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RESEARCH ARTICLE

# Thiocolchicoside-Assisted Green Synthesis of AgNPs: Evaluation of Anti-Inflammatory Efficacy and Embryonic Toxicology Evaluation

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Abstract: ***Background***: Nanomedicine focuses on nanoparticles for both prevention and treatment, with silver nanoparticles AgNPs being particularly popular because of their specific physical, chemical, and biological features, which include antiviral, antifungal, anti-inflammatory, and anticancer activity. To synthesise AgNPs, this work takes an environmentally benign strategy and uses green leaf extract. It also assesses the embryonic toxicity and cytotoxic implications of Thiocolchicoside, an analgesic caused by AgNPs. ***Methods:*** AgNPs were synthesised using an environmentally benign technique that included lowering silver ions with green leaf extract. AgNPs were validated by UV-vis spectroscopy at 410 nm, while FTIR analysis indicated polyphenols, polysaccharides, and proteins. Wild-type zebrafish embryos were gathered, rinsed in E3 medium, and then treated with five distinct amounts of Thiocolchicoside-mediated AgNPs. To evaluate toxicity, embryo growth was observed using a stereo microscope. ***Results:*** AgNPs (10, 20, and 50 mg/ml) considerably reduced bacterial growth against Staphylococcus aureus and Klebsiella sp., indicating that they could serve as antimicrobial substances. Thiocolchicoside-mediated AgNPs demonstrated a dose-associated decrease in embryonic survival and hatching rates. Cytotoxic assessment found no toxicity on day one; however, survival decreased in a dose-related manner from day two forward. ***Conclusion:*** Silver nanoparticles made from green leaf extract were characterised by utilising a variety of methods of analysis and showed antibacterial activity against Klebsiella sp. and Staphylococcus aureus. Additionally, Thiocolchicoside-mediated AgNPs demonstrated a dose-dependent increase in toxicity to zebrafish embryos and nauplii.

Keywords: Thiocolchicoside, Silver nanoparticles (AgNPs), Green synthesis, Anti-inflammatory activity, Cytotoxicity, Embryotoxicity

## INTRODUCTION

Nanotechnology has evolved as a game-changing topic having enormous applications in medical, ecological studies, and materials engineering. Silver nanoparticles (AgNPs) have received a lot of interest because of their powerful antibacterial, anti-inflammatory, antioxidant, and anticancer capabilities [1]. Previously, chemical and physical procedures have been utilised to synthesise silver nanoparticles, but these methods generally require hazardous chemicals, large amounts of energy, and adverse ecological implications [2]. In recent times, green synthesis methodologies have become a sustainable option, using herbal extracts, microbes, or natural substances as minimising and stabilising forces [3]. The process of green synthesis provides a cleaner, safer, and more environmentally responsible approach to nanoparticle creation. This approach additionally eliminates hazardous chemicals, but also inserts biologically active substances into the nanoparticles, which could improve their beneficial effects[4]. TCC's molecular composition, which is high in hydroxyl and methoxy groups, may promote effective silver ion reduction while also increasing the physiological roles of AgNPs [36][5].

The integration of TCC into the production of AgNPs could provide twofold functionality—acting as a substance synthesis agent and a biological promoter[6]. The destructive ability of these nanoparticles must be evaluated before they can be used in biomedical applications, especially cancer therapy or drug delivery systems. Cytotoxicity evaluations assist in evaluating the safe limits and therapeutic opportunities of the synthesised nanoparticles [7]. Because silver nanoparticles are known to be proven to induce cytotoxic effects on several cancer cell lines, identifying their particular toxicity is critical [8][37]. When coupled with a therapeutic molecule such as TCC, these particulates may exhibit improved or changed cytotoxicity profiles, based on the type of cell and dosage[9][38]. In addition, embryonic toxicity is an important consideration when assessing the biological safety of nanomaterials, especially assuming they are meant for pharmaceutical or medical applications. Early-stage toxicity can affect regular early growth and could indicate for a long time dangers of nanoparticle ingestion [10, 11]. Laboratory

species such as zebrafish embryos offer an ideal in vivo framework for such assessments because of their genetic resemblance to individuals, fast growth, and clear embryos that allow actual time monitoring[12] [13].

The present investigation intends to synthesise silver nanoparticles utilising thiocolchicoside as a reduction and capping agent by an ecological process, evaluate their anti-inflammatory potential, and examine their cytotoxic and embryotoxic implications [14]. To determine the nanoparticles' biological significance in inflammatory illnesses, their anti-inflammatory activity will be tested using conventional models in vitro[15] [16]. Embryonic contaminants are being examined using zebrafish (Danio rerio) embryos, focusing on death rate, physical defects, hatching delay, and cardiac rhythm changes [17]. This will offer a thorough toxicological profile of the synthesised nanoparticles, helping establish what is appropriate for biological usage [18]. The rationale for selecting TCC stems from its dual role as a drug substance and a natural stabiliser. Despite herbal extracts, which might fluctuate in their substance, a well-characterized chemical such as TCC delivers predictable synthesis results. [19]. It lays the groundwork for additional investigations into comparable bioactive compounds for nanoparticle-mediated medication delivery, inflammatory surveillance, and regenerative healthcare [20].

# MATERIALS AND METHODS

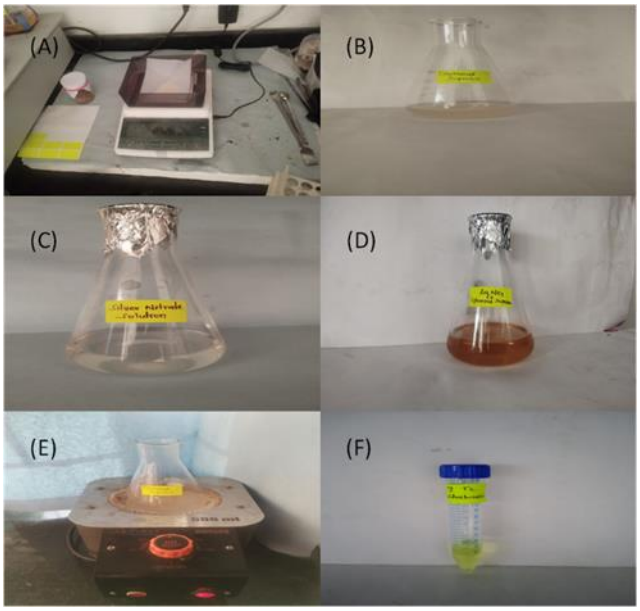


Figure 1: Thiocolchicoside is used to facilitate the green production of silver nanoparticles. A digital balance was used to weigh 100 mg of glorious supera leaves (B) Glorious superba powder combined with 100ml of pure water (C) Aquatic mixture has been warmed on the mantle at 60-70 degrees Celsius for 15-20 minutes (D) 1mM of silver nitrate (0.034 g) in 50 millilitres of purified water (E) The first solution was mixed with 50ml of filtered herbal combination (F), which included 100 mg of thiocolchicoside weighed and mixed with 1ml of purified water.

**Thiocolchicoside preparation**

100 mg of thiocolchicoside was weighed first, then diluted in 1ml of distilled water, mixed thoroughly, and stored properly for future use.

**Silver Nitrate Solution Preparation**

0.5 g of magnificent superba plant powder was weighed and combined with 50 millilitres of purified water. The plant extract was cooked for 15 to 20 minutes at 50 degrees Celsius and then filtered through a cotton cloth. Take 25 ml of plant extract and 2 millimolars of silver nitrate (0.034gm g) and combine with 75 ml of purified water. Stir in a stirrer at 500 rpm for 30 minutes, then add 1 ml of STPP (sodium tripolyphosphate) and shake for 24 hours. Take the solution, centrifuge it at 8000 rpm for 10 minutes, and remove the pellet while discarding the supernatant. Separate the pellet and store it safely for future use [21].

**Anti-inflammatory activity**

The study of green silver nanoparticles generated from Glorious superba used two tests: the bovine serum albumin denaturation assay and the egg albumin denaturation assay.

**Bovine serum albumin**

Bovine serum albumin (0.45 mL) and various concentrations of silver nanoparticles mediated by Glorious superba (10-50 g/mL) were mixed. A pH correction of 6.3 was performed. It was subsequently kept at room temperature for 10 minutes before being incubated for 30 minutes in a 55°C water bath. The control group used was diclofenac sodium, while the control group comprised dimethyl sulfoxide. The samples were then spectrophotometrically assessed at 660 nm.

The calculation of protein denaturation percentage was performed using the formula: % Inhibition = (Absorbance of control - Absorbance of sample/Absorbance of control) x 100[22].

**Egg albumin denaturation assay**

The egg albumin denaturation assay was performed by mixing 0.2 mL of fresh egg albumin with 2.8 mL of phosphate buffer. Silver nanoparticles (10-50 µg/mL) were incorporated into the resultant mixture via Glorious Superba. pH was set to 6.3. After 10 minutes of standing at room temperature, the solution was incubated for 30 minutes in a water bath at 55°C. The normative reference was diclofenac sodium, and the untreated group was given dimethyl sulfoxide. The materials were then spectrophotometrically analysed at 660 nm [23].

The proportion of protein denaturation was calculated through the subsequent equation.: Inhibition = (As/Ac - 1) × 100 (As = absorbance of sample, Ac = absorbance of control).

**Membrane stabilization assay**

The in vitro membrane stabilisation assay is a typical method for evaluating a compound's ability to stabilise membranes in in vitro studies. This assay assesses an ingredient's capacity to keep the cell membrane intact by preventing disruption and dispersion of the contents of cells. Human red blood cells (RBCs), PBS, Tris-HCl buffer (50 mM, pH 7.4), silver nanoparticles (10-50 µg/mL), a centrifuge tube, and a UV-Vis spectrophotometer are used [24].

**Preparation of RBC suspension**

Fresh person's blood was taken in a sterile anticoagulant tube and centrifuged at room temperature for 10 minutes to remove the RBCs from the other constituents. Following supernatant removal, the RBCs were rinsed three times with PBS. RBCs were reconstituted in Tris-HCl buffer to form a 10% (v/v) suspension

**Assay procedure**

Every centrifuge tube was filled with 1 mL of RBC solution using a pipette. The tubes were then filled with various amounts of silver nanoparticles. After agitation, the tubes were allowed to remain at 37°C for 30 minutes. To isolate the RBCs, the tubes have been placed in a centrifuge and spun at 1000 × g for 10 minutes at room temperature. A UV-Vis spectrophotometer was used to assess the absorbance of the effluent at 540 nm. The haemolysis suppression percentage was calculated using the following equation: % inhibition = [(Absorbance control − Absorbance sample)/Absorbance control] ×100.

**Cytotoxic effect**

**Brine Shrimp Lethality Assay**

2 g of iodine-free salt was weighed and dissolved in 200 millilitres of purified water. This study employed six enzyme-linked immunosorbent assay (ELISA) wells, with 10-12 mL of treated saline water in every well. After progressively adding 10 nauplii to every well, various quantities of CTLA nanogel (5 µg/mL, 10 µg/mL, 20 µg/mL, 40 µg/mL, and 80 µg/mL) were applied to the wells. The sixth well was used as a control and did not receive the nanogel. The plates were then incubated for 24 hours at the ambient temperature, allowing the intended effects of the nanogel on the nauplii to occur. After twenty-four hours, the ELISA plates were thoroughly inspected and counted for the number of live nauplii present, which was determined using the following formula: Number of dead nauplii/Number of dead nauplii + Number of live nauplii × 100 [25].

**Zebrafish Embryonic Toxicology Studies**

Zebrafish embryos were placed in water used for cultivation and incubated at 26°C. At 4 hours post-fertilization (sphere stage), randomised embryos were kept alive in 10 mL of zebrafish culture water. Good health embryos were placed in 96-well plates with 0.2 mL of culture water. To every well, 0.1 mL of Silver nanoparticles (0 to 150 µg/mL) was introduced. Three separate experiments were added, and the embryos in the culture media were deemed the control. The plates were then incubated at 26°C, and the growth conditions of the embryos and zebrafish larvae were monitored during various fertilizing times. The hatching and mortality rates in percentages were obtained every 12 hours based on the total number of surviving embryos. A microscope was utilised to study nanoparticle-induced embryo dysfunction [26].

# RESULTS AND OBSERVATIONS:

Perception of sight. The biological concentrate of *Glorious Superba* with silver nanoparticles was visible [Figure 1]. It developed into an earthy coloured kind of extract, as evidenced by the formation of silver nanoparticles.

**Bovine Serum Albumin Assay**

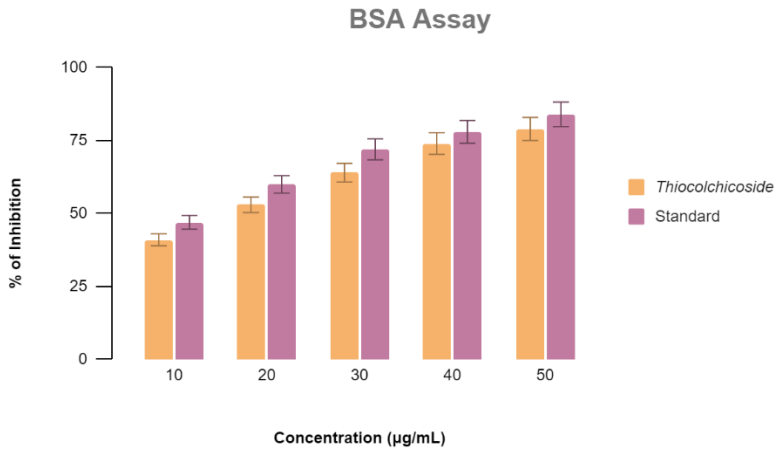


Figure 2: Thiocolchicoside's anti-inflammatory efficacy as measured by bovine serum albumin assay

The BSA assay graph compares the anti-inflammatory activity of thiocolchicoside to a reference medication at dosages that vary from 10 to 50µg/mL. The two samples show a dose-dependent increase in protein degradation reduction. Thiocolchicoside has a slightly lower but equivalent inhibition to the reference at all doses. At 50 µg/mL, it exhibits above 80% inhibition, showing substantial anti-inflammatory properties. These findings indicate thiocolchicoside's ability to stabilise proteins under inflammatory circumstances.

**Egg Albumin Assay**

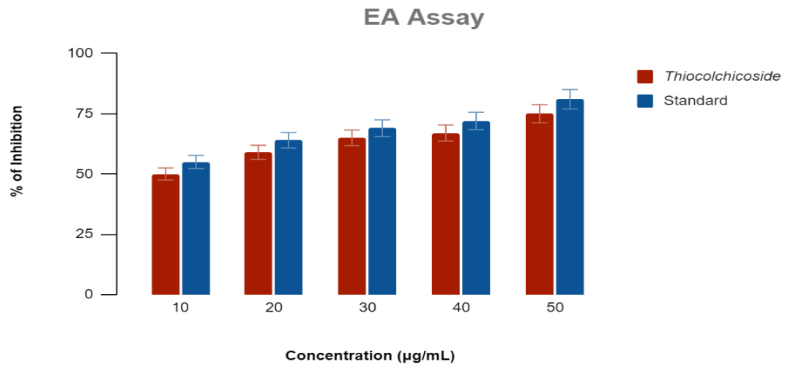


Figure 3: Thiocolchicoside's anti-inflammatory efficacy as measured by egg albumin denaturation assay

The EA (Egg Albumin) assay chart compares the anti-inflammatory activity of thiocolchicoside to a reference medication at various doses (10-50 µg/ml). Both compounds suppress protein denaturation in a dose-dependent manner. Thiocolchicoside exhibits similar restriction to the standard, achieving close to 75% at 50 µg/mL. The standard has somewhat greater action at each dose, although the variance is small. These data demonstrate that thiocolchicoside has strong anti-inflammatory potential, comparable to traditional medications.

**Membrane Stabilization Assay**

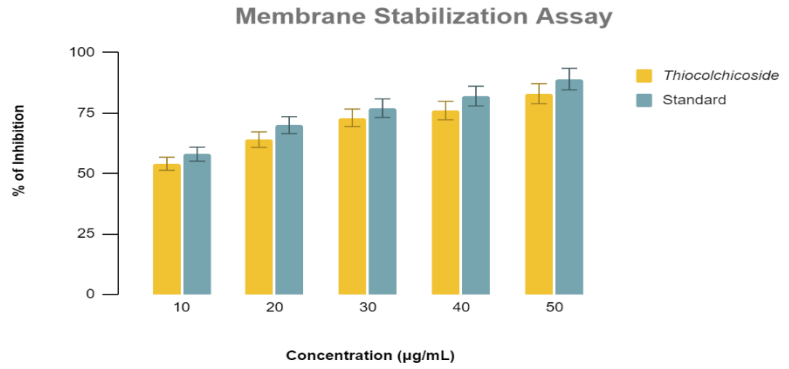


Figure 4: Thiocolchicoside's anti-inflammatory activity assessed using the membrane stabilisation assay

The membrane stabilisation assay graph demonstrates a dose-associated increase in inhibition for both thiocolchicoside and the reference drug. Thiocolchicoside increases membrane stabilisation from around 55% at 10 µg/mL to around 85% at 50 µg/mL. Despite the standard demonstrating somewhat better suppression at each concentration, thiocolchicoside remains nearly in efficacy. This shows that thiocolchicoside has substantial anti-inflammatory capabilities, as it efficiently prevents red blood cell lysis. In general, the findings suggest its potential as a membrane stabilising agent.

**Bovine Serum Albumin Assay**

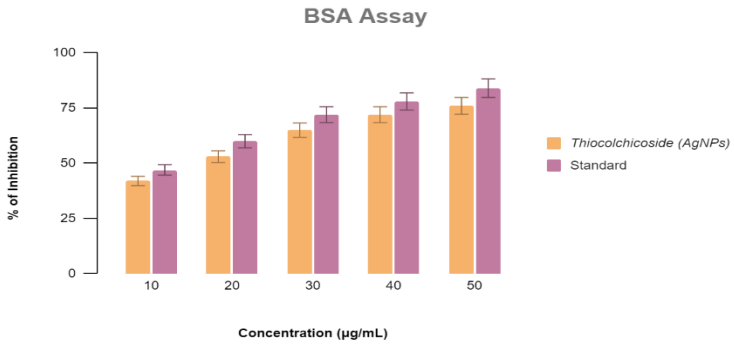

Figure 5. The anti-inflammatory effect of synthesised thiocolchicoside-mediated silver nanoparticles was tested utilising bovine serum albumin.

The BSA Assay graph compares the anti-inflammatory properties of thiocolchicoside-assisted silver nanoparticles (AgNPs) to a standard. Inhibition increases with dose, from 10 to 50 µg/mL. Thiocolchicoside (AgNPs) inhibits approximately 80% at 50 µg/mL, showing significant protein stabilisation. While the standard regularly displays somewhat more activity, the difference is negligible. These findings imply that thiocolchicoside-mediated AgNPs have a significant anti-inflammatory capability

**Egg Albumin Assay**

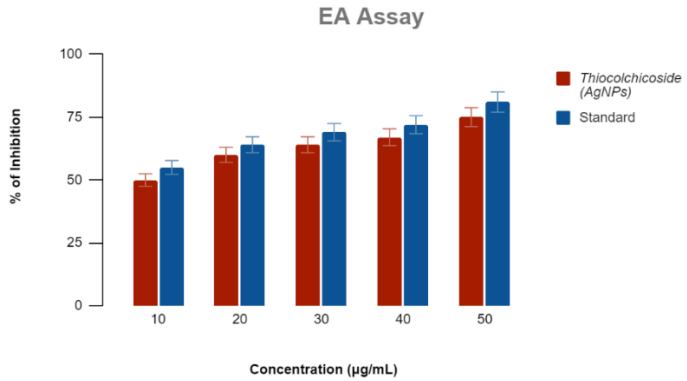


Figure 6. The anti-inflammatory effect of thiocolchicoside-mediated silver nanoparticles was investigated utilising an egg albumin denaturation assay.

The EA Assay graph compares the anti-inflammatory effects of thiocolchicoside-assisted silver nanoparticles (AgNPs) to a conventional medication. Both groups showed a strong dose-related rise in reduction from 10 to 50 µg/mL. Thiocolchicoside (AgNPs) inhibits roughly 75% at 50 µg/mL. Although conventional medicine typically outperforms the nanoparticles, their efficacy is equivalent. This shows that thiocolchicoside-AgNPs have potential protein stabilising and anti-inflammatory effects.

**Membrane Stabilization Assay**

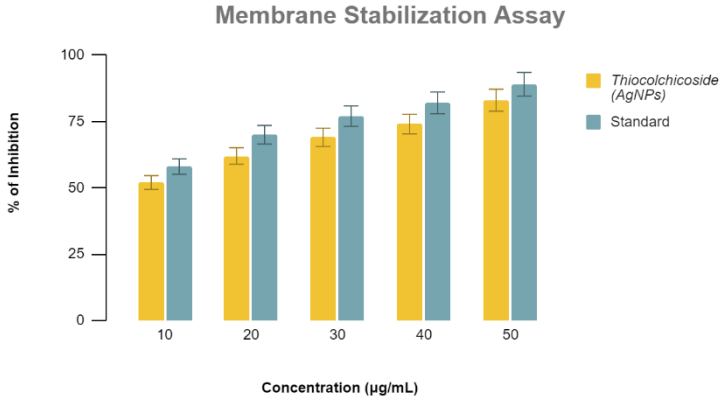


Figure 7 Anti-inflammatory effectiveness of synthesised thiocolchicoside-mediated silver nanoparticles utilising membranes stabilising test

The Membrane Stabilisation Assay graph compares the anti-inflammatory capability of thiocolchicoside-assisted silver nanoparticles (AgNPs) with a conventional medication. From 10 to 50 µg/mL, the percentage of inhibition gradually increases with dose. At a concentration of 50 µg/mL, thiocolchicoside (AgNPs) inhibits membrane stabilisation by approximately 83%. While the standard has a little more inhibition, the AgNPs function similarly well. This suggests that thiocolchicoside-AgNPs have potent anti-inflammatory action via membrane stabilisation.

**Cytotoxic Assay-Thiocolchicoside+AgNPs**

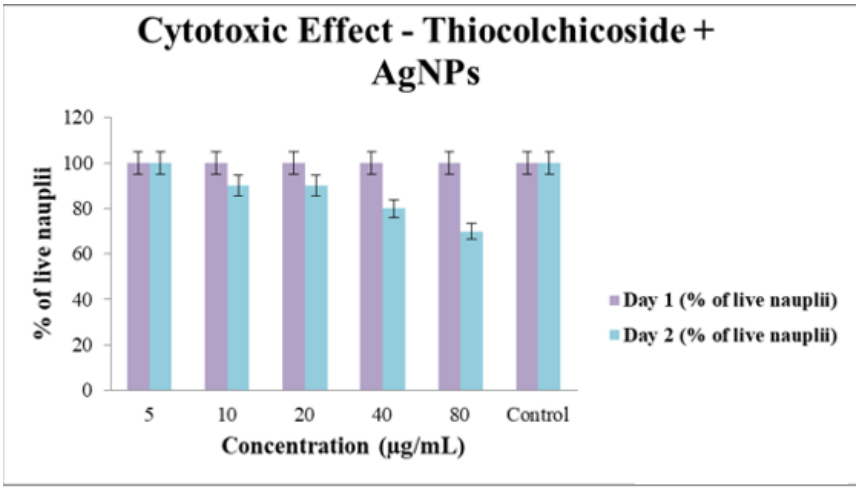


Figure 8 represents the Cytotoxic effect of the activity Thiocolchicoside and silver nanoparticles on Days 1 and 2activity Figure 5. The study shows the harmful effects of Thiocolchicoside-assisted silver nanoparticles (AgNPs) on brine shrimp nauplii at various concentrations (5-80 µg/mL) over 2 days. The percentage of living nauplii declines as you raise the quantity, showing a dose-dependent cytotoxic effect. A more dramatic drop in longevity is found on Day 2 than on Day 1, indicating a time-dependent effect. At 80 µg/mL, survival decreases substantially, particularly on Day 2, when it attains a low of approximately 70%. In contrast, the control group retains good rates of life on each day, indicating that the untreated medium is biocompatible.

**Cytotoxic Effect-Thiocolchicoside**

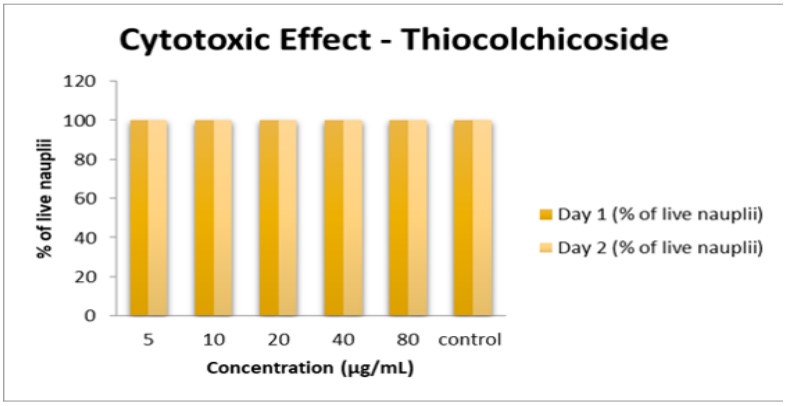


Figure 9 represents the Cytotoxic effect of Thiocolchicoside in Days 1 and 2 Activity

The study examined the cytotoxic effects of Thiocolchicoside alone on brine shrimp nauplii at doses that varied from 5 to 80 µg/mL over two days. On both Days 1 and 2, the percentage of live nauplii is always elevated across every concentration. This suggests that Thiocolchicoside does not cause considerable cytotoxicity even at greater doses. The outcomes are similar to the control group, indicating high biocompatibility. Overall, Thiocolchicoside appears to be non-toxic to nauplii within the circumstances examined.

**Embryonic Toxicology**

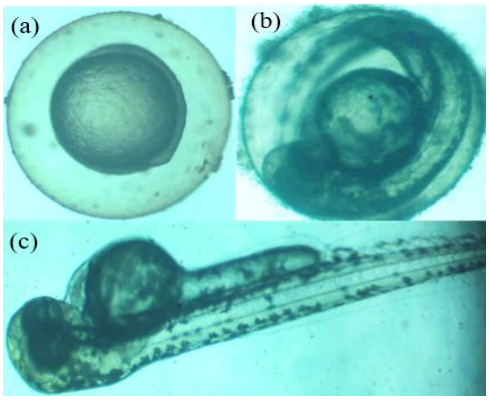

Figure 10 (a) represents embryogenic activity on day 1. (b) represents embryogenic activity on day 2. (c) represents embryogenic activity on day 3

shows key developmental stages of zebrafish embryos. Figure 2(a) depicts the initial cleavage stage, with a visible blastodisc within the chorion. Figure 2(b) depicts the somite stage, in which the embryo begins to elongate and coil around its yolk. Figure 2(c) shows a hatched larva with a fully formed head, eyes, tail, and body pigmentation. These steps aid in the assessment of toxicity during development as well as the impacts of drugs or nanoparticles in research.

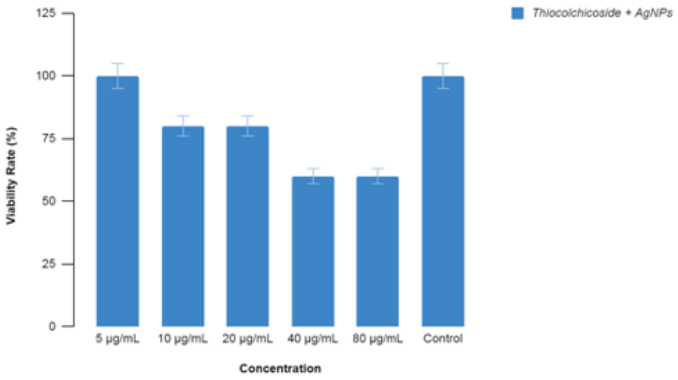


Figure 11 represents the Viability rate (%) of Glorious superba mediated silver nanoparticles with thiocolchicoside

Figure 3. The graph shows the viability rate of cells exposed to Thiocolchicoside-assisted silver nanoparticles (AgNPs) at various concentrations (5-80 µg/mL). The viability rate declines gradually as one raises concentration, demonstrating a dose-dependent cytotoxic effect. Lower dosages (5 µg/mL) maintain high viability (~100%), while higher doses (40 and 80 µg/mL) reduce the survival rate to about sixty percent. The control group has the greatest survival, indicating low spontaneous cell mortality. These findings indicate that, whereas small quantities are rather safe, greater concentrations of the nanocomposite cause considerable cytotoxicity.

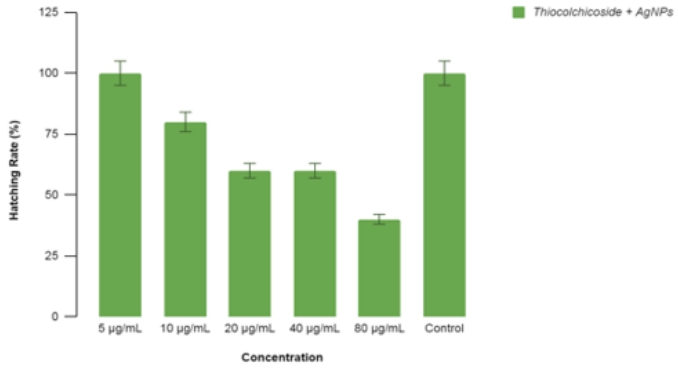


Figure 12 represents the Hatching rate (%) of *Glorious superba* mediated silver nanoparticles with thiocolchicoside

Figure 4 demonstrates the hatching rate of brine shrimp nauplii subjected to Thiocolchicoside-assisted silver nanoparticles (AgNPs) at different doses. The greatest amount (80 µg/mL) resulted in the lowest hatching rate (~40%), indicating a clear dose-related decline. At lesser dosages (5 µg/mL), the hatching rate is comparable to the control (~100%), showing negligible toxicity. As dosage rises, embryonic growth becomes more impeded, implying that bigger doses may impair basic processes in the body. These data indicate the nanocomposite's potential embryotoxicity at high dosages.

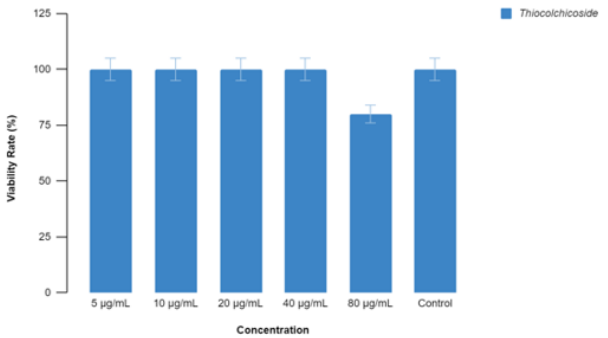

Figure 13: Thiocolchicoside amounts of 5, 10, 20, and 40 µg/mL were used to determine the survival rate of zebrafish nauplii.
Figure 7. This graph shows the survival rate of brine shrimp nauplii administered with Thiocolchicoside alone at various quantities (5-80 µg/mL). At lower dosages (5-40 µg/mL), survival stays high and comparable to the control group (~100%), showing minimal cytotoxic consequences. At the maximum dosage (80 µg/mL), survivability decreases by around 80%, indicating moderate toxicity at higher dosages. Error bars are modest, indicating reliable and consistent outcomes. These data demonstrate that Thiocolchicoside, when isolated, is substantially biocompatible with nauplii under the circumstances examined.

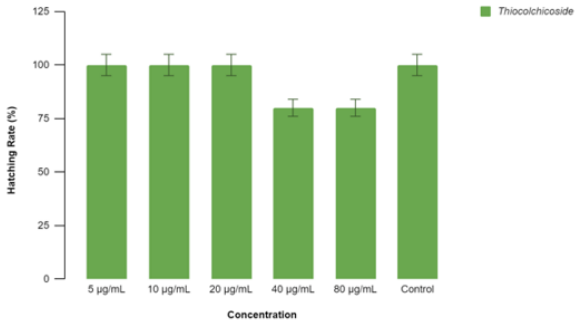


Figure 14: Thiocolchicoside levels ranging from 5, 10, 20, and 40 µg/mL were used to determine the hatching rate of zebrafish nauplii.

Figure 8 represents the hatching rate of brine shrimp nauplii subjected to various levels of Thiocolchicoside. At dosages of 5, 10, and 20 µg/mL, the hatching rate stays elevated and almost equal to the control (~100%), showing low embryotoxicity. However, at 40 and 80 µg/mL, hatching rates decrease to roughly 80%, indicating minimal harm at higher dosages. Despite this, the total effect is minimal, with no significant drop. These results show that thiocolchicoside only is relatively safe for embryonic growth in nauplii, even at high doses.

# DISCUSSION

The current study emphasises the effective green synthesis of silver nanoparticles (AgNPs) utilising Glorious superba leaf extract together with Thiocolchicoside (TCC), an anti-inflammatory molecule, and assesses their anti-inflammatory, cytotoxic, and embryotoxic capabilities [27]. The synthesis procedures were eco-friendly, as evidenced by the colour change caused by UV-Vis absorption at 410 nm [28]. The use of TCC in the environmentally friendly synthesis procedure looks to give dual benefits, acting as both a reducing/stabilizing agent and a bioactive molecule that increases the nanoparticles' pharmaceutical characteristics [29]. Nanoparticles demonstrated comparable activity to the standard medicine (diclofenac sodium), particularly at 50 µg/mL, indicating their potential as anti-inflammatory drugs.
[30]. In regard to cytotoxicity, brine prawn lethality tests found that TCC was harmless at all evaluated dosages. However, TCC-assisted AgNPs demonstrated a concentration- and time-varying raised toxicity[31]. This finding implies that silver ions participate in cytotoxic behaviour, and their association with TCC may further alter responses in cells. On Day 1, negligible toxicity was found. However, by Day 2, larger doses (≥40 µg/mL) dramatically lowered survival rates among nauplii, showing dependent on time effect presumably due to slow nanoparticle absorption or prolonged initiation of toxicity [32].

These results are consistent with prior studies revealing the cytotoxic and antibacterial properties of AgNPs, but also underline the requirement of cautious choice of dose for biological purposes [33]. Medicinal uses must be properly optimised to maintain effectiveness and biological safety [34]. The findings further back up the assumption that model species like zebrafish offer an effective and morally sound approach for early toxicological screenings, delivering real-time developmental facts that match well with higher vertebrates[35]. Future research should look into the molecular processes behind the identified harmful effects, as well as analyse the long-term consequences on organogenesis and gene expression.
Conclusion

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Ethics approval and consent to participate
All the data was available from public databases and there is no need for ethics approval and consent.
Credit authorship contribution Statement:
Renu Avajjiravelu: Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data

curation. Karthikeyan Shanmugam: Validation, Methodology, and editing with review of the manuscript, Data curation, and Visualization.Santhoshkumar Jayakodi: Investigation, Validation, Review and editing. Rajeshkumar Shanmugam: Writing – review & editing, Validation, Supervision, Methodology, Investigation, Data curation, Conceptualisation.

Consent for publication
Not applicable.
Conflict of interest
The authors state no conflict of interest
Declaration of competing interest
The authors declare that they have no conflict of interest.

# CONCLUSION

This study effectively demonstrated the green synthesis of silver nanoparticles (AgNPs) utilising Glorious superba leaf extract and Thiocolchicoside (TCC), resulting in nanoparticles that have potent anti-inflammatory characteristics and significant biological impacts. The synthesised AgNPs demonstrated essential dose-dependent antibacterial and anti-inflammatory activity, with TCC increasing effectiveness in therapy. Cytotoxicity tests showed that, while TCC only was non-hazardous, TCC-mediated AgNPs demonstrated a concentration- and time-varying cytotoxic response, especially at greater dosages. Embryotoxicity tests utilising zebrafish embryos additionally demonstrated that increased amounts of the nanocomposite caused prolonged hatching, decreased survival, and morphological abnormalities. These results emphasise the combined potential and precautionary constraints of employing TCC-assisted AgNPs in medical fields. While minimal amounts provide medicinal advantages, higher doses raise safety issues, emphasising the importance of careful dose optimisation and additional molecular studies to ensure safe clinical implementation.

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