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Research Article

Evaluation of Anti-Diabetic Activity of Zinc Oxide Nanoparticles of *Gymnema sylvestre* Extract on Wistar Rats

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Abstract

Diabetes mellitus is described as a metabolic disorder, which was characterised by high blood glucose levels, which were resulted due to insufficient insulin secretion. There were major complications related to diabetes mellitus. To evaluate the antidiabetic effect of *Gymnema sylvestre* Zinc Oxide Nanoparticles. Streptozotocin (STZ) of 50mg/kg is used for the induction of diabetes into Wistar rats. The animals were divided into 6 groups. Group-I represented as Control; Group-II represented as diabetic control (STZ); Group-III represented as 5 mg/kg body weight of glibenclamide + diabetes; Group-IV represented as *Gymnema sylvestre* extract + diabetes; Group-V represented as 100 µg *Gymnema sylvestre* Zinc Oxide nanoparticles + diabetes; group-6- *Gymnema sylvestre* Zinc Oxide Nanoparticles 200 µg + diabetes. The GsZnONPs were inspected to morphological properties using UV spectrophotometer and FTIR. The GsZnONPs shown characterization peak at 300nm. The study has shown that GsZnONPs treated group has shown reduced glucose concentration. The histopathological alterations were also studied in all experimental groups. The pathology results of GsZnONPs (200 µg) group has shown the regeneration of islets cells of pancreas. The research study of GsZnONPs has proved that *Gymnema sylvestre* ZnO NPs exerts antidiabetic properties.

Keywords: Diabetes, *Gymnema sylvestre*, Zinc Oxide nanoparticles

INTRODUCTION:

The occurrence of diabetes is predicted to be doubled from 171 million in 2000 to 366 million in 2030 globally with maximum increase in India. It is concluded that by 2030 in India diabetes mellitus may rise to 79.4 million individuals. Currently India faces potential burden that diabetes may impose upon the country¹. Diabetes is a chronic metabolic syndrome that causes multiple organ dysfunction, mortality and morbidity². Diabetes Mellitus (DM) is characterized by hyperglycaemia that arise from either the deficiency in insulin secretion or the insulin activity. DM is of three types -Type-1DM (non-insulin dependent), Type-2 DM (insulin dependent) and Gestational Diabetes³.

Type-1 DM occurred by insulin deficiency which is due to the destruction of β cells in the pancreas by cell mediated autoimmune process³. Type-2 DM is characterized by the imbalance between insulin levels and insulin sensitivity which causes a functional deficit of insulin⁴. Gestational Diabetes is a type of diabetes that occurs during pregnancy. The development of diabetes is still unknown, but some studies indicate that HLA antigens play a vital role in its development, specifically HLA DR³⁻⁵. Several factors are responsible for the occurrence of diabetes but the patient has high potential of hyperglycaemia. Insulin resistance is caused due to high levels

of fatty acids and proinflammatory cytokines, which finally leads to impaired glucose transport and increase in fat breakdown. As there was decreased production of insulin, the body will respond by more glucagon generation, thus leading to hyperglycaemia. The complete occurrence of disease is seen when the patient does not produce enough insulin to overcome the insulin resistance.⁶ The complication of diabetes include stroke, myocardial infraction and coronary artery disease. Other complications like retinopathy, neuropathy and neuropathy have major impact on patients life.

Signs and symptoms:

In DM the signs and symptoms will develop steadily and progress chronically. It will not show signs like major acute diseases.

The major risk factors include:

- Positive diabetes family history.
- Above 35years age.
- Overweight.
- Hypertension.
- Recent weight gain.

- Sedentary lifestyle.⁷

Nanoparticles

Nanoparticles are ultrafine particle is usually defined as a particle of matter that is between 1 -100 nanometres(nm) in diameter. Nanoparticles can be synthesized using various approaches including chemical, physical, and biological approaches. Thus, there is an increasing demand for “green nanotechnology.” (In green synthesis of ZnO nano-particles using plants, plant extracts can be used as reducing agent, capping agent or both. Different plant parts are used in green synthesis and the flavonoids, resins, tannin, saponins, alkaloids acts as reducing agents for ZnO Nano particles.⁸

Zinc oxide:

Many studies have shown the activity of trace metals in glucose metabolism and their connection with the diabetes. The maintenance of blood glucose levels can be achieved by the zinc, magnesium and chromium and they can be used in the treatment of diabetes ⁹⁻¹¹. In our body a number of 300 enzymes were activated by zinc and it also has a important role in various metabolic pathways like glucose metabolism ¹² and biosynthesis of insulin, its secretion and storage(¹³). Zinc transporters were present in the pancreatic cells which helps in insulin secretion ^{14,15}.

Applications of ZnO nanoparticles:

- Zinc oxide nanoparticles have bioavailability, biocompatibility, and high solubility.
- FDA approved them as new and potent anti-cancer therapy.
- They possess potent antioxidant
- Used in reproductive dysfunction for males
- They have cytoprotective effects
- Used in sunscreens and ointments.
- Has immunomodulatory effect.
- Have anti-bacterial
- Anticancer property ¹⁶

Gymnema sylvestre:

The plant belongs to the family apocynaceae. It was majorly distributed in Africa, India, Sri lanka, and Australia. *Gymnema* is gregarious woody climber, much branched, running over the tops of tall trees. Young stems and branches are pubescent. Leaves are 3-5 cm long and up to 3 cm broad, ovate-elliptic, acute or shortly acuminate, pubescent on both sides; base rounded or heart shaped with 6-13 mm long pubescent petioles. ¹⁷



Figure 1: *Gymnema sylvestre*

METHODS AND MATERIALS:

Preparation of plant extract:

The *Gymnema sylvestre* leaves were collected washed and shade dried, powdered and were passed through 40 meshes. The dried powder was subjected to soxhlet extraction with petroleum ether.

Extraction with petroleum ether:

100gms of drug leaf powder was packed into a clean Soxhlet extraction unit. One litre of petroleum ether (60-80°C) was added and extracted for 3-6 hrs till all the components are soluble in petroleum.

Petroleum extract is collected and distilled in a distillation unit. Then a net weight of 25gms of petroleum ether extracts were obtained ¹⁸

Synthesis of *Gymnema sylvestre* ZnO nanoparticles:

ZnO particles are prepared by using sol-gel method. Take a beaker with 0.2M ZnCl solution add 2ml acetic acid. Sodium hydroxide solution was poured drop by drop to the solution containing zinc acetate with a constant stirring by magnetic stirrer. Stirring is done when the solution attains pH=7. Now add the petroleum ether extract to the solution and stir it for one hr. Filtering, drying, calcination done at 500c for 2hr in muffle furnace ^{19,20}

Characterization of ZnO nanoparticles:

UV-Visible analysis:

The UV-visible absorption spectrum of ZnO nanoparticles is 250-800nm ²¹. The ZnO nanoparticles were measured by using spectroscopy. UV-spectral analysis has been done by using (LABINDIA ANALYTICAL UV3200).

Fourier transforms infrared (FTIR) spectroscopy:

The FTIR analysis is used to evaluate the functional groups. The characteristic peak is ranging from 400-4000cm⁻¹ on (Bruker ALPHA II FTIR spectrometer). FTIR analysis is performed to estimate the presence of functional groups of synthesized ZnO nanoparticles.

Invivo evaluation of antidiabetic activity:

Rat information:

The experimental animal model used for this study is male Wister rats, which are 8-12 weeks old. They were purchased from the Jeeva Life Sciences Laboratory Animal Centre, Uppal, Hyderabad. The animals used were weighed approximately 150-200gms. A strict hygiene condition was maintained with humidity between 40-60% and a 12hrs light and 12hrs dark cycle was maintained. A constant temperature of 23±1°C was maintained. The animals were fed with a standard diet (laboratory pellets) and water ad libitum in the laboratory animal house during the study. The rat's cage was contained with corncob bedding material.

Animals ethical committee:

All protocols for animal experimentation have been approved by the Institutional Animal Ethics Committee (approved No.CPCSEA/IAEC/JLS/19/02/23/153) and experiments conducted in accordance with Committee guidelines for control and monitoring of experiments on animals (CPCSEA, India).

Induction of diabetes:

Type-II diabetes was induced to the overnight-starved rats by intraperitoneal injection of 200µl citrate buffer (pH = 4.5) solution-containing streptozotocin (STZ) 50mg/kg. After 30

mins of STZ administration, the rats were fed with food and water freely. After 7hrs, a drink with 20% glucose solution was given to prevent death due to hypo-glycaemic shock for 24hrs. 2-3 days after the STZ administration, the animals were screened for diabetes. Glucose concentration in animals was measured by using tail puncture method and animals with >250mg/dl were selected for the study.²²⁻²⁴

Treatment Protocol:

After induction of diabetes, the rats were divided into 6 groups, each group containing 4 rats each.

Group1: Normal control

Group2: Diabetic control

Group3: Diabetic rats treated with glibenclamide (5mg/kg body wt.)

Group4: Diabetic rats to be treated with *Gymnema sylvestre* extract.

Group5: Diabetic rats treated with nanoparticles of *Gymnema sylvestre* (100µg)

Group6: Diabetic rats treated with nanoparticles of *Gymnema sylvestre* (200µg)

Treatment was started from 4th day of STZ administration up to 21 days. The blood glucose levels were checked on 0,7,14, 21 days of study by using glucometer (Roche diabetes care India Pvt ltd).

Oral glucose tolerance test:

After 21 days of ZnO nanoparticles containing plant extract administration, oral glucose test was performed to all groups. 2gm/kg of fed was given to all animals and glucose levels were checked after 1hr. blood samples were collected by using tail vein method before and after administration of plant extract with nanoparticles. The biological parameters were estimated on 0, 30, 60, 90, 120 mins.

Statistical analysis:

The values are expressed as Mean ± Standard Error Mean (SEM) obtained from the study. The data was subjected to the analysis of variance (one-way ANOVA) to determine the significance and confirmed by dunett's test.

RESULTS:

The formation of ZnO nanoparticles in the solution of 0.2M Zinc chloride and aqueous extract of *Gymnema sylvestre* the change in the colour to brown colour.



Figure 2:

(a) Colour change of ZnCl₂ solution on reduction to ZnO nanoparticles.

(b) Pellet formed after centrifugation of reaction mixture.

(c) ZnO NPs after drying.

UV visible analysis

The synthesis of ZnO NPs from *Gymnema sylvestre* was initially conformed by the colour change of the reaction mixture from

colourless to brown colour indicated the synthesis of ZnO NPs preliminarily. Then, the synthesized nanoparticles were exhibits as strongest UV absorbance peak at 300 nm.

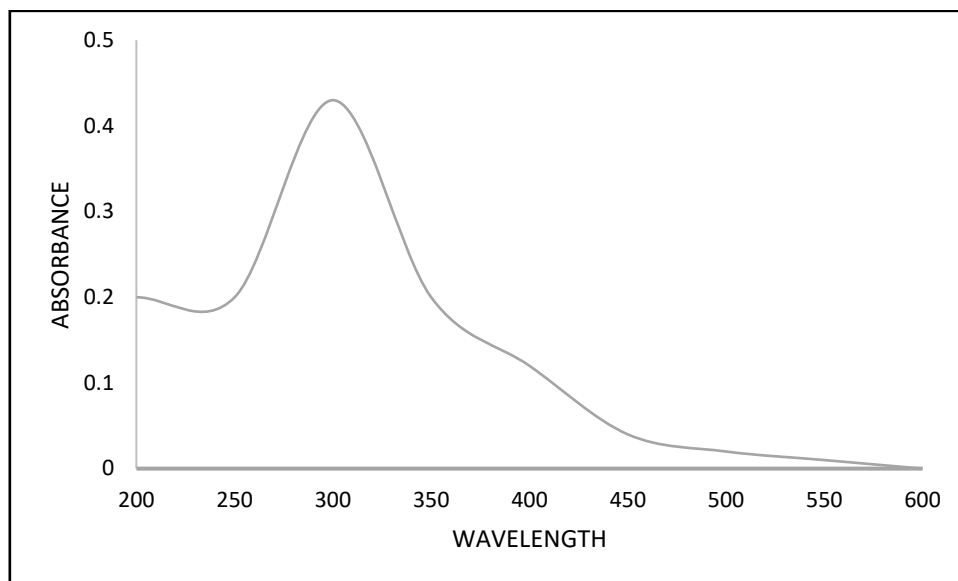


Figure 3: UV absorbance of GsZnONPs

FTIR spectrum analysis of ZnO nanoparticles:

Active and functional biomolecules are present in the ZnO NPs synthesized from *G.sylvestre*, which are identified and analysed from FTIR spectrum and it is shown. The FTIR spectrum exhibits the peaks at 3415 cm^{-1} were allotted to the extending vibrations of hydroxy groups; primary and secondary amines groups were presented in the synthesized nanoparticles respectively. The presented peaks were directly equivalent to protein and enzymes molecules or polysaccharides are found in

the cell biomass. The peak at 2926, 2854, 2358 and 2330 cm^{-1} were owed to symmetric and asymmetric stretching shaking of sp^3 hybridized. The peak at 1612 and 1313 cm^{-1} were allocated to $\text{C}=\text{O}$ extending vibrations of the carbonyl group in ketones, aldehydes and functional carboxylic acids. Moreover, the peak at 1163 and 1055 cm^{-1} were allocated to vibration of $\text{-C}=\text{C}$ -aromatic ring stretching. In addition to this band at 995 cm^{-1} resembles to metal binding interact with carboxylic ($\text{M}\leftrightarrow\text{C}\equiv\text{O}$) groups, this functional group might be acts template, reducing agent and capping of nanocrystals¹⁸.

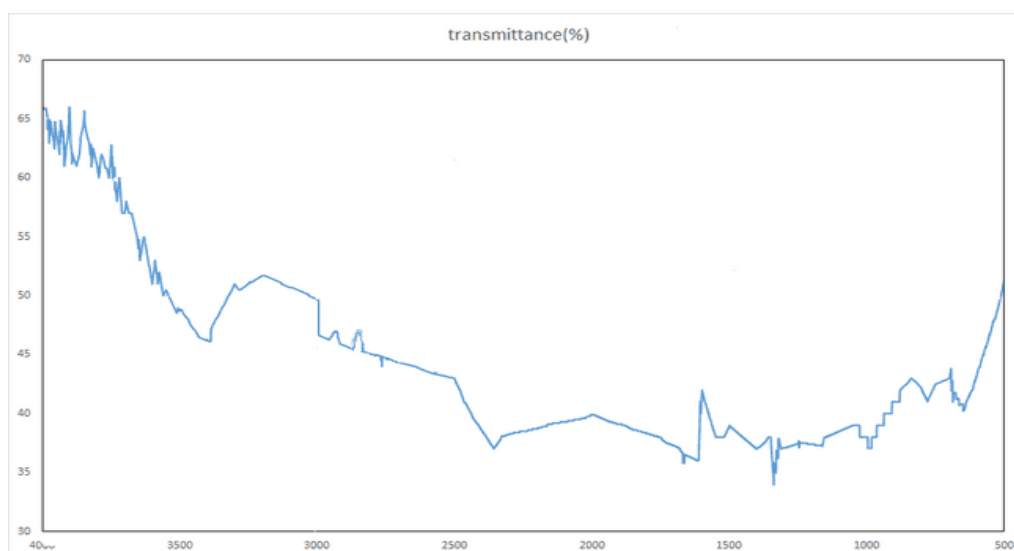


Figure 4: FTIR

Oral glucose tolerance test:

All the 6 groups are given 2gm/kg was fed after 1hr of administration and glucose levels were checked blood samples were collected from the tail vein just prior to administration

and after administration of extract and nanoparticles. The biological parameters are estimated on 0,30,60,90,120 mins.

Effect of *G.sylvestre* leaf extracts and ZnO nanoparticles in oral glucose tolerance test after 21 days of treatment. The graph represents mean SD (n=6).

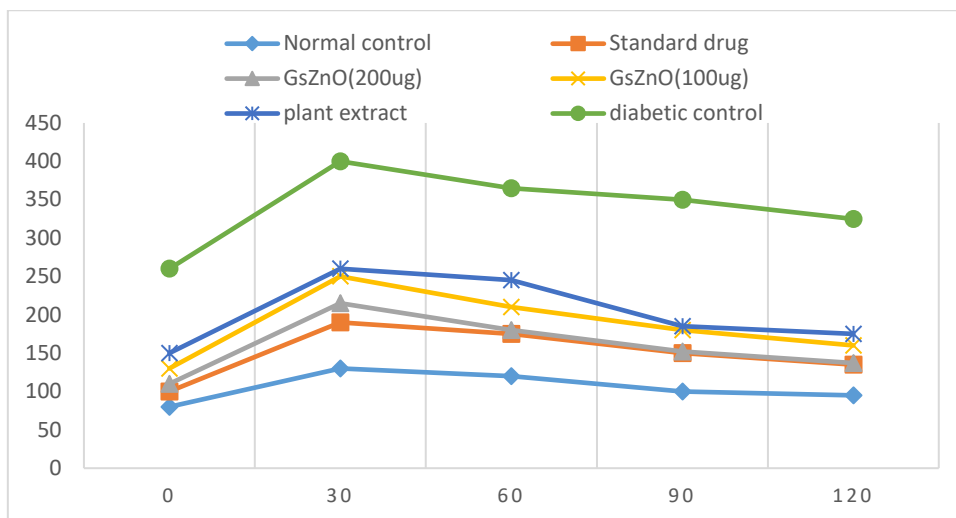


Figure 5: Oral glucose test

Blood glucose level of animals after STZ injection

At the end of 21 days of treatment the decrease in blood glucose levels with the glibenclamide and plant extract nanoparticles when compared to the diabetic control group.

Table: 1 Effect on the blood glucose levels in the normal and diabetic rats:

Day	Normal control	Diabetic control	Standard group	G.sylvestre extract	Nanoparticles of G.sylvestre (100µg)	Nanoparticles of G.sylvestre (200µg)
0	92.67±1.97	290.76±2.32	272.5±1.19*	258.1±2.11*	248.92±0.97*	236.45±3.49*
7	90.45±0.47	305.55±2.32	196.82±1.20*	223.5±2.04*	211.9±3.24*	202.3±1.27*
14	87.5±0.94	368±4.20	139±1.34*	146.85±1.65*	185.57±164*	134.72±2.62*
21	90.1±0.34	390.75±3.36	106.72±1.94*	139.65±1.13*	145.72±2.15*	119.75±4.01*

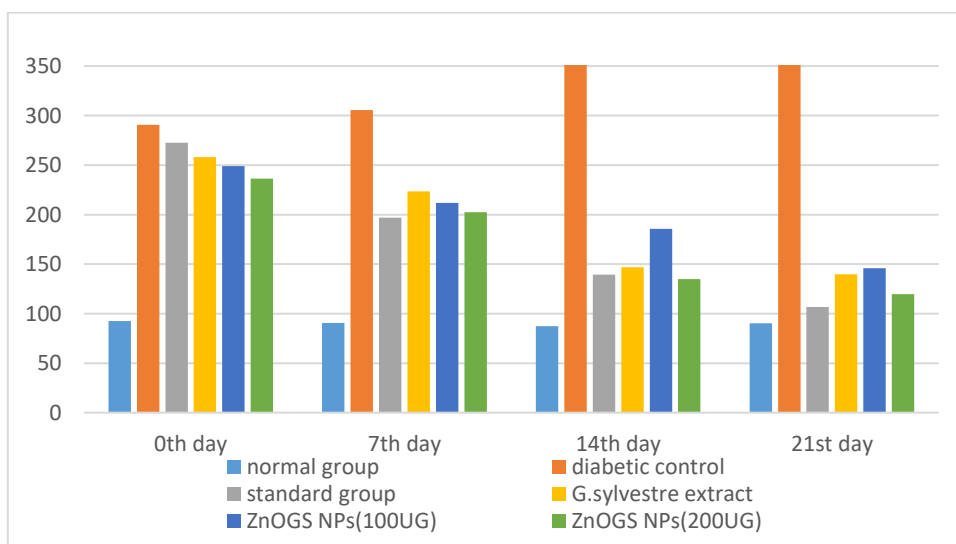


Figure 6: Effect on the blood glucose levels in the normal and diabetic rats

Effect of G.sylvestre extract and ZnOGsNPs on STZ induced rats before and after treatment. Each column represents mean±SD (n=6) .The level significance *P<0.05, **P<0.01, ***p<0.0001. When compared with diabetic control group.

At 0, 7, 14, 21 days, blood was collected through tail vein method and glucose levels were noted.

Statistical analysis were performed by comparing diabetic control with remaining groups.

The blood glucose levels of each group have been estimated during the treatment. The glucose levels are measured there is significantly decrease in blood glucose levels in the animals treated with nanoparticles compared to diabetic control. GsZnONPs (200 µg) were shown to decrease blood glucose levels than the plant extract alone.

Effect of body weight after the treatment

Effect of various plant extract treatment in mean body weight of the non-diabetic rats in comparison with diabetic rats. Induction of diabetes with stz is associated with the characteristic loss of body weight, which is due to loss of tissue

proteins. The treatments were given at dose orally for a period of 21 days

Effect of extract of *G. sylvestre* and ZnOGsNPs on body weight before and after treatment. Each column represents mean ± SD (n=6). All the values were found to be significant when compared to diabetic control at *P<0.05.

Table: 2 Changes in the body weight before and after treatment in normal and diabetic rats

	Normal control	Diabetic control	Standard group	G.sylvestre extract	Nanoparticles of G.sylvestre (100µg)	Nanoparticles of G.sylvestre (200µg)
Before treatment	208±2.85	208±2.21	198±1.89	205±1.68	200±2.28	205±1.98
After treatment	212±1.87	168±1.68	210±3.21*	200±1.47*	195±1.12*	210±2.17*

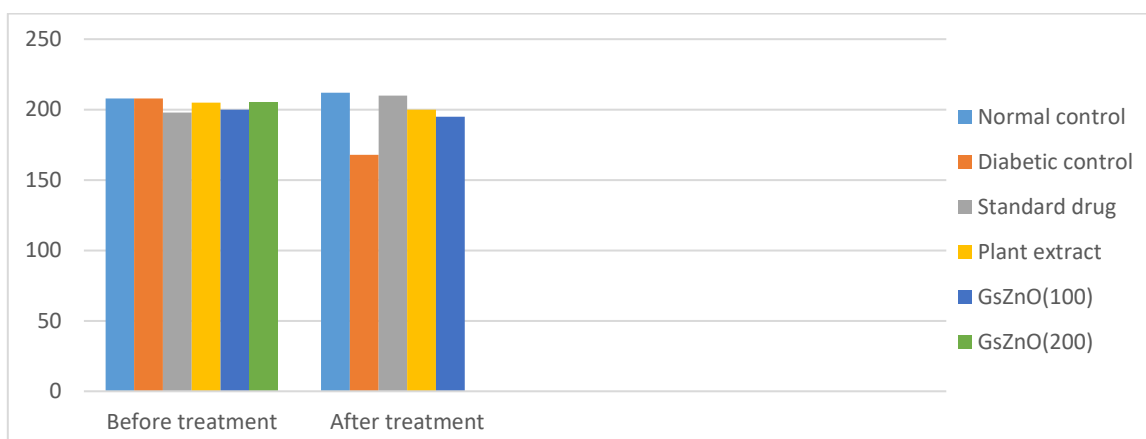


Figure 7: Changes in the body weight before and after treatment in normal and diabetic rats.

HISTOPATHOLOGY REPORT:

Pancreas

Histopathological examination of pancreatic tissue revealed that in untreated normal rats, the islets of Langerhans as well as circumscribed masses surrounded by deeply stained pancreatic exocrine acini appeared normal.

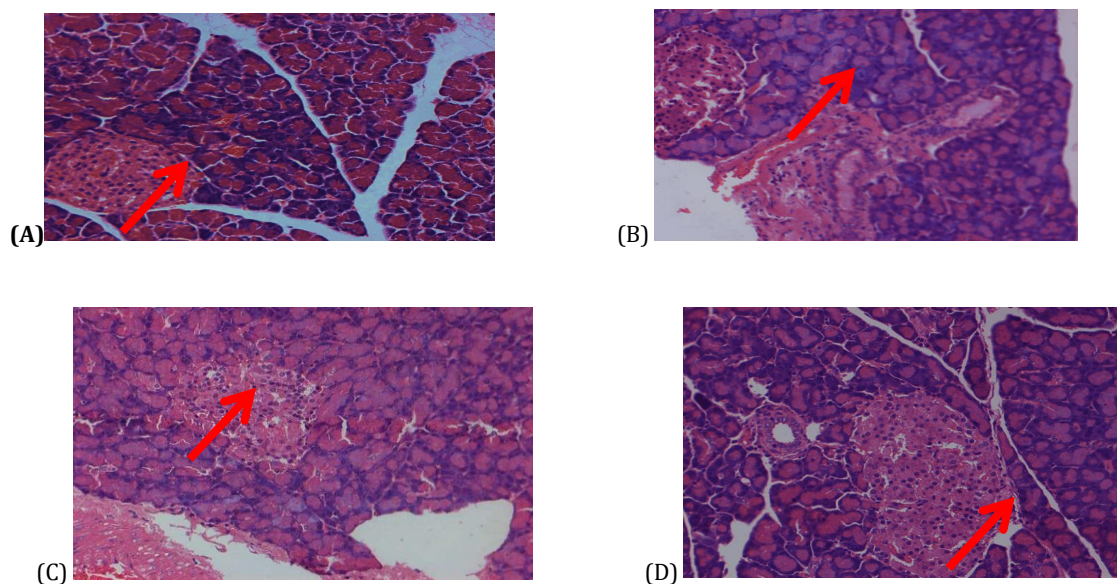


Figure 8: Histopathology report

- A. Pancreas- Normal Control → Beta cells in islets of pancreas appeared normal – arrow
- B. Pancreas – Diabetic Control → Periductular fibrosis sin which thickening of ductular region of glandular pancreas
- C. Standard Pancreas→ Mild to moderate degenerative changes and haemorrhages noticed in the islet cells in glandular pancreas.
- D. Pancreas- nanoparticles of plant extract 200µg → Glandular pancreas containing islets cells appeared normal. Collecting ducts in the glandular pancreas appeared normal.

DISCUSSION:

Diabetes mellitus is a complex chronic health condition caused by various genetic and environmental factors. It causes elevated blood glucose levels that are resulted due to decreased production of Insulin. These decreased insulin levels are improved by *Gymnema sylvestre*. The glucose levels were high in diabetic condition. 24 animals were taken and diabetes were induced by streptozotocin.

In this study, animals were separated into 6 groups: normal control, diabetic control, standard control, *G.sylvestre* plant extract, GsZnONPs (100 µg), GsZnONPs (200 µg).

The gymnemic acid, a component of *Gymnema sylvestre* leaf extract, exerts hypoglycemic effects by increasing secretion of insulin, promotes regeneration of islet cells. It increases utilization of glucose.

The zinc oxide nanoparticles increase serum insulin levels and also increases expression of mRNA in insulin gene.

The GsZnONPs NPs (200 µg) has shown significant antidiabetic activity when compared to *G.sylvestre* plant extract (200mg) alone, as zinc oxide NPs increase pancreatic cell proliferation leads to potent antidiabetic potential with small dose of plant extract.

CONCLUSION:

Reduction of the zinc oxide nanoparticles by *G.sylvestre* extract resulted in formation of stable nanoparticles. The use of ZnONp's for the treatment of rats with streptozotocin induced diabetes reduced serum blood glucose concentrations. In stz induced, there is decrease in the bodyweight by treating with the ZnONp's there is remarkable improvement in the body weight. The present work indicates the phytochemically synthesized zinc oxide nanoparticles of *Gymnema sylvestre* as a hypoglycemic treatment for diabetes mellitus.

ACKNOWLEDGMENTS:

Not applicable

AUTHORS CONTRIBUTION:

SG contributed to design of the study, performed the experiment, analysed the data and prepared the manuscript. MK contributed in the supervision of the experiment and analysed the data. SAK and SK contributed in the analysis of data. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST:

The authors declare that they have no conflicts of interests.

ETHICAL APPROVALS:

All protocols for animal experimentation have been approved by the Institutional Animal Ethics Committee (approved No.CPCSEA/IAEC/JLS/19/02/23/153) and experiments conducted in accordance with Committee guidelines for control and monitoring of experiments on animals (CPCSEA, India).

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