

# Assessment of Anti-Inflammatory and Antioxidant Properties of Triphala Mediated Gold Nano particles-An *In-vitro* Study

# Lichi Ashwin Solanki<sup>\*</sup>, Swapna Sreenivasagan

Department of Orthodontics and Dentofacial Orthopaedics, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, 162, Poonamallee High Road, Chennai-600077, Tamil Nadu, India

# ABSTRACT

Background: conventional methods used for synthesis of nanoparticles use toxic chemicals, thus leading to toxicity in the environment. Hence, we need to shift to "green synthesis". Previously done have shown excellent antimicrobial properties of the gold nanoparticles reinforced with amla extract. Hence, this study was conducted to assess the antiinflammatory and antioxidant properties of copper and graphene oxide Nano composite reinforced with triphala extract. Gold causes cell wall lysis by leakage of intracellular substances hence is an excellent antimicrobial.

Aim: Aim of the study was phyto assisted preparation of gold nanoparticles and evaluation of its anti-inflammatory and antioxidant properties.

Material and methods: Anti-inflammatory and antioxidant properties of the nanoparticle were assessed using Bovine Serum Albumin (BSA) and DPPH Assay respectively at 10 µL, 20 µL, 30 µL, 40µL, 50 µL.

Results: Values for anti-inflammatory property of nanoparticles were higher than the standard values at 40 $\mu$ L, 50  $\mu$ L concentrations. Percentage of inhibition was highest at 40  $\mu$ L (86%) and 50  $\mu$ L (84.6%). The values for antioxidant property of nanoparticles were found to be higher than the standard values at concentrations except at 40 $\mu$ L, 50  $\mu$ L. Percentage of inhibition was highest at 20  $\mu$ L (86.2%).

Conclusion: Within the limits of the study it can be concluded that triphala mediated gold nanoparticles have exceptional anti-inflammatory and antioxidant properties and further can be incorporated in dental material or can be used to coat suture materials to improve their properties.

Key words: Gold, Characterisation, Green synthesis, Nanoparticle, Anti-inflammatory property, Antioxidant property

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Corresponding author: Lichi Ashwin Solanki

e-mail : lichisolanki17@gmail.com

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# INTRODUCTION

Nanotechnology is an emerging technology and has led to a new revolution in every field of science. Nanoparticles have gained a lot of importance in the research community in recent years. This technology has been used in the fields of optics, electronics, and biomedical and materials sciences [1]. Potent antimicrobial, anticancer, antioxidant agents, drug and gene delivery, etc are some of the highlighted advantages of the nanoparticles in recent years [2–4]. Nanotechnology deals with nanoparticles that are atomic or molecular aggregates characterized by size less than 100 nm. These are actually basic elements derived by modifying their atomic and molecular properties [5,6].

Several conventional methods are used for synthesis of zinc oxide nanoparticles like chemical reduction [7], laser ablation [8], solvothermal, inert gas condensation [9,10], sol-gel method [11]. Even though less time is utilized for synthesizing large quantities of nanoparticles using conventional physical and chemical methods, toxic chemicals are required as capping agents to maintain stability, thus leading to toxicity in the environment. "Green synthesis" offers numerous benefits of eco friendliness and compatibility for biomedical applications, where toxic chemicals are not used for the synthesis protocol. The use of agricultural wastes [9] or plants and their parts [10], has emerged as an alternative to chemical synthetic procedures because it does not require elaborate processes such as intracellular synthesis and multiple purification steps or the maintenance of microbial cell cultures.

Gold nanoparticles (AuNP) are superior owing to their nontoxicity, biocompatibility, use in drug and bactericidal activity [7,12,13]. Contact killing property of gold was studied widely in recent years. Studies have shown that increased bacterial intracellular oxidative stress in the bacterial cell wall due to release of ions from the copper surface which results in bacterial cell lysis [14]. Synthesis of gold nanoparticles is highly technique sensitive due to its high incidence of oxide layer formation on the nanoparticle surface which will result in reduced antibacterial property [15]. Tripahala is known for its excellent for anti-inflammatory and the barrier properties [16].

The objective of this study was to use triphala extract to synthesize gold nanoparticles and to evaluate its antiinflammatory and antioxidant properties as its excellent potency against oral aerobes was already proven in the previous studies.

# MATERIAL AND METHODS

# Preparation of amla extract

Freshly collected triphala were thoroughly washed multiple times in distilled water. Seed was taken out and the pulp was cut into small pieces using a sterile knife and was grounded into small particles by means of a mortar and pestle. Triphala extract was prepared by 1 grams of amla pulp with 100 ml distilled water to make 1 molar solution of amla extract [16].

#### Synthesis of CuGO nano-composite

NP synthesis was done by mixing 50 ml of both 1M solutions of gold nanoparticles as mentioned in the previous steps. The nanoparticle solution was stirred overnight on an orbital shaker followed by a magnetic heated stirrer till colour change was observed. UV-vis spectrometric readings were taken hourly to check the synthesis of copper-graphene oxide nano composite. The resultant mixture was centrifuged and AuNP nanoparticle was obtained [16].

# Anti-inflammatory activity

#### **Test group**

10 µL, 20 µL, 30 µL, 40 µL and 50 µL of the nanoparticle solution was taken in 5 test tubes respectively. To each test tube 2 ml of 1% Bovine Serum Albumin (BSA) was added. 390 µL, 380 µL, 370 µL, 360 µL and 350 µL of distilled water was added to the test tube containing 10 µL, 20 µL, 30 µL, 40 µL and 50 µL of nanoparticles respectively.

# **Control group**

2 ml of gold chloride was added to 2 mL of BSA solution.

# Standard group

10  $\mu$ L, 20  $\mu$ L, 30  $\mu$ L, 40  $\mu$ L and 50  $\mu$ L of Diclofenac Sodium was taken in 5 test tubes respectively. To each test tube 2 mL of 1% Bovine Serum Albumin (BSA) was added. The test tubes were incubated at room temperature for 10 minutes. Then they were incubated in water bath at 55°C for around 10 minutes. Absorbance was measured at 660 nm in UV Spectrophotometer.

% Inhibition was calculated using the following formula:

% of inhibition = Control OD – Sample OD Sample OD100

#### RESULTS

Anti-inflammatory assay showed the following values at the end of the study:

Anti-inflammatory property of nanoparticles was higher than the standard values at  $40\mu$ L, 50  $\mu$ L concentrations. Percentage of inhibition was highest at 40  $\mu$ L (86%) and 50  $\mu$ L (84.6%) (Figure 1).

Antioxidant test showed the following values for the Nano composite: The values for antioxidant property of nanoparticles were found to be higher than the standard values at concentrations except at  $40\mu$ L,  $50\mu$ L. Percentage of inhibition was highest at  $20\mu$ L (86.2%) (Figure 2).

# DISCUSSION

Effective drug delivery systems with the ability to improve the therapeutic profile and efficacy of therapeutic agents are one of the key issues faced by modern medicine. Advances in nano science and nanotechnology, enabling the synthesis of new Nanomaterials, have led to the development of a number of new drug delivery systems.

There has been a rapid evolution of nanoparticle synthesis recently as compared to the early part of the



Figure 1: Bar diagram depicting the anti-inflammatory value on y-axis vs. different concentrations of gold nano particle. Green colour represents the test group and blue represents the standard group.



Figure 2: Bar diagram depicting the antioxidant value on y-axis vs. different concentrations of gold nano particle. Green colour represents the test group and blue represents the standard group.

century [17]. Earlier conventional methods were used for the synthesis of the nanoparticles. Even though less time is utilized for synthesizing large quantities of nanoparticles using conventional physical and chemical methods, toxic chemicals are required as capping agents to maintain stability. These methods resulted in toxicity in the environment due to use of toxic chemicals. Green Synthesis method was proposed and is widely used all over the world to avoid the use of such toxic chemicals. It is an eco-friendly method as well as it is highly cost effective [18]. We therefore undertook this study to evaluate the cytotoxicity of the copper and graphene oxide Nano composite reinforced with amla extract. Antibacterial properties of the same composite were found to be excellent against oral microbes in the previous studies [16].

Triphala is known since ancient times for its medicinal value and is commonly used in Ayurvedic medicine. Triphala is a rich source of vitamin C which is a highly essential vitamin in maintenance of epithelial homeostasis. Superoxide radicals (O2–) have been implicated in several pathological disorders and are responsible for elevated oxidative stress. Studies have shown that amla extract acts as a very good antioxidant by scavenging the reactive oxygen species and protects the antioxidant enzymes like SOD required for the cellular Defence.

Gold has been known for its antibacterial activity. Gold nanoparticles impart the antioxidant effect by inhibition of chain reaction, decomposition of peroxides, binding of transition metal ion catalysts, radical scavenging activity and inhibition of continued hydrogen abstraction. The properties of absorbing, neutralizing these free radicals or quenching singlet and triplet oxygen are few crucial factors that are responsible for the antioxidant activity. The highest antioxidant activity is attributed due to the presence of various bio-reductive groups of the phytochemicals present on the surface of the AuNPs. Gold not only is an excellent antioxidant but also an effective anti-inflammatory agent [12,19]. Remarkable physicalchemical properties, including a high Young's modulus, high fracture strength, and excellent electrical and thermal conductivity, fast mobility of charge carriers, large specific surface area and biocompatibility are few of the major advantages of graphene nanoparticles. Gold is studied in the medicinal field for over 40 years for its potential for being the ideal drug delivery properties. Hence the combination of gold with triphala was selected to be tested for its anti-inflammatory and antioxidant properties in the current study.

# CONCLUSION

Within the limits of this study it can be concluded that gold nanoparticle has exceptional anti-inflammatory and antioxidant properties. With a possibility to be included in the dental materials to improve the properties.

# **CONFLICT OF INTEREST**

The author declares that there are no conflicting interests.

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