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In-vivo Anti-Diabetic Efficacy of Silver Nanoparticles from Marine Brown Seaweed *Colpomenia sinuosa* on Alloxan Stimulated Hyperglycemic Activity in Wistar Albino Rats

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ABSTRACT

Diabetes mellitus is a chronic disease of the endocrine system characterized by elevated blood glucose levels, and disturbances in carbohydrate, fat and, protein metabolism. In the present study, the anti-diabetic efficacy of biosynthesized silver nanoparticles from marine brown seaweed *Colpomenia sinuosa* has been evaluated. The anti-diabetic effect of the silver nanoparticles biosynthesized from the *Colpomenia sinuosa* was assessed by inducing diabetes in the experimental Wistar albino rats through Alloxan monohydrate, a chemical that ultimately results in hyperglycemia (increase in the fasting blood sugar level) at a dosage of 50 mg/kg body weight given orally for about 28 days. The outcome of treatment with silver nanoparticles (50 mg/Kg i.p.) biosynthesized from *Colpomenia sinuosa*, were estimated using various biochemical parameters. The fasting blood glucose levels have reduced in the affected animals to near-normal levels. The retention of the level of the enzymes involved in diabetes, hematological analysis, decrease in the levels of total cholesterol, triglycerides, low-density lipoprotein, and phospholipids in the silver nanoparticles treated animals compared to the levels in normal control animals, exhibited significant anti-diabetic activity as compared to glipizide.

1. Introduction

Diabetes mellitus is a metabolic disorder characterized by a loss of glucose homeostasis by disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both [1, 2]. The WHO has estimated that 3% of the world's population have diabetes and the prevalence is expected to double by the year 2025 to 6.3% [3]. Management of diabetes without any side effects is still a challenge to the medical community. The use of the drugs is restricted by their pharmacokinetic properties, secondary failure rates, and accompanying side effects [4]. Thus, searching for a new class of compounds is essential to overcome diabetic problems and there is continuous research searching for alternative drugs [5, 6]. According to Sharma and Garg, 2009 [7], synthetic antioxidants for diabetes are suspected to be carcinogenic. As a result, there is a need to search for effective, safe, and better anti-diabetic agents. The brown and red seaweeds which contain α -glucosidase and α -amylase inhibitors have been reported to possess anti-diabetic effects [8, 9]. The unusual allenic bond and oxygenic functional group of fucoxanthin an accessory pigment of Marine brown seaweeds *Fucus*, *Dictyota*, and *Laminaria* reported to possess strong antidiabetic activity [10], the brown seaweeds belonging to the genus *Ecklonia* and family *Lessoniaceae* possess different phlorotannins such as eckol, dieckol, 6,6'-bieckol, phlorofucofuroeckol-A, phloroglucinol, and 7-phloroeckol which exhibit antidiabetic activities mainly through the inhibitory action of α -amylase and α -glucosidase enzymes [11]. The extracts from macroalgae such as *Rhodomela confervoides*, *Gracilaria textortii*, *Plocamium telfairiae*, *Ecklonia kurome*, *Dictyopteris divaricata*, *Ulva pertusa*, and *Enteromorpha intestinalis* reported for the strong inhibitory activity against alpha-glucosidase [12-14]. Thus the increase in the percentage of diabetes mellitus and the requirement for alternative medications is of immense importance; one promising approach is the use of silver nanoparticles by the green synthesis method which has long been used for dressing wounds, in creams and known to contain antibacterial activity with minimal toxicity can be used in medicine with low risk. Biosynthesis of silver nanoparticles from marine seaweeds and plants has been reported to exhibit anti-

diabetic activity [16-19]. In the present study, the assessment of silver nanoparticles biosynthesized from marine seaweed *Colpomenia sinuosa* were analyzed on alloxan stimulated hyperglycemic activity in Wistar albino rats *in vivo*.

2. Experimental Methods

2.1 Collection and Preparation of Seaweed Extract

The marine brown seaweed *Colpomenia sinuosa* (Mertens ex Roth) Derbes and Solier were collected from the intertidal regions of Leepuram, Kanyakumari District (Latitude 8°14'23.10" N, Longitude 77°20'04.02" E); South East Coast of Tamilnadu. Collected seaweed was washed with seawater for eliminating impurities such as sand, rocks, epiphytes, and epifauna. The washed samples were preserved with 5-10% formaldehyde in seawater and transported to the laboratory in a box containing slush ice. The fumes of the formaldehyde would help to fix and preserve the seaweed material. In the laboratory, the samples were washed thoroughly in running tap water to remove salt and washed three times using distilled water which may remove metallic compounds and it was shade dried at room temperature (37 °C) for 10 days. The dried seaweed materials were crushed by using mortar and pestle to get the powder form and it was stored in an air-tight container. About 1 g of crushed seaweed powder was added with 100 mL of distilled water in a 250 mL conical flask and boiled for 5-10 minutes at 60-80 °C. The crude extract was collected and stored at 4 °C for experimental use [20].

2.2 Biosynthesis of Silver Nanoparticles

The crude extract of the experimental marine brown seaweed *Colpomenia sinuosa* (Mertens ex Roth) Derbes and Solier was used for the synthesis of silver nanoparticles. Silver nitrate (AgNO_3) (SD fine) was used for the synthesis of silver nanoparticles and double-distilled, deionized water was used for all the experiments. The silver nanoparticle formation was carried out by taking 500 mg of dry, shade dried powder samples of *Colpomenia sinuosa* in a 250 mL Erlenmeyer flask with 10^{-3} M aqueous AgNO_3 solution and was incubated at room temperature. The pH was checked during the reaction, and it was found to be 5.09. Nearly 95% of bio reduction of AgNO_3 - ions occurred within 24 hrs at stirring conditions. The biosynthesis of silver nanoparticles was characterized by UV-Vis

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spectroscopy; size and morphology by employing SEM and TEM, structure from X-ray diffraction (XRD) technique, stability of silver nanoparticles from Thermogravimetric analysis (TGA), and biomolecules involved in the capping agent of silver nanoparticles from Fourier transform infrared (FT-IR) spectroscopy which was published earlier by our team [21].

2.3 Animal and Experimental Design

The Wistar strain of male albino rats weighing between 180–200 g was used for the present study. They were housed in micro nylon boxes in a controlled environment (25 °C and humidity 65–70%) and 12 hours dark/light cycles with a standard laboratory diet and water ad libitum. Animals were well acclimatized to the standard environmental conditions of temperature (22 ± 5 °C) and humidity (55 ± 5%) and 12 hrs light/dark cycles throughout the experimental period.

2.4 Chemical Induction of Diabetes Mellitus

Alloxan monohydrate was obtained from Sigma Aldrich S.D Fine. Chem. Ltd, Mumbai, India. Glipizide was obtained from Micro Labs, Hosur, India. Diabetes mellitus was induced in Wistar albino rats by a single intraperitoneal injection of a freshly prepared solution of Alloxan monohydrate (150 mg/kg body weight) in physiological saline after overnight fasting 12 hrs [22]. Alloxan is commonly used to produce diabetes mellitus in experimental animals due to its ability to destroy the β -cells of the pancreas possibly by generating excess reactive oxygen species such as H_2O_2 , O_2 , and OH. The development of hyperglycemia in rats is confirmed by plasma glucose estimation 72 hrs post alloxan injection. Rats with fasting plasma glucose levels of 200–260 mg/dL were used for this experiment. All animal experiments were carried out as per protocol (IAEC/KMCP/155/2014) approved by the Institutional Animal Ethics Committee

2.5 Anti-Diabetic Study Procedure

The experimental group comprised 24 rats (4 groups comprising 6 animals per group). Diabetes was induced by injecting alloxan monohydrate 3 days before the commencement of the experiment. The details of the 4 groups as follows:

Group-I: [Normal untreated control] consists of normal rats treated with normal saline orally at 10 ml/kg. Group-II: [Diabetic control] Diabetic control received 150 mg/kg of Alloxan monohydrate intraperitoneally (i.p). Group-III: [Positive control] Diabetic rats given Glipizide (10 mg/kg i.p) for 28 days orally. Group-IV: [Treatment group] Diabetic rats treated with silver nanoparticle biosynthesized from *Colpomenia sinuosa* at a dose of (50 mg/kg) daily using an intra-gastric tube for 28 days.

2.6 Sample Collection and Analysis

After 28 days of treatment, body weight, blood glucose, haemoglobin, glycosylated haemoglobin, plasma insulin, total cholesterol, triglycerides, HDL-cholesterol, and phospholipids were determined using standard procedures. Blood was collected from the eyes (venous pool) by sino-ocularpuncture [22] in EDTA coating plasma tubes for the estimation of blood parameters.

2.7 Statistical Analysis

All the experimental outcomes were expressed as the mean ± S.E.M. The biochemical parameters and hematological were subjected to statistical analysis by one-way Analysis of Variance to determine the significant difference between the groups was done with Graph pad Prism software. All Pairwise Multiple Comparison Procedures by Student-Newman-Keuls Method. If the statistical significant difference was $p < 0.05$ data accepted for assessment.

3. Results and Discussion

The antidiabetic activity of silver nanoparticles biosynthesized from the experimental marine seaweed *Colpomenia sinuosa* was analyzed through in vivo activity. The toxicity studies revealed the non-toxic nature of silver nanoparticles biosynthesized from *Colpomenia sinuosa*. There was no lethality or any toxic reactions found at the end of the study period.

3.1 Effect of Biosynthesized Silver Nanoparticles on Blood Glucose and Body Weight

The levels of initial and final blood glucose and change in body weight, in normal and treatment control animals in each group are given in Table 1. The mean body weight of diabetic rats (G₂) was 181 ± 4.65 g which significantly decreased as compared to normal control rats (G₁) (229 ± 8.15 g). The bodyweight of diabetic control rats treated with

biosynthesized nanoparticles from *Colpomenia sinuosa* (234 ± 8.20 g) (G₄) at a dose of 50 mg/kg significantly increased the body weight as compared to the normal control animals in 28 days.

The fasting blood glucose level was significantly ($p < 0.001$) increased 216.35 ± 6.55 mg/100 mL in diabetic animals which exhibited hyperglycemia and had a significant ($p < 0.0001$) increase in the blood glucose level as compared to normal animals (87.65 ± 3.62 mg/100 mL) (non-diabetic group). However, the level of fasting blood glucose, returned to the near normal range in diabetic rats treated with silver nanoparticles biosynthesized from *Colpomenia sinuosa* (130.40 ± 5.15 mg/100 mL) at a dose of 50 mg/kg. It was also observed that the mean in the normal group ($p < 0.001$) was nearly constant throughout the experiment and was significantly different from those Alloxan induced diabetic mice group (116.22 ± 4.50 mg/100 mL) (G₂). From the results, it is observed that the silver nanoparticles biosynthesized from the experimental marine seaweed *Colpomenia sinuosa* were able to restore the body weight, blood glucose level as well as glycogen content that of the normal group (G₁).

Table 1 Effect of silver nanoparticles biosynthesized from *Colpomenia sinuosa* on initial and final body weight and blood glucose in normal and treated animals

S. No.	Treatment	Body weight (g)		Blood glucose (mg/100mL)	
		Initial	Final	Initial	Final
1	Normal control (G ₁)	221±7.25	229±8.15	83.60±3.25	87.65±3.62
2	Diabetic control (G ₂)	213±6.85	181±4.65** ^(a)	85.75±3.85	216.35±6.55** ^(a)
3	Positive control (G ₃)	231±7.50	221±7.25	87.50±4.20	116.22±4.50** ^(b)
4	<i>C. sinuosa</i> control (G ₄)	221±7.30	234±8.20	83.78±3.58	130.40±5.15** ^(b)

G₃ - Positive control (Glipizide), G₄ - Treatment control (AgNPs from *Colpomenia sinuosa* at 50 mg/kg). Values are expressed as mean ± SEM. ** (a) Values are significantly different from normal control G₁ at $p < 0.001$. ** (b) Values are significantly different from Diabetic control G₂ at $p < 0.01$.

3.2 Effect of Biosynthesized Silver Nanoparticles on Total Haemoglobin, Haemoglobin and Plasma Insulin

The levels of total haemoglobin, glycosylated haemoglobin and plasma insulin in normal rat and treatment control animals in each group are given in Table 2. The level of total haemoglobin (12.85 ± 1.50 g/100 mL), plasma insulin (33.52 ± 2.85 µU/mL), and glycosylated haemoglobin (0.33 ± 0.06%) of normal animals were significantly low when compared to total haemoglobin (16.05 ± 0.65 g/100 mL), plasma insulin (53.65 ± 1.65 µU/mL) and glycosylated haemoglobin (0.97 ± 0.14%) of the Alloxan induced diabetic control rats. The Alloxan induced diabetic rats treated with biosynthesized silver nanoparticles from *Colpomenia sinuosa* at a dose of 50 mg/kg showed the level of total haemoglobin (11.32 ± 0.90 g/100 mL), glycosylated haemoglobin (0.48 ± 0.09%), and plasma insulin (24.65 ± 2.26 µU/mL), returned to near normal range which was comparable with the positive control Glipizide and the normal control of the Wistar rats.

Table 2 Effect of biosynthesized silver nanoparticles on haemoglobin, glycosylated haemoglobin, and plasma insulin in normal and treated animals

S.No.	Treatment	Haemoglobin (g/100 mL)	Glycosylated haemoglobin HbA ₁ (%)	Plasma insulin (µU/mL)
2	Diabetic control (G ₂)	16.05 ± 0.65** ^(a)	0.97 ± 0.14** ^(a)	53.65 ± 1.65** ^(a)
3	Positive control (G ₃)	12.05 ± 1.15** ^(b)	0.42 ± 0.07** ^(b)	28.50 ± 2.45** ^(b)
4	<i>C. sinuosa</i> (G ₄)	11.32 ± 0.90** ^(b)	0.48 ± 0.09** ^(b)	24.65 ± 2.26** ^(b)

G₃ - Positive control (Glipizide), G₄ - Treatment control (AgNPs from *Colpomenia sinuosa* at 50 mg/kg). Values are expressed as mean ± SEM. ** (a) Values are significantly different from normal control G₁ at $p < 0.001$. ** (b) Values are significantly different from Diabetic control G₂ at $p < 0.01$.

3.3 Effect of Silver Nanoparticles on Serum Total Cholesterol (TC), Triglycerides (TG), High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL), and Phospholipids

The level of serum total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL-C), low-density lipoprotein (LDL), and phospholipids of normal and experimental animals in each group are shown in Table 3. Total cholesterol (218.22 ± 6.82 mg/dL), triglycerides (155.62 ± 4.55 mg/dL), phospholipids (205.27 ± 6.30 mg/dL), and low-density lipoprotein (37.97 ± 2.30 mg/dL) (LDL) levels were higher, whereas high-density lipoprotein (HDL-C) level was observed to be low (30.55 ± 1.22 mg/dL) in Alloxan induced diabetic rats as compared to normal rats. The treatment of normal and alloxan-induced diabetic rats with silver nanoparticles biosynthesized from *Colpomenia sinuosa* at a dose of 50 mg/kg for 28 days resulted in low levels of total cholesterol, triglycerides, low-density lipoprotein (LDL), and phospholipids level, whereas, increase in HDL-C level was observed when compared to Alloxan induced diabetic rats.

Table 3 Serum lipids of normal and biosynthesized silver nanoparticles of experimental groups

S.No	Control	Total Cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL-C (mg/dL)	Phospho-lipids (mg/dL)	LDL (mg/dL)
1	G1	82.92 ± 2.52	86.50 ± 2.45	48.29 ± 1.75	120.66 ± 2.40	15.36 ± 1.45
2	G2	218.22 ± 6.82** ^(a)	155.62 ± 4.55** ^(a)	30.55 ± 1.22** ^(a)	205.27 ± 6.30** ^(a)	37.97 ± 2.30** ^(a)
3	G3	108.86 ± 3.25** ^(b)	92.81 ± 2.50** ^(b)	43.91 ± 1.40	145.43 ± 3.90	20.26 ± 1.85** ^(b)
4	G4	122.62 ± 3.56** ^(b)	105.76 ± 2.90** ^(b)	39.44 ± 1.35** ^(b)	160.66 ± 4.05** ^(b)	28.43 ± 1.90** ^(b)

G₁ – Normal Control, G₂ – Diabetic Control, G₃ – Positive control (Glipizide), G₄ – Treatment control (AgNps from *Colpomenia sinuosa* at 50 mg/kg). Values are expressed as mean ± SEM. ** (a) Values are significantly different from normal control G₁ at p < 0.001. ** (b) Values are significantly different from Diabetic control G₂ at p < 0.01.

3.4 Histopathological Studies of Pancreas

Histopathological studies revealed Islets of Langerhans of normal cell population in the pancreas of the non-diabetic Wistar rats. Extensive damage was observed and the reduction in the number of cells of Islets of Langerhans was seen in the pancreas of the diabetic rats due to the induction of Alloxan monohydrate. Severe swelling of cells was also observed in the pancreas of the diabetic Wistar rats. The restoration of the normal cellular population in the size of Islets of Langerhans was observed with hyperplasia due to the induction of Glipizide, where the cells were mildly swelled. The partial restoration of normal cellular population and moderately enlarged size of β-cells with hyperplasia were observed when treated with AgNPs biosynthesized from *Colpomenia sinuosa* dose of 50 mg/kg (Fig. 1).

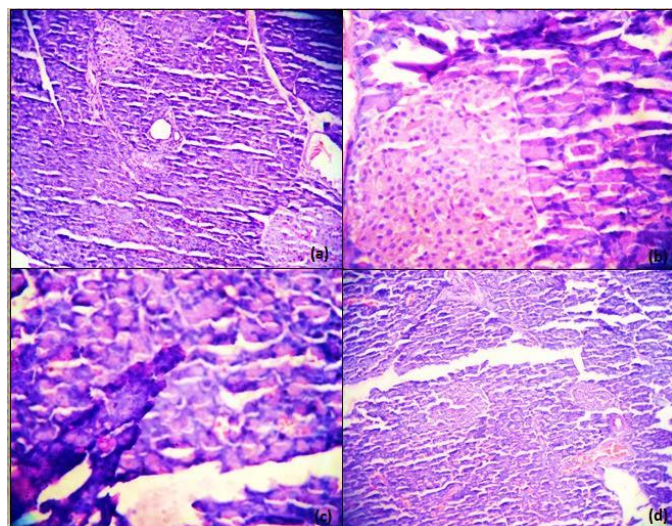


Fig. 1 Cross-section of pancreas stained with Haematoxylin and Eosin (Magnification 400 ×). A) – Normal Control, b) – Diabetic Control, c) – Positive control (Glipizide) and d) – Treatment control (AgNps from *Colpomenia sinuosa* at 50 mg/kg)

In the present study, an attempt has been made as an introductory approach to evaluate the biosynthesized silver nanoparticles from *Colpomenia sinuosa* and to analyze them for their in vivo antidiabetic activity. The various parameters like body weight, blood glucose, haemoglobin, glycosylated haemoglobin, plasma insulin, and serum lipids were investigated in Alloxan induced Wistar rats at 50 mg/kg body weight, which were compared with the positive control Glipizide induced rats, normal control rats, and rats treated with biosynthesized silver nanoparticles from the experimental seaweed.

The bodyweight of Alloxan-induced diabetic Wistar rats decreased significantly as compared to the positive control Glipizide treated and normal control rats, whereas, the blood glucose levels of Alloxan-induced diabetic rats were observed at high levels as compared to the blood glucose level of Glipizide treated and normal control rats. The retention of body weight and blood glucose to normal level was observed in rats treated with silver nanoparticles biosynthesized from experimental seaweed *Colpomenia sinuosa* which were more or less similar to that of the positive control Glipizide treated rats and normal control rats [35–37].

The haemoglobin, glycosylated haemoglobin and plasma insulin levels of Alloxan induced rats were observed at high levels as compared to that of the positive control Glipizide treated and normal rats. The retention of normalcy in the level of haemoglobin, glycosylated haemoglobin, and plasma insulin was observed in rats treated with biosynthesized silver nanoparticles of the experimental seaweed, which were more or less in comparison with the positive and the normal control rats. In uncontrolled

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or poorly controlled diabetes, increased glycosylation of several proteins including haemoglobin and glycosylated haemoglobin (HbA1C) was observed in patients with type II diabetes mellitus [38, 39]. The excess of glucose present in the blood reacts with haemoglobin to form glycosylated haemoglobin during type II diabetes and is proportional to the fasting blood glucose level [40, 41]. Hence, the estimation of glycosylation of haemoglobin proves to be a well-established parameter in the management and prognosis of the disease [18, 42, 43].

The characteristic features of diabetic dyslipidemia occur due to high plasma triglyceride, low HDL cholesterol and an increased LDL-cholesterol concentration in the serum. Excessive glucose utilization causes hyperglycemia and mobilization of fatty acids from adipose tissues. The lipid changes associated with diabetes mellitus may be attributed to the increase in the flux of free fatty acids into the liver which results in excess fatty acid accumulation in the liver and gets converted to triglycerides, resulting in impaired insulin, fatty acid release, and hepatic VLDL (Very low-density lipoprotein) cholesterol production risking the elevation coronary heart disease (CHD). The lower level of serum lipid concentration in the diet or drug therapy acts as a prognostic measure [44–46].

The high level of serum lipids like total cholesterol (TC), triglycerides (TGL), High-density lipoprotein-C (HDL-C), low-density lipoprotein (LDL) and phospholipids in the Alloxan induced Wistar rats was observed as compared to the positive control (Glipizide treated) and normal control rats. The retention of the normalcy of the serum lipids was observed in Wistar rats treated with biosynthesized silver nanoparticles from experimental seaweed *Colpomenia sinuosa* which were more or less comparable to the values of positive and normal control rats. The abnormal levels of serum lipids in diabetic patients may be due to increased mobilization of free fatty acids from the peripheral fat depots since insulin inhibits the hormone-sensitive lipase. However, glucagon, catecholamines, and other hormones in diet and the human metabolism, enhances lipolysis [18, 47, 48]. The section of the pancreas from the Islets of Langerhans of male Wistar rats appears to be normal along with the cells of islets, whereas, the swelling of a section of the pancreas and a decrease in the volume of cells was observed in Alloxan induced Wistar rats. The retention of normal cell volume with minimal swelling of the section of the pancreas in rats treated with biosynthesized nanoparticles from the experimental seaweeds was compared to the positive control Glipizide rats at 10 mg/kg body weight [18].

4. Conclusion

The results of present work reveals that the biosynthesized silver nanoparticles from brown seaweed *Colpomenia sinuosa* exhibited good anti-hyperglycemic activity in Wistar albino diabetic rats. The enhancement in body weight, hematological analysis, and decreased levels of the total cholesterol, triglycerides, low-density lipoprotein, and phospholipids along with regeneration of β-cells of the pancreas in the treatment group may have a possible effect and value in diabetes treatment. The histopathological results showed moderate swelling and a decrease in the number of islets cells which shows a good sign of the study undertaken.

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