

Pharmacological Activity of Biosynthesized Gold Nanoparticles from Brown Algae- Seaweed *Turbinaria conoides*

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Abstract

The purpose of *in vitro* testing in the present study is to demonstrate the anti-diabetic activity and anti-inflammatory activity of biosynthesized gold nanoparticle (AuNp) from *Turbinaria conoides*. The anti-diabetic activity by different *in vitro* methods method, anti-inflammatory activity by HRBC membrane stabilization assay and anticoagulant activity by calculating prothrombin time was carried out in the concentration range of (50 µl/ml, 100 µl/ml and 200 µl/ml) respectively. The inhibition rate of gold nanoparticle from *Turbinaria conoides* exhibited significantly maximum activity of 57.3% at concentration of 200 µg/ml and minimum level inhibition of 42.9% in 50 µl when compared with the standard drug Glinil that showed inhibition of maximum 85.6% and minimum 77.1%.

Keywords

Turbinaria conoides, Gold nanoparticle, Hypoglycemic activity, Anti-inflammatory activity

Introduction

Diabetes mellitus is a chronic hyperglycemic metabolic disorder. According to World Health Organisation (WHO) the number of diabetes cases was increasing gradually worldwide. Type 2 diabetes mellitus is mainly due to insulin resistance or insulin deficiency. Due to its severe complications it leads to nephropathy, cardio vascular disease, neuropathy, myopathy, ketoacidosis, etc. There are more medicines available in the market for the treatment of diabetes mellitus, but none of the drug is completely effective and safe. Opioids drug and Non-Steroidal Anti-inflammatory Drugs (NSAIDS) due to its side effects and potency are not used for any inflammatory diseases [1]. Haemostasis is the process of bleeding arrest due to vasoconstriction, abnormal obstruction and helps for wound healing. In the adult cardiovascular disorders including coronary thrombosis, arteriosclerosis, cerebral haemorrhage constitutes a serious medical issues. Some NSAIDS drugs such as aspirin and indomethacin are used as antithrombotic agents. Many researchers are in search of new drug development for the treatment of diabetes and associated inflammatory disorders. Among natural resources marine flora has amazing scope for discovery of new drugs [2].

Marine organisms offer a great opportunity to provide rich nutritional supplements for controlling diabetes. It has been reported that incorporation of seaweeds into a balanced diet helps to minimizing of diabetes nearly 10-15% in western countries comparing to India. Marine brown algae *Turbinaria conoides* belongs to the group of Sargassaceae. *Turbinaria conoides* helps to cure children's

fever, antibacterial and pesticide. *Turbinaria conoides* rich in bioactive compounds such as flavonoids, reducing sugar, steroids, phenols, sulfated polysaccharides includes neutral glucan, guluronic, alginic acid, minerals, and unsaturated fatty acids [3].

Nanomedicine makes vital impact in treating various deadly diseases. Many pharmacological industries paying attention towards nanotechnology based drug development in the last few years. Among other nanoparticles, gold nanoparticles received a great attention on anti-inflammatory activity which subsequently inhibits expression of various inflammatory reactions. The gold nanoparticles induce the endothelial cells which provide therapeutic response for chronic inflammation disease, towards antioxidative effect. Therefore the biologically synthesized gold nanoparticles from *Turbinaria conoides* have been initiated against anti-diabetic, anti-inflammatory activity and anticoagulant activity through *in vitro* studies [4].

Materials and Methods

Preparation of extract

The leaves of *Turbinaria conoides* were collected from Mandapam coast region (Figure 1). 5 gm of leaves were weighed and washed a few times with double distilled water to wipe out the waste and dirt materials. The leaves were cut into fine pieces and were boiled in an Erlenmeyer flask with 100 ml of sterile double distilled water for 15 min, then the algae extract broth was filtered through Whatman No. 1 filter paper and stored at 4 °C for further analysis and used within seven days.



Figure 1: *Turbinaria conoides*.

Biosynthesis and characterization of gold nanoparticles

10 ml of algae extract and 100 ml of 1 mM aqueous solution of gold chloride were mixed together and kept at 37 °C. The reductions of gold ions to nanoparticles were confirmed by change in color into pinkish ruby red of the surrounding medium which was detected visually. The size and shape of the algae mediated synthesized gold nanoparticles was determined by Scanning Electron Microscope (Hitachi, Model: S- 3400N).

In vitro anti-diabetic activity

Blood sample collection

The 5 ml of blood sample was collected from diabetic patients and pinch amount of EDTA was added, which was centrifuged at 5,000 rpm for 5 min. Then, the pellet was discarded and supernatant of haemoglobin was collected and stored in vials. Using the supernatant, *in vitro* anti-diabetic activity was done by following methods.

Non-enzymatic glycosylation of hemoglobin method

The biosynthesized gold nanoparticle from *Turbinaria conoides* was investigated for anti-diabetic activity by calculating the degree of non-enzymatic haemoglobin glycosylation, which was measured at 520 nm [5]. In phosphate buffer (0.01 M, pH 7.4), glucose (2%), haemoglobin (0.06%) and gentamycin (0.02%) solutions were prepared and 1 ml each of above solution was mixed. Gold nanoparticle of *T. conoides* was weighed and dissolved in DMSO to gain stock solution and then 50, 100 and 200 µg/ml solutions were prepared. 1 ml of each concentration was added to above mixture. The mixture was incubated in dark condition at room temperature for 72 hrs. The degree of glycosylation of haemoglobin was read colorimetrically at 520 nm. Metformin was used as a standard drug.

Percentage of inhibition was calculated as follows:

$$\% \text{ inhibition} = \frac{As - Ac}{As} \times 100$$

Ac is Absorbance of Control, As is Absorbance of Sample

Glucose uptake in yeast cell method

Yeast cells preparation was followed by the standard procedure [6]. The commercial baker's yeast was washed by repeated centrifugation (3,000 × g; 5 min) with distilled water until the supernatant fluids were clear and a 10% (v/v) suspension was prepared in distilled water. To 1 ml of glucose solution (5, 10 and 25 mM), the various concentrations of AuNP (50-200 µg) were added and incubated at 37 °C for 10 mins. Then added 100 µl of yeast suspension reaction, vortexed and further incubated for 1 hour at 37 °C. After 60 minutes, the tubes were centrifuged at 2,500 × g, for 5 min and from the supernatant glucose were estimated. Glinose was used as standard drug.

The increased glucose uptake by yeast cells percentage was determined by the formula-

$$\text{Increase in glucose uptake (\%)} = \frac{\text{O.D of the sample} - \text{O.D of the control}}{\text{O.D of the sample}} \times 100$$

Where, O.D of the control is the absorbance of the control reaction (containing all reagents except the test sample), and O.D of the sample is the absorbance of the test sample. All the experiments were carried out in thrice.

α-Amylase inhibition method

An enzyme α-amylase hydrolyses alpha-bonds of large alpha connected polysaccharide to yield glucose and maltose [7]. In this

method, we have used 1 ml of substrate-potato starch (1% w/v) and 1 ml of test solution (Glinil drug/AuNP) of 3 different concentrations such as 50, 100 and 200 µg/ml. To, 1 ml of α-amylase enzyme (1% w/v), 2 ml of acetate buffer (0.1 M, 7.2 pH) was added. The mixture was incubated for 60 minutes and finally 0.1 ml of iodine-iodide which acts as an indicator was added (635 mg iodine and 1 gm potassium iodide dissolved in 250 ml distilled water) in the mixture. The absorbance was read at 565 nm under UV-Visible spectroscopy.

$$\text{Inhibition of } \alpha\text{-amylase (\%)} = \frac{\text{O.D of the sample} - \text{O.D of the control}}{\text{O.D of the sample}} \times 100$$

Where, O.D of the control is the absorbance of the control reaction (containing all reagents except the test sample), and O.D of the sample is the absorbance of the test sample. All the experiments were carried out in thrice.

In vitro anti-inflammatory assay

In this study *in vitro* anti-inflammatory assay was carried out using the human red blood cell (HRBC) membrane stabilizing assay. Fresh blood was collected from healthy human volunteer who was not taken any NSAIDS for 2 weeks prior to the experiment. Equal volume of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) was mixed to the collected blood sample and centrifuged at 3,000 rpm. After centrifugation packed cells were washed with isosaline and made upto 10% suspension. The synthesized gold nanoparticles of *Turbinaria conoides* 1 mg was macerated with 10 ml of hyposaline (0.36% NaCl) as a drug, and centrifuged at 3000 rpm. Different concentrations of drugs in the range of 50, 100 and 200 µg/ml were prepared using distilled water. 1 ml of phosphate buffer, 2 ml hyposaline and 0.5 ml of HRBC suspension were added to each above concentrations and simultaneously test solutions was also added and incubated at 37 °C for 30 min which was centrifuged at 3,000 rpm for 20 min [8]. Finally, hemoglobin content in the supernatant solution was recorded at 560 nm spectrophotometrically for all the concentrations. The Hydrocortisone drug was used as standard.

In vitro anticoagulant activity

In this method 0.2 ml of plasma was taken and 0.1 ml of test sample of different concentration and different volume of CaCl₂ (25 mM) were added together in a clean fusion tube and incubated in water bath at 37 °C for 15 mins. EDTA & Sodium citrate were taken as standard. The test solution was replaced by same volume of 0.9% saline which act as a control. For every 5 seconds the clotting time was recorded with stopwatch by tilting the tubes for determination of prothrombin time [9, 10].

Statistical Analysis

All the results were carried out in triplicates and data were examined by ANOVA followed by Duncan's multiple comparisons test for significant differences using SPSS 14.0 software. Values were considered significant at $p \leq 0.05$. Graphs were plotted using Origin 8.1 software.

Result and Discussion

The gold nanoparticles was generally used for various biological and medicinal applications due to its size, biocompatible nature and targeting biomolecule. The aim of the present study is to focus the synthesis of gold nanoparticle from *Turbinaria conoides* and to study the *in vitro* anti-diabetic activity by 3 various methods such as non-enzymatic glycosylation of haemoglobin, glucose uptake in yeast cells and α-amylase inhibition method and also *in vitro* anti-inflammatory activity and anticoagulant activity by standard methods.

In vitro anti-diabetic activity

In the present study *in vitro* anti-diabetic assay by non-enzymatic glycosylation of haemoglobin of gold nanoparticle of *Turbinaria conoides* at different concentration (50, 100 and 200 µg/ml) respectively was evaluated. The concentration of 200 µg/ml gave the maximum inhibition level (65.3%) and minimum level of inhibition was observed in 50 µg/ml concentration (44.5%). The standard drug of metformin inhibition was performed the maximum rate of inhibition 87% in 200 µg/ml and minimum rate inhibition 68% in 50 µg/ml concentration respectively (Table 1 and Figure 2).

Table 1: *In vitro* anti-diabetic activity by non-enzymatic glycosylation of hemoglobin method.

Sample	Concentrations (µg/ml)			
	Abs	50 µg/ml	100 µg/ml	200 µg/ml
Blank		% of inhibition	% of inhibition	% of inhibition
Standard (Metformin)	0.080 ± 0.006	68.4	82.6	87.5
Gold nanoparticle		44.5	60.2	65.3

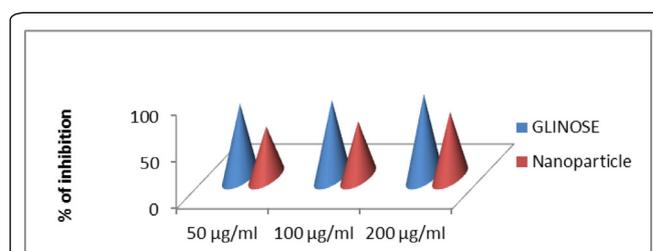


Figure 2: *In vitro* anti-diabetic activity by non-enzymatic glycosylation of hemoglobin method.

The *in vitro* anti-diabetic activity by glucose uptake in yeast cells of gold nanoparticle of *Turbinaria conoides* at different concentration (50, 100 and 200 µg/ml) respectively was evaluated. The result suggests that the gold nanoparticle of *Turbinaria conoides* exhibited significantly maximum level inhibition was recorded 69.7% in 200 µg/ml and minimum level of inhibition 54.1% in 50 µg/ml. In standard drug of Glinose the rate of glucose inhibition of maximum 88.9% is observed in 200 µg/ml. In yeast cells system, the rate of glucose transport across cell membrane was noted. The amount of glucose remaining in the medium after a specific time serves

as an indicator of the glucose uptake by the yeast cells [11, 12]. The rate of uptake of glucose into yeast cells was linear in all the glucose concentrations (Table 2 and Figure 3).

Table 2: Percentage of inhibition for glucose uptake in yeast cells.

Sample	Concentrations (µg/ml)			
	Abs	50 µg/ml	100 µg/ml	200 µg/ml
Blank	0.141 ± 0.016	% of inhibition	% of inhibition	% of inhibition
Standard (Glinose)		78.7	82.5	88.9
Biosynthesis gold nanoparticle		54.1	59.4	69.7

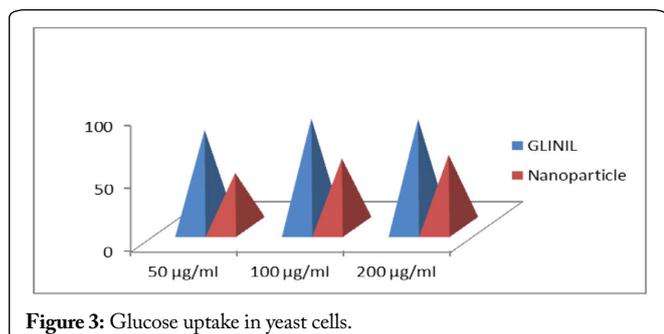


Figure 3: Glucose uptake in yeast cells.

The α -amylase inhibition method are carried out in gold nanoparticle of *Turbinaria conoides* in the above concentration respectively. The inhibition rate in gold nanoparticle of *Turbinaria conoides* exhibited significantly maximum level activity 57.3% in 200 µg/ml and minimum level of inhibition 42.9% in 50 µg/ml. In standard drug of Glinil the rate of glucose inhibition maximum level 85.6% and minimum level 77.1% (Table 3 and Figure 4). Because of significant α -amylase inhibition, these vegetables consumed regularly by rural people in places where insulin is not available, these plants may offer some control against diabetes.

Table 3: % of inhibition for α -amylase inhibition assay.

Sample	Concentrations (µg/ml)			
	Abs	50 µg/ml	100 µg/ml	200 µg/ml
Blank	0.136 ± 0.020	% of inhibition	% of inhibition	% of inhibition
Standard (Glinil)		77.1	86.2	85.6
Nanoparticle		42.9	54.4	57.3

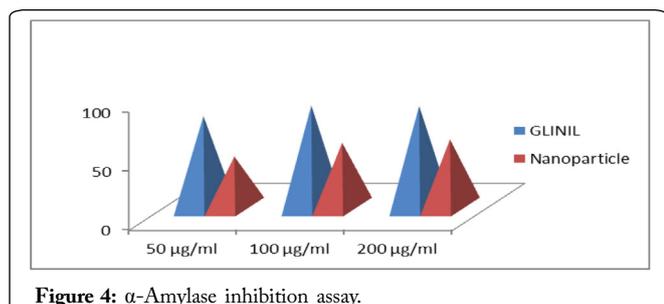


Figure 4: α -Amylase inhibition assay.

In vitro anti-inflammatory and anticoagulant activity

The *in vitro* anti-inflammatory activity by HRBC membrane stabilization method was studied for the synthesized gold nanoparticle of *Turbinaria conoides*. In HRBC method, nanoparticle at a concentration 200 µg/ml showed maximum percentage of inhibition (73.31 ± 0.89%) in hypotonic solution and minimum protection was observed at 50 µg/ml (60.33 ± 0.72%). All the results were matched with standard hydrocortisone which showed above 81% to 88% protection (Table 4 and Figure 5).

Table 4: *In vitro* anti-inflammatory activity by HRBC membrane method.

Con. µg/ml	Prevention of lysis %	
	Hydrocortisone (Std drug)	Gold Nanoparticle
50	81.41 ± 0.28	60.33 ± 0.72
100	83.05 ± 0.25	71.38 ± 0.67
200	88.26 ± 0.54	73.31 ± 0.89

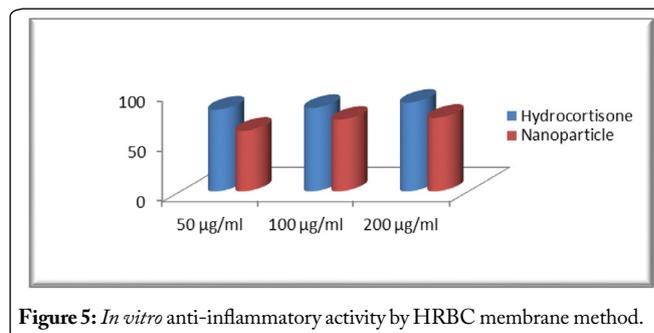


Figure 5: *In vitro* anti-inflammatory activity by HRBC membrane method.

The *in vitro* anticoagulant activity by plasma recalcification method was carried out. The test samples showed significant anticoagulant activity based on the concentration dependent manner [13, 14]. The standard drug EDTA and Sodium citrate showed very good anticoagulant activities for more than one hour (Table 5).

Based on the above results, it was concluded that the gold nanoparticle of *Turbinaria conoides* has significant anti-inflammatory activity (membrane stabilization property), anti-diabetic activity (Non-enzymatic glycosylation hemoglobin, glucose uptake in yeast cells and α -amylase inhibition methods) and anticoagulant activity. Hence, based on further pharmacological and biochemical investigations the mechanism of action will be clearly elucidated and helpful in exploring seaweeds such as *Turbinaria conoides* as a therapeutic use in anti-inflammatory, anti-diabetic and anticoagulation research [15, 16].

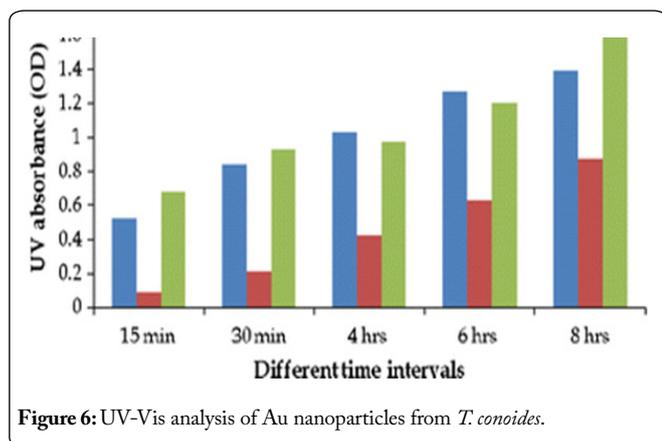
Characterization of biosynthesised gold nanoparticles from *T. conoides*

UV-Visible spectra recorded at different time intermissions for the reaction with aqueous chloroauric acid solution displayed an initial increase in the absorbance, which decreased with higher incubation period and became constant

Table 5: Effect of nanoparticle on prothrombin time of normal human plasma.

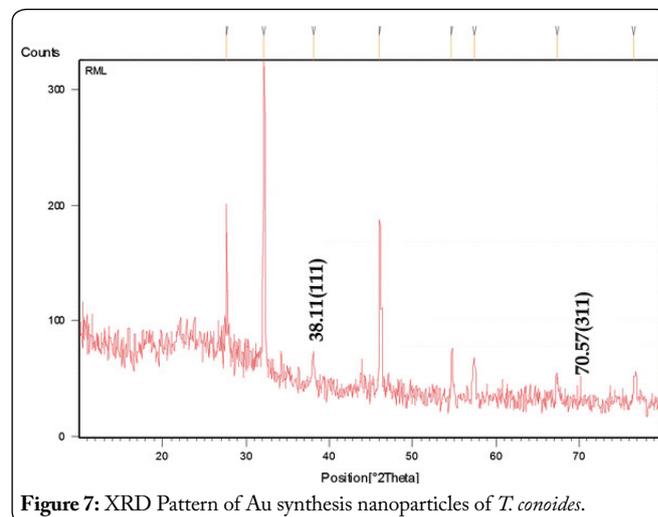
Test Samples	Concentrations (in ppm)	Prothrombin Time (in minutes)
EDTA	500	1:22:09
	1000	1:35:26
	1500	1:29:31
Sodium citrate	500	1:21:16
	1000	1:46:28
	1500	1:55:34
Isosaline (control)	500	6:43
	1000	2:45:39
	1500	2:38:48
Gold nanoparticle	500	23:45
	1000	45:42:00
	1500	30:27:00

giving a maximum absorbance at 599.78 nm after three hours of incubation. The formation of gold nanoparticles in the reaction mixture was confirmed by the formation of color and efficient reduction of the Au^{3+} to Au^0 . The formation of AuNPs was confirmed by colored solution which measures the absorbance against distinct wavelength [17, 18]. The rate of AuNPs formation the kinetics of the reaction with respect to time was studied with the help of UV-Vis spectroscopy. The slow reduction of Au^{3+} to Au^0 was detected using a gradual increase in the intensity of absorbance band without any shift with increasing time from spectra. No significant change in the intensity from spectra also proposes that the reduction is going over upto 8 hrs (Figure 6).

**Figure 6:** UV-Vis analysis of Au nanoparticles from *T. conoides*.

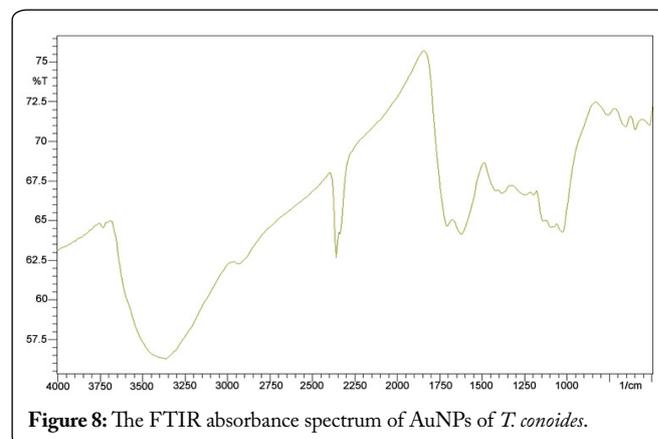
The suspension of Au nanoparticles was dried inside a vacuum chamber for 24 hours so that a small amount of dry Au nanoparticles can be obtained for X-ray diffraction (XRD) analysis. The XRD curve established that the nanoparticles are nothing but Au. Elucidation of this XRD pattern exposes the existence of diffraction lines at low angles (5° to 75°). From 20° to 80° at 2 theta angles the diffracted intensities have been recorded. Figure 7 represents the diffracted pattern which are significantly resembles to pure AuNPs. Due to the spherical

structure of nanoparticles, in the spectra clear peaks are not detected. Broadening peak and noise were related to the effect of the nano particles by SEM analysis and also the occurrence of numerous crystalline biological molecules in the plants extracts that acts as reducing agent. The present of material in nano range was determined as the width of the peak increases size of particle size decreases.

**Figure 7:** XRD Pattern of Au synthesis nanoparticles of *T. conoides*.

The FTIR spectra of AuNPs with absorption peaks at 3432, 1631, 1599, 1384, 1351, 1030 and 760 were detected (Figure 8). The spectra obtained to characterize the interaction between HAuCl_4 and plant extract showed strong peak at 3432.0 that corresponds to the presence of OH group (stretch H bonded, strong broad) along with the above mentioned peaks. Many peaks at 2959, 2925, 2804, 2718, and 2649 were also detected which correspond to C-H group (stretch strong), C-H group (variable), C-O group (strong), =C-H group (strong), C=C group (variable). The reducing property of the extract is based on the OH group which gain highest absorption peak at 3432.

The SEM images of gold nanoparticles were shown in figure 9. SEM images of gold nanoparticles clearly explain the morphology and size of the nanoparticles within the range of $< 1 \mu\text{m}$, with spherical and cubic shape.

**Figure 8:** The FTIR absorbance spectrum of AuNPs of *T. conoides*.

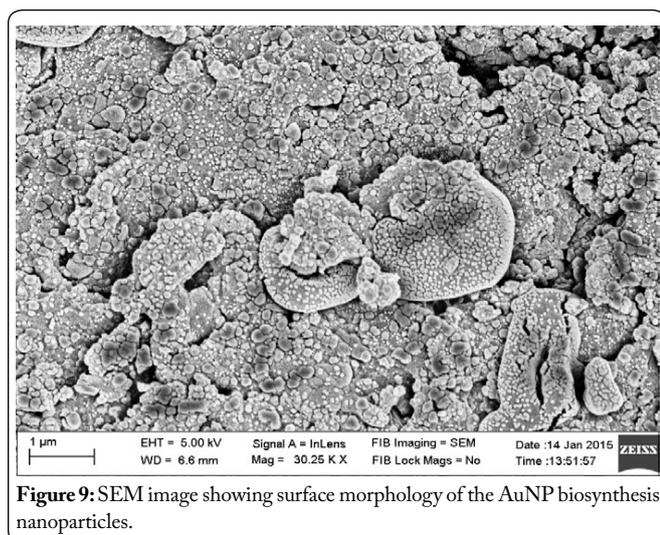


Figure 9: SEM image showing surface morphology of the AuNP biosynthesis nanoparticles.

Conclusion

In this present study we evaluated the anti-diabetic, anti-inflammatory and anticoagulant activity of biosynthesised gold nanoparticles. The anti-diabetic action of isolated gold nanoparticle from *Turbinaria conoides* can also be attributed due to the presence of polyphenolic compounds which acts as reducing agent in the formation of gold nanoparticles. *Turbinaria conoides* plays potentially important role in managing diabetes via the inhibition of α -amylase and non-enzymatic glycosylation of haemoglobin. It was suggested that further studies are required to clarify the mechanism of anti-diabetic and anti-inflammatory activity potential.

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