

**Type of Manuscript: Original study**

## **FREE RADICAL SCAVENGING AND ANTI-INFLAMMATORY ACTIVITY OF CHLOROGENIC ACID MEDIATED SILVER NANO PARTICLES**

**Running Title:** Free radical scavenging and Anti-inflammatory activity of Chlorogenic acid mediated Silver nano particles

### **ABSTRACT**

#### **Introduction:**

Nanotechnology is a field of research and innovation concerned with building 'things' - generally, materials and devices on the scale of atoms and molecules. It is a booming field of this 21st century. The role of Nano technology is becoming very crucial in nearly every aspect of life ranging from cosmetics to advanced bio technological approaches. In recent years, silver nano particles have gained considerable attention in the field of medicine. The green synthesis of nano particles eliminates the generation and use of hazardous substances and thus sustains non toxicity. Chlorogenic acids are phenolic compounds formed by the esterification of cinnamic acids. They exhibit various pharmacological Properties. Our study deals with the green synthesis of Chlorogenic acid mediated silver nano particles and assessment of their anti inflammatory and Anti oxidant properties.

#### **Aim:**

The present study aims at assessing the Anti oxidant and anti inflammatory property of chlorogenic acid mediated silver nano particles and investigating the efficacy of Chlorogenic acid mediated Silver nano particles.

#### **Materials and Methods:**

The methodology includes Green synthesis of Chlorogenic acid mediated Silver nano particle synthesis followed by tests for Anti inflammation and Anti oxidation.

#### **Results:**

Both the Anti inflammatory and Anti oxidation activity of the chlorogenic acid mediated silver nano particles had shown a proportionate increase in activity with increasing  $\mu$ l concentration.

#### **Conclusion:**

Chlorogenic acid mediated silver nano particles have shown significant anti inflammatory and anti oxidation activity and they are considered as potent anti inflammatory and Anti oxidant agents.

**Keywords:** Chlorogenic acid, Silver nano particles, Anti inflammatory, Anti oxidant, Nanotechnology

## **1.Introduction:**

Nanotechnology is an incredibly growing field in the era of modern science. It is a booming field of this 21st century. The role of Nano technology is becoming very crucial in nearly every aspect of life ranging from cosmetics to advanced bio technological approaches. Nanotechnology is the field of science that mainly consists of the processing of separation, consolidation and deformation of materials by one atom or one molecule<sup>1</sup>. In simple terms, Nanotechnology is the design, characterisation, production and application of structures, devices and systems by controlled manipulations of size and shape at the nanometer scale. Basically Nanotechnology can be defined as manipulation of matter on an atomic and supramolecular scale. It deals with the synthesis and fabrication of materials at the nano scale level ( 1 - 100 nm )<sup>1</sup>. This is the scale at which the materials exhibit some unexpected and unusual new properties that cannot be defined by the classical laws of physics<sup>2</sup>. In recent years, silver nano particles have gained considerable attention in the field of medicine. Silver nano particles have become a focus of interest because they play a significant role in biological systems, living organisms, and medicine<sup>3</sup>. Silver nano particles are nano-scale particles that are made of silver between 1nm and 100 nm in size<sup>4</sup>. They are largely composed of silver oxide. Silver nano particles are greatly known for their application in drug delivery, diagnosis and treatment of various diseases<sup>5</sup>. They have got a large surface to bulk silver atom ratio. The anti bacterial, anti fungal, anti inflammatory, anti oxidation properties of them are studied by various researchers<sup>6</sup>. They have got excellent anti microbial properties that extend their applications nearly in every sphere of life. The Silver nano particles interact with sulphhydryl groups of Proteins and DNA of the bacteria<sup>7</sup>. They cause unwinding of DNA, alter the hydrogen bonding and cause interference to Cell wall synthesis and cell division<sup>8</sup>. They have got increased surface area that permits the coordination of various ligands<sup>9</sup>. Their quantum effects attribute to their unique mechanical and physicochemical properties<sup>10</sup>. Silver nano particles provide additional mechanical, optical, chemical and biological peculiarities that recommend them for the design, obtaining, evaluation, and clinical assessments of performance enhanced bio materials and medical devices<sup>11</sup>. But it's short lived longevity caused by particle aggregation and its high cost serve as hindrance in the chemical production of the particles<sup>9</sup>. This has paved the way for evolution of green synthesis of silver nano particles. Here the biological organisms reduce the silver ions in solution into colloidal nano particles. The green method also eliminates the generation and use of hazardous substances and thus sustains non toxicity<sup>12</sup>. In our study , the green synthesis of silver nano particles are mediated through Chlorogenic acids. Chlorogenic acids are phenolic compounds formed by the esterification of cinnamic acids<sup>13</sup>. They exhibit various pharmacological Properties. It's Anti oxidant , anti obesity, anti viral, anti hypertension, antipyretic and anti inflammatory properties are being studied by various researchers<sup>14</sup>. Chlorogenic acid is a biologically active polyphenol which is soluble in ethanol and acetone<sup>15</sup>. Chlorogenic acids are present in abundance in green coffee beans<sup>16</sup>. They are also found in potatoes, peaches, prunes and bamboo<sup>17</sup>. It's wide availability makes them economical and aids in easy application. In addition, It has been found that the Chlorogenic acids have the capability to modulate lipid metabolism and glucose in both genetically and healthy metabolic related disorders<sup>18</sup>. It is speculated that Chlorogenic acids can perform

crucial roles in lipid and glucose metabolism regulation and thus help to treat many disorders such as hepatic steatosis, cardiovascular disease, diabetes and obesity as well <sup>19</sup>. So, they serve as a good alternative to synthetic antibiotics. In our study, the anti-inflammatory and anti-oxidation properties of Chlorogenic acid mediated silver nano particles are studied. Inflammation is the protective response of the body to a variety of etiological factors like Infective agents, Immunological agents, Physical changes, Chemical changes and inert materials. Though inflammation is protective in nature, they may cause considerable harm to the body as well. Anaphylaxis to bites by insects or reptiles, drugs, chronic rheumatoid arthritis, fibrous bands and adhesions in intestinal obstruction are to name a few. So, It becomes really very important to treat an Inflammatory response. Phenolic compounds are able to inhibit either the production or the action of pro-inflammatory mediators, resulting in an anti-inflammatory effect. Chlorogenic acids are rich in phenolic compounds <sup>20</sup>. On the other hand, Oxidation is a chemical reaction that can produce free radicals, thereby leading to chain reactions that may damage the cells of organisms<sup>21</sup>. Anti oxidants are the compounds that prevent oxidation<sup>21</sup>. The human Anti oxidant system consists of small Anti oxidants, Anti oxidant proteins, ROS-metabolizing enzymes, as well as many regulator proteins that mediate adaptive responses to oxidant stress <sup>22</sup>. Chlorogenic acids are said to have good anti oxidation property<sup>14</sup>. So, the present study aims at assessing the Anti oxidant and anti-inflammatory properties of chlorogenic acid mediated silver nano particles and investigating the efficacy of Chlorogenic acid mediated Silver nano particles.

## **2. Materials and Method:**

### **2.1 Synthesis of nano particles:**

0.1 gram of the Chlorogenic acid extract was added to 100 ml of distilled water. The solution was heated for minutes. Then 2 milli molar Silver nitrate was added to the solution and kept in the shaker for nano particle synthesis (Figure 1).

### **2.2 Anti inflammatory activity:**

2ml of 1% bovine albumin was mixed with the Chlorogenic acid extract of different concentrations ( 10-50 g/ ml ).Then distilled water (390-350l) was added to Chlorogenic acid extract of varying concentrations( 10-50 g/ ml ) And it is incubated at room temperature for 10 minutes. Then the mixture was heated at 55 degree Celsius for 20 min in a water bath and was cooled to room temperature. The absorbance value was recorded at 660 nm. An equal amount of plant extract was replaced with DMSO for control. Diclofenac sodium was used as a standard. All the tests were done in triplicate.

### **2.3 Anti oxidant activity:**

2ml of the Chlorogenic acid extract in the concentration range of 10 - 50 g / ml was prepared in 50% methanol solution and was added to equal volume of 0.1 mM of DPPH solution. The reaction mixture was incubated for 20 min in dark at room temperature. Absorbance value was measured spectrophotometrically at 517 nm. The methanol solution was used as a blank. Methanol solution mixed with 0.1 mM of DPPH solution had been used as a control. Ascorbic acid was used as a standard. The IC<sub>50</sub> Value was calculated. % inhibition was calculated using the below mentioned equation

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample} \times 100}{\text{Absorbance of control}}$$

### 3. Results and Discussion:

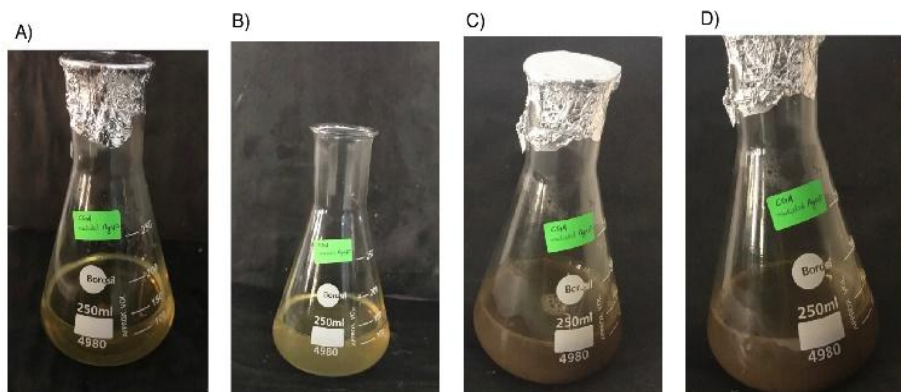


Figure :1 A) Mixture of Chlorogenic acid extract with 100ml distilled water B) Chlorogenic acid mediated silver nano particle synthesis C) After 33 hours D) After 72 hours

#### 3.1 Anti inflammatory activity:

From the Figure-2, We can infer that the chlorogenic acid mediated silver nano particles showed about 40% protein denaturation inhibition at 10 $\mu$ l concentration, 51% inhibition at 20 $\mu$ l concentration, 69% inhibition at 30 $\mu$ l concentration, 78% inhibition at 40 $\mu$ l concentration and 88% inhibition at 50 $\mu$ l concentration.

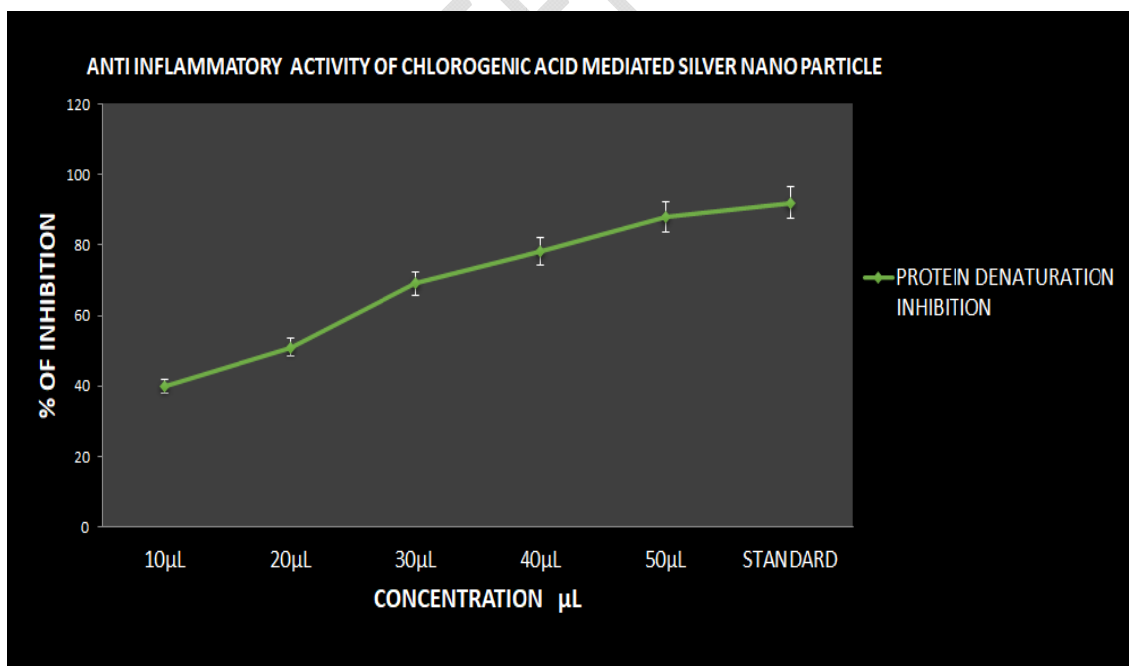
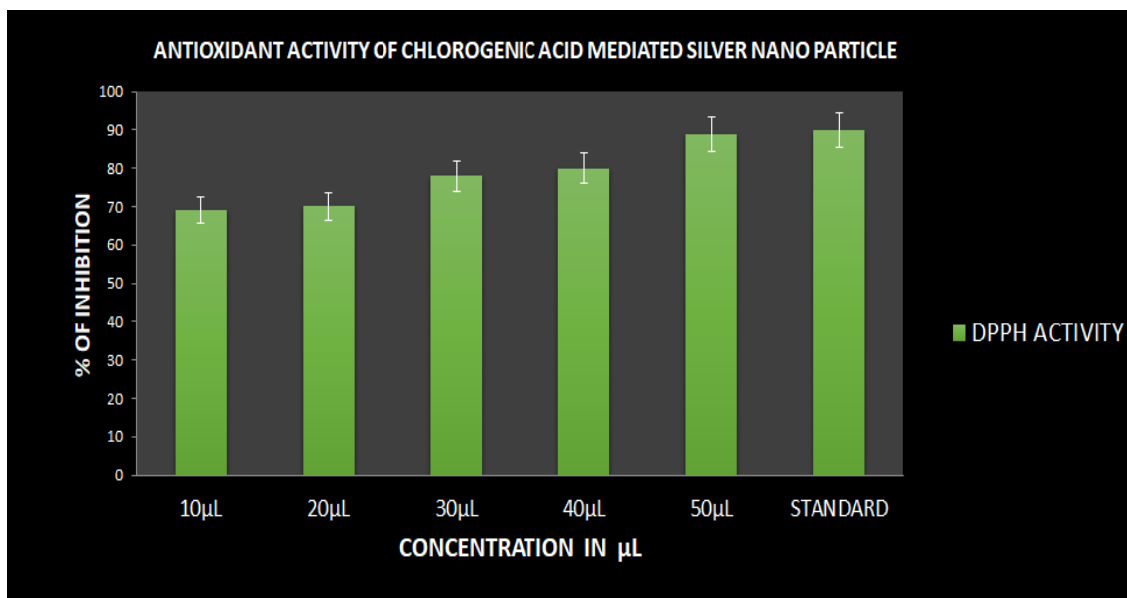


Figure-2: Anti inflammatory activity of Chlorogenic acid mediated Silver nano particles

#### 3.2 Antioxidant activity:

Similarly, Figure-3 shows the results of Anti oxidation activity. The DPPH activity is analysed. At 10 $\mu$ l concentration the DPPH activity is about 69%, At 20 $\mu$ l concentration it is

about 70%, At 30 $\mu$ l concentration it is 78% , At 40 $\mu$ l concentration the DPPH activity is 80% and at about 50 $\mu$ l concentration it is about 89%.



**Figure 3: Anti oxidant activity of Chlorogenic acid mediated Silver nano particles**

Both the Anti inflammatory and Anti oxidation activity of the chlorogenic acid mediated silver nano particles had shown a proportionate increase in activity with increasing  $\mu$ l concentration. They are almost equal to the standards taken. This shows their significance in anti inflammation and anti oxidation activities. The silver nano particles synthesized using herbal extracts and herbal formulations show very good anti-inflammatory and Anti oxidant activities using biochemical procedures<sup>23 24 25</sup>.

#### 4. Conclusion:

From our Results, We can conclude that Chlorogenic acid mediated silver nano particles have got significant Anti inflammatory and anti oxidation activity and they are considered as potent Anti inflammatory and Anti oxidant agents. In recent times, the world is shifting towards the herbal cures because of the pronounced cumulative and irreversible reactions of modern therapeutic drugs. Plants have got an extraordinary ability to synthesize aromatic substances which are the useful phenols or their oxygen substituted derivatives. The maintenance of good health of the people in most of the developing countries forms the normative basis of the usage of traditional medicines. On that note, It is rightly to say that our study paves the way for further research and development of a new drug that can be used as a good alternative to the already existing therapeutic drugs.

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