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Research Article

Evaluation of Anti-Diabetic Activity of Silver Nanoparticles Synthesized from Ethanolic Extract of *Phyllanthus niruri* on Wistar Rats

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Abstract

Diabetes is a metabolic disorder characterized by hyperglycemia resulting from either the deficiency in insulin secretion or the action of insulin Diabetes can affect numerous different organ systems in the body and, over time, can lead to serious complications. Complications are microvascular or macrovascular. To evaluate the anti-diabetic effect of *Phyllanthus niruri* silver nano-particles. Streptozotocin (STZ) of 50mg/kg is used to induce diabetes in wistar rats. The wistar rats were distributed to six different 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 *phyllanthus niruri* extract + diabetes; Group-V represented as 100 µg *Phyllanthus niruri* silver nanoparticles + diabetes; group-6- *Phyllanthus niruri* silver nanoparticles150µg + diabetes. The silver nanoparticles were inspected for the morphological and optical properties investigated using UV spectrophotometer, SEM and FT-IR techniques. The AgNPs has shown characteristic peak at 428nm.The study revealed that the AgNPs treatment reduced serum blood glucose concentrations remarkable improvement in the body weight, Abnormal high serum levels in diabetes treatment with AgNp's they reached to normal in STZ-challenged diabetic rats. The histo-pathological alterations were also studied in all the experimental animals. The pathology results revealed that the AgNPs treatment induces the regeneration of islets cells of pancreas in the experimental rats. The research study proved that the *Phyllanthus niruri* AgNPs exerts anti-diabetic properties.

Keywords: Diabetes, STZ, P*hyllanthus niruri*, silver nanoparticles

INTRODUCTION

Diabetes is a metabolic disorder characterized by hyperglycemia resulting from either the deficiency in insulin secretion or the action of insulin. Types of diabetes milletus include-Type 1Diabetis milletus (T1DM)-insulin dependent diabetes milletus,Type 2 Diabetes milletus (T2DM)-non – insulin dependent diabetes mellitus ,Gestational DM (GDM)[1]

Type 1 diabetes milletus is also known as insulin dependent diabetes milletus usually affects children and people below thirty years of age, but can also affect older adults. Type 1 diabetes is characterized by loss of insulin secretion due to idiopathic attack or autoimmune destruction of insulin-secreting beta cells of the islets of Langerhans in the pancreas. Therefore; it is mainly treated by insulin replacement therapy[2] . Type -2 diabetes milletus It predominantly affects adults above thirty years of age although many cases have recently been diagnosed amongst obese children. Type 2 diabetes has also been known as non-insulin-dependent diabetes mellitus (NIDDM) In T2DM, it is due to insulin resistance and relative insulin deficiency[3]. Gestational diabetes mellitus (GDM) occurs when glucose intolerance is first observed during pregnancy. The pathogenesis of GDM still remains largely unknown; nonetheless studies have shown involvement of dysregulation and defects in the insulin signaling pathway, resulting in reduced glucose uptake and transport in skeletal muscles and adipocytes. Other specific types are those in which the underlying defect or disease process can be identified in a relatively specific manner. They include disease of the exocrine pancreas, such as fibro calculous pancreatopathy or secondary to use of medicines such as corticosteroids[4].

Diabetes can affect many different organ systems in the body and, over time, can lead to serious complications. Complications from diabetes can be classified as microvascular or macrovascular. Microvascular complications include nervous system damage (neuropathy), renal system damage (nephropathy) and eye damage (retinopathy)[5]. Macrovascular complications include cardiovascular disease, stroke, and peripheral vascular disease. Peripheral vascular disease may lead to bruises or injuries that do not heal, gangrene, and, ultimately, amputation[6].

Nanoparticles

Nanoparticles are ultrafine particle is usually defined as a particle of matter that is between 1 and 100 nanometres (nm) in diameter. Nanoparticles can be synthesized using various approaches including chemical, physical, and biological approaches[7,8]. Although chemical method of synthesis requires short period of time for synthesis of large quantity of nanoparticles capping agents are added for size stabilization of the nanoparticles. Chemicals used for nanoparticles synthesis and stabilization are toxic and lead to non-eco-friendly byproducts[8,9]. The need for environmental nontoxic synthetic protocols for nanoparticles synthesis leads to the developing interest in biological approaches which are free from the use of toxic chemicals as byproducts. Thus, there is

an increasing demand for "green nanotechnology." Many biological approaches for both extracellular and intracellular nanoparticles synthesis have been reported till date using microorganisms including bacteria ,plants[10]. In green synthesis of AgNPs 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 silver nano particles. The plants extracts may work both as reducing and capping agents in AgNPs synthesis[11,12].

METHODS AND MATERIALS

Preparation of plant extract

The *P.niruri* plant materials was collected washed and shade dried at room temperature for 2 days .The dried plants were grinded to small pieces .The extraction process was carried out with the help of soxhlet apparatus 50 gms of powder is subjected to sohxlet extraction with 500ml of ethanol the extraction process is repeated until the required quantity is obtained. The extract is dried by using the rota evaporator.

Synthesis of nanoparticles

Silver nitrate was purchased from merck india ltd, and used for the study .The 50 ml of fresh leaf extract was added into the aqueous solution of 1mM silver nitrate[13]. the solution was centrifuged ate the rate of 11000 rpm for 20 mins to form small nanoparticles and the supernatant is decanted and the nanoparticles are dried for further use.

Characterization of silver nanoparticles

UV Visible analysis

The formation of silver nanoparticles was measured by UV spectroscopy. The bio-reduction of Ag^{+} in aqueous solution was measured by UV spectrum of the reaction medium at different nanometers. UV-spectral analysis has been done by using a (schimadzu UV-2450 spectrophotometer).

Scanning electron microscope (SEM)

SEM analysis is done to know the size and shape of the silver nanoparticles. The VEGA-3 SEM was used to classify the particle size, the morphology of nanoparticles.

Fourier transforms infrared (FTIR) spectroscopy

The silver nanoparticles were subjected to FTIR analysis to determine the functional groups the characteristic peaks in ranging from 400-4000 cm-1 on a Bruker FTIR spectrometer .The FTIR analysis was used to characterize the nature of capping ligands that stabilizes the silver nanoparticles formed. The FTIR absorption peaks indicated various bio-active components present in the AgNPs of extract.

Invivo evaluation of antidiabetic activity

Rat information

The experiment was carried out on healthy Eight- to eleven-week-old wistar male rats weighing approximately 150-200gm animals were purchased from the jeeva life sciences Laboratory Animal Centre, Uppal, Hyderabad. The animals were housed under strict hygienic conditions at 25 ± 2∘C temperature with 44–56% relative humidity and were subjected to a 12h light/12h dark cycle, fed with standard diet and water ad libitum in the Laboratory Animal House during the study. Each cage contained rats with corncob bedding material.

Licence

All protocols for animal experimentation have been approved by the Institutional Animal Ethics Committee (approved No.CPCSEA/IAEC/JLS/14/02/21/65) and experiments conducted in accordance with Committee guidelines for control and monitoring of experiments on animals (CPCSEA, India).

Induction of Diabetes

Rats were fasted for overnight. STZ (sigma-aldrich) is dissolved in freshly prepared phosphate buffered solution (PBS- 7.4). STZ of dose 50 mg per kg body weight were injected intraperitonially (i.p.) 200 μl of sterile PBS containing streptozotocin (STZ) 50 mg was used to induce diabetes in each rat. After 30 minutes administration of STZ, food and water were allowed freely to the animals. Following 6 hours administration of STZ, 5% glucose solution in a volume of 1 mL/kg was given to the animals for the next 24 hours to prevent death secondary to hypoglycemic shock. Animals were screened for diabetes after 2 days of STZ injection Glucose concentration was measured in a blood sample obtained from tail puncture (one touch glucometer) and those with fasting blood glucose level >250 mg/dL were included in the study. Control rats were injected with PBS only[14,15,16].

Dosage preparation

Dosage was prepared by mixture of aqueous extract the fusion extracts are given to rats by cannula.

Treatment Protocol

The animals are divided into 6 groups each group contain 6 animals.

Group1: normal control

Group2: diabetic control

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

Group4: diabetic rats to be treated with *P.niruri* extract.

Group5: diabetic rats treated with nanoparticles of *P.niruri*(100µg)

Group6: diabetic rats treated with nanoparticles of *P.niruri*(150µg)

Treatments are given orally after 4th day of STZ administration upto 21 days. The blood glucose levels are checked by glucometer (one touch) on 0,7,14,21day of study

Oral glucose tolerance test

Oral glucose test was performed after 21 days administration of AgNPs and plant extract to respective groups All the 6grps 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

Statistical analysis

The values are expressed as mean ± standard deviation (SD) 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 AND DISCUSSIONS

The formation of silver nanoparticles in the solution of 1mMsilver nitrate and aqueous extract of *Phyllanthus niruri* the change in the colour to brown colour.

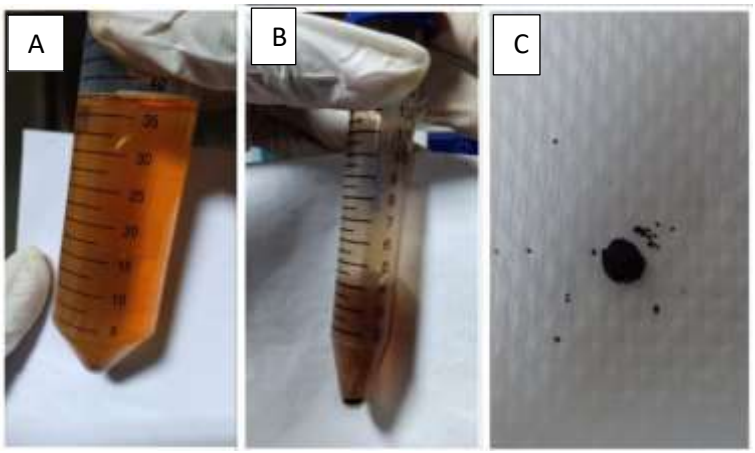

Figure 1: (a) colour change of $AgNO_3$ solution on reduction to silver nanoparticles (b) pellet formed after centrifugation of reaction mixture (c) nanoparticles after drying.

UV visible analysis

Reduction of silver ions into silver nanoparticles during exposure to plant extracts was observed as an outcome of the colour modify. The colour modify is due to the surface Plasmon resonance phenomenon. The metal nanoparticles have free electrons, which give the spr absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave[17]. The sharp band of silver nanoparticles were observed around 428 nm in case of *Phyllantus niuri* extract(fig:2). So we confirmed that the extract has more potential to reduce silver ions into silver nanoparticles, which lead us for further research on synthesis of silver nanoparticles from *Phyllantus niuri* extract.

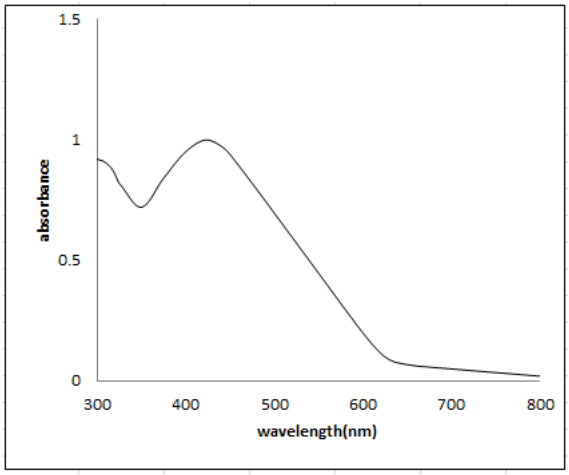

Figure 2: UV absorbance of *Phyllantus niuri* silver nanoparticles

Scanning Electron Microscopical (SEM) analysis of AgNPs

SEM analysis was carried out to understand the topology and the size of the AgNPs, which showed the synthesis of higher density poly-dispersed spherical AgNPs of various sizes[18]. The SEM image showing the high-density silver nanoparticles synthesized by the *Phyllanthus niruri* extract further confirmed the development of silver nanostructures. Most of the nanoparticles aggregated and only a few of them were scattered, as observed under SEM. The SEM analysis showed the particle size between 40-70 nm as well the cubic, face-centred cubic structure of the nanoparticles. The average size is 55nm. This result strongly confirms that *Phyllanthus niruri* extract might act as a reducing and capping agent in the production of silver nanoparticles and some chelating action also available in the solution.

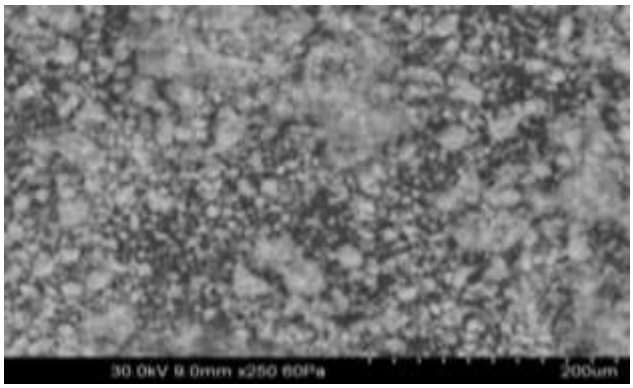

Figure 3: High resolution scanning electron microscopic (SEM) image of silver nanoparticle (AgNPs) ranged between 40-70nm.

FTIR spectrum analysis of AgNPs

FTIRs analysis was used to characterize the nature of capping ligands that stabilizes the silver nanoparticles formed. FTIR spectral analysis showed array of absorbance bands in 400 cm -1 -4400 cm -1. Organic functional groups are available in the air dried silver nanoparticles. The spectral bands were prominent for *Phyllanthus niruri* at 2915, 2565,2325 cm -1 (Si-H silane), 1657 cm -1 (C=O), 1525, 1475 cm -1 (C=C), 1065 cm -1 (Alcohols, ether, carboxylic acid, esters), 927cm - 1 (Alkenes), 862 cm -1 (Phenyl ring substitution rings) This organic group presence is due to Silver Particles reduction through biological sources. These data suggests that the stabilizing agents may be alkaloids, phenols, terpenes and polyols present in the aqueous leaf extract of *Phyllanthus niruri*. FTIR spectrum was examined to identify the possible biomolecules responsible for capping and efficient stabilization of the Ag nanoparticles synthesized by *Phyllanthus niruri* extract. The analysis of IR spectrum also provided an idea about biomolecules bearing different functionalities which are present in the underlying system.

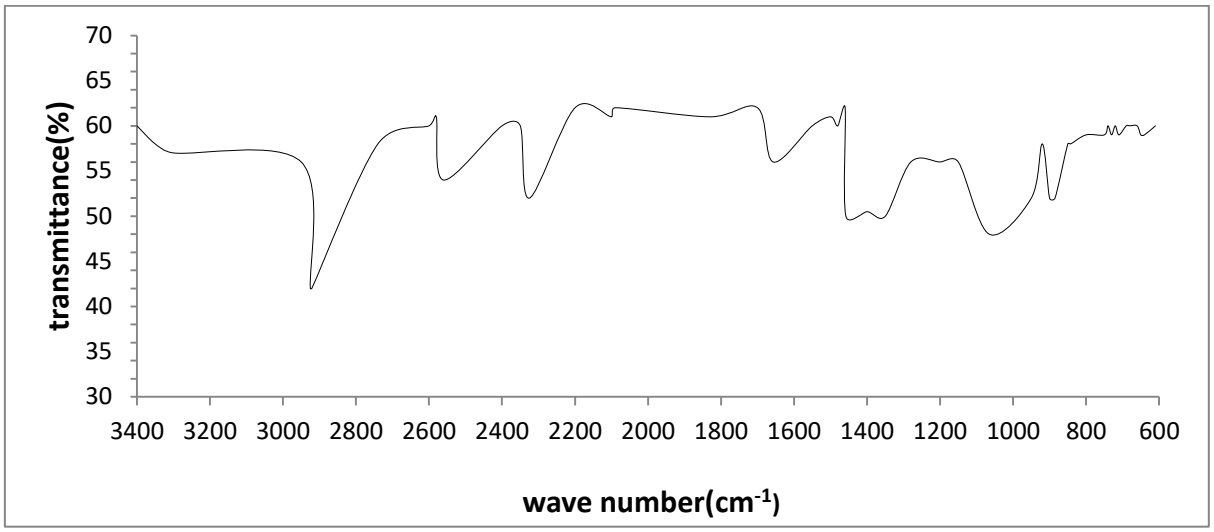

Figure No: 4 FTIR spectrum of silver nanoparticle synthesized by reduction of Ag+ ions by *phyllanthus niruri* extract

The Anti diabectic activity of AgNPs

The blood glucose levels of each group have been estimated during the treatment (Table:1) the blood glucose levels are checked there is pronouncedly decrease in the blood glucose level in the animals treated with nanoparticles compared to the diabetic control. AgNPs were shown to have reduced blood glucose levels than the plant extract alone.

The glucose tolerance level is significantly lowered in the *P.niruri* AgNPs among the treated groups the *P.niruri* AgNPs shown to improve the blood glucose level compared to the glibenclamide treated group(Fig:5). in treatment of diabetes, blood glucose concentration is considered as a routine bio chemical marker to monitor the disease condition.

The weight loss is the major aspect in the diabetes due to muscle wasting. In (Table:2) demonstrate the level of changes in the body weight of the rats before and after treatment.in our study the diabetic control (Group- II) showed significant weight loss compared to normal control. There is significant increase in the body weight in theP.*niruri* AgNPs treated groups.

Table 1: Blood glucose levels(mg/dl) in different groups

Groups / Days	Normal control	Diabetic control	Standard	Phyllanthus niruri extract	AgNPs of Phyllanthus niruri(100µg)	AgNPs of Phyllanthus niruri(150µg)
0	88.77±1.47	278.33±7.68	266.5±4.46	264.16±4.11	269.83±2.71	261.33±3.61
7	90.83±1.77	310.16±3.13	193.16±2.03	228.66±2.13	210.33±1.79	203.16±2.33
14	88.16±1.86	385±2.03	139±2.13	204±1.15*	186±1.63**	140±1.86**
21	90.55±1.38	403.66±2.68	101.01±1.91***	183.66±2.13***	143.33±2.21***	112.66±2.98***

Effect of *Phyllanthus niruri* extract AgNPs on STZ induced rats before and after treatment. Each coloumn represents mean±SD (n=6). The significance *P< 0.05; **P<0.01; ***P< 0.001.

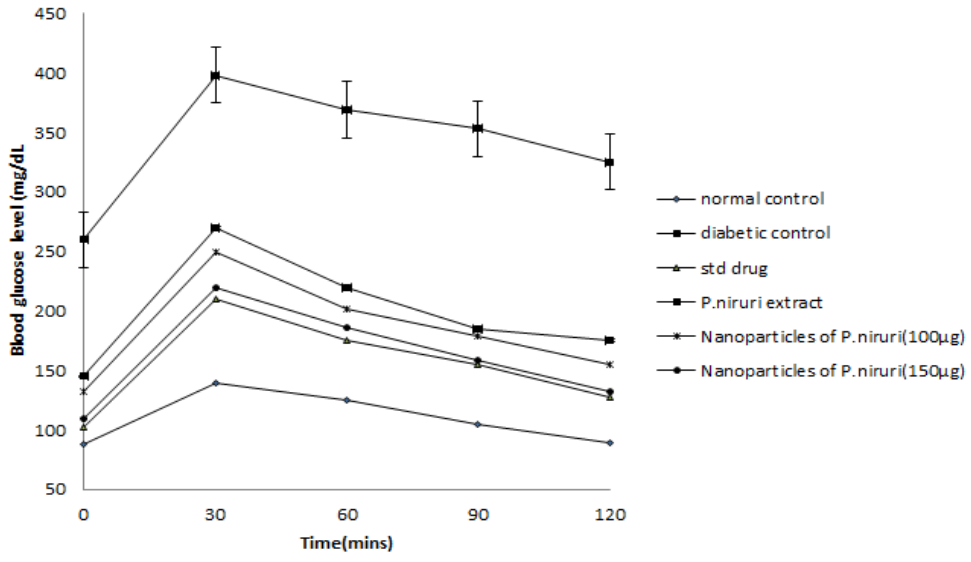

Figure 5: Effect of *P.niruri* leaf extracts and AgNPs in oral glucose tolerance test after 21 days of treatment.

The Total cholesterol (TC) and Triglycerides (TG) levels were also elevated in diabetic rats (Table 3,4) there is decrease in total cholesterol levels in treated groups when compared to the diabetic animals. The triglycerides levels also decreased in the treated groups compared to the diabetic control. Administration of AgNp's brought back the levels of serum lipids to near normal values.

Table 2: Body weight of animals before and after treatment in different groups

groups	Pre-Treatment	Post-treatment
Normal control	198.67±7.21	218± 8.96
Diabetic control	209.54±16.54	168±4.6
glibenclamide	220.67± 8.96	245.69 ± 7.89*
Plant extract	234.67±15.6	262.99± 6.5*
Nanopartricles of *Phyllanthus niruri* (100µg)	212.12± 9.8	249.33± 4.5
Nanopartricles of P*hyllanthus niruri* (150µg)	228.34±12.4	261.66 ± 8.9

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

Table 3: Total cholesterol (mg/dl) in different groups

Groups / Days	Normal control	Diabetic control	standard	Phyllanthus niruri extract	AgNPs of Phyllanthus niruri(100µg)	AgNPs of Phyllanthus niruri(150µg)
0	65±1.67	85±1.45	90±2.88	89±1.65	93±2.34	87±2.44
21	67±2.12	142±2.02	68±1.62***	82±1.97*	75±1.89**	70±1.34***

Effect of oral administration of AgNPs of *P.niruri* on serum cholesterol, Triglycerides level before and after the treatment .All values were found to be significant with diabetic control at *P< 0.05; **P< 0.01; ***P< 0.001.

Table 4: Triglycerides mg/dl in different groups

Groups / Days	Normal control	Diabetic control	standard	Phyllanthus niruri extract	AgNPs of Phyllanthus niruri(100µg)	AgNPs of Phyllanthus niruri(150µg)
0	47±1.84	88±2.56	90±1.34	89±3.55	92±1.98	88±3.45
21	49±1.98	130±1.78	51±2.65**	78±2.67*	72±2.65*	54±2.35**

Triglycerides level before and after the treatment .All values were found to be significant with diabetic control at *P< 0.05; **P< 0.01; ***P< 0.001

Histopathology report

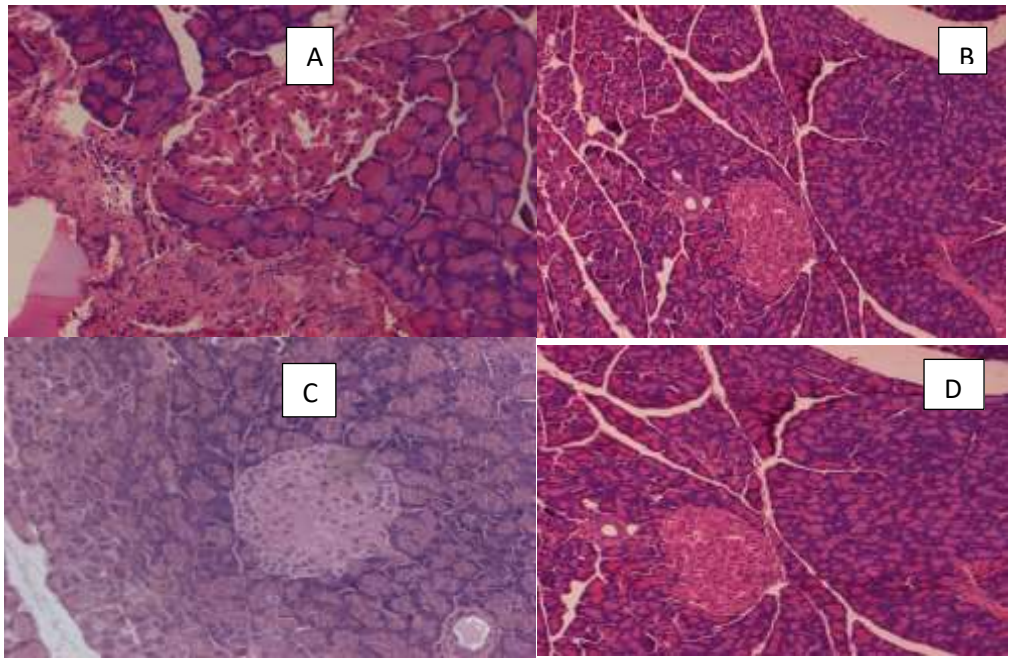

Figure 6: Histopathology of Pancreas
A-normal control, B- diabetic control, C- standard drug, D- nanoparticles of *P.niruri*(150µg)

CONCLUSION

Reduction of the silver nanoparticles by *Phyllanthus niruri* extract resulted in formation of stable nanoparticles. The use of AgNp'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 AgNp's there is remarkable improvement in the body weight. Abnormal high serum levels in diabetes treatment with AgNp's they reached to normal .

The present work indicates the phytochemically synthesized silver nanoparticles of *Phyllanthus niruri* as a hypoglycemic treatment for diabetes milletus.

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Not Applicable

Authors contribution:

MK contributed to the design of the study, performed the experiment, analyzed the data and prepared the manuscript. 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/14/02/21/65) and experiments conducted in accordance with Committee guidelines for control and monitoring of experiments on animals (CPCSEA, India).

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