Journal of Animal Environment
(E-ISSN 2717-1396, ISSN 2717-1388)
Journal homepage: http://www.aejournal.ir

Original Research Paper

Thrombolytic, Anticoagulant, and Cytotoxicity Assessment of Rutin-Incorporated Chitosan Nanocomposite: Embryonic Toxicology Evaluation

Ashvikka V.H[1], Steffy Dominic[2], Renu Vajjiravelu[3], Santhoshkumar Jayakodi[3], Rajeshkumar Shanmugam[1*], R. Kowsalya[4], Alagendran Subbarayalu[5], Arjun Pandian[6]

[1]Nanobiomedicine Lab, Department of Anatomy, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai - 602105, Tamil Nadu, India
[2]Department of Physiology, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai 602 105, Tamil Nadu, India
[3]Department of Biotechnology, Saveetha School of Engineering, Saveetha Institute of Medical and Technical Sciences, Chennai, Tamil Nadu 602105, India.
[4]Centre for Applied Research, Saveetha School of Engineering (SSE), Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Thandalam, Chennai – 602105, Tamil Nadu, India.
[5]Clinical Neuroscience and Phytomedicine Laboratory, Department of Research, Saveetha College of Nursing (SCON), Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Thandalam, Chennai – 602105, Tamil Nadu, India.
[6]Tissue Culture, Natural Products & Microbial Biotechnology Research Laboratory, Saveetha Institute of Basic Medical Sciences (SIBMS), Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Thandalam, Chennai – 602105, Tamil Nadu, India.

Key Words

Anticoagulant, Biocompatibility, Cytotoxic effects, Embryotoxic, Thrombolytic

Abstract

This study investigates the thrombolytic, anticoagulant, embryotoxic, and cytotoxic effects of a Rutin-chitosan nanocomposite using zebrafish embryos as a model. Rutin, a flavonoid with thrombolytic and antioxidant properties, is combined with chitosan, a biocompatible and biodegradable biopolymer that enhances its bioavailability. This synergistic formulation holds promise for cardiovascular health, thrombotic disorder management, and biomedical applications. Chitosan nanoparticles incorporating rutin were synthesized using ionic gelation. Thrombolytic activity was assessed by introducing the nanocomposite to clotted blood drops, while anticoagulant effects were evaluated in treated blood samples. Zebrafish embryos were exposed to 0–200 µg/mL of the nanocomposite, and survival, development, and heart rate were monitored up to 96 hpf. Cytotoxicity was tested using the brine shrimp assay. The Rutin-chitosan nanocomposite effectively prevented clot formation and enhanced clot lysis in a concentration-dependent manner. It exhibited minimal cytotoxicity and embryotoxicity at low concentrations in zebrafish embryos, suggesting its potential for safe medical applications in anticoagulation and antioxidation. The Rutin-chitosan nanocomposite exhibits potent thrombolytic and anticoagulant properties while demonstrating minimal cytotoxicity at lower concentrations. Its biocompatibility and efficacy make it a promising therapeutic option for thromboembolic diseases. Further in vivo and clinical studies are needed to validate its medical applications.

** Corresponding Author's email: rajeshkumars.sdc@saveetha.com*
Received: 16th August, 2026; Revised: 30th August, 2026; Accepted: 2nd September, 2026
(DOI): 10.70102/AEJ.2026.18.4s.76

Introduction

Nanotechnology is a rapidly developing topic in biotechnology, with numerous applications. Nanoparticles (NPs) have transformed various fields, including medicine, nutrition, and energy. Nanotechnology's application in medicine, particularly medication delivery, has demonstrated numerous advantages. Nanoparticles can lower medicine toxicity and negative effects on patients [1]. The development of a blood clot in venous or arterial vessels that obstructs normal blood flow is known as thrombosis. For blood to circulate freely within the vessels, a delicate equilibrium among blood cells, platelets, plasma proteins, coagulation factors, inflammatory agents, and the endothelial lining of the venous and arterial lumens is essential [2] . The biggest cause of arterial thrombosis is the collapse of an atherosclerotic layer, which arises as a result of the accumulation of lipid deposits and lipid-laden macrophages (foam cells) inside the arterial wall [3]. Platelet-rich thrombosis occurs after plaque rupture. Small cells in the bone marrow produce these small anucleate cells [4]. A developing body of research supports a link between venous and arterial thrombosis. Age, obesity, smoking, diabetes mellitus, hypertension, hypertriglyceridemia, and metabolic syndrome are all risk factors for both types of cardiovascular disorders [5]. The coagulation pathway, a series of events leading to hemostasis, facilitates rapid healing and prevents spontaneous bleeding, RUNX is anticipated to grow into a novel therapeutic target [6]. Targeting anticoagulation or clot prevention at different phases in the coagulation cascade can result in some overlap at different sites. Fibrin clot formation can be effectively prevented by direct thrombin and direct factor Xa inhibitors [7]. Rutin is a flavonol present in various plants, including buckwheat, tea, and apples. It serves as an important nutritional component in food products. Research has demonstrated that rutin possesses neuroprotective properties in cases of brain ischemia [8]. The administration of rutin has been shown to reduce ischemic brain apoptosis by inhibiting p53 expression and lipid peroxidation, as well as enhancing endogenous antioxidant defense enzymes [9]. Chitin, an essential element of fungal cell walls and the exoskeletons of insects and crustaceans, is the second most frequently found natural polysaccharide, following cellulose [10].

The cationic character of chitosan is peculiar because most polysaccharides in acidic environments have a neutral or negative charge. Because of this property, Chitosan can mix with additional negatively charged natural or synthetic polymers to generate electrostatic force complexes or multilayered structures [11]. Chitosan derivatives have been developed with the goal of improving chitosan qualities such as solubility and biodegradability, as well as introducing novel functionalities or properties [12]. One naturally occurring flavonoid that can be found in fruits, seeds, grains, peels, roots, teas, and wines is called rutin, or vitamin. With a wide range of pharmacological effects, such as anti-inflammatory, anti-cancer, anti-allergic, antiviral, antioxidant, and neuroprotective qualities, it is a secondary metabolite generated from plants [13]. As a result, it can either prevent or lessen the damage associated with oxidative stress, inflammation, autophagy, and apoptosis. This is the definition of its anticancer activity. The direct toxicity of natural agents to tumor cells seems to be caused by their pro-oxidant properties and the generation of free radicals [14]. Silver nanoparticles, nanocrystals, carbon nanotubes, and maize starch nanoparticles have all recently been found to contain rutin. Rutin silver nanoparticles served as a nano-anticoagulant and improved the stability and water solubility of rutin [15]. Zebrafish embryos are perfect for toxicity research because of their short life cycle, high fertility, and genetic resemblance to humans. They are also translucent, which makes it easier to observe developmental processes. To evaluate the toxicity of bioactive substances, unrefined plant extracts, and different experimental methods, this model is widely used [16]. The comprehensive sequencing of the zebrafish genome has facilitated advanced genetic research using tools like CRISPR/Cas9 and other nucleases. These genetic manipulation techniques enable researchers to simulate human disease conditions in vivo and gain valuable insights into the genetic factors underlying human diseases [17]. Zebrafish are an important source of data for vertebrate study because of their large datasets, which link precise findings at the structural, functional, and behavioral levels to

biochemical, genetic, and cellular theories [18]. A popular technique for determining the cytotoxicity of bioactive compounds is the brine shrimp lethality bioassay. It entails using the brine shrimp, a simple zoological model organism, to assess the fatal effects of various chemicals [19]. This bioassay is widely used to assess the toxicity of a wide range of chemicals, such as pesticides, medicines, heavy metals, and natural plant extracts [20]. It is used as a preliminary screening method for toxicity evaluation before more involved studies involving mammalian animal models are carried out [21].

Materials and Methods

Preparation of a nanocomposite

The nanocomposite is obtained by preparing rutin and chitosan and then incorporating rutin into chitosan. Rutin is prepared by diluting 100 mg of rutin with 1 mL of distilled water. Chitosan is obtained by mixing 500 mg of chitosan along with 1 mL of glacial acetic acid and 49 mL of distilled water. In the end, the nanocomposite is created by mixing 1 mL of rutin with 4 mL of chitosan (Fig 1).

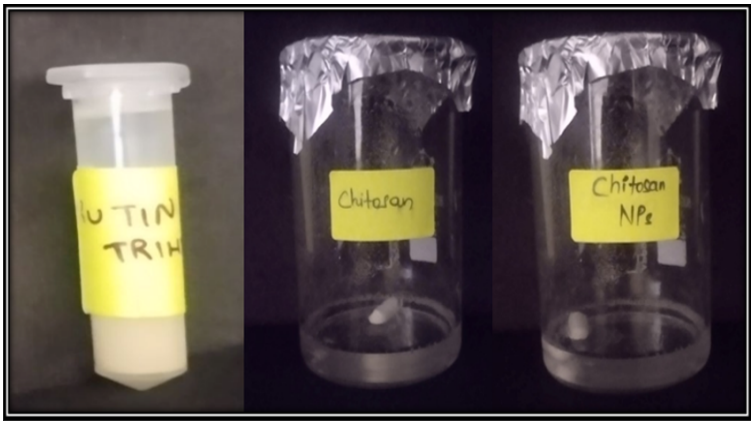


Fig. 1. Schematic presentation of the preparation of Chitosan Nanoparticles

Thrombolytic Activity:

A single drop of blood was collected onto a sterile glass slide and allowed to clot by incubating at room temperature for 45 minutes. Following clot formation, a solution containing rutin-incorporated chitosan nanoparticles at a concentration of 20 µg/ml was added. The outcomes were then assessed against the control group, which had no solution applied. The glass slide was incubated for 90 minutes at room temperature, and the incubation hours were recorded to monitor clot lysis.

Anticoagulant activity:

The anticoagulant activity of rutin and chitosan nanocomposite was tested on freshly obtained human blood at room temperature. This investigation added 1 mL of chitosan nanoparticle suspension (50 µg/mL) to 1 mL of fresh human blood (vial B). Vial A contained 10 mL of blood without any additions. the two blood samples were kept at room temperature for 1 hour to check for any changes.

Cytotoxic effect

Brine shrimp lethality assay

Saltwater preparation:

After weighing, two grammes of iodine-free salt were allowed to dissolve in 200 mL of distilled water. After preparing the six-well ELISA plates, ten to twelve mL of saline water were added. Each well-received 10 nauplii was progressively added (5, 10, 20, 40, and 80 µg/mL). After that, the appropriate concentration of chitosan nanoparticles was added. The plates were incubated for a whole day. After 24 hours, the ELISA plates were checked and reported for the number of live nauplii that were present.

Percentage of dead nauplii = (Number of dead nauplii / (Number of dead nauplii + Number of live nauplii)) × 100

Toxicology evaluation of Rutin incorporated chitosan nanocomposite

Fish maintenance and chitosan nanocomposite exposure:

We acquired wild-type zebrafish from nearby Indian vendors. The fish were housed in separate

tanks with regulated temperature, light/dark cycle (14:10 h), and pH (6.8–8.5). The fish were fed dry bloodworms from a commercial source or optimum food twice daily. Zebrafish embryos were created by crossing three males and one female in each breeding tank. A minimum of three rounds of washing in newly produced E3 medium without methylene blue were subsequently performed on viable eggs. In this study, fertilized eggs were placed in culture plates with six, twelve, and twenty embryos per 2 mL solution.

Both the control and experimental groups were replicated 3 times each. To begin the experimental treatment, a new stock solution containing five different concentrations of the rutin-chitosan nanocomposite was introduced directly to the E3 medium. In order to disperse the nanoparticles and maintain a pH of 7.2 – 7.3, the solution was sonicated for 15 minutes. After fertilization, embryos that were in good health were subjected to different concentrations of chitosan nanocomposite (5, 10, 20, 40, and 80 µg/mL) for 96 hours. The embryos were treated in E3 media containing the chitosan nanocomposite with rutin integrated into it. The experiment also included control groups. Every 12 hours, dead embryos from the groups exposed to nanoparticles were extracted. To block off light, foil was placed over each experimental plate, and they were all maintained at 28°C.

Zebrafish embryo evaluation:

Zebrafish embryos exposed to various concentrations of chitosan nanocomposite (5, 10, 20, 40, and 80 µg/mL) were seen to show different developmental patterns from 24 to 78 hours after fertilization (hpf) using a stereo microscope. Every 24 hours, systematic observations and records were made about the occurrence of anomalies, hatching rates, and embryonic death. Specifically, aberrant embryos were photographed and documented using a COSLAB light microscope (Model: HL-10A). In comparison to control settings, the study sought to evaluate the effects of chitosan nanocomposite exposure on zebrafish embryos, with particular attention paid to mortality rates, hatching success and the frequency of developmental anomalies.

Results and Discussion

Thrombolytic activity

The prepared nanocomposite to the blood clot formed on the slide at various concentrations, it demonstrated a dose-dependent effect on clot lysis. This activity was compared to that of the control sample. The graph represents the percentage of clot lysis at various concentrations, and the percentage of clot lysis was found to be higher at 50 µg/mL, which was more effective than the control, the only limitation is that it takes a few minutes to act completely as the concentration increases (Fig 2A & B).

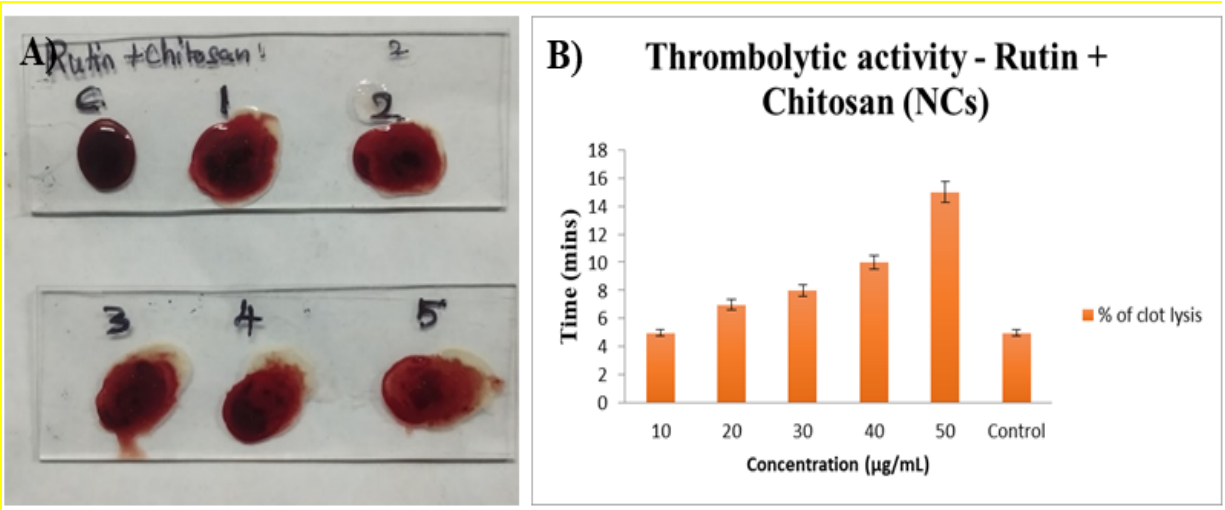


Fig. 2. A) Thrombolytic activity (% clot lysis) of Rutin–Chitosan nanocomposites at different concentrations compared with control and B) Graph representation of the thrombolytic activity.

Anticoagulant activity

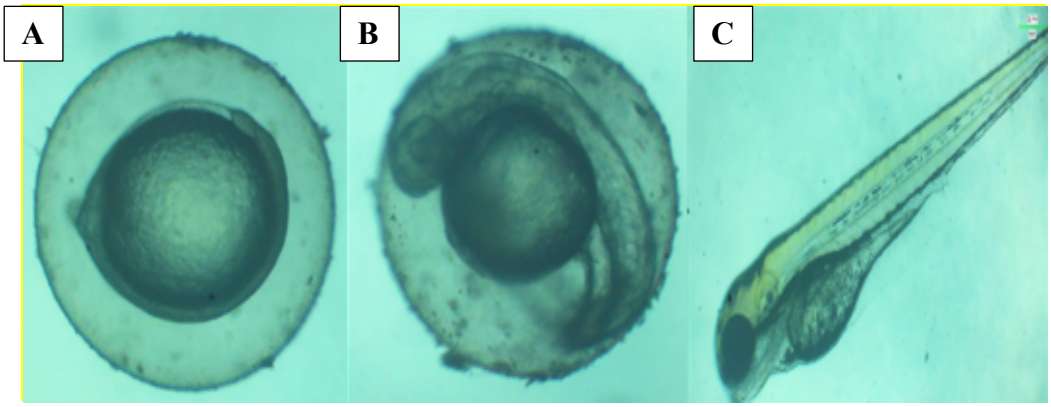
The nanocomposite was added and left for a few minutes. Then the vial with the nanocomposite was not clotted, but the blood in the control was clotted. This claims that the rutin combined with chitosan NPs prevents the formation of blood clotting and proves to be an efficient anticoagulant (Fig 3).

Fig. 3. Anticoagulant activity of Rutin–Chitosan nanocomposites

For this, two vials were filled with blood; one acted as a control, and another vial with blood.

Embryonic toxicology using zebrafish

The embryos had been administered with different quantities of the produced extract before 4 hpf, or the sphere stage, and their defects in development were noted (Fig 4). The survival rate reflects the harmful effects of the test and control groups, which are untreated and treated, respectively. No major changes were seen during the exposure at dosages of 5, 10, and 20 µg/mL. On the other hand, exposure to higher quantities of 10 µg/mL resulted in fatality rates.



Day 1 **Day 2** **Day 3**

Fig. 4. Microscopic images of Embryonic Toxicology evaluation of Rutin-incorporated Chitosan NCs as A) Day 1, B) Day 2, and C) Day 3

Hatching rate

In this work, chitosan nanoparticles were applied to zebrafish embryos for a full day, and the outcome was a dose-dependent reduction of embryo hatching at different concentrations. At a dose of 5µg/ml, 100% of the zebrafish embryos hatched. At 10µg/ml of nanoparticle concentration, the hatching rate dropped to 75%. The hatching rate dropped to 55% when the concentration was raised to 20µg/ml. At 40µg/ml and 80µg/ml concentrations, less than 30% of the eggs hatched. Thus, the study demonstrates that chitosan nanoparticles at varying concentrations caused dose-dependent suppression of embryo hatching (Fig 5).

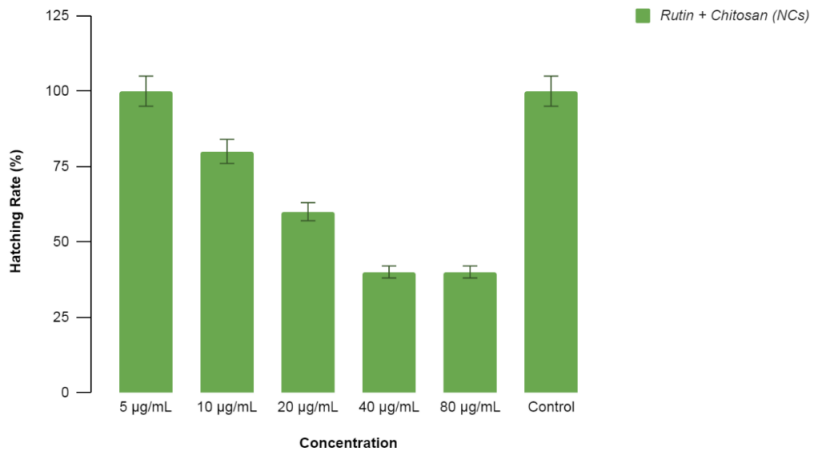


Fig. 5. Hatching rate (%) of brine shrimp exposed to varying concentrations of Rutin–Chitosan nanocomposites compared with untreated control.

Viability rate

When the concentration was 5µg/ml, the viability rate was 100%. However, as the concentration increased, the viability rate decreased gradually. When the concentration was 10, the viability rate was 70%. The viability rate was 60% and 55% for 20 and 40 µg/ml concentration, respectively, and the viability rate was highly reduced to 30% with 80µg/ml concentration (Fig 6).

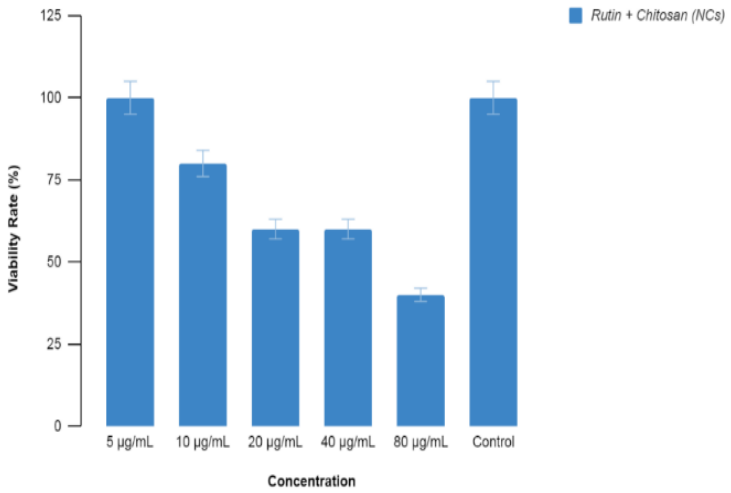


Fig. 6. Viability (%) of Rutin–Chitosan nanocomposites at various concentrations compared with untreated control.

Cytotoxic effect

On day 1, the % of live nauplii was 100%, even with varying concentrations of the chitosan nanoparticles, as mentioned in the graph. On day **2,** there was a decrease in the % of live nauplii when compared to the control and the percentage on day one. When the concentration of chitosan nanoparticles was 5µg/ml and 10 µg/ml, the %of live nauplii was reduced to 90%. When the concentration was 20µg/ml and 40µg/ml, it was reduced to 80% and with 80 µg/ml, the % reduced to 65% of live nauplii (Fig 7).

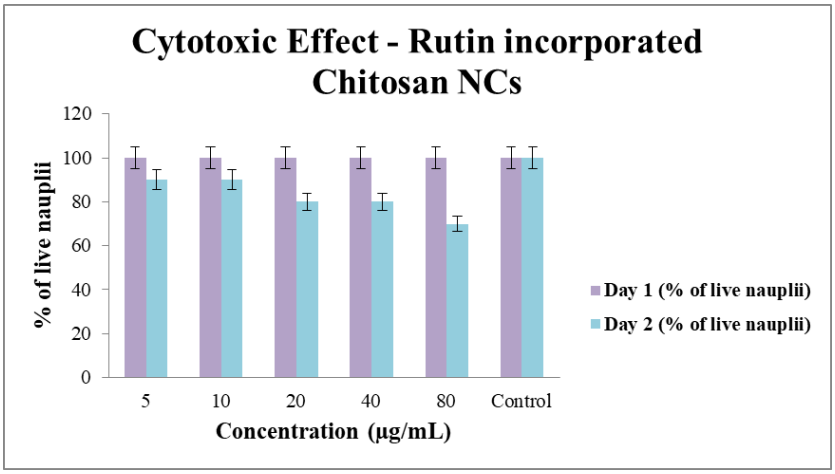


Fig. 7. Cytotoxic effect of Rutin–Chitosan nanocomposites at various concentrations compared with untreated control.

Previous studies have established that rutin prolongs activated partial thromboplastin time (aPTT), prothrombin time (PT), and closure time (CT), and inhibits thrombin activity, a crucial procoagulant protein. Another mechanism through which rutin exerts antithrombotic effects is by inhibiting protein disulfide isomerase, also used for dental tissue engineering [22-26]. In an animal model of electric current-induced arterial thrombosis, combining rutin with zinc chelation effectively reduced arterial occlusion without increasing bleeding risks [23]. Compared to free rutin, rutin-loaded AgNPs prolonged the suppression of mouse tail thrombosis, thereby addressing rutin's short half-life issue while mitigating bleeding risks [24,27-29]. The choice of stabilizer significantly influenced the properties of the nanocrystals, resulting in enhanced permeability and a more potent in vivo anti-inflammatory effect compared to both free rutin hydrogel and commercial diclofenac sodium gel [30-32, 25]. Zebrafish embryos sustain themselves through yolk absorption during the egg phase and are easily assessed for abnormalities within individual microplate wells over consecutive days [33-34, 26].So in this study, we can claim that the rutin and chitosan nanoparticles exhibited a greater antithrombotic and anticoagulant activity effectively when compared with the previous studies done on various nanoparticles and also with different rutin combinations for this same activity. Embryonic toxicology evaluation and cytotoxic effect the result indicates that the nanocomposite prepared was acting in a dose-dependent method so lesser or minimal the concentration there is little or no toxicity in its use and further research can be done to be used in various medical applications. It also showed efficacy due to the incorporation of rutin in chitosan than the previous research studies. The current study involving the nanocomposite preparation demonstrates comparatively lower cytotoxicity than the previously mentioned investigations.

Conclusion

In conclusion, combining nano chitosan with rutin in a composite provides a unique method with potential thrombolytic and anticoagulant properties. Rutin, which is recognized for its antiplatelet and antithrombotic effects, may be more effective when combined with a nano chitosan matrix due to enhanced dispersion and delivery. This compound can break blood clots through thrombolytic activity and prevent their development through anticoagulant actions. If the nano chitosan rutin composite proves effective, it might be a useful addition to the arsenal of therapies for thrombotic diseases, thereby enhancing the prognosis and quality of life for patients. Zebrafish can be utilized as a toxicology model to reveal developmental toxicity mechanisms because of their strong resemblance to mammals. Compared to adult zebrafish, zebrafish embryos and larvae were more vulnerable to toxins. Zebrafish embryos were used to demonstrate the embryonic toxicity of nanoparticles; the results showed that the percentage of hatching and viable embryos was 100%, and that the concentration had a similar

effect to the control at lower concentrations. However, as the concentration was gradually increased, the percentage of hatching and viable embryos drastically decreased. Hence, using chitosan nanoparticles at a basic or lower concentration to avoid cytotoxic and embryotoxic effects is safe. Because it exhibits lower toxicity than other nanoparticles currently utilized in the medical area, it can, therefore, be employed as an alternative in a variety of medicinal applications.

Funding

This study did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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:

AV: Writing-Review & editing, Writing Original Draft, VR: Methodology, Data curation, Visualization, SD: Drew the figures and pictures. PE: Helped in writing the manuscript and revised it. SJ: Validation and editing with review of the manuscript. RS: Designed protocols for all the experiments, supervised, proofread, analyzed the data, and revised the reviewer's comments, investigation, and Conceptualization, AS and AP: Review, Validation, Data curation. All authors reviewed and discussed the results, provided comments on the manuscript, and approved its final published version.

Consent for publication

Not applicable.

Conflict of interest

The authors state no conflict of interest

Declaration of competing interest

The authors declare no conflicts of interest.

Reference

1. Dhanvanth, M. and T. Uma Maheshwari, S Rajeshkumar. Analysis Of Dispensing Equipment for Topical Gel Formulations For Management Of Oral Potentially Malignant Disorders. Int J Dentistry Oral Sci, 2021. **8**(8): p. 3698-3701.
2. Setia, A., et al., Nanoparticles for Thrombus Diagnosis and Therapy: Emerging Trends in Thrombus-theranostics. Nanotheranostics, 2024. **8**(2): p. 127.
3. Mackman, N., Triggers, targets and treatments for thrombosis. Nature, 2008. **451**(7181): p. 914-918.
4. Pauline, C. R., Akshita, Pavithra, T., Kannan, K., & Sivaperumal, P. (2025). Characterisation and biological activity of silver nanoparticles from (*Rhizophora Mucronata*) mangrove extract. Nano Life, 15(03), 2450018.
5. Fernández, J.A., et al., Serum amyloid A4 is a procoagulant apolipoprotein that it is elevated in venous thrombosis patients. Research and practice in thrombosis and haemostasis, 2020. **4**(2): p. 217-223.
6. Vimalraj S, Sekaran S. RUNX Family as a Promising Biomarker and a Therapeutic Target in Bone Cancers: A Review on Its Molecular Mechanism(s) behind Tumorigenesis. Cancers. 2023; 15(12):3247.
7. Wang, Y., X. Xu, and W. Zhu, Anticoagulant therapy in orthopedic surgery–a review on anticoagulant agents, risk factors, monitoring, and current challenges. Journal of Orthopaedic Surgery, 2024. **32**(1): p. 10225536241233473.
8. Ganeshpurkar, A. and A.K. Saluja, The pharmacological potential of rutin. Saudi pharmaceutical journal, 2017. **25**(2): p. 149-164.
9. Khan, M.M., et al., Rutin protects the neural damage induced by transient focal ischemia in rats. Brain research, 2009. **1292**: p. 123-135.
10. Cheung, R.C.F., et al., Chitosan: an update on potential biomedical and pharmaceutical applications. Marine drugs, 2015. **13**(8): p. 5156-5186.
11. Venkatesan, J. and S.-K. Kim, Chitosan composites for bone tissue engineering—an overview. Marine drugs, 2010. **8**(8): p. 2252-2266.
12. Aranaz, I., R. Harris, and A. Heras, Chitosan amphiphilic derivatives. Chemistry and applications. Current Organic Chemistry, 2010. **14**(3): p. 308-330.

13. Subash, H. A., Santhosh, K., Kannan, K., & Pitchiah, S. (2024). Extraction of keratin degrading enzyme from marine Actinobacteria of *Nocardia* sp and their antibacterial potential against oral pathogens. Oral Oncology Reports, 9, 100184.
14. Joseph, T.M., et al., Nanoparticles: Taking a unique position in medicine. Nanomaterials, 2023. **13**(3): p. 574.
15. Jeyaram, M., Jeyaram, Y., Ayyadurai, T., Gurusamy, M. (2026). Exploration of Streptomycetes as Biocontrol Agents Against Plant Pathogens. In: Kaur, T., Kumar, H., Manhas, R.K. (eds) Streptomycetes: Biological Candidates for Sustainable Agriculture. Springer, Singapore. https://doi.org/10.1007/978-981-95-5803-2_6
16. Ivanova, D.G. and Z.L. Yaneva, Antioxidant properties and redox-modulating activity of chitosan and its derivatives: Biomaterials with application in cancer therapy. BioResearch open access, 2020. **9**(1): p. 64-72.
17. Ayyadurai, T., Ravi, S., & Annamalai, A. (2026). Steroidogenic Enzyme Dysregulation in Polycystic Ovary Syndrome: Mechanistic Insights and Emerging Therapeutic Strategies. The Journal of Steroid Biochemistry and Molecular Biology, 107088.
18. Anbazhagan, V., Natrajan, A., Arul, J. et al. Strategic development of a buffalo farming framework and optimization of the foraging behaviour using mathematical models. Trop Anim Health Prod 58, 424 (2026). https://doi.org/10.1007/s11250-026-05211-6
19. Jancyhelan, J., Thirumurugan, A., Alagumanian, S., & Vignesh, K. (2026). Eco-friendly biosynthesis of silver nanoparticles using marine algal extract and their biomedical applications. Results in Chemistry, 103606.
20. Modarresi Chahardehi, A., H. Arsad, and V. Lim, Zebrafish as a successful animal model for screening toxicity of medicinal plants. Plants, 2020. **9**(10): p. 1345.
21. Ansori, A.N., et al., Application of CRISPR-Cas9 genome editing technology in various fields: A review. Narra J, 2023. **3**(2): p. e184.
22. Meda SSR, Joseph NM, Lakshmikanthan M, Ayyadurai T, Muthu S. Comparative Efficacy of Mandelic Acid Peel versus Topical Kojic Acid 2% in the Treatment of Periorbital Melanosis. Int J Drug Deliv Technol. 2026;16(15s): 118-123. DOI: 10.25258/ijddt.16.15s.14.
23. Meda SSR, Srinivasan VR, Lakshmikanthan M, Ayyadurai T, Muthu S. Topical Prostaglandin Analogues in Androgenetic Alopecia: A Systematic Review of Clinical Evidence. Int J Drug Deliv Technol. 2026;16(15s): 108-117. DOI: 10.25258/ijddt.16.15s.13.
24. Vimalraj, S., Saravanan, S. Tooth-derived stem cells integrated biomaterials for bone and dental tissue engineering. Cell Tissue Res, 2023; 394, 245–255.
25. Harwig, J. and P. Scott, Brine shrimp (*Artemia salina* L.) larvae as a screening system for fungal toxins. Applied microbiology, 1971. **21**(6): p. 1011-1016.
26. Ayyadurai, T., Mohan, P. K., Annamalai, A., Kamaraj, C., & Waheeb, M. Q. (2026). Biogenic silver nanoparticles synthesis from *Guilandina bonduc* L. seed kernel extract with antibacterial, antioxidant and anticancer potential against ovarian Cancer cells. Results in Chemistry, 103246.
27. Rajeshkumar, S., et al., Herbal Formulation Mediated Synthesis of Silver Nanoparticles and Its Antifungal Activity Against *Candida albicans*. Indian Journal of Public Health Research & Development, 2019. **10**(11).
28. B, BB., Sivaperumal, P., Ganapathy, D., & Kamala, K. (2025). Evaluating the efficacy of doripenem against *Staphylococcus aureus* in vancomycin-resistant strains. Microbial Pathogenesis, 202, 107449.
29. Choi, J.-H., et al., Anti-thrombotic effect of rutin isolated from *Dendropanax morbifera* Leveille. Journal of Bioscience and Bioengineering, 2015. **120**(2): p. 181-186.
30. Liao, X., et al., Enhanced inhibition of protein disulfide isomerase and anti-thrombotic activity of a rutin derivative: rutin: Zn complex. RSC advances, 2023. **13**(17): p. 11464-11471.
31. Wu, H., et al., Rutin-loaded silver nanoparticles with antithrombotic function. Frontiers in bioengineering and biotechnology, 2020. **8**: p. 598977.
32. Thirumurugan, A., Padmanaban, M., Kumar, D. S., Govindharaju, R., Padmavathy, S., Karthikeyan, V., ... & Karthik, S. (2026). Phytochemical and antioxidant properties of *Kedrostis foetidissima* and its larvicidal potential

against mosquitoes. South African Journal of Botany.
33. Hassan, A.S. and G.M. Soliman, Rutin nanocrystals with enhanced anti-inflammatory activity: Preparation and *ex vivo/in vivo* evaluation in an inflammatory rat model. Pharmaceutics, 2022. **14**(12): p. 2727.
34. Soni M, Pitchiah S, Suresh V, Ramasamy P, Sivaperumal P. Fabrication and partial characterization of silver nanoparticles from mangrove (*Avicennia marina*) leaves and their antibacterial efficacy against oral bacteria. Cureus. 2024 Jan 11;16(1)