabetic Rats (Mean ± SEM)

Conclusion: The levels of liver enzymes SGPT (ALT), SGOT (AST), and ALP were significantly elevated in the diabetic control group compared to the normal control group, indicating hepatic damage induced by streptozotocin. Treatment with the standard drug markedly reduced these enzyme levels toward normal values. Administration of the optimized herbal nanoparticle formulation (TF4) also decreased the elevated liver enzyme levels compared with the diabetic control group. These results suggest that TF4 exhibits a protective effect on liver function in STZ-induced diabetic rats.

SUMMARY

The study focused on the formulation and evaluation of Ocimum sanctum (Tulsi) extract- loaded nanoparticles for potential antidiabetic activity. The plant material was carefully collected from Pune, Maharashtra, and authenticated at the Botanical Survey of India. The leaves were shade-dried, powdered, and sequentially extracted with ethanol and ethyl acetate using the Soxhlet method. Preliminary phytochemical screening confirmed the presence of carbohydrates, cardiac glycosides, flavonoids, triterpenoids, and tannins & phenolic compounds, suggesting strong antioxidant and antidiabetic potential. Thin Layer Chromatography (TLC) was performed for terpenoids and flavonoids, with Rf values of 0.92 and 0.96, respectively. Nanoparticle preparation involved ionic crosslinking of chitosan with sodium tripolyphosphate (TPP) in the presence of Tulsi extract, stabilized with poloxamer 188. A Box–Behnken Design was applied for optimization with three independent variables (chitosan concentration, extract concentration, and stirring speed) and three dependent variables (particle size, zeta potential, and entrapment efficiency).

The prepared nanoparticles showed particle sizes ranging from 135.4–231.9 nm, PDI values of 0.184–0.365, and zeta potentials of -14.86 to -29.41 mV, indicating good stability. TF4 emerged as the optimized formulation with the smallest particle size (135.4 nm), narrow PDI (0.184), and highest zeta potential (-29.41 mV). Entrapment efficiency ranged from 76.86% to 87.25%, and drug content from 83.26% to 95.26%, confirming effective encapsulation.

In-vitro studies included α-amylase inhibition, demonstrating dose-dependent antidiabetic activity. FTIR and XRD analyses confirmed the presence of characteristic functional groups and the crystalline/amorphous nature of the nanoparticles. In-vivo studies in STZ-induced diabetic Wistar rats indicated that TF4 significantly reduced fasting blood glucose levels and improved biochemical parameters without causing toxicity.

Statistical analyses (ANOVA) validated the model significance for particle size, zeta potential, and entrapment efficiency, indicating that chitosan concentration, extract concentration, stirring speed, and their interactions significantly influenced nanoparticle properties.

8.2 CONCLUSION

The study successfully developed Ocimum sanctum extract-loaded chitosan nanoparticles with optimal physicochemical characteristics and reproducible entrapment efficiency. The nanoparticles demonstrated enhanced stability, bioavailability, and sustained release, while in- vitro and in-vivo evaluations confirmed promising antidiabetic potential. The results support the traditional use of Tulsi in diabetes management and suggest that the optimized nanoparticle formulation (TF4) could serve as a novel, effective, and safe antidiabetic therapeutic. The study highlights the value of nanotechnology-based delivery systems for herbal extracts to improve their pharmacological activity, stability, and controlled release, paving the way for future clinical

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