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Research Article
Genotoxic Attribute of Bioconjugate of Tyr-AuNps Synthesized by *Streptomyces tuirus* DBZ39

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Abstract
Background and Objective: Nanotechnology is a cumbersome field used in industrial, medical and environmental applications. Abundant information regarding the genotoxicity of gold nanoparticles is available, but limited information is available about the genotoxicity of enzymes and bioconjugates of gold nanoparticles from microbial sources. The present investigation reveals the genotoxicity of bioconjugate of tyrosinase and gold nanoparticles from *Streptomyces* sp. **Materials and Methods:** Tyrosinase and gold nanoparticles were isolated from *Streptomyces tuirus* DBZ39 and employed for the development of bioconjugates by the flocculation assay method. Methyl thiazole (MTT) assay, transmission electron microscopy, Dynamic Light Scattering (DLS) and UV-vis absorption spectroscopy were used for the investigation. **Results:** Bioconjugate showed a significant genotoxic effect at a concentration of 18 µL and at least 10 µL. Bioconjugate of tyrosinase and gold nanoparticles by *Streptomyces tuirus* DBZ39 revealed a more expressed genotoxic effect than nanoparticles alone. The efficacy of gold nanoparticles was expected to improve the bonding of tyrosine due to their catalytic properties. **Conclusion:** This innovative concept of the application of Tyr-AuNps bioconjugate can be used for the development of powerful weapons in medicinal fields. The introduction of bioconjugates of enzymes and nanoparticles in the field of drug design and cancer imaging will be a fundamental breakthrough in medicine.

Key words: *Streptomyces turius*, gold nanoparticles, tyrosinase, flocculation assay, bioconjugate, genotoxicity, trypan blue viability test

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Microorganisms have been exposed to various types of pollutants in the environment. Metal ions are non-biodegradable and persistent, causing toxicity and inhibit microbial growth. However, microorganisms can survive at high metal ion concentration due to their ability to fight metal stresses. Mechanisms include the efflux system; variation of solubility and toxicity through reduction, bioaccumulation, precipitation of metals[1,2]. These metal-microbe interactions have an important role in many biotechnological applications, including the fields of bioremediation and biomineralization, Recently microorganisms have been discovered as potential biofactories for the synthesis of metal nanoparticles[3-5]

The introduction of nanotechnology has increased the use of nano-material-based products in daily life. However, the major as well as the consequences of this rapidly evolving field of nanotechnology have potential adverse human health effects due to exposure to commonly used nanoparticles. Nanoparticles and tyrosinases are being widely used and the number of products that are being consumed by humans. This widespread application has raised concern as there is little information about the potential risk of nanoparticles. Cancer is one of the world's most devastating diseases. Current cancer treatments are mainly surgical intervention, radiation therapy and chemotherapy drugs. These cause toxicity to patients even after killing healthy cells. The introduction of nanotechnology in the fields of drug design and cancer imaging is generally considered a fundamental breakthrough in medicine. The emerging trend of using nanoparticles as a drug carrier has exploited the potential of nanoparticles to revolutionize cancer therapy[5].

Gold nanoparticles have gained considerable attention due to their attractive physicochemical properties. Due to its effective antimicrobial properties and low toxicity to mammalian cells, gold nanoparticles have become one of the most commonly used nanomaterials in consumer products[6]. Nanoparticles are at the forefront of the rapidly developing field of nanotechnology. New properties of nanoparticles such as magnetic, optical, electronic, electrical and catalytic[7], which differ depending on their size, shape and structure, play an important role in the development of nanosciences and nanotechnology. Microbial nanotechnology is the science that deals with the use of microorganisms for the synthesis of nanoparticles. Due to their special properties, remarkable plasmon resonance and non-toxicity, gold nanoparticles are used in numerous areas of biological research[8,9].

Bioconjugation is a lengthy research area in which two biomolecules are bound to a hybrid bioconjugate while maintaining the individual properties and creating a single unit with two complementary functions[10]. The chemistry of a suitable bioconjugate must be compatible with such environments while maintaining the function of biomolecules. Conjugates are typically formed by adding different but reactive complementary functional groups to each of the two biomolecules. The desired bioconjugate can be obtained by mixing two modified biomolecules. Such bioconjugates can be used in numerous research areas. The literature discloses the development of bioconjugates using various enzymes and metal nanoparticles for important applications[11]. Various criteria are available for the development of bioconjugates using enzymes and nanoparticles for various applications[12]. The method for the production of bioconjugates from enzymes and nanoparticles reveals the electrochemical theory, in which enzymes and nanoparticles from different sources are involved[13]. Recent studies have shown an improvement in the performance of enzyme electrodes through the introduction of gold nanoparticles[14,15]. The use of biomolecule-nanoparticle hybrid systems as functional units can be considered for various applications[16].

Tyrosinase is a multi-catalytic enzyme and is involved in many enzymatic reactions. It has two enzymatic activities: monophenol monooxygenase and ortho diphenol oxidoreductase. It mainly uses molecular oxygen to catalyze these two different enzymatic reactions, the ortho-hydroxylation of monophenols to oxidize O-diphenols (monophenolase activity) and o-quinones (diphenolase activity)[17]. Tyrosine is widespread in plants, animals and microorganisms. Highly pure preparations of the enzyme were mainly obtained from mushrooms[18]. Tyrosinase plays an important catalytic role in medical diagnostics, pharmacy, cosmetics, environmental analysis and biosensors.

The development of bioconjugate from enzyme nanoparticles has recently been an active research area due to a large number of applications. Tyrosinase-Gold-Nanoparticle-Bioconjugate is rarely used for the detection of genotoxicity because good selectivity and stability have their advantages compared to free enzymes. The conjugation of tyrosinase and gold nanoparticles, offers a suitable platform for tyrosine for correct genotoxicity[19]. The study aimed to investigate the genotoxicity of the bioconjugate, to independently look at the potential applications of tyrosinase and gold nanoparticles and both as functional components simultaneously.

MATERIALS AND METHODS

Study area: This research project was conducted from June 2019-January, 2020. At A-DBT Research laboratory, Gulbarga University, Gulbarga, India and Jouf University, Kingdom of Saudi Arabia.

Preparation of Tyr-AuNps bioconjugate: According to the protocol, the flocculation assay method was employed for the development of the bioconjugate of tyrosinase and gold nanoparticles[20,21]. A standard dilution of 1:39 dilutions of tyrosinase and gold nanoparticles was prepared and mixed in 10 mM sodium bicarbonate solution and stored in the dark for 40 min. About 200 µL of 2M NaCl was separately added to the sample and again placed in a dark place for incubation for 20 min. This was done for the sample, except for the reference sample in which the salt solution was replaced with 50 µL of water. The UV-vis absorption of the incubated sample was measured between 400-800 nm. Bioconjugate of tyrosinase-gold nanoparticles was characterized by Dynamic Light Scattering (DLS). Various concentrations of bioconjugates of Tyr-AuNps were prepared to range from 10, 12, 14, 16 and 18 µL to examine genotoxicity.

Genotoxic activity of Tyr-AuNps bioconjugate

Preparation of base slide: Prepare 1% (500 mg 50 mL^{-1} PBS) and 1.0% Normal Melting Agarose (NMA) (500 mg 50 mL^{-1} Milli Q water). Microwave or heat and agarose dissolve until close to boiling. For Low Melting Point Agarose (LMPA), pour 5 vial samples into vials of vials and refrigerate until required. When necessary, briefly melt the agarose in the microwave. Place the LMPA vials in a 37°C dry/water bath for temperature and cooling.

Dip the slide in methanol and burn on a blue flame to remove machine oil and dust.

While the NMA agarose is hot, submerge the conventional slides to a third frosted area and drain gently. To remove agarose, wipe the bottom of the slide and place the slide in a tray on a dry surface to dry. The slide can be air dried or heated at 500°C for quick drying. Store slides at room temperature until needed; Avoid high humidity conditions.

Cell isolation/treatment: In coated slides, mix 75 µL of LMPA (0.5%, 370°C) with ~5-10 lymphocytes in ~5-10 µL and add equal amounts of diluted blood and 1% LMPA, place the coverslip and place the slide on a resting tray. Rest on ice pack until the agarose layer hardens (~5-10 min).

Gently slide off the coverslip and add a third agarose layer (80 µL of LMPA) to the slide. Replace the coverslip and return to the slide tray until the agarose layer hardens (~5-10 min).

Isolated lymphocytes: Mix 20 µL whole blood with 1 mL Rosewell Park Memorial Institute (RPMI) 1640 in a microcentrifuge tube, add 1008 µL Ficoll histopaque to the bottom of the blood/media mixture. Spin for 3 min at 2000$\times$g. Remove 100 µL of the bottom/top of the Ficoll layer of media, 1 mL media and mix, lymphocyte pellet to spin for 3 min. Pour off the supernatant, re-suspend the pellet in 75 µL LMPA and process as above.

Separating lymphocytes and treating *in vitro*: Total 1 mL of blood is taken from a healthy donor by venipuncture. Lymphocytes are isolated from it using histopaque-1077. Briefly, blood is diluted 1:1 with PBS or RPMI (without FBS) and layered on a 600 µL histopack and centrifuged at 800 XG for 20 min. The 3-5 buffy coat is aspirated in 3-5 mL of PBS/RPMI and centrifuged at 250$\times$g for 10 min to pellet lymphocytes. The pellet is resuspended in ~1 mL RPMI and coated on a hemocytometer, G.K, chemicals, A.P, India. Approximately 2×10^4 cells 100 µL^{-1} were taken for each dose of the test material.

One milliliter of each dose is made into a medium (without FBS) and lymphocytes are added to it, inversely binding the eppendorf to mix cells and test materials.

The eppendorf tube is properly washed with alcohol and placed in an incubator at 37°C for 3 hrs.

After treatment, the cells are centrifuged at 3000 rpm for 5 min. To replenish lymphocytes. The test substance is aspirated and discarded. The pellet is then reproduced in 100 mL of PBS and 10 mL removed for the Trypan Blue viability test[22-27].

Total 100 mL of 1% LMPA is added and 80 mL of suspension is suspended on the base slide and a coverslip is placed over it. Place the slide on the ice pack until the agarose layer hardens (~5-10 min).

Gently slide off the coverslip and add a third agarose layer (90 µL LMPA) to the slide. Replace the casing and return to the slide tray until the agarose hardens later (~5-10 min).

Remove the cover and slowly pour the lower slide into a cold, freshly made lysing solution. Protect from light and refrigerator for a minimum of 2 hrs.

Bone marrow: Perfusion of the femur (mouse) with 1 mL of cold mining solution (20 mM EDTA, HBSS with 10% DMSO) in a micro centrifuge tube. Mix 5 µL 75 µL^{-1} LMPA and process accordingly.

Solid organization/tissue: Place a small piece of an organ/tissue in 1 mL cold Hank's Balanced Salt Solution (HBSS) containing 20 mL EDTA/10% DMSO. Mix into fine pieces, arrange 5-10 75l with 75 Al LMPA, remove and mix and process accordingly.

Cell cultures: Remove media and replace with mincing solution, scrape cells into the cell lining using a Teflon scraper for a yield of approximately 1×10 cells mL^{-1}. Remove and mix 5-10 µL of 5-10 LLPA per cell suspension and process accordingly or remove the media and add 0.005% trypsin to the cells. Keep cells at 370°C for 5 min to separate cells (a very low concentration of trypsin-0.005% was used because higher concentrations increase DNA damage. Medium to quench trypsin with FBS, add 10,000 cells in 10 µL or lower volume according to 75 µL LMPA and process accordingly.

Suspension ii. Suspension culture: Add ~ in 10,000 cells in 10 less or less: 5: l LMPA and process accordingly.

DNA damage assessment: To visualize DNA damage, observations with EtBr-stained DNA were carried out using a 40×objective on a fluorescence microscope.

Although any image analysis system may be suitable for quantifying SCGE data, the present investigation uses that of Kinetic Imaging, Ltd. (Liverpool, UK) developed Komet 5 image analysis software to assess the quantitative and qualitative extent of DNA loss. A CCD was attached to the camera. By measuring the length of DNA migration in cells and the percentage of DNA migrated. Finally, the program calculates the end time. Typically 50-100 randomly selected cells per sample were analyzed.

RESULTS AND DISCUSSION

Genotoxicity of bioconjugates of Tyr-AuNps: In a broad spectrum, several studies available so far have reported genotoxicity for various Ag NPs and some of these also reveal potential size-dependent effects[28]. With this in mind, an attempt was made in the current study to assess the genotoxicity of bioconjugates of Tyr-AuNps mediated by *Streptomyces tuirus* DBZ39 and to understand the mechanisms involved. The potential genotoxicity of *bioconjugates of Tyr-AuNps* on a human lymphocyte is presented in Table 1. Bioconjugate solutions of Tyr-AuNps tested for genotoxic activity include bioconjugates with a size of 109.5 nm characterized by Dynamic Light Scattering (Fig. 1). Different concentration of bioconjugate solution was employed to record the degree of genotoxic activity. Comet parameters such as tail limit moment, olive tail moment and a tail length of DNA were recorded at different concentrations

Table 1: Genotoxic activity of bioconjugate of Tyr-AuNps synthesized by *Streptomyces tuirus* DBZ39

	Tail DNA	Tail extent moment	Olive tail moment	Tail length
Control	11.345	0.9	0.39	7.71
	8.975	0.54	0.33	6.5
	12.05	0.695	0.395	5.075
SE	10.79	0.711667	0.371667	6.428333
Mean	1.610877	0.180578	0.036171	1.318961
10 µL	20.84	5.988	2.148	12.19
	12.9	2.8	2.688	10.9.
	10.92	2.66	2.69	8.10
SE	14.623333	3.816	2.508666	10.396666
Mean	7.31166667	1.908	1.254333	5.198333
12 µL	28.10	6.85	5.699	14.910
	27.99	7.588	5.41	18.859
	28.898	6.899	5.888	16.104
SE	28.329333	7.112333	5.665666	16.624333
Mean	14.164666	3.556166	2.832833	8.3121666
14 µL	32.100	7	2.899	15.710
	26.989	6.859	2.18	13.98
	27.195	6.710	1.88	14.810
SE	28.761333	6.856333	2.319666	14.833333
Mean	14.380666	3.428166	1.159833	7.416666
16 µL	29.72	7.15	5.589	15.979
	21.99	5.919	3.919	15.979
	29.13	7.15	5.59	17.199
SE	26.946666	6.739666	5.032666	16.385666
Mean	13.473333	3.369833	2.516333	8.192833
18 µL	30.5105	6.918	5.87108	15.13711
	27.14	6.818	5.188	16.22
	28.99	7.713	5.888	16.83
SE	28.880166	7.149666	5.649026	16.062366
Mean	14.440083	3.574833	2.824513	8.031183

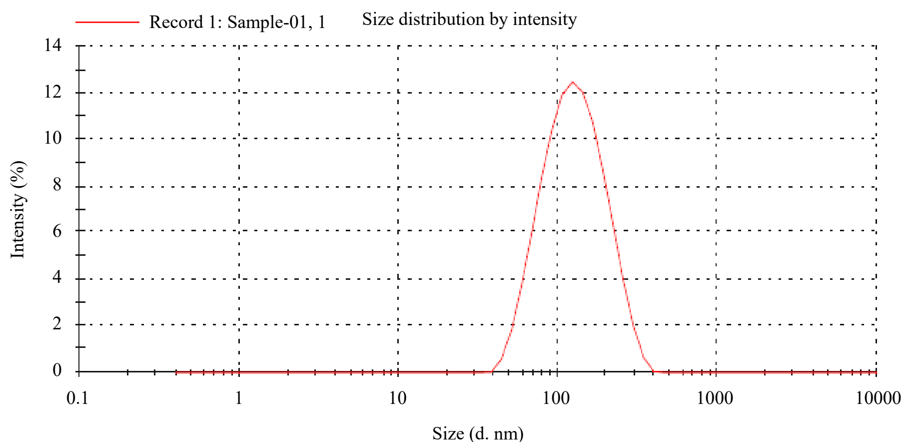

Fig. 1: Dynamic light scattering for the confirmation of bioconjugate of Tyr-AuNps

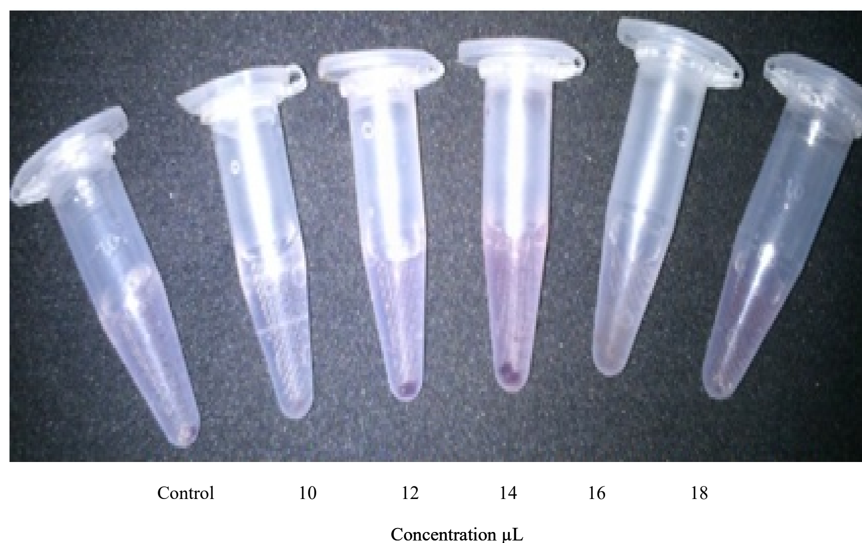

Fig. 2: Different concentration of bioconjugate of Tyr-AuNps for genotoxicity

10, 12, 14, 16 and 18 µL of bioconjugate (Fig. 2). The considerable genotoxic activity was observed at the 10 µL (14.62%) followed by 12 µL (28.32%), 14 µL (28.76) and 16 µL (26.94%) concentration of bioconjugate treatment (Table 1). A significant TAIL DNA inhibition of 28.88% was recorded with an 18 µL solution of bioconjugate of Tyr-AuNps (Table 1). The irregularity in the dose-response may, however, be due to the formation of agglomerates with increasing treatment concentration. This is an important part of regulatory norms because damage to the genome may promote carcinogenesis or have an effect on reproduction. The present investigation correlates with Souza *et al.*[28] explained the genotoxicity of Ag NPs at particle sizes of 10 and 100 nm and observed a clear induction of DNA breaks for both sizes, while only a moderately increased MN induction was observed. Huk *et al.*[29] investigated the size-dependent genotoxicity of Ag NPs using A549 cells and found that 50 nm particles are the most effective in inducing DNA strand breaks. Small-sized Ag NPs were also the most genotoxic in a comprehensive study by Guo *et al.*[30] Probe size (20, 50 and 100 nm) and coating-(citrate and polyvinylpyrrolidone) dependent genotoxicity of Ag NPs[31]. A high genotoxic potential of small Ag NPs has also been shown in other studies[32]. Overall, a significant number of *in vitro* studies show genotoxicity of Ag NPs, possibly due to variations in parameters such as agglomeration, cell dose and Ag ion release in different studies. Genotoxicity is less researched and controversial in animal experiments[33]. A recent review summarized findings from 16 *in vivo* studies and concluded that genotoxicity was reported in most of them[34].

Therefore, the goal of the present investigation was to provide an update of the genotoxic effects of bioconjugates. According to the literature, no report is available on the genotoxic effects of bioconjugates and that to bioconjugates of tyrosinase and gold nanoparticles. However, the testing strategies employed in traditional toxicological studies, such as nanotoxicity studies, fall short to meet many challenges.

CONCLUSION

The use of nanoparticles in cancer medicine as medicine is an ideal phenomenon. The next major milestone goal will be the employment of bioconjugates of enzymes and nanoparticles to cancer cells. One possible reason to explain the specific binding of the bioconjugate to enzymes and nanoparticles to cancer cells, but other body cells, may not be due to morphological differences between cancer cells and other body cells. Cancer cells differ in pore size compared to other cells and hence the size-controlled targets of the bioconjugate of tyrosinase and gold nanoparticles may prove effective in the case of cancer treatment.

SIGNIFICANCE STATEMENT

This study discovers the potential genotoxic effect of bioconjugate of Tyr-AuNps that can be beneficial as the emerging trend of using nanoparticles in drug carrier to revolutionize cancer therapy. This study will help the researcher to uncover the critical area of genotoxic activity of enzymes and nanoparticles together as bioconjugate that many researchers were not able to explore. Thus, employing bioconjugate of Tyr-AuNps for genotoxicity against different cell lines considered a fundamental breakthrough in cancer therapy.

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