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RESEARCH ARTICLE

ANTIOXIDANT AND CYTOTOXIC ACTIVITY OF CHITOSAN MEDIATED NANOPARTICLES SYNTHESIZED FROM *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

In this study, chitosan nanoparticles were synthesized from chitosan polymer by ionic gelation method. The chitin was first extracted from *Saccharomyces cerevisiae* and then deacetylated to chitosan. The presence and characterization of chitosan nanoparticles was investigated by UV-Visible Spectroscopy (UV) and scanning electron microscopy (SEM). The antioxidant property of these chitosan nanoparticles were studied and compare with that chitosan polymer. CSNPs showed higher antioxidant activity than chitosan. The cytotoxicity of this chitosan nanoparticles against CHO cell line were studied which showing very low toxic. The antioxidant property was studied by radical scavenging (DPPH) assay and cytotoxic activity was evaluated against A-431 osteosarcoma cell line by MTT assay. The characteristics of the synthesized nanoparticles suggest their application as a potential Antioxidant and Cytotoxic activity.

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INTRODUCTION

Nanoparticles exhibit many unique electronic, catalytic, optical, and other physical and chemical properties that their bulk counterparts do not have (Sun Y, 2013). In recent years, bio- or green-synthesis of nanoparticles has become an emerging science (Krishnaraj *et al.,* 2010 and Zargar *et al.,* 2011). The fabrication of silver nanoparticles has been widely studied, and their stability and dispersion are crucial. Stabilizers such as surfactants, micelles, ligands, and polymers have been widely utilized in the literature (Ahmed *et al.,* 2006; Zou *et al.,* 2006 Pinto *et al.,* 2010 and Baruah *et al.,* 2013) Recently, incorporating metal nanoparticles into polymers has been proposed. Immobilizing metal nanoparticles on matrix polymers called nanometal polymer hybrids efficiently prevents nanoparticles' aggregation (Prozorova *et al.,* 2014). Polymers can act as matrix materials to help metal nanoparticles' growth control and stabilization (Laudenslager *et al.,* 2008). Various polymers have been employed for the synthesis of silver nanoparticles–polymer composites (Ahmad *et al.,* 2009). In addition, polymers [eg, chitosan, gelatin, (Regiel *et al.,* 2013; Bin *et al.,* 2011 and Fouda *et al.,* 2013). liposome, (Barani *et al.,* 2010) poly (lactic acid), (Fortunati *et al.,* 2012) polymethylmethacrylate, (Muzalev *et al.,* 2012) poly (vinyl alcohol), (Pencheva *et al.,* 2012) polyvinylpyrrolidone, (Xu *et al.,* 2008 and Slistan-Grijalva *et al.,* 2008) can be used as reducing or stabilizing agents for the synthesis of silver nanoparticles, without other reducing agents (Wei *et al.,* 2009 and Tran *et al.,* 2010) Chitin is a characteristic compound found in fungi and some animals. In animals, chitin mainly exists in the shells of crustaceans and mollusks, in the backbone of squids and in the cuticle of insects.Chitin is apolysaccharide, made of N-acetyl-D-glucosamine units connected by β (1→4) linkage. When the acetyl-D-glucosamine units in chitin lose acetyl groups, the molecule is called chitosan. Chitosan is widely recognized for its potent antimicrobial activity with, broad spectrum, and high killing rate but low toxicity toward mammalian cells. Chitosan acts as water binding agent and inhibits various enzymes. It inhibits bacterial activity by inhibiting RNA and protein synthesis (El-Diasty, *et al.,* 2012). Various factors play role in antimicrobial activity of chitosan. Till date, no report has present about biosynthesis of NPs utilizing Chitason of Yeast and their Antioxidant and Cytotoxic activity. The resulted green-synthesized nanoparticles were examined by ultraviolet-visible spectroscopy (UV-Vis), Transmission Electron microscopy (TEM), Fourier transform infrared (FTIR) spectroscopy, and dynamic light scattering spectroscopy (DLS) to determine their size and charge. The Antioxidant and cytotoxic activities of silver nanoparticles were also evaluated.

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MATERIALS AND METHODS

Materials

Baker's yeast (*Saccharomyces cerevisiae*) were purchased from local market (Mumbai, India). DMSO, ammonium molybdate, MTT, RPMI were purchased from Hi Media (Mumbai, India). Chinese Hamster ovary cells were purchased from Ruparel College, Biotech department (Mumbai, India). All other chemicals and medium used were of analytical grade.

Isolation of chitosan from yeast cells

Dry yeasts were activated by adding them into warm water for 5 mins. The froth indicated that yeast cells were in active condition. An active yeast cells were suspended in 1 mol l M NaOH solution (1 : 30 w/v) and autoclaved at 121°Cfor 15 min. Alkali-insoluble fractions were collected after centrifugation at 12 000 g for 15 min, washed with distilled water and recentrifuge to a neutral pH (pH 7). Further extracted the residues using 2% acetic acid (1 : 40 w/v) at 95°C for 8 h. Centrifuged the extract slurry at 12 000 g for 15 min and insoluble acid were discarded. The pH of supernatant fluid were adjusted to 10 with 2 M NaOH, the solution was centrifuged at 12 000 g for 15 min and washed the precipitated chitosan with distilled water, 95% ethanol (1: 20 w/v) and acetone (1: 20 w/v), respectively and dried at 60°C to a constant weight.

Determination of chitosan percentage yield

The percentage yield of chitin was determined by taking the dry weight of *Saccharomyces cerevisiae* before treatment and the dry weight of prepared chitosan in percentage. The percentage yield was calculated from the weight of chitosan obtained as a percentage of chitin before deacetylation.

Synthesis of Chitosan Nanoparticles

Chitosan solution was prepared of 2.5 mg/ml by dissolving the polymer in 1% (w/v) acetic acid aqueous solution for 0.5 hrs under magnetic stirring. The pH of solution was adjusted to 5.0-6.0 using 1 mol/L NAOH. Chitosan solution was stirred for 0.5 hr at room temperature. Finally, dissolved sodium tripolyphosphate (TPP), the counter ion in pure water to prepare a 1mg/ml solution, added in to the chitosan solution under mild magnetic stirring to form chitosan nanoparticles. Centrifuged the nanoparticles solution at 18000 rpm and 4°C for 30 minutes, after which the nanoparticles were collected at the bottom, extensively washed 3 times with water to remove the TPP and the acetic acid, and finally lyophilized and Stored at 4°C- 8°C (Yu-Lan *et al*., 2011).

Characterization of Chitosan Nanoparticles

Chitosan nanoparticles were characterized by SEM (Scanning Electron Microscopy) by Philips XLD 3D model, CIRCOT, Matunga East, Mumbai, to examine the particle size and surface morphology. Where CSNPs are coated with gold metals film and magnified under 15000X.

Antioxidant activity of CSNPs by phosphomolybdenum assay

The antioxidant activity of chitosan was evaluated by the green phoshomolybdenum complex formation according to the method of Prieto (1999).

Reagent Preparation: Reagent was prepared by adding 0.588 ml of sulfuric acid, 0.04 gm ammonium molybdate and 0.36 gm sodium phosphate. The final volume was made up to 10 ml with Distilled water.

Procedure: 25, 50, 75, 100, 200, 300, 400 and 500 micrograms of chitosan nanoparticles dissolved in 1ml of DMSO (Dimethylsulfoxide). 100 microliter from the prepared sample was taken and 1 ml of reagent solution was added to it and incubated in a boiling water bath at 950C for 90 min. After 90 min., the absorbance of the solution was read at 695 nm. The phosphomolybdenum reduction potential (PRP) of studied extract were reported in percentage.

Cytotoxicity of chitosan nanoparticles against CHO cell line by MTT assay

2 x 103 cells/well seeded in 96 well plate and incubated in RPMI medium for 24 hour to form a monolayer. The medium was removed and replaced by fresh medium containing treatment with different concentration of sample provided. Along with that, a column of control well (medium control) and incubated for 24 hours. After 24 hours, medium were removed and cells were treated with MTT for around 4 hours. On completion of incubation MTT was removed and cells were treated with DMSO with constant shaking for 15 min. and readings were taken at 595 nm in ELISA microplate reader.

RESULTS AND DISCUSSION

Chitosan synthesis from *Saccharomyces cerevisiae*

In this study chitosan has been successfully prepared from *Saccharomyces cerevisiae*. The synthesis of chitosan involves various chemical steps. Pretreatment methods were done using 1N NaOH and 2% acetic acid. The alkali and acid treatments remove proteins and minerals from chitin respectively and deacetylates simultaneously. These methods give advantages for obtaining of higher quality chitosan. Chitin is not soluble but chitosan, the deacetylated product of chitin, is soluble in very dilute acids like acetic acid, lactic acid, formic acid etc. the deacetylation experiment using 2 N NaOH was done to reduce acetyl group from molecular structure, because the presence of acetyl group prevents to make the solution of chitosan. 10 gm (dry weight) of *Saccharomyces cerevisiae gives* 0.9 gmchitosan and percentage yield of chitosan is 0.81%.

Preparation and characterization of chitosan nanoparticles

Chitosan nanoparticles can be prepared using many methods such as ionic gelation method, emulsion cross- linking, and spray drying. In this study, ionic gelation method was applied because the method is easy and fast to be carried out. This simple technique involves electrostatic interaction between positively charged amino group of chitosan and negatively charged polyanions. Formation of nanoparticles occurs spontaneously through the formation of intra- and intermolecular cross- linkages under a constant stirring at room temperature (Liang *et al*, 2012).

The chitosan nanoparticles prepared in the experiment exhibit a white powdered shape and are soluble in deionized water. The synthesized chitosan nanoparticles were characterized by

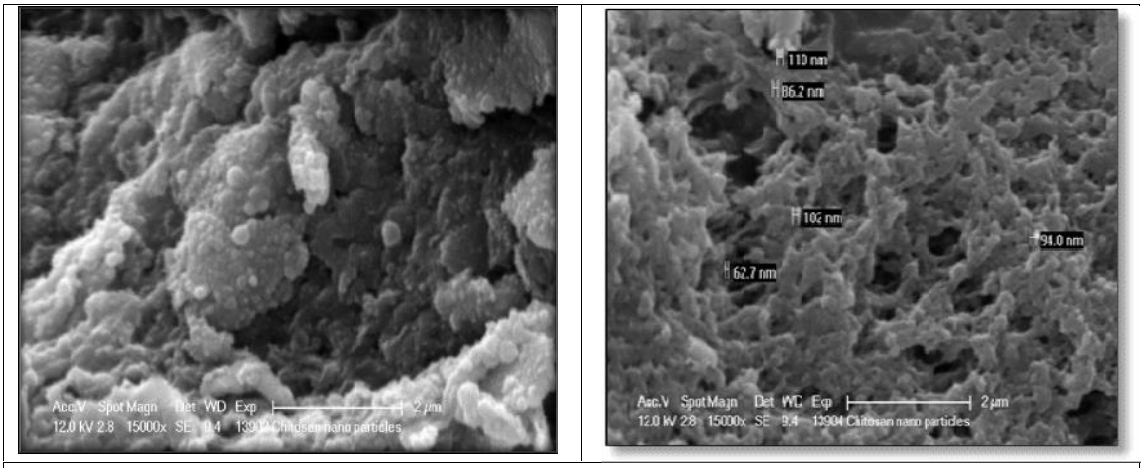

Fig.1. Scanning electron microscopy photograph of chitosan nanoparticles

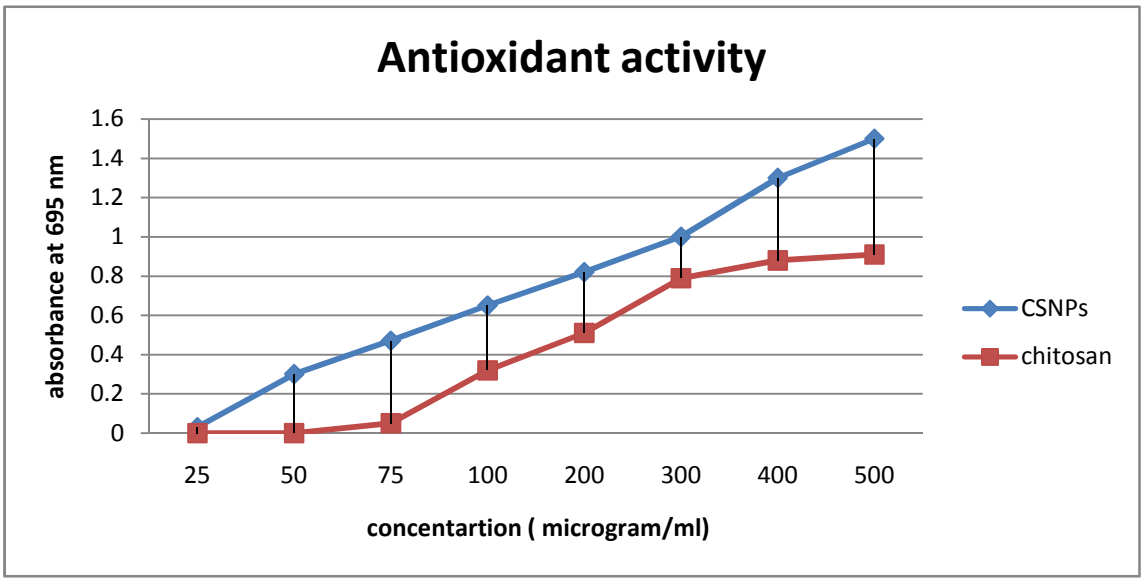

Fig.2.Antioxidant activity of chitosan nanoparticles by Phoshomolybdenum assay

Table 1. Cytotoxicity of chitosan nanoparticles against CHO cell line by MTT assay

	Concentrations of CSNPs (mcg./ml)							
	Media Control	6.25	12.5	25	50	100	200	400
1	0.245	0.207	0.225	0.269	0.301	0.273	0.279	0.241
2	0.277	0.209	0.265	0.293	0.297	0.27	0.283	0.267
3	0.275	0.236	0.279	0.308	0.313	0.285	0.301	0.301
4	0.234	0.222	0.247	0.297	0.304	0.318	0.278	0.281
Average	0.25775	0.2185	0.254	0.29175	0.30375	0.2865	0.285	0.2725

scanning electron microscopy. SEM was used for the determination of the particle size and the morphological structure of the prepared polymer matrix. The coating of chitosan nanoparticles were done by gold metal and magnified under 15000X. It was observed that chitosan/TPP has average particle size of 60- 110 nm. Fig.1 shows the size of chitosan nanoparticles. Particle size of chitosan nanoparticles is depending on concentration of chitosan and TPP, their mass ratios, and drying methods. Fig.1 shows the SEM picture of chitosan nanoparticles.

Antioxidant activity of chitosan nanoparticles

Antioxidant of chitosan and CSNPs was studied by phoshomolybdenum assay. In which antioxidant activity of chitosan and CSNPs gradually increases as increase in concentration. It was found that CSNPs shows greater antioxidant activity than chitosan polymer. The antioxidant properties of chitosan are reported to the correlated to its structural characteristics, including Molecular Weight (MW), Degree of Deacetylation (DD) as well as sources of chitosan. The MW and DD may also present some synergistic effects on the biological activities of chitosan. Different results may obtain from different free radical systems. Yen and coworkers investigated the antioxidant properties of chitosan prepared from crab shells and shiitake stipes. Both studies pointed out that the antioxidant activities of chitosan increased with increase of DD during preparation. The longer N-deacetylation time results in more amino groups on C-2 positions which contribute significantly to the antioxidant activities.

Cytotoxicity of chitosan nanoparticles against CHO cell line by MTT assay

In this study, the exponentially grown Chinese Hamster Ovary cells were treated with various concentrations of chitosan nanoparticles ranging from 6.25 to 400 and the cell viability was measured by the MTT assay. The inhibition of cell viability by chitosan nanoparticles was observed in a dose-dependent manner.

Conclusion

In the present study, we synthesized silver nanoparticles using chitosan isolated form yeast. The successful formation of silver nanoparticles was evaluated using The UV-Vis spectroscopy and SEM. The SEM image shows that the diameter of the fabricated silver nanoparticles was 60- 110 nm. The UV-Vis spectroscopy shows a characteristic peak at approximately 410 nm, confirming the size of silver particle is within the nanoscale range. In this study we found that chitoson mediated silvelnanoparticls found good antioxidant and cytotoxic properties, suggesting that it may be useful natural antioxidants for health preservation against different oxidative stress associated with degenerative diseases. In fact, antioxidant evaluation is essential for AgNPs before its use in vivo models and also human applications. In depth study will be required to understand the real mechanism behind anticancer activity of the synthesized silver nanoparticles.

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