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Antioxidant, Anti-Inflammatory, And Embryonic Toxicology Evaluation Of Iron Oxide Nanoformulated Sinapic Acid Using Cinnamon

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ABSTRACT

Introduction: Iron oxide nanoparticles (Fe_2O_3 NPs) are gaining attention in biomedicine due to their magnetic properties, stability, and low toxicity. This study explored a green synthesis method using sinapic acid and cinnamon extract to produce Fe_2O_3 NPs and evaluated their antioxidant, anti-inflammatory, cytotoxic, and embryonic toxicity profiles.

Materials and Methods: Fe_2O_3 NPs were synthesized by mixing iron chloride with cinnamon extract, while sinapic acid was added via sonication. The nanoparticles were characterized using different characterization techniques. Antioxidant and anti-inflammatory activities were assessed through various in vitro assays. Cytotoxicity was tested using brine shrimp, and embryonic toxicity was studied in zebrafish embryos.

Results: The green synthesized nanoparticles showed strong anti-inflammatory and antioxidant effects, comparable to standard drugs. Cytotoxicity was low, with high survival in brine shrimp, and over 80% embryo viability was observed at all concentrations.

Discussion and Conclusion: The eco-friendly synthesis produced biocompatible Fe_2O_3 NPs with promising therapeutic properties. Their strong bioactivity and low toxicity suggest potential for use in drug delivery and treatments for oxidative stress and inflammation. Further in vivo studies are needed to confirm their clinical relevance.

Keywords: *Iron oxide nanoparticles, Sinapic acid, Green synthesis, Cinnamon extract, Antioxidant activity, Anti-inflammatory activity, Biomedical applications, Nanomedicine.*

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1. INTRODUCTION

Iron oxide nanoparticles have attracted significant interest for biomedical use owing to their distinctive magnetic behavior, adaptable surface chemistry, high stability, and low toxicity (1). Their superparamagnetic behavior enables targeted drug delivery and imaging when guided by external magnetic fields, enhancing therapeutic precision. Furthermore, Fe_2O_3 NPs have shown promise as MRI contrast agents by improving image clarity and diagnostic sensitivity at targeted sites(2).

Incorporating bioactive compounds into Fe_2O_3 nanoparticles has emerged as an effective strategy to enhance their therapeutic potential(3). Sinapic acid (SA), a naturally occurring phenolic compound found in plants such as cereals and

berries, has shown notable antioxidant, anti-inflammatory, and antimicrobial properties(4,5). SA scavenges free radicals and modulates inflammatory pathways, including the NLRP3 inflammasome, a key driver of inflammatory responses in various chronic diseases. Despite these promising properties, the clinical use of sinapic acid is limited by its poor bioavailability and stability under physiological conditions(6). Nanoformulating SA with Fe_2O_3 NPs may improve its solubility, bioavailability, and therapeutic efficacy, allowing for targeted delivery to inflamed or oxidative stress sites and enhancing its pharmacological performance(7).

In green nanotechnology, cinnamon has gained attention as an eco-friendly reducing and stabilizing agent in nanoparticle synthesis. Rich in phenolic and flavonoid compounds, cinnamon act as both a reducing agent, facilitating the conversion of iron salts into Fe_2O_3 NPs, and a stabilizing agent, capping the nanoparticles to prevent aggregation(8). The bioactive properties of cinnamon, including its antioxidant and anti-inflammatory effects, may further enhance the therapeutic profile of the sinapic acid-loaded Fe_2O_3 NPs. Additionally, this green synthesis method correlates with sustainable practices, avoiding the use of toxic reagents and contributing to environmentally conscious nanomedicine(9).

This study aims to evaluate the antioxidant, anti-inflammatory, and embryonic toxicity profile of sinapic acid nanoformulated with Fe_2O_3 NPs synthesized using cinnamon extract. The antioxidant and anti-inflammatory properties are of particular relevance in chronic diseases associated with oxidative stress and inflammation, where they have been implicated in the progression of conditions such as cancer, neurodegenerative diseases, and cardiovascular disorders(10). Additionally, embryonic toxicology testing is essential to ensure the biocompatibility and safety of this nanoformulation for potential applications in regenerative medicine and prenatal health(11).

This present study contributes to the development of safe, biocompatible nanoformulations with the potential for improved therapeutic efficacy in managing oxidative stress, inflammation, and ensuring embryonic safety. By leveraging the benefits of Fe_2O_3 NPs, sinapic acid, and cinnamon, this study provides an innovative approach to nanomedicine with applications in antioxidant therapies, anti-inflammatory treatments, and safer biomedical applications.

2. MATERIALS AND METHODS

Preparation of Extract

Dried cinnamon (1 g) was added and heated in 100 mL distilled water at 70 °C for 20 minutes, filtered (Whatman No. 1), and the extract was stored at 4 °C for nanoparticle synthesis.

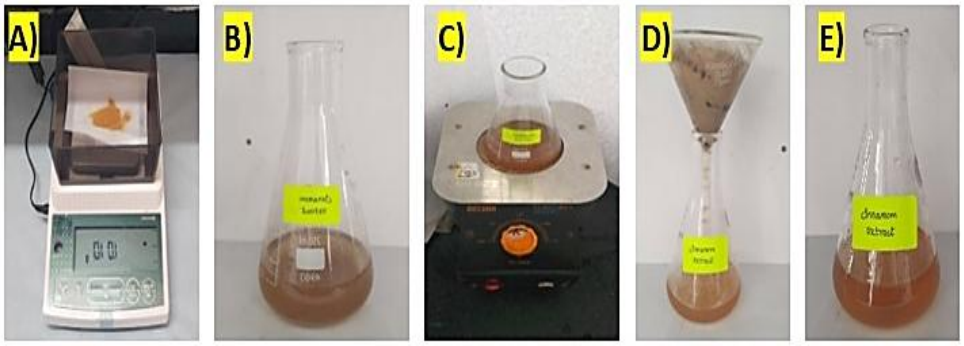

Figure 1: Preparation of the aqueous cinnamon extract. (A) Dried cinnamon bark, (B) cinnamon in distilled water, (C) heating the mixture on a mantle (D) filtering the extract and (E) the final filtered extract stored for use.

Preparation of green synthesized nanoparticles

The iron salt was dissolved in 50 mL of distilled water, then combined with 50 mL of the prepared extract, which acted as a natural reducing and stabilizing agent. The mixture was stirred at ~600 rpm for 24–48 hours to promote nanoparticle formation.

Synthesis progress was monitored via UV–Vis spectrophotometry by identifying the characteristic Fe_2O_3 absorbance peak. After synthesis, the suspension was centrifuged at 8000 rpm for 10 minutes. The resulting pellet containing Fe_2O_3 NPs was collected, stored in airtight tubes, and used for subsequent characterization and applications.

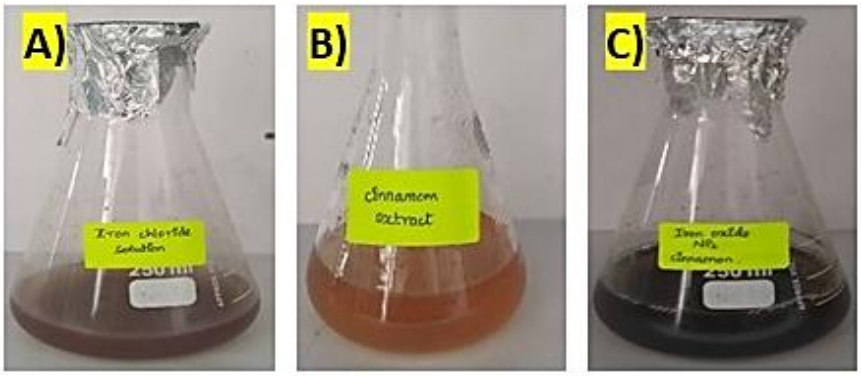

Figure 2: Synthesis of iron oxide nanoparticles using cinnamon extract. (A) Iron(III) chloride solution, (B) cinnamon extract, and (C) the iron oxide nanoparticles obtained via the cinnamon-mediated green synthesis.

Preparation of Fe_2O_3–Sinapic Acid Nanoformulation

The Fe_2O_3–sinapic acid nanoformulation was prepared by loading sinapic acid onto pre-synthesized Fe_2O_3 nanoparticles. A stock solution was prepared by dissolving 100 mg of sinapic acid in 1 mL of DMSO and 4 mL of PBS, then stirred on an orbital shaker for 1 hour to ensure complete dissolution.

Next, 1 mL of this stock was combined with 1 mL of the Fe_2O_3 NP suspension and subjected to sonication for 30 minutes to facilitate the binding of sinapic acid onto the nanoparticle surfaces and ensure a stable dispersion. The final nanoformulation was stored for subsequent characterization and bioactivity assays, leveraging both the therapeutic potential of sinapic acid and the functional properties of Fe_2O_3 nanoparticles.

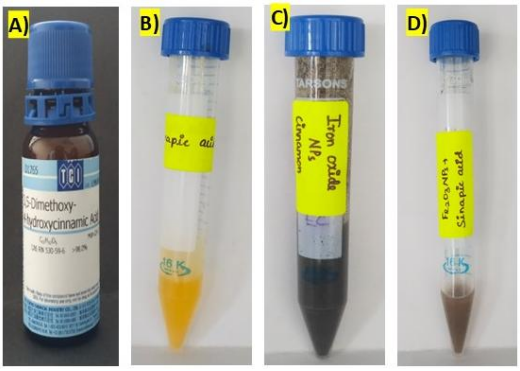

Figure 3: Preparation of the Fe_2O_3–sinapic acid nanoformulation. (A) Sinapic acid powder, (B) sinapic acid dissolved in DMSO + PBS, (C) synthesized Fe_2O_3 nanoparticles, and (D) the final nanoformulation.

Biomedical applications:

The antioxidant (DPPH, H_2O_2, FRAP, ABTS, and NO scavenging), anti-inflammatory (BSA and egg albumin denaturation, HRBC membrane stabilization), cytotoxic (brine shrimp lethality), and zebrafish embryonic toxicology assays were performed based on standard protocols adopted from previously published research works, with appropriate modifications. These methods involved dose-dependent evaluations of the Fe_2O_3–sinapic acid nanoformulation and comparisons against established reference standards such as ascorbic acid and diclofenac sodium(12–14).

Statistical Analysis:

Statistical significance between treatment groups and the control was assessed using one-way ANOVA, followed by Tukey's post hoc test for pairwise comparisons when $p < 0.05$. All analyses were conducted using a significance threshold of $p < 0.05$.

3. RESULT

Antioxidant Activity Evaluation

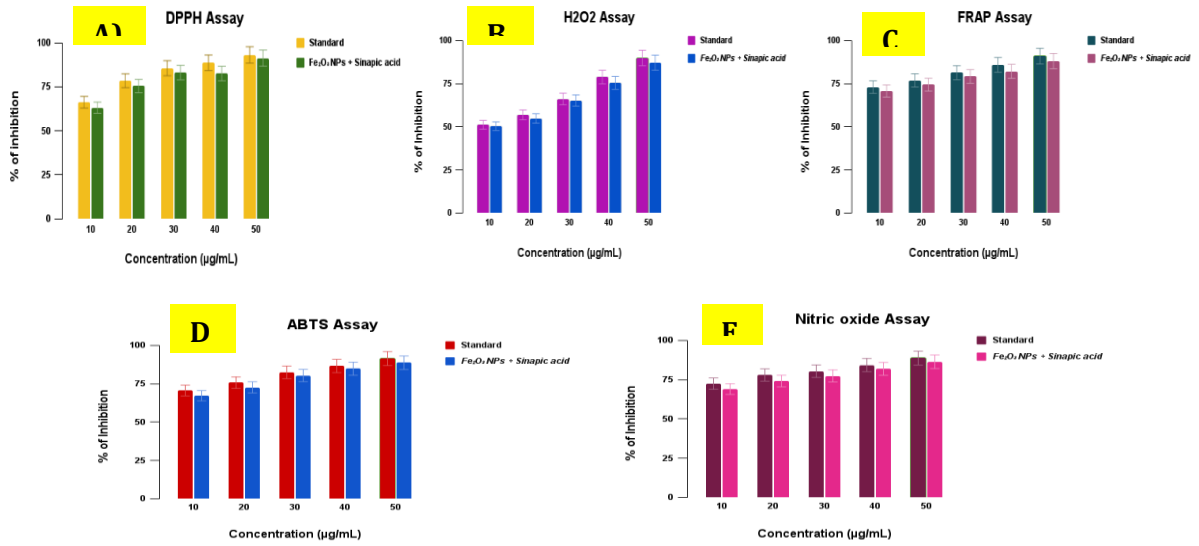

Figure 4: Fe_2O_3 nanoparticles -sinapic acid nanoformulation antioxidant activity evaluated through (A) DPPH assay, (B) H_2O_2 assay, (C) FRAP assay, (D) ABTS assay, and (E) Nitric oxide assay.

In the DPPH assay, Fe_2O_3 NPs showed increasing inhibition from 67% to 85% across 10–50 µg/mL, closely mirroring ascorbic acid's 70–88% range. Similarly, the H_2O_2 assay showed Fe_2O_3 NPs achieving 56–84% inhibition, slightly lower than ascorbic acid (60–90%), yet still demonstrating strong peroxide scavenging ability.

The FRAP assay confirmed high ferric reducing power, with Fe_2O_3 NPs achieving 70–88% activity, nearly matching ascorbic acid's 72–90%. In the ABTS assay, inhibition ranged from 75% to 87% for Fe_2O_3 NPs, again closely following the standard's 78–90% activity. The Nitric Oxide assay showed effective scavenging, with inhibition increasing from 72% to 86%, compared to 74–88% for ascorbic acid.

Overall, Fe_2O_3–sinapic acid nanoparticles demonstrated strong and consistent antioxidant activity across all tested assays. While slightly trailing ascorbic acid in some cases, their performance was comparable, highlighting their broad-spectrum radical scavenging potential and supporting their suitability for biomedical use in oxidative stress-related conditions.

Anti-inflammatory Activity Evaluation

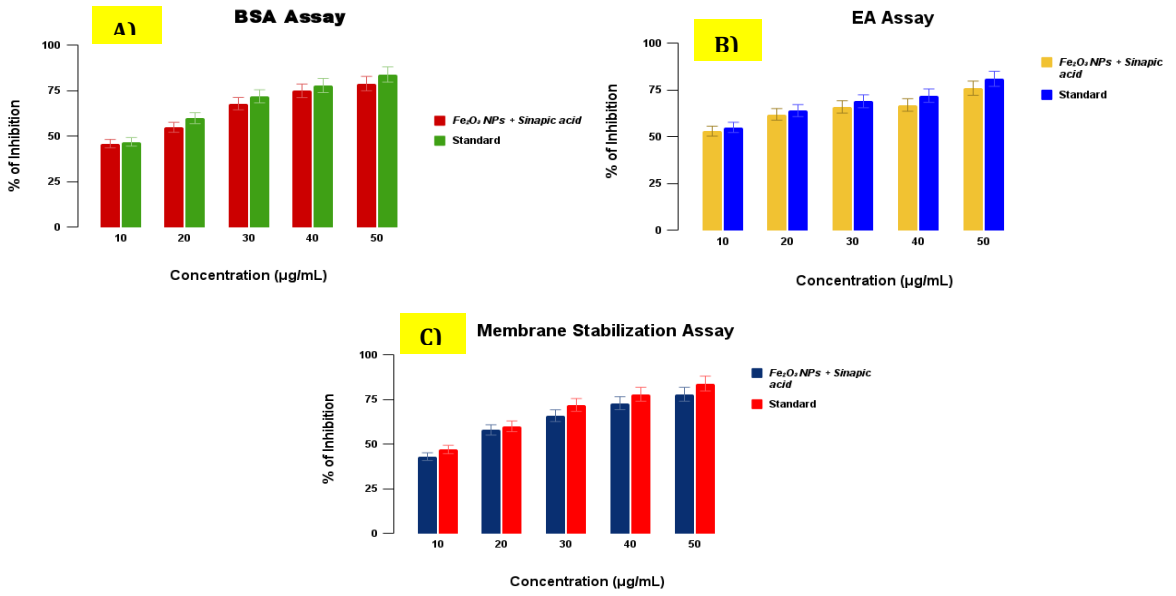

Figure 5: Fe_2O_3 nanoparticles with sinapic acid nanoformulation anti-inflammatory activity assessed using (A) BSA denaturation assay, (B) Egg albumin denaturation assay, and (C) Membrane stabilization assay.

In the BSA denaturation assay, Fe_2O_3 NPs showed inhibition ranging from 55% at 10 µg/mL to 77% at 50 µg/mL, closely matching diclofenac sodium's 58–80%. Similarly, the EA denaturation assay demonstrated inhibition increasing from 52% to 74%, with diclofenac slightly higher at 55–78%. The Membrane Stabilization assay showed inhibition values for Fe_2O_3 NPs rising from 50% to 75%, compared to 53–78% for diclofenac sodium. These results highlight the nanoparticles' effectiveness in stabilizing cell membranes and preventing protein denaturation, both of which are relevant mechanisms in inflammation control. Overall, the Fe_2O_3–sinapic acid nanoformulation exhibited significant anti-inflammatory activity across all tests. While slightly less potent than diclofenac, the nanoparticles still demonstrated substantial efficacy, supporting their potential as a natural, biocompatible alternative for inflammation management.

Cytotoxicity Evaluation of Fe_2O_3 NPs with Sinapic Acid

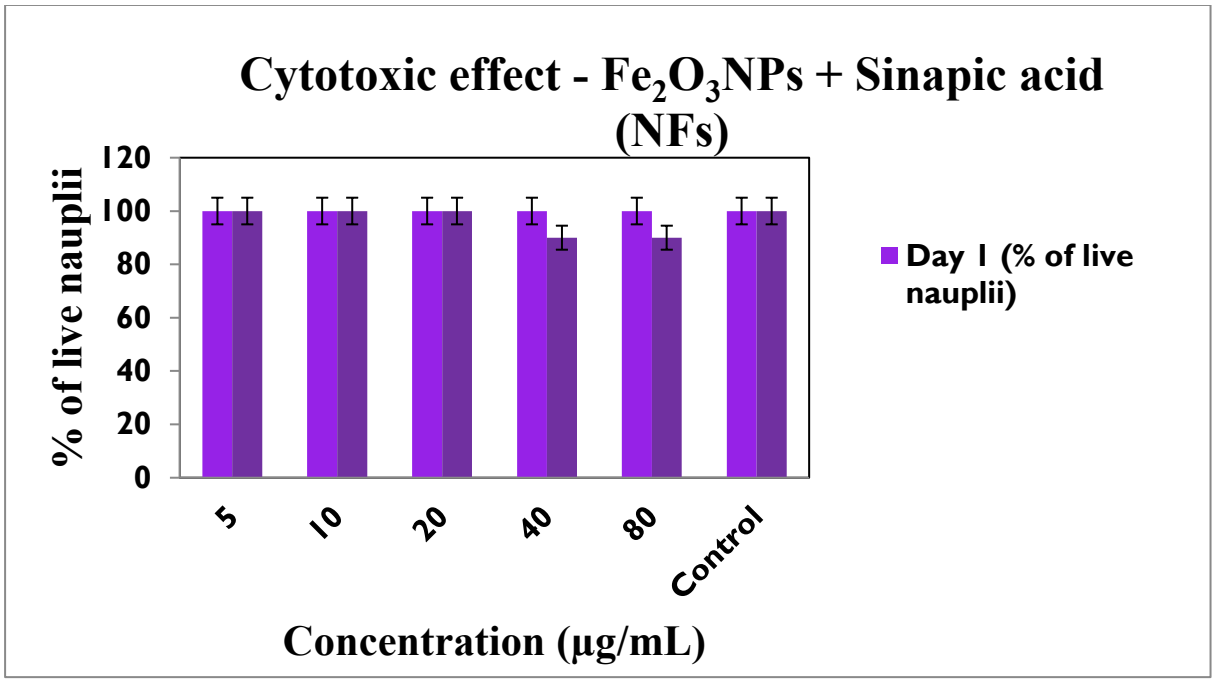

Figure 6: Fe_2O_3 nanoparticles with sinapic acid (NFs) cytotoxic effect assessed using the brine shrimp lethality assay.

The cytotoxic effect of Fe_2O_3 NPs formulated with sinapic acid was evaluated using the brine shrimp lethality assay. The percentage of live nauplii was assessed after exposure to various concentrations (5-80 µg/mL) of the nanoformulation over two days, as shown in Figure 6. The results indicated minimal cytotoxicity across all tested concentrations, with survival rates consistently high and comparable to the control group. On Day 1, the percentage of live nauplii ranged from 95% to 100% across all concentrations, demonstrating limited immediate toxicity. By Day 2, slight reductions in live nauplii were observed, with survival rates slightly decreasing but remaining above 90% at all concentrations. The findings suggest that Fe_2O_3 NPs -sinapic acid nanoformulation exhibit low cytotoxicity, even at the highest tested concentration of 80 µg/mL. This result supports the biocompatibility of the nanoformulation, indicating its potential safety for biomedical applications, particularly in therapeutic contexts where prolonged exposure is expected.

Zebrafish Embryonic Toxicology Evaluation

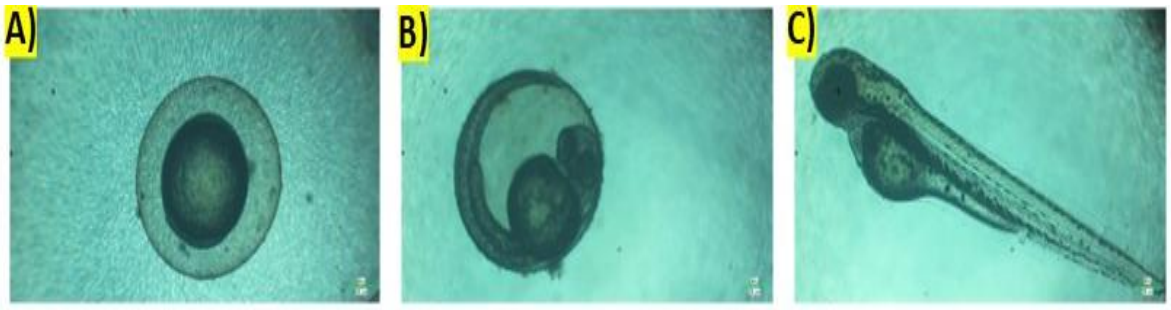

Figure 7: Developmental stages of zebrafish embryos exposed to Fe_2O_3 nanoparticles -sinapic acid nanoformulation. (A) Cleavage stage, (B) Organogenesis stage, and (C) Hatched larvae.

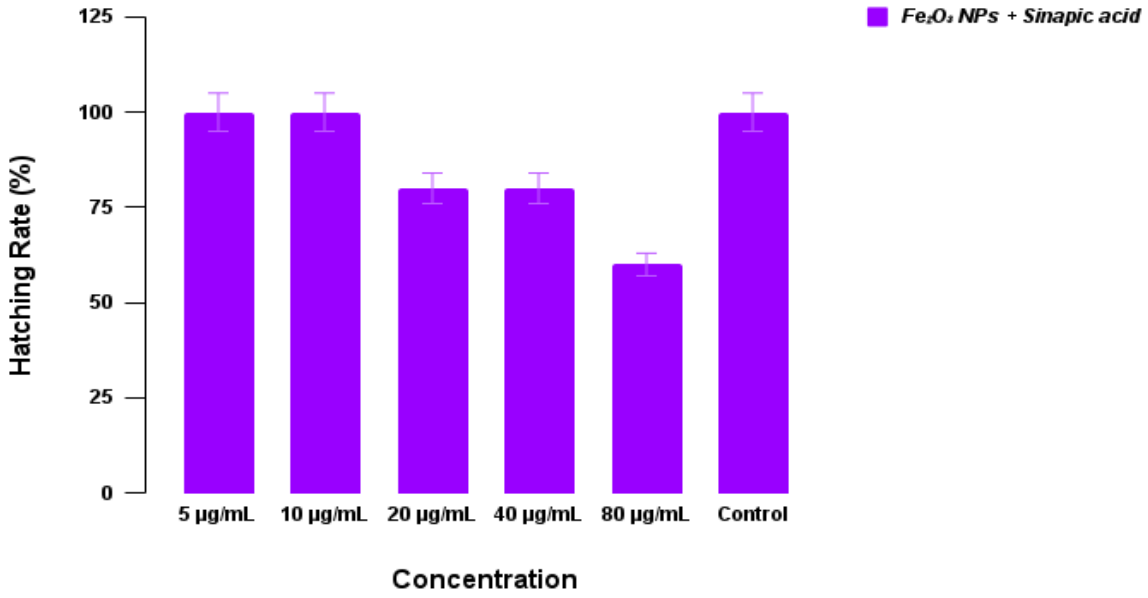

Figure 8: Hatching rate (%) of zebrafish embryos exposed to Fe_2O_3 nanoparticles with sinapic acid at various concentrations

The embryonic hatching rate of zebrafish exposed to varying concentrations of sinapic acid-incorporated Fe_2O_3 nanoparticles (Fe_2O_3 NPs) demonstrated a dose-dependent decline in viability which was depicted in figure 8. At lower concentrations of 5µg/mL and 10µg/mL, the hatching rates remained high and comparable to the control group, with minimal deviation, indicating negligible toxicity at these doses. Specifically, the hatching rate at 5µg/mL was nearly equivalent to the control, suggesting good biocompatibility.

However, a marked reduction in hatching rate was observed at 20 µg/mL and 40 µg/mL, with values dropping significantly, indicating increased embryotoxicity. The most pronounced decline was recorded at 80µg/mL, where the hatching rate fell to nearly 55%, highlighting a clear cytotoxic effect at higher nanoparticle concentrations. In contrast, the control group exhibited a consistently high hatching rate above 95%, reinforcing the adverse impact of elevated concentrations.

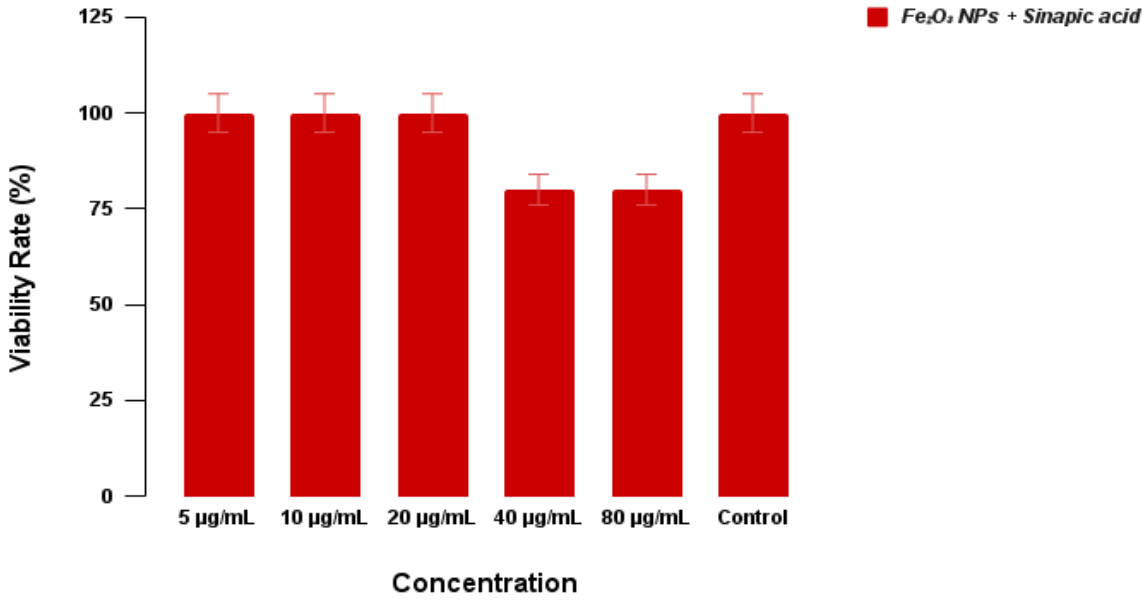

Figure 9: Viability rate (%) of zebrafish embryos exposed to Fe_2O_3 nanoparticles with sinapic acid at various concentrations

The results, depicted in Figure 8 & 9, indicate a concentration-dependent response, with slight reductions in viability observed at higher concentrations. At lower concentrations (5 and 10 µg/mL), the viability rate remained close to that of the control group, maintaining over 95% survival. As the concentration increased to 20 and 40 µg/mL, a modest decline in viability was noted, with survival rates dropping to approximately 90% and 85%, respectively. The most significant reduction in viability was observed at 80 µg/mL, where the viability rate approached 80%, indicating a mild toxic effect at this higher concentration. Overall, the findings suggest that Fe_2O_3 NPs, even when formulated with sinapic acid, exhibit low embryotoxicity across a broad concentration range. This limited toxicity highlights the potential biocompatibility of the formulation, supporting its prospective use in biomedical applications where minimal embryotoxicity is desired.

4. DISCUSSION

This study comprehensively examined the antioxidant, anti-inflammatory, cytotoxic, and embryotoxic properties of iron oxide nanoparticles (Fe_2O_3 NPs) synthesized using cinnamon extract and functionalized with sinapic acid. The results demonstrate their strong biomedical potential, showcasing improved stability, functionality, and biocompatibility for therapeutic and diagnostic applications.

The Fe_2O_3 NPs formulated with sinapic acid displayed significant antioxidant capabilities across multiple assays (DPPH, H_2O_2, FRAP, ABTS, and nitric oxide scavenging), with inhibition values closely matching those of ascorbic acid(15,16). This robust antioxidant activity is attributed to sinapic acid's potent radical-scavenging ability and the nanoparticle's high surface reactivity, which together enhance its efficacy in neutralizing oxidative stress(17). The anti-inflammatory potential was similarly promising, as demonstrated in BSA and egg albumin denaturation assays, as well as membrane stabilization tests, where Fe_2O_3 NPs showed inhibition rates comparable to diclofenac sodium. These results emphasize the dual antioxidant and anti-inflammatory functionality, beneficial for treating inflammation-related diseases with minimal side effects(16).The Fe_2O_3 NPs demonstrated low cytotoxicity in the brine shrimp lethality assay across concentrations of 5–80 µg/mL, with survival rates consistently high, indicating biocompatibility(18). This is corroborated by the low embryotoxicity observed in zebrafish embryos, where viability remained above 80% even at the highest concentration tested(19). The hatching rate analysis further supported this, showing minimal impact at 5–10 µg/mL and a modest decline at higher concentrations, with the lowest hatching rate recorded at 80 µg/mL. The mild toxicity at higher concentrations may be attributed to the nanoparticles' surface properties, modified by sinapic acid, which mitigates oxidative damage by capping and stabilizing the Fe_2O_3 NPs effectively(20,21,22).

The synthesis approach using sinapic acid and cinnamon extract as reducing and stabilizing agents underscores a sustainable, eco-friendly method for producing stable, biocompatible Fe_2O_3 NPs(23). The antioxidant properties of these natural agents not only stabilize the nanoparticles but also contribute to their bioactivity, providing enhanced therapeutic benefits. This method avoids toxic chemicals, aligning with green chemistry principles and supporting the development of nanoparticles that are safe for clinical use(24,25).

5. LIMITATION AND SCOPE OF THE STUDY

The study's limitations include variability in the green synthesis process, where natural extracts may introduce batch inconsistencies, impacting the reproducibility of nanoparticle characteristics. Additionally, the lack of detailed structural characterizations, such as zeta potential and DLS analysis, restricts understanding of surface properties and dispersion stability in physiological conditions(26). While in vitro and embryonic assays confirmed low toxicity, further in vivo studies are essential to evaluate safety, pharmacokinetics, and therapeutic effectiveness in complex biological systems. The observed tendency for aggregation in Fe_2O_3 NPs could also pose challenges in maintaining stability within biological environments, potentially requiring surface modifications for enhanced dispersion. Moreover, scaling up production while ensuring environmental sustainability in waste management and byproducts remains a challenge(27).

In terms of scope, Fe_2O_3 NPs synthesized via green methods show promise across biomedical applications, including drug delivery, antioxidant therapies, and anti-inflammatory treatments. Their magnetic properties and biocompatibility also make them suitable for diagnostic imaging, particularly as MRI contrast agents, with further potential in theranostics for simultaneous therapy and diagnostics(28). This eco-friendly synthesis approach could support the development of sustainable nanomaterials, expanding the possibilities for environmentally benign production methods. Enhancements in nanoparticle stability, such as polymer coatings or functionalization with specific ligands, could also improve targeted delivery for medical applications. Furthermore, the biocompatibility and low toxicity of these Fe_2O_3 NPs open avenues in regenerative medicine, especially in tissue engineering and wound healing, making them versatile candidates for a range of innovative biomedical applications(29).

6. CONCLUSION

The comprehensive characterization and biological evaluation of Fe_2O_3 NPs -sinapic acid nanoformulation and cinnamon extract indicate their significant antioxidant and anti-inflammatory properties, and minimal toxicity, making them suitable for biomedical applications. These findings support the potential use of these nanoparticles in drug delivery, diagnostics,

and therapies targeting oxidative stress and inflammation-related conditions. Further in vivo studies and application-specific testing are recommended to advance their development in clinical nanomedicine, demonstrating the effectiveness of green synthesis in producing functional and biocompatible nanoparticles.

7. ACKNOWLEDGEMENTS

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Compliance with ethical standards

Conflict of interest: The Authors declare no conflict of interest.

Ethical issues: None

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