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Drug Discovery

To Cite:
Elias A, Habbu PV, Iliger S. Biosynthesis of gold nanoparticles from *amaranthus gangeticus* and its anti-diabetic activity on streptozotocin induced rats. *Drug Discovery* 2024; 18: e21dd2009 doi: https://doi.org/10.54905/dissi.v18i42.e21dd2009

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Peer-Review History
Received: 12 August 2024
Reviewed & Revised: 16/August/2024 to 16/November/2024
Accepted: 20 November 2024
Published: 27 November 2024

Peer-Review Model
External peer-review was done through double-blind method.

Drug Discovery
pISSN 2278–540X; eISSN 2278–5396

Biosynthesis of gold nanoparticles from *Amaranthus gangeticus* and its anti-diabetic activity on streptozotocin induced rats

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ABSTRACT

Introduction: Diabetes mellitus is a metabolic disease that causes high blood sugar which causes impairment in parts of the body like nerves, eyes, kidneys, and other organs. The treatment using Oral antihyperglycemics can cause many adverse effects and if given in combination, can lead to drug-drug interactions. Innovative ant-oxidising substances might be introduced to produce therapeutic action on the exact target organs to overcome these issues. Nowadays, metal nanoparticles are highly used in the human health care system due to their excellent biocompatibility and constancy, low cost of methods, and environmentally friendly impressions. *Materials and Methods:* In the current study, green methods have been adopted to generate the gold nanoparticles (AuNPs) using the *Amaranthus gangeticus* plant which is proven as an antidiabetic plant and used to synthesise the green AuNPs from the leaf extract of *A.gangeticus* using 1 mM Gold chloride solution. The synthesised herbal-mediated AuNPs were introduced to different characterization techniques such as UV Spectroscopy, FTIR analysis, X-ray diffraction analysis (XRD), transmission electron microscope (TEM), and Scanning electron microscope (SEM) respectively. The antidiabetic effect of the produced AuNPs was also tested in vivo. *Results:* The induction of diabetes using STZ showed increased blood glucose, cholesterol, triglycerides, LDL, VLDL, and massive loss in body weight. These changes were reversed following the dealing of diabetic rats for 28 days and showed significant inhibition at a dose range of 1 mg/kg AuNPs related to the plant extract-tested group. *Conclusion:* These obtained results suggested that plant-mediated AuNPs have shown promising antidiabetes when correlated to the crude extract.

Keywords: Diabetes mellitus, gold nanoparticles, blood glucose, body weight, plant-mediated AuNPs.

1. INTRODUCTION

Diabetes is a condition where the body either produces insufficient amounts of insulin or does not react appropriately to it. This results in excessively high blood

sugar (glucose) levels and long-term problems that impact the neurological system and other organs (Lawrence et al., 2008). Diabetes has a significant genomic factor, with environmental factors also playing a role. Although the condition is heterogynous, once it has fully emerged, there seems to be a consistent phenotype. Whatever the pathologic reason, insulin resistance in the early stages of diabetes targets tissue primarily in the liver, skeletal muscle, and adipocytes (Pandey et al., 2011). Hyperglycaemia, or the liver producing too much glucose, and poor glucose uptake by peripheral tissue, particularly muscle, are linked to insulin resistance in the tissue (Khosla et al., 2000).

Type 1, Type 2, and gestational diabetes are the three primary forms of the disease. Type 1 diabetes primarily affects children and teenagers, while it can strike anybody at any age. Because the body generates little to no insulin, daily insulin injections are necessary to control blood glucose levels in people with Type 1 diabetes (Jeyam et al., 2021). Adults are more likely to have type 2 diabetes, which makes up almost 90% of all cases of diabetes. The body does not use the insulin it generates efficiently in people with Type 2 diabetes. The key to treating Type 2 diabetes is leading a healthy lifestyle, which includes frequent exercise and eating a balanced diet. To maintain stable blood sugar levels, the majority of people with Type 2 diabetes will eventually need insulin and/or oral medicines (Lee et al., 2021).

High blood glucose levels during pregnancy are a hallmark of gestational diabetes mellitus (GDM), a type of diabetes that can cause problems for the mother and the fetus. GDM raises the risk of Type 2 diabetes in later life for both the woman and her unborn child, even though it usually goes away after pregnancy (Lain and Catalano, 2007). In allopathic medicine, the majority of individuals with Type 2 diabetes mellitus (DM) are treated with metformin as their first line of treatment. Parenteral insulin is also occasionally used to treat DM. The main goal of the many allopathic medications that have been created and are now being used to treat Type 2 DM is glycemic control. It has not been demonstrated that any of these drugs provide a full recovery (Kumar et al., 2021). Numerous plants have been utilized to treat diabetes mellitus in the Indian medical system along with other traditional medical systems around the world.

Blood glucose levels are regulated, diabetes complications are decreased, and patients' lifespans and quality of life are enhanced by the usage of medicinal herbs (Al-Rowais, 2002). Formulating medications directly from medicinal plants has several drawbacks, including a lack of standardization, poor stability, an unpleasant taste, and decreased absorption that results in limited bioavailability (Wickramasinghe et al., 2022). The process of producing, modifying, and using nanomaterials—known as nanotechnology—is showing promise in a number of domains for both therapeutic and diagnostic uses (Elobeid et al., 2022). Usually, hazardous reagents are used as reducing agents in chemical processes to create nanoparticles, which might result in hazardous byproducts that are bad for the environment.

To transform metal ions into metal nanoparticles, recent studies have investigated the use of plant extracts as a safer, substitute supply of reducing agents. Current research is examining their possible effectiveness as antidiabetic and anticancer drugs (Jagessar, 2020). Due to their therapeutic qualities, gold nanoparticles are frequently utilized in conventional medicine to treat a variety of long-lasting syndromes. To develop nanomedicines, the Plant-mediated synthesis technique proved efficient, affordable, and dependable (Guo et al., 2020). The researchers are widely using the non-safe compound streptozotocin to bring diabetes in animal models (Ibrahim and Abd-El-Maksoud, 2015).

Nevertheless, no one has reported research on the *A.gangeticus-mediated* Gold metallic nanoparticles, and the way to its anti-diabetic activity. In this work, we examined the anti-diabetic properties of gold nanoparticles from A. gangeticus in in vivo models of diabetes produced by streptozotocin. The physical characteristics of the gold nanoparticles, which were created via green-mediated techniques, were examined. In this study, the synthesized A. gangeticus gold nanoparticles were compared to glibenclamide, the usual medication.

2. MATERIALS AND METHODS

Collection, authentication and extraction of Plant Material

The Tamil Nadu district of Tirunelveli is where the leaves of Amaranthus gangeticus were gathered. V. Chelladurai, a retired research officer in botany with the Central Council for Research in Ayurveda & Siddha, recognized and verified the specimen. After being shade-dried, the healthy leaves were ground into a coarse powder with an electric blender. Using a Soxhlet system, the powdered leaf material was extracted using solvents in increasing order of polarity. For the extraction procedure, solvents including petroleum ether (PEAG), chloroform (CEAG), ethanol (EEAG), and water (AEAG) were employed. After the solvent was removed using distillation,

each extract was dried off and placed in a desiccator for further examination. Based on the total phenolic content of the aforementioned extracts, the ethanolic extract was selected, and the phenolic-rich fraction was then used to create gold nanoparticles (AuNPs).

Green Synthesis and Characterization of AuNPs

Gold nanoparticles (AuNPs) were synthesized by mixing 10 ml of phenolic-rich fractions from *Amaranthus gangeticus* (Ag) with 90 ml of an aqueous 1 mM chloroauric acid ($HAuCl_4$) solution under vigorous stirring at room temperature. Within 5 minutes, the pale-yellow solution turned ruby red, indicating the formation of gold nanoparticles (Geetha et al., 2013). X-ray diffraction (XRD), fluorescence spectrometry, and UV-V is spectroscopy were used to examine the Ag-AuNPs. Fourier transform infrared (FT-IR) spectroscopy, scanning electron microscopy (SEM), and transmission electron microscopy (TEM) were used to further establish their properties (Wang et al., 2019).

In-Vivo Anti-Diabetic Activity

Experimental Animals Acclimatization & Selection

Four-week-old adult Wistar rats from Soniya College of Pharmacy's Animal House in Dharwad participated in the study. Five rats per cage were kept in controlled environments with a natural 12-hour light-dark cycle and a constant temperature of 25 ± 2 ºC. They were fed a typical chow diet and allowed unlimited access to purified water. The IAEC Committee accepted all of the study's methods, and all tests and experiments were carried out in accordance with OECD standards.

Acute Toxicity

Doses of 0.5 and 1 mg per kg of body weight were used to evaluate the acute toxicity of AuNPs. Observations for behavioral, physiological, and neurological alterations as well as mortality were documented throughout a 72-hour period with daily monitoring. In accordance with OECD 423 criteria, rats were also used in an acute toxicity test for Amaranthus gangeticus leaves (Lalit et al., 2019). Up to a dosage of 2000 mg/kg, no group showed signs of death.

Induction of diabetes

50 mM sodium citrate solution (pH 4.5) was used to dissolve the drug streptozotocin (STZ). The rats received a dosage of 55 mg/kg body weight of the solution intraperitoneally. To verify the occurrence of diabetes mellitus, fasting blood sugar (FBS) levels were assessed after three days.

Experimental protocol

Seven groups of adult Wistar rats were randomly divided up. Eight rats were distributed to each group. Streptozotocin (STZ) was administered via the abdomen to the experimental rats (but not to the control groups) at a dosage of 55 mg/kg in citrate buffer in order to cause diabetes.

Group 1: The control group (standard diet and water was administered).
Group II: The STZ-treated rats (negative control)
Group III: The *A.gangeticus* mediated AuNPs (0.5 mg/kg body weight).
Group IV: The *A.gangeticus* mediated AuNPs (1 mg/kg body weight).
Group V: The Phenolic fraction of *A.gangeticus* extract (200 mg/kg body weight).
Group VI: The Phenolic-rich fraction of *A.gangeticus* extract (400 mg/kg body weight).
Group VII: The standard drug Glibenclamide (0.5 mg/kg body weight).

All group rats received AuNPs, plant extract, and glibenclamide orally for 28 days (Pari et al., 2000).

Samples collection

After the experiment, animals were given a 12-hour fast before being given a light ether anesthetic to put them to sleep and have blood drawn from the eye's retro-orbital venous plexus using heparinized capillary tubes. Three different types of tubes were used to collect blood samples: One with heparin for plasma separation, one with sodium fluoride for fasting blood sugar measurement, and one dry and clean tube without anticoagulant for serum separation. In a cooling centrifuge, each tube was spun for ten minutes at 3000 rpm.

After being split into Eppendorf tubes, plasma and serum were kept at -30°C until they were needed again. He rats were put to death by cervical dislocation at the conclusion of the study procedure. After being removed, the pancreas, liver, and kidneys were promptly washed with a cold phosphate buffer solution. After that, the tissues were weighed, measured, and stored for later examination (Shaheen et al., 2016).

Quantitative measurement of biological parameters

The glucometer's disposable strip was filled with the blood samples. In test rats, the glycaemic levels were measured. To get the serum samples, the blood samples were centrifuged at 2000 rpm for 5 minutes. In the serum of test rats, the AST and ALT levels were measured. It was discovered what serum alkaline phosphatase was. The concentrations of serum proteins were measured. A modified JAFFE test kit technique was used to measure the serum creatinine levels. The remaining blood sample was stored in a heparinized container in order to extract the plasma. The insulin levels in the experimental animals were determined using the plasma and the ELISA method. The glycosylated hemoglobin (HbA1c) was measured using a commercially available kit (Drabkin and Austin, 1932).

Establishing the conditions for oxidative stress and antioxidants

The pancreas was removed and minced in ice-cold buffer to gather the supernatant for the lipid peroxidation and antioxidant tests. Together with the activity of antioxidant enzymes such glutathione peroxidase, catalase (CAT), and superoxide dismutase (SOD), lipid hydroperoxide levels were also assessed. A UV spectrophotometer was used to examine antioxidant qualities and indicators of oxidative stress.

Histopathological study

After being removed, a sample of pancreatic tissue was preserved in 10% formal saline solution and treated appropriately. The tissues were embedded in paraffin following fixation. Hematoxylin and eosin was used to stain the preserved tissues after they were sectioned at 5-micrometer intervals. Following a light microscope examination of the tissue slices, pictures were taken (Selvan et al., 2008).

Statistical study

Software called SPSS version 22 was used to examine the data. Significant differences between groups were identified using a one-way ANOVA combined with Duncan's test. The findings are shown as mean ± SD, and a p-value of less than 0.05 is regarded as statistically significant.

3. RESULTS AND DISCUSSION

Synthesis and Characterization of Gold Nanoparticles

A.gangeticus was identified as a novel precursor capable of biosynthesis-based reduction of gold ions into gold metals and stabilisation of the resulting nanoparticles. According to Figure 1(a), the absorbance wavelength of AuNPs is produced between 520 and 570 nm (Ghramh et al., 2019; Al-Radadi et al., 2024). To pinpoint the potential biomolecules in charge of the Au nanoparticles' reduction, capping, and effective stabilisation, ATR FTIR measurements were made. SEM and TEM methods were used to study the gold nanoparticles' size and form. It was discovered that the nanoparticles were monodispersed and ranged in size from 25 to 200 nm on average. At different magnifications, white particles were visible (Figure 1(b & c)).

Acute Toxicity Test

In a short-term toxicity evaluation, the green-synthesised AuNPs and the plant extract of Amaranthus gangeticus were determined to be non-toxic. At the doses of 0.5 mg/kg and 1 mg/kg, which were shown to be the optimal dose based on acute toxicity testing, no mortality was detected in the green-produced AuNPs treated rats throughout the experiment.

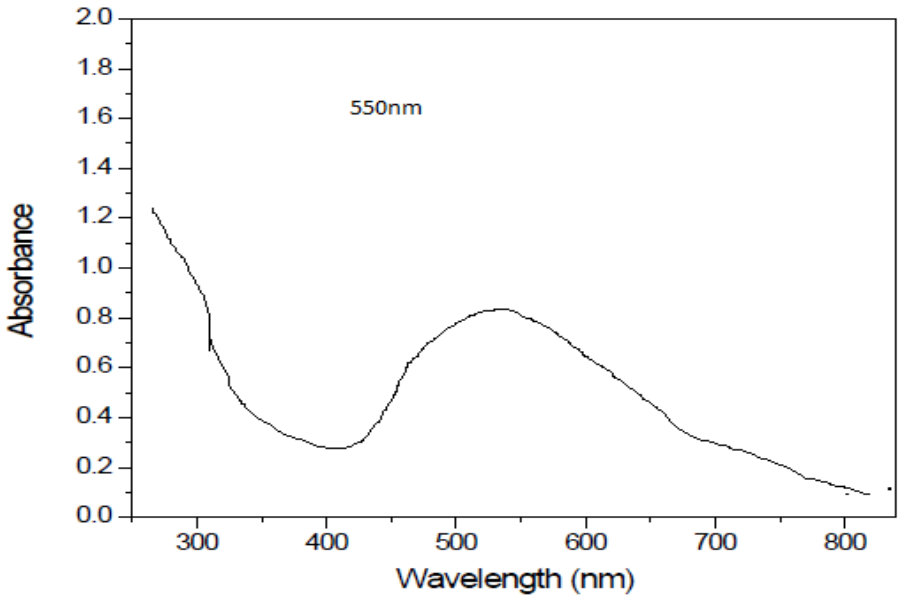

Figure 1(a) UV-VIS absorption spectra of Au NPs synthesized by Amaranthus gangeticus

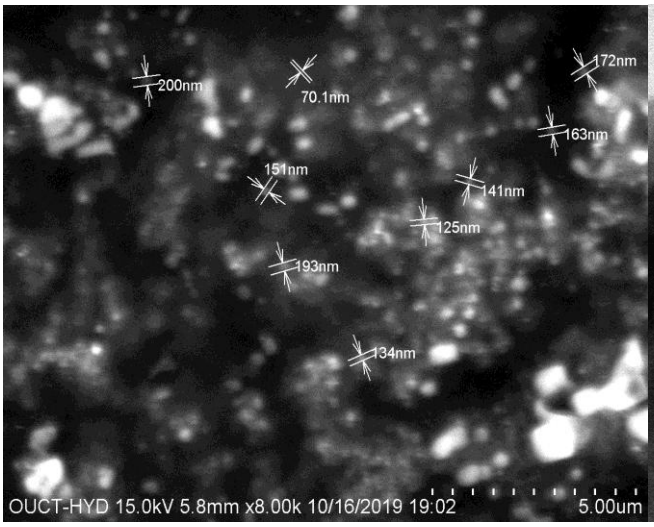

Figure 1(b) SEM Monograph of Au NPs

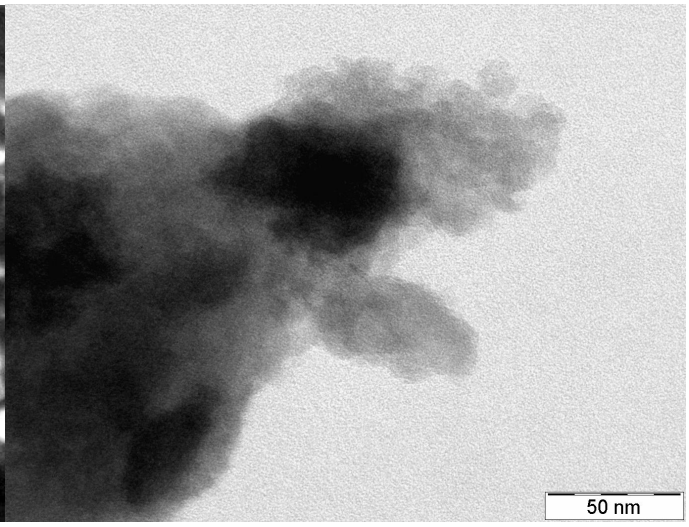

Figure 1(c) SEM Monograph of Au NPs

Induction of Experimental Diabetes

In this experiment, streptozotocin (STZ) was used to create diabetes. The inducing agent was dissolved in 50 mM citrate buffer at a pH of 4.5. The prepared streptozotocin solution at 55 mg/kg dose was given through an intraperitoneal route which was fasted overnight for 14 hrs before administration. To prevent hypoglycemic shock-related mortality, the rats were given glucose solution for roughly 24 hours after receiving STZ injections for 6 hours. The rats were examined for the presence of Diabetes Mellitus 72 hours after the STZ injection. Rats were eligible for the trial if their FBG levels were greater than 150 mg/dl.

Blood Glucose Level

Amaranthus gangeticus plant extract, together with its Ag NPs and Au NPs, had a substantial impact on blood glucose levels in STZ-induced diabetic mice, as seen in (Table 1). The administration of glibenclamide, Amaranthus gangeticus plant extract (200 and 400 mg/kg), and its AuNPs (0.5 mg/kg & 1 mg/kg) greatly reduced blood glucose levels. Both dosages (0.5 and 1 mg/kg) of AG-mediated AuNPs showed much higher activity than the Amaranthus gangeticus plant extract alone.

Table 1 Effect of *Amaranthus gangeticus* plant extract and its AuNPs on Blood Glucose Level in Streptozotocin induced diabetic rats.

	Blood Glucose Levels (mg/dL) (Mean ± SEM)		
Treatment groups	**Initial**	**14th Day**	**28th Day**
Normal rats	93.66±1.73	95.21± 1.02	95.21± 1.02
Diabetic control rats (1%v/v Tween 80)	163.33±1.86	172.33±2.50	177.31±1.03a
Diabetic + Glibenclamide (10mg/kg)	161.33±1.26	116.60±1.2***	101.71±1.10***
Diabetic + *A.gangeticus* extract 200mg/kg	162.33±1.50	135.24±1.21***	122.37±1.15***
Diabetic + *A.gangeticus* extract 400mg/kg	161.21±2.50	129.20±1.25***	117.31±1.21***
Diabetic + AG Mediated AuNPs 0.5mg/kg	162.48±1.26	131.23±1.10***	122.71±1.20***
Diabetic + AG Mediated AuNPs 1mg/kg	163.13±1.05	122.17±1.11***	111.37±1.25***

All values are expressed as mean ± SEM, n=6, *p<0.05, **p<0.01, ***p<0.001 as compared to the control group (One-way Analysis of Variance (ANOVA) followed by multiple comparisons Tukey's test.

Outcome on Diverse Biological Parameters

The liver function of diabetic rats was assessed using alkaline phosphatase (ALP), aspartate transaminase (AST), and alanine transaminase (ALT). Similar to this, estimations of protein and creatinine levels were used to study renal function. In type 2 diabetes patients, the glycemic state is typically correlated with the rise in liver enzymes (Mandal et al., 2018). Numerous liver enzymes, such as ALT, AST, and ALP, were found to be elevated in diabetic rats in this investigation. Elevated transaminase may have helped diabetes ketogenesis and gluconeogenesis develop (Jiang et al., 2020). On the other hand, treatment with AuNPs and plant extract from A. gangeticus significantly reduced the activity of hepatic transaminase in diabetic rats. At both doses (0.5 and 1 mg/kg), the AG-mediated AuNPs' activity was much higher than that of the A.gangeticus plant extract.

This suggests that in diabetes, may function as a hepatoprotective agent. In diabetes, hyperglycemia-induced renal impairment, blood protein and creatinine levels are dramatically increased (Mirmohammadlu et al., 2015). The study's diabetic rats had higher blood creatinine and protein levels, which may indicate that their kidneys are less effective in filtering out waste and pollutants. On the other hand, diabetic rats given AuNPs plus plant extract from Amaranthus gangeticus had noticeably reduced blood levels of creatinine and protein, suggesting that kidney function was protected. Notably, the Ag-mediated AuNPs were more successful in lowering blood creatinine and protein levels (Table 2).

Table 2 Outcome of *Amaranthus gangeticus* plant extract and its AuNPs on serum biochemical parameters

Treatment groups	**AST (IU/L)**	**ALT (IU/L)**	**ALP (IU/L)**	**Protein (IU/L)**	**Creatinine (IU/L)**
Normal rats	0.89±0.0915	2.414±0.3321	122.1 ± 1.293	0.049 ± 0.12	0.61 ± 0.57
Diabetic control rats (1%v/v Tween 80)	1.50±0.0915a	3.944±0.3344a	239.9 ± 1.891a	0.092 ± 0.23a	1.96 ± 0.13a
Diabetic + Glibenclamide (10mg/kg)	1.40±0.106*	2.308±0.0763***	143.4 ±1.161***	0.035 ± 0.25 **	1.57 ± 0.36**
Diabetic + *A.gangeticus* extract 200mg/kg	1.25±0.1108***	0.632±0.0483***	172.9 ± 1.211**	0.082 ± 0.17	0.92 ± 0.11***
Diabetic + *A.gangeticus* extract 400mg/kg	1.18±0.0872***	0.610±0.0650***	151.2 ±1.121***	0.076 ± 0.18	0.88 ± 0.45***
Diabetic + AG Mediated AuNPs 0.5mg/kg	1.56±0.1051	0.926±0.0442***	152.2 ±1.110***	0.066 ± 0.29c	0.72 ± 0.12***
Diabetic + AG Mediated AuNPs 1mg/kg	1.15±0.133***	0.610±0.0725***	138.9 ±1.290***	0.088 ± 0.21	0.67 ± 0.23**

All values are expressed as mean ± SEM, n=6, ap<0.001 as compared to the normal group *p<0.05, **p<0.01, ***p<0.001 as compared to the control group (One-way Analysis of Variance (ANOVA) followed by multiple comparisons Tukey's test.

Impact on the Liver's Oxidant-Antioxidant Status

The degree of oxidative stress caused in diabetic rats was evaluated using lipid peroxidation (LPO) tests, glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD), and enzymatic antioxidants. Reactive oxygen species (ROS) are produced when glucose is oxidized in diabetes mellitus. These ROS intensify lipid peroxidation, which raises the number of free radicals and produces lipoxidation end products (Asmat et al., 2016). Protein aggregation, which results in DM vascular problems and liver damage, is caused by lipid peroxidation (Pitocco et al., 2013). Hyperglycemia is associated with a rise in plasma MDA, a byproduct of lipid peroxidation and a sign of the production of free radicals. Increased ROS levels in type 2 DM may cause a hypercoagulable condition, and data suggests that oxidation products build up before DM onset (Papachristoforou et al., 2020).

The antioxidant defenses are weakened by hyperglycemia. For instance, streptozotocin-treated rats displayed reduced levels of hepatic SOD, CAT, and GPx, which is consistent with the current study (Pottathil et al., 2020). Free radical eradication from the tissues is a crucial role SOD, CAT, and GPx perform. The SOD, CAT, and GPx activities in the liver and kidneys of diabetic rats treated with *Amaranthus gangeticus* plant extract and its AuNPs considerably increased in the current study. This research highlights how Amaranthus gangeticus plant extract, particularly its phenolic content and AuNPs, can raise antioxidant levels and reduce the activity of free radicals. According to the study, diabetic rats had far lower amounts of GSH than normal rats.

Table 3 Effect of Amaranthus gangeticus plant extract and its AuNPs on Oxidant–Antioxidant Status in Liver

Treatment Groups	LPO (n moles/mg of protein)	CAT (µmol/mg of protein)	Glutathione (µmol/mg of protein)	SOD (unit/mg of protein)
Normal rats	21.21 ± 2.110	15.116 ± 0.321	5.291 ± 0.218	14.113 ± 0.121
Diabetic control rats (1%v/v Tween 80)	79.31 ± 2.110a	4.516 ± 0.315a	1.691 ± 0.131a	3.213 ± 0.312a
Diabetic + Glibenclamide (10mg/kg)	55.05 ± 1.095***	16.54 ± 0.569***	4.871±0.135***	14.10 ± 0.401***
Diabetic + *A.gangeticus* extract 200mg/kg	47.15 ± 2.659***	13.48 ± 0.906***	4.597 ± 0.316***	10.02 ± 0.716***
Diabetic + *A.gangeticus* extract 400mg/kg	34.99 ± 1.883***	14.48 ± 0.705***	5.263 ± 0.227***	14.02 ± 1.240***
Diabetic + AG Mediated AuNPs 0.5mg/kg	45.71 ± 1.108***	12.66 ± 0.615***	4.532 ± 0.569***	8.619 ± 0.9837***
Diabetic + AG Mediated AuNPs 1mg/kg	32.37 ± 3.467***	15.16 ± 0.635***	6.032 ± 0.219***	15.12 ± 0.6637***

All values are expressed as mean ± SEM, n=6, ap<0.001 as compared to the normal group; ***p<0.001 as compared to the control group (One-way Analysis of Variance (ANOVA) followed by multiple comparisons Tukey's test.

This is probably because they produce more free radicals, which encourage GSH to be converted to its oxidized form (Rahal et al., 2014). Hepatic GSH levels were higher in diabetic rats treated with Amaranthus gangeticus plant extract and its AuNPs than in control rats. According to Table 3, this implies that the plant extract and its AuNPs may either increase the production of GSH or lessen oxidative stress, which normally lowers GSH levels. As a cofactor for antioxidant enzymes, GSH scavenges free radicals and speeds up xenobiotic detoxification, both of which contribute to protection (Lushchak, 2012).

Effect on Plasma Insulin and glycosylated hemoglobin Levels

In normal control rats, there were no variations in plasma insulin levels. Nevertheless, during the study, diabetic rats' plasma insulin levels dramatically dropped. In diabetic rats, plasma insulin levels significantly rose in response to glibenclamide or Amaranthus gangeticus plant extract and its AuNPs (Table 4). When compared to the diabetic control group, the groups treated with glibenclamide (10 mg/kg), AuNPs, and plant extract from Amaranthus gangeticus had noticeably greater hemoglobin (Hb) levels. The normal control group had the highest Hb level. For these therapies, no dose-dependent effects were seen (Table 4).

Table 4 Effect of *Amaranthus gangeticus* plant extract and its AuNPs on Glycosylated hemoglobin HbA1c in % (mmol/mol OR mg/dl) values and Insulin level

Treatment groups	HbA1c in % (mmol/mol OR mg/dl)	Insulin level (µU/l)
Normal rats	5.06 ± 0.05	25.2 ±0.5
Diabetic control rats (1%v/v Tween 80)	7.73 ± 0.12	13.2 ±0.6
Diabetic + Glibenclamide (10mg/kg)	5.40 ± 0.23**	24.0 ± 0.3**
Diabetic + *A.gangeticus* extract 200mg/kg	7.30 ± 0.26*	22.3 ±0.4**
Diabetic + *A.gangeticus* extract 400mg/kg	7.33 ± 0.28*	21.1 ±0.7*
Diabetic + AG Mediated AuNPs 0.5mg/kg	6.86 ± 0.03*	20.2 ±0.2*
Diabetic + AG Mediated AuNPs 1mg/kg	5.46 ± 0.03**	23.5 ±0.1**

All values are expressed as mean ± SEM, n=6, **p<0.005 as compared to the control group (One-way Analysis of Variance (ANOVA) followed by multiple comparisons Tukey's test).

Effect on Organ Body Weight Ratio

When compared to rats without diabetes, the liver weights of diabetic rats in the treated groups were noticeably lower. Conversely, there were no appreciable variations in the kidney weights of diabetic rats in the treated groups and non-diabetic rats (Table 5).

Table 5 Effect of *Amaranthus gangeticus* plant extract and its AuNPs on organ body weight ratio.

Treatment	Organ weight (gram)		*organ-to-body weight ratio*	
	Kidney	Liver	Kidney	Liver
Normal rats	1.57±0.06	7.52±0.31	0.60±0.02	3.29±0.36
Diabetic control rats (1%v/v Tween 80)	1.31±0.21	6.58±0.24	0.50±0.13	2.69±0.44
Diabetic + Glibenclamide (10mg/kg)	1.59±0.12	8.51±0.14	0.63±0.07	3.29±0.71
Diabetic + *A.gangeticus* extract 200mg/kg	1.67±0.06	8.55±0.14	0.68±0.11	3.49±0.23
Diabetic + *A.gangeticus* extract 400mg/kg	1.55±0.01	8.48±0.41	0.73±0.17	3.58±0.41
Diabetic + AG Mediated AuNPs 0.5mg/kg	1.53±0.03	8.68±0.24	0.72±0.16	3.20±0.71
Diabetic + AG Mediated AuNPs 1mg/kg	1.54±0.04	8.528±0.31	0.63±0.23	3.31±0.21

All values are expressed as mean ± SEM, n=6, *p<0.01 as compared to the control group (One-way Analysis of Variance (ANOVA) followed by multiple comparisons Tukey's test).

Histological assessment

The *Amaranthus gangeticus* plant extract and its AuNPs treated groups showed minor periacinar inflammation with a regular insular pancreas pattern, in contrast to the regular control group, which had a regular insular pancreatic pattern as determined by the histological analysis. Glibenclamide-treated rats had fewer necrotic pancreatic cells and reduced periacinar inflammation in comparison to the diabetes control group (Figure 2). An attempt was made to evaluate acute toxicity and look into the antidiabetic potential of gold nanoparticles produced from A. gangeticus. According to the present study's findings, the diabetes treatment groups' body weight rose, most likely as a result of better glycemic control. The groups treated with extract and AuNPs showed similar levels of food intake and weight increase (Ara et al., 2023).

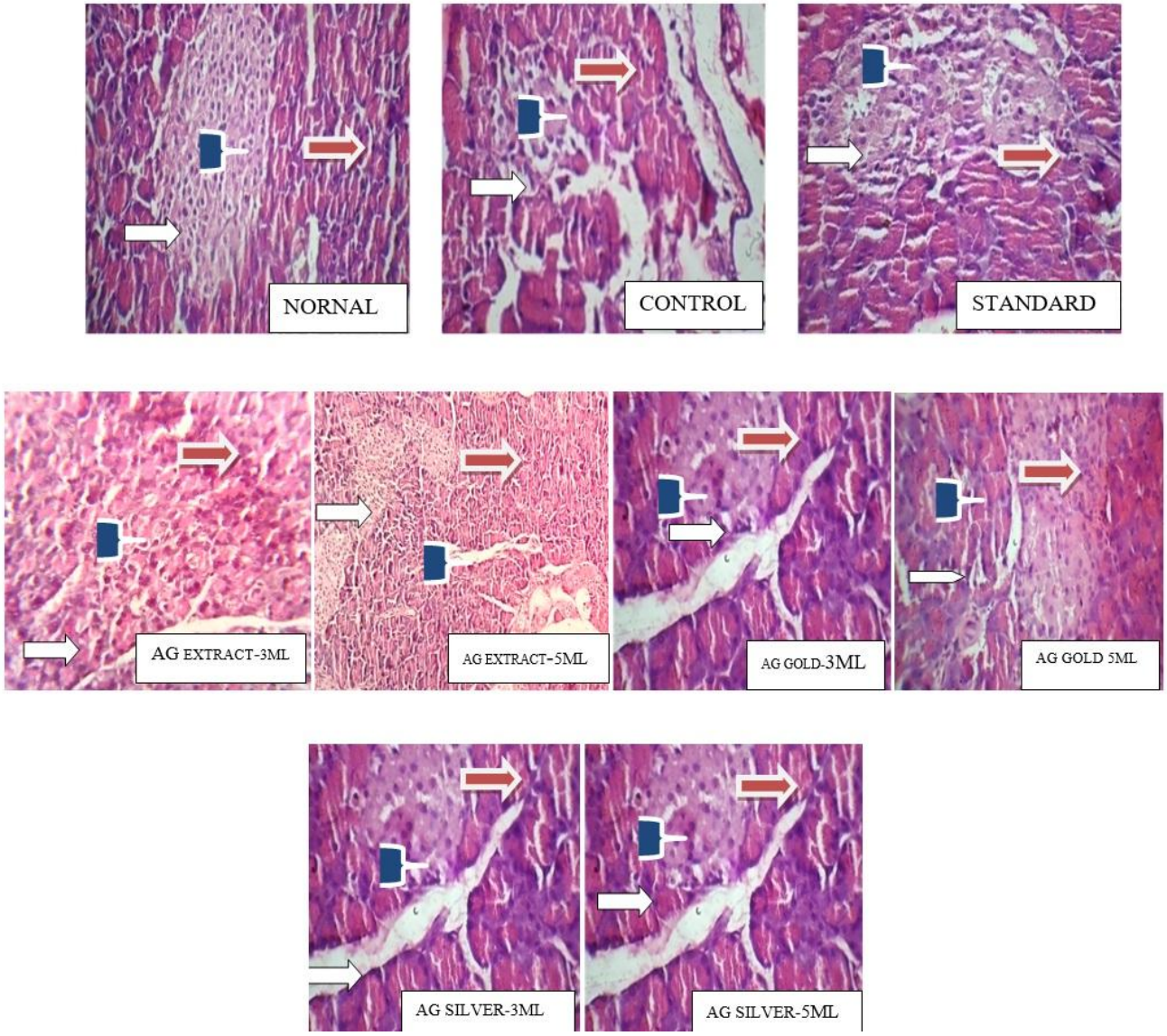

Figure 2 Effects of Amaranthus gangeticus plant extract and its AuNPs on pancreas against Streptozocin-induced diabetic rats.
Normal: Pancreatic islet of rat showing an intact cluster of β-cells and architecture.
Control: Diabetic control group animals showed destruction of pancreatic islets of β-cells, islet, shape and number of islets are reduced.
Standard: Pancreatic islet of glibenclamide-treated diabetic rat showing reduced degranulation architecture, moderately restored pancreatic cells, islet, shape and islets numbers are increased
Treatment: Amaranthus gangeticus plant extract and its AuNPs treated diabetic rat showing regeneration of the β-cells and restoration of architecture, shape and numbers of islets

One of the most vital organs, the liver is in charge of metabolism, cleansing, and storage. ALP, SGOT, and SGPT are important indicators for evaluating liver function. Liver necrosis and increased levels of these markers were seen in the diabetic animal group. Enzyme leakage from the liver cytosol may be the cause of the rise in these markers. The A. gangeticus and standard groups were among the treatment groups where this was markedly the opposite (Khan et al., 2023). These results demonstrated the preparation's improved impact and were in line with histopathology findings from the group treated with the reference medication, glibenclamide. It

has been scientifically demonstrated that a number of plant-based gold nanoparticle compounds have antidiabetic properties. The antidiabetic potential of AuNPs, which were biologically generated from the phenolic-rich fraction of the ethanolic extract of A. gangeticus leaves, was examined in this work.

4. CONCLUSION

The development of new technology has made it possible to investigate the biological characteristics of nanoparticles in a way that is safe for the environment. The phenolic-rich portion of ethanolic leaf extracts of Amaranthus gangeticus was used to create Au-NPs. This study set out to assess these drugs' antidiabetic benefits. Because the natural substances that cover the biosynthesized gold nanoparticles also have antidiabetic qualities, the nanoparticles showed considerable antidiabetic potential. The finished nanomaterial showed a synergistic antidiabetic effect. Consequently, AuNPs made from A. gangeticus may have the potential to produce gold nanoparticles for a range of therapeutic uses at a reasonable cost. As far as I'm aware, this paper is the first to mention using extract from A. gangeticus for the environmentally friendly manufacture of gold nanoparticles and their antidiabetic properties.

Ethical approval

The study was approved by the appropriate institutional Animal Ethical Committee (Reg No 112/ PO/Re/S/99/ CPCSEA) (Ref-SETCPD/ IAEC/ SEP/2021/09) Soniya Educational Trust's College of Pharmacy, Dharwad and certify that the study was performed by the ethical standards. Meantime, the ethical guidelines for plants & plant materials are also followed in the study for plant collection, identification & experimentation.

Authors' contributions

Akila Elias: Contributed to the design and implementation of research, to the analysis of the results and the writing of the manuscript.
Prasanna V Habbu: Involved in planning and supervising the work
Sudhir Iliger: Contributed to the interpretation of the results.

Acknowledgement

The abstract of the above article has been published with the entitled "Biosynthesis of Gold Nanoparticles from Amaranthus gangeticuss. and Its Anti-Diabetic Activity on Streptozotocin-Induced Rats" in Proceedings. DOI:10.3390/proceedings2024103086.

Informed consent

Not applicable

Conflicts of interests

The authors declare that there are no conflicts of interests.

Funding

The study has not received any external funding.

Data and materials availability

All data associated with this study are present in the paper.

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