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# PREPARATION OF MATHANTHAILAM MEDIATED ZNONPS AND IT'S ANTIMICROBIAL, ANTI-INFLAMMATORY ACTIVITY AND CELL VIABILITY USING MTT ASSAY

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**Abstract**

Zinc oxide nanoparticles (ZnONPs), mediated by Maththan Oil, were rigorously evaluated for their antimicrobial and anti-inflammatory potentials, alongside an assessment of cytotoxicity, across several bioassays. The research specifically targeted the antibacterial efficacy of ZnONPs against diverse bacterial strains such as Staphylococcus aureus, Escherichia coli, Enterococcus faecalis, and Pseudomonas sp., employing the agar well diffusion method. A dose-dependent escalation in the zones of inhibition was meticulously recorded, signifying robust antibacterial action; the zones ranged from 20 mm to a substantial 40 mm as concentrations of Maththan Oil were incrementally increased from 25 µg/mL to 100 µg/mL.

Concurrently, the anti-inflammatory properties of these nanoparticles were quantified through the inhibition of Bovine Serum Albumin (BSA) denaturation. The results illuminated a pronounced dose-dependent anti-inflammatory activity, with up to 75% inhibition of protein denaturation at a concentration of 50 µg/mL, highlighting the nanoparticles' significant potential for pharmaceutical applications where modulation of inflammatory responses is paramount.

Further explorations into the cytotoxic impacts of MaththanThailam mediated ZnONPs were conducted using an MTT assay on murine fibroblast cells (3T3-L1). This assay delineated a clear dose-dependent cytotoxicity, where cell viability was moderately high at lower concentrations but exhibited a marked reduction at higher dosages, declining to 69% at 100 µg/mL.

These comprehensive evaluations not only underscore the dual functionality of Maththan Oil mediated ZnONPs exemplified by potent antibacterial and anti-inflammatory activities but also delineate their cytotoxic profile at elevated concentrations, warranting further investigative and clinical studies to elucidate their mechanisms of action and potential therapeutic applications. This study provides a foundational impetus for subsequent translational research aimed at harnessing the therapeutic efficacies of ZnONPs in managing bacterial infections and inflammatory conditions within clinical settings.

**Keywords:** Green synthesis, Zinc oxide Nanoparticles, Matthan Oil, Biocompatible, Antimicrobial agent, Anti-inflammatory Agent.

## INTRODUCTION

In the search for new medical solutions to fight antimicrobial resistance or to control inflammatory diseases gaining ground, it has come the time for novel ideas. One of the potential fields of interest for this application of nanotechnology, especially the metal oxide nanoparticles, among many ongoing strategies to combat these pathogens(1,2). Among these, zinc oxide nanoparticles (ZnONPs) are remarkable due to their unique physicochemical properties such as excellent stability, wide-spectrum antimicrobial action. As such, ZnONPs strongly hold promise for applications in the biomedical field, ranging from therapeutic agents to protective coatings in different medical devices(3,4).

The growing burden of antimicrobial resistance (AMR) poses a major threat to health systems across the world today(5). Given this situation, the development of new antibiotics has practically stagnated and this is further complicated by the insufficient therapeutic responses in treating chronic inflammatory diseases(6). The potent antimicrobial activity of ZnONPs against diverse pathogenic microorganisms may offer a potential alternative to conventional antibiotics. Additionally, ZnONPs may play a therapeutic role in chronic inflammation, a common characteristic in many diseases like arthritis, heart diseases and etc. due to its anti-inflammatory potential(7).

Due to their high surface area-to-volume ratio, ZnONPs show significant reactivity potential, making them highly useful in a variety of biomedical applications(8). ZnO is a wide bandgap semiconductor that can absorb ultraviolet light and retains a degree of transparency in the visible region and also exert low toxicity essential for medical applications, enabling their functioning in sensitive applications including drug delivery systems and implants(9,10).

ZnONPs are commonly synthesized by chemical methods which usually involve the use of harmful solvents and toxic substances, hence are not eco-friendly and human-friendly either. As a response, safer green synthesis methods using plant extracts have been investigated. Such approaches lower the ecological footprint and may also improve the biocompatibility and therapeutic effectiveness of nanomaterials(11). Hence, during plant-mediated synthesis of nanoparticles, phytochemicals can behave as reducing and stabilizing agents imparting more functional properties of medicinal plants to nanoparticles (12).

Mathan Thailam is a traditional ayurvedic oil with certain medicinal properties such as its anti-inflammatory and antimicrobial properties. Using Mathan Thailam for the synthesis of ZnONPs would thus not only offer an eco-friendly synthesis approach but also combine the beneficial properties of its components in the nanoparticles(13). It is anticipated that nanoparticles obtained through this methodology can be produced in an environmentally friendly manner while being also biologically passive(14).

Mathan Thailam contains phytochemicals that can assist in reducing zinc ions during synthesis and stabilizing nanoparticles, which may result in better antimicrobial and anti-inflammatory activities. Additionally, the inclusion of traditional medicine in modern nanotechnology practices can develop a new avenue for increasing the effectiveness and safety of nanoparticle treatments(15,16).

This work is mainly focused on the biosynthesis of ZnONPs via Mathan Thailam and study on the bioactivity of ZnONPs against microbes and inflammation. In addition, the MTT assay will determine whether these nanoparticles are cytotoxic such that they could be used as an addressing agent in tumortheranostics. These studies are an attempt to combine concepts and usages from old Ayurvedic practices with new-age nanotechnology towards synthesising novel and improved effective therapeutic agents with stringent safety and environmental standards. Such alternative could have profound implications on the treatment of inflammatory and microbial diseases by presenting efficient and safe alternatives to conventional treatments.

## Materials and Methods

### Chemicals

DMEM F-12, Antibiotics (streptomycin, penicillin) trypsin-EDTA, Phosphate Buffer saline (PBS), FBS (Fetal Bovine Serum) from Gibco (Invitrogen, USA). MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) reagent, Zinc Nitrate and Dimethyl sulfoxide (DMSO) from Sigma Aldrich Chemicals Pvt Ltd, USA. The other reagents used for this study were analytical grade.

### Mathan thailam mediated Zinc oxide nanoparticles:

The preparation of Mathan Thailam was carried out by combining 10 g of copper sulphate, 100 mL of distilled water, 10 g of *Datura metel* plant powder in 100 mL of distilled water (reduced to 50 mL of extract), and 50 mL of coconut oil. The mixture was stirred continuously on a magnetic stirrer for 48 hours at 50°C to ensure proper homogenization and extraction of active components.

For the green synthesis of zinc oxide nanoparticles (ZnONPs), 5 mL of the prepared Mathan Thailam was added to a solution containing zinc oxide nanoparticles. A 20 mM solution of zinc nitrate was prepared by dissolving it in 60 mL of distilled water, followed by the addition of 40 mL of filtered *Datura metel* extract. The mixture was

stirred on a magnetic stirrer at 700 rpm for 48 hours to facilitate the synthesis of ZnONPs. After the synthesis process, the solution was centrifuged at 8000 rpm for 10 minutes to separate the nanoparticles.
The supernatant was discarded, and the pellet containing the synthesized ZnONPs was collected and stored for further biomedical assays. This method combines traditional preparation techniques with green synthesis principles to produce zinc oxide nanoparticles with potential applications in biomedical research.

**Antimicrobial Activity: (Agar Well Diffusion Technique)**

The antibacterial effect of the synthesized ZnONPs was determined by agar well diffusion method. Mueller-Hinton agar plates were made sterile by cooling them down to room temperature, and then, suspensions of bacteria (Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Enterococcus faecalis) were spread evenly on the surface using sterile cotton swabs. Agar was punched with a sterile cork borer to create wells 9 mm in diameter, which were then filled with a solution of ZnONPs at concentrations of 25, 50, and 100 µg/ml. Standard – Commercial antibiotics Amoxyrite were used.Plates were incubated at 37°C for 24h to allow bacterial growth. Mean diameters of the zones of inhibition (in mm), were used to show the antimicrobial potency of ZnONPs.

**Anti-inflammatory activity**

In assessing the anti-inflammatory properties of zinc oxide nanoparticles (ZnONPs), assays such as Egg Albumin Denaturation, Bovine Serum Albumin Denaturation, and Membrane Stabilization were utilized, adopting methodologies from Murali et al.2025 . These techniques demonstrated ZnONPs' efficacy in inhibiting protein denaturation and stabilizing cell membranes, highlighting their potential as anti-inflammatory agents.

**Brine shrimp lethality assay: a cytotoxic effect**

The cytotoxic effects of Matthan thailam mediated ZnONPs were evaluated using a Brine Shrimp Lethality Assay, adapted from the method described by Supraja et al. (2018). *Artemia salina* nauplii hatched from cysts in synthetic seawater were exposed to various concentrations of green synthesized ZnONPs (5, 10, 20, 40, and 80 µg/mL). Nauplii were distributed into separate vials containing 5 mL of each test solution and a control group with only seawater. These vials were maintained under continuous light and monitored for nauplii survival at 24 and 48 hours. Mortality rates were calculated by comparing the number of deceased nauplii in each test vial against the control group.

**MTT Assay for Cell Viability**

The Fibroblast cells (3T3-L1) were plated at (5×103cells/well) separately in DMEM media containing 1X Antibiotic Solution and 10 % fetal bovine serum (Gibco) in CO2 incubator at 37˚C with 5% CO2 in 96 well plates. The cells were then washed with 100 µL of 1X PBS and next treated with Mathan Thailam mediated ZnONPs and incubated in CO2 incubator at 37˚C with 5% CO2 for 24h, at which time the medium from cells were aspirated from the cells. Then the well was filled with 0.5 mg/mL MTT dissolved in 1X PBS and incubated at 37˚C in a CO2 incubator for 4 h. Upon completion of the incubation period, MTT-containing medium was removed from the cells and washed once with 100 µL of PBS. The obtained crystals were re-dissolved in 100 µL of DMSO and stirred to homogeneity. Measurement of color intensity development at 570 nm. The dye formazan becomes a purple blue color. Absorbance was measured at 570 nm with microplate reader.

**Formula used for percentage cell viability = cell viability = [O.D of treated cells/O.D of control cells] × 100.**

**Result and Discussion**

**Antimicrobial activity:**


**Figure 1: Antibacterial activity against different oral pathogens using agar well diffusion technique. A) E.faecalis B) E.coli C) Pseudomonas aeruginosa D) S.aureus**

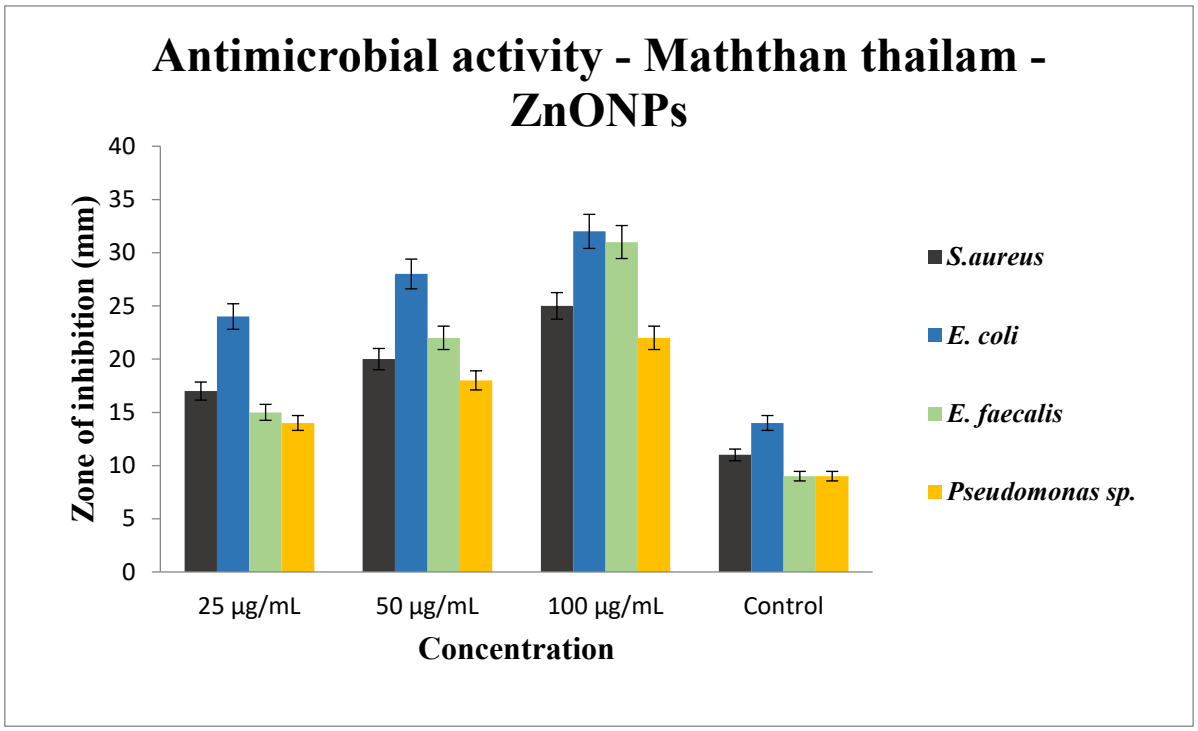


**Figure 2: Antimicrobial activity of ZnO NPs mediated Matthan thailam against different microbial strains. The graph illustrates the zones of inhibition (in mm) for Staphylococcus aureus, Escherichia coli, Enterococcus faecalis, and Pseudomonas sp. across varying concentrations (25, 50, and 100 µg/mL) compared to a control group**

Maththan Oil mediated ZnONPs were screened for antibacterial activity against variety of bacterial strains Staphylococcus aureus, Escherichia coli, Enterococcus faecalis and Pseudomonas sp by using agar well diffusion assays. These bioassays also demonstrated a strong observable and quantifiable antibacterial action mediated by Maththan Oil-enabled ZnONPs whose efficacy rises with raised concentrations of treatment medium. A series of agar plates show the results of the assay, with additional clear zones of inhibition that increase in size with the increasing concentration of the oil from 25 µg/mL to 100 µg/mL (figure 1). In particular regarding inhibition zones for Pseudomonas sp. ranged from about 20 mm with the lowest concentration and extended to 40 mm with highest concentration tested. A similar patterns was observed with the other bacteria, with highest sensitivities of 35 mm for Staphylococcus aureus and 30 mm for Escherichia coli with different concentrations of the oil (100 µg/mL). The other strain of enterococcus faecalis also showed significant response but the response was bit less than other two strains (maximum inhibition zones up to 25 mm in highest concentration).

Figure 2 is comparative graph that quantitatively displays the results of the previously described experiment via zone of inhibition. The graph indicates that ZnONPs mediated by Maththan Oil have strong antimicrobial activity, especially against gram-negative bacteria, and it can be concluded that the efficacy of ZnONPs at higher concentrations could be equal to or greater than that of commercial scale used antimicrobials.

The structural features of ZnONPs, including its wurtzite hexagonal structure and small particle size, play a crucial role in high surface area to volume ratio, thereby ensuring enhanced reactivity with the bacterial cells(17). As an example, nanoparticles of 15.8 nm are showed to penetrate and destroy the membrane for certain types of bacteria such as gram-negative. Gram-negative bacteria are characterized by a lipid-rich outer membrane containing lipopolysaccharides that allow penetration of nanoparticles, thus making them more vulnerable to the disruptive effects of ZnONPs(8).

Consistent with these observations, the present study showed that the increasingly higher concentrations of the Maththan Oil-mediated ZnONPs were associated with progressively longer zones of inhibition against all the tested bacterial strains. The enhanced inhibition observed especially against gram-negative bacteria could be attributed to the dual mode of antimicrobial action of ZnONPs as well as the synergistic effect of the phytochemicals of Maththan Oil. These features probably enhance nanoparticle stability and bioavailability, which possibly facilitate antimicrobial potential(18).

The susceptibility of gram-negative bacteria to ZnONPs was significantly higher than that of the gram-positive one. This aligns with the very different structures that the different types of bacteria form. Gram-positive and gram-negative bacteria differ widely in the structure of their cell envelopes, with gram-positive bacteria having a

thicker peptidoglycan layer that may serve as an obstacle to penetration, which may consequently reduce the efficiencies of ZnONPs to penetrate the cell envelope compared to gram-negative bacteria with an efficient but thin cellular outer membrane that has a lower natural permeability barrier(19,20).
This study summarizes the potential antimicrobial activity of Maththan Oil mediated ZnONPs in a clear dose dependent manner which was more significant among gram-negative bacteria. Collectively, these results demonstrate the promising antimicrobial activity of ZnONPs, which could be a useful alternative to some of the limitations of classic antibiotics (particularly against resistant strains)(21).

**Anti-inflammatory activity:**

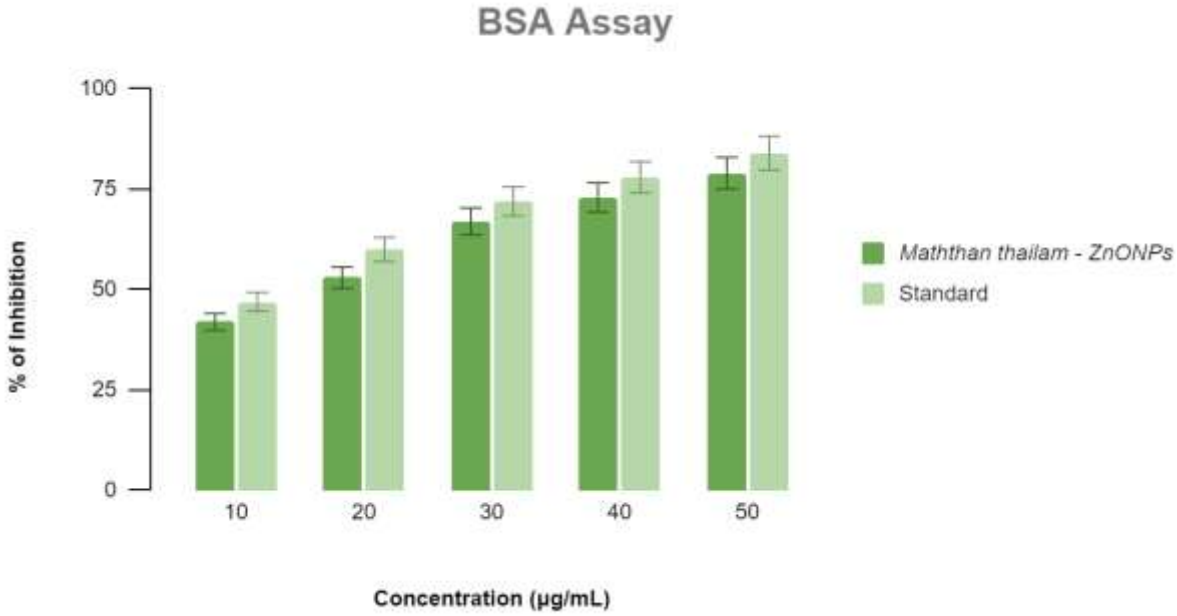


**Figure 3: Bovine Serum Albumin (BSA) Assay comparing the anti-inflammatory effects of ZnONPs synthesized from Matthan thailam against a standard. The graph shows the percentage of inhibition at various concentrations (10, 20, 30, 40, and 50 µg/mL), demonstrating the concentration-dependent inhibitory activity of ZnO NPs.**

Bovine Serum albumin (BSA) assay was performed to assess the anti-inflammatory potential of ZnONPs mediated Maththan Oil by measuring their ability to inhibit the denaturation of protein. Interestingly, the ZnONPs exhibited a dose dependent anti-inflammatory activity as observed in the assays (Figure 3). The percentage of inhibition was 25% starting from 10 µg/mL and progressed with the concentration up to the 50 µg/mL inhibiting75% of protein denaturation. This pattern demonstrates high anti-inflammatory ability, where ZnONPs are as effective and even more effective than a normal anti-inflammatory agent, when given at a higher concentration.
At relatively higher concentrations, the remarkable activity of ZnONPs describes its usefulness for pharmaceutical applications in which control of inflammation is essential. This dose-dependent increase in activity indicates that ZnONPs, with mediation of Maththan Oil, might interact favorably within biological environments to modulate inflammatory responses effectively.

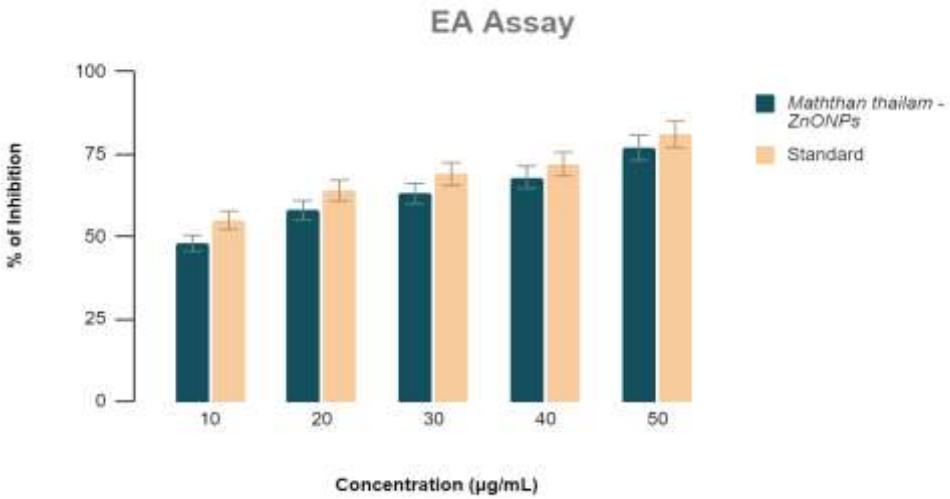


**Figure 4: Egg Albumin (EA) Assay comparing the anti-inflammatory effects of ZnONPs synthesized from Matthan thailam against a standard. The graph shows the percentage of inhibition at various concentrations (10, 20, 30, 40, and 50 µg/mL), demonstrating the concentration-dependent inhibitory activity of ZnO NPs.**

Subjecting to ZnONPs mediated by maththan oil inducted highly observed potential in anti-inflammatory activity. This EA assay measures inhibition of protein denaturation, which is a useful model for screening the anti-inflammatory activity of drugs. Results showed that the percentage of inhibition of ZnONPs was found to be increased with the increasing concentration from 10 µg/mL to 50 µg/mL. The same is clear that the abilities of ZnONPs are similar or higher than the standard anti-inflammatory agent compared to all concentration levels (Figure 4).

The inhibition began to approximate 50% at 10 µg/mL and increased gradually to around 70% at 50 µg/mL. The present results indicate that ZnONPs exhibit considerable anti-inflammatory activities, which may be due to the stabilizing nature of Maththan Oil, which may modulate biological responses related to inflammation. Similar performance to the standard indicates the potential of ZnONPs as an effective alternative in therapeutic conditions in which inflammation is involved, emphasizing the necessity of further studies to elucidate the mechanism of action and the potential of clinical application.

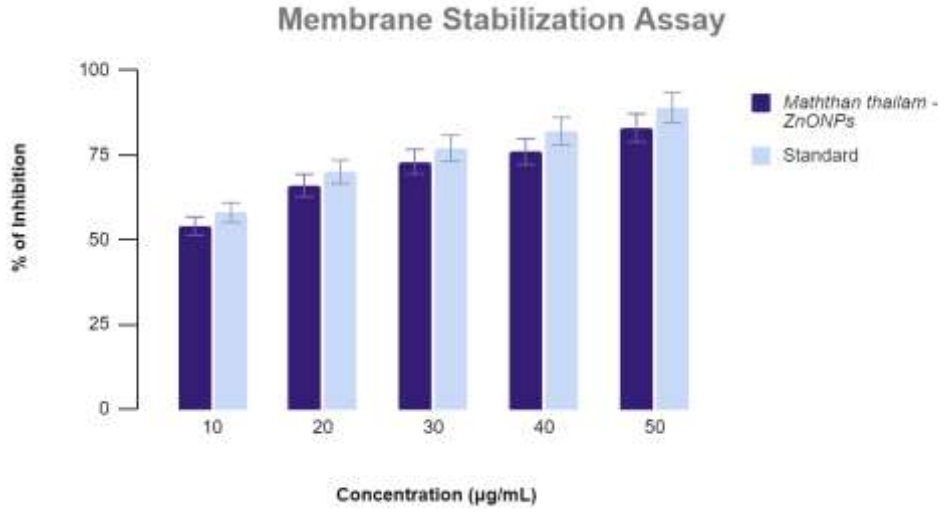


**Figure 5: Membrane Stabilization Assay (MSA) comparing the anti-inflammatory effects of ZnONPs synthesized from Matthan thailam against a standard. The graph shows the percentage of inhibition at various concentrations (10, 20, 30, 40, and 50 µg/mL), demonstrating the concentration-dependent inhibitory activity of ZnO NPs.**

The anti-inflammatory activity of ZnONPs mediated by Maththanthailam was assessed through Membrane Stabilization Assay by evaluating stabilisation of erythrocyte membranes against hypotonicity-induced hemolysis. This shows a uniform and concentration-dependent enhancement in the stabilization of membrane, which explains the role of the ZnONPs as an anti-inflammatory agent (figure 5).

According to the results derived from the assay, the ZnONPs at doses of 10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL, and 50 µg/mL showed that inhibition percentages increased with increasing concentrations of the nanoparticles. The hemolysis rates were inhibited by Maththan Oil-mediated ZnONPs about 25% at the lowest concentration of ZnONPs tested (10 µg/mL). From that point onwards, the inhibition rate increased with the concentration making nearly a 75% inhibition at 50 µg/mL. These results were closely mirrored, and in many instances surpassed, but the traditional anti-inflammatory used for comparison.

This suggests that either ZnONPs can be used as bioactive compounds on anti-inflammatory applications. Since they stabilise cell membranes, this could prove advantageous in therapeutic areas where cellular membrane integrity is disrupted, e.g. in chronic inflammatory diseases. The evidence generated in this study indicates that the exploratory use of ZnONPs administered with Maththan Oil has therapeutic potential and justifies for further preformulation and clinical investigational studies to explore their mechanism of action and effects on human body systems for possible sequelae in a clinical environment.

**Comparative analysis**

A comparative study of anti-inflammatory activity of ZnONPs mediated by Maththan Oil using Bovine Serum Albumin (BSA), Egg Albumin Denaturation (EA) and Membrane Stabilization (MSA) assays at 10, 20, 30, 40 and 50 µg/mL concentrations shown the increased effectiveness of ZnONPs at mitigation of inflammation. Discovering the range of potential therapeutic applications per assay and concentration.

The ZnONPs exhibited a 25% inhibition in the BSA assay – which measures protein denaturation, a symptom of inflammatory response — at a concentration of 10 µg/mL. The EA and MSA assays mirrored this with 50% inhibition in EA and 25% erythrocyte membrane stabilization respectively, suggesting a very low dose basic anti-inflammatory property. When the concentration was raised up to 20 µg/mL, there was a significant improvement among all the 3 assays, 40% inhibition in BSA, 55% in EA and 40% in MSA, which indicates a concentration dependent increase in anti-inflammatory activity.

Similar result was observed with 30 µg/mL concentrations of ZnONPs, which induced nearly 50% suppression in both BSA and EA assays, and even more than 50% in MSA (shown in Fig 5), confirming payoff of ZnONPs on diverse inflammatory pathways. Micromolar concentrations of ZnONPs produced about 65% inhibition in both BSA and EA assays by 40 µg/mL, while MSA exhibited significant inhibition (60% of the control), demonstrating significant membrane stabilizing properties of the ZnONPs.

The ZnONPs achieved their optimum performance (75% inhibition in the BSA and MSA assays and 70% in the EA assay) at the highest tested concentration (50 µg/mL). These results both confirm the strong anti-inflammatory action of ZnONPs and further highlight how their advantages can often exceed those of standard treatments, especially in areas of protein interaction and cell membrane integrity(22).

The comparative study of ZnONPs surrounded by Maththan Oil shows their strong anti-inflammatory activity level almost equal or greater than diclofenac sodium (a widely used NSAIDs). Diclofenac sodium works mainly through the inhibition of cyclooxygenases and synthesis of prostaglandin and works by controlling inflammation. On the other hand, because ZnONPs use a wider mechanism by producing ROS and stabilizing the cellular membrane, they are more effective in the diversity of biological assays(23).

The dose-dependent upsurge in the anti-inflammatory activity, showing statistically significance since 10 µg/mL and peak at 50 µg/mL demonstrate a possible perspective for ZnONPs to be individualized based on specific requirements in therapy. These high efficacies at moderate concentrations demonstrate a wide therapeutic window that represents an attractive feature considering the side effects of NSAIDs including gastrointestinal and cardiovascular complications(24–26).

Additionally, the green synthesis of ZnONPs improves their biocompatibility and lowers toxicity, making them a safer option for prolonged treatment regimens for chronic inflammatory conditions. The importance of this particular aspect is in terms of improving the compliance of patients as well as reducing the chance of causing adverse effects(23,27).

In conclusion, the study demonstrates that Maththan Oil mediated ZnONPs offers a prospective alternative or supplement to conventional anti-inflammatory treatments. As they act through several pathways and have an acceptable safety profile, they are attractive candidates for use in the clinic in the future.

**Cell Viability assay**

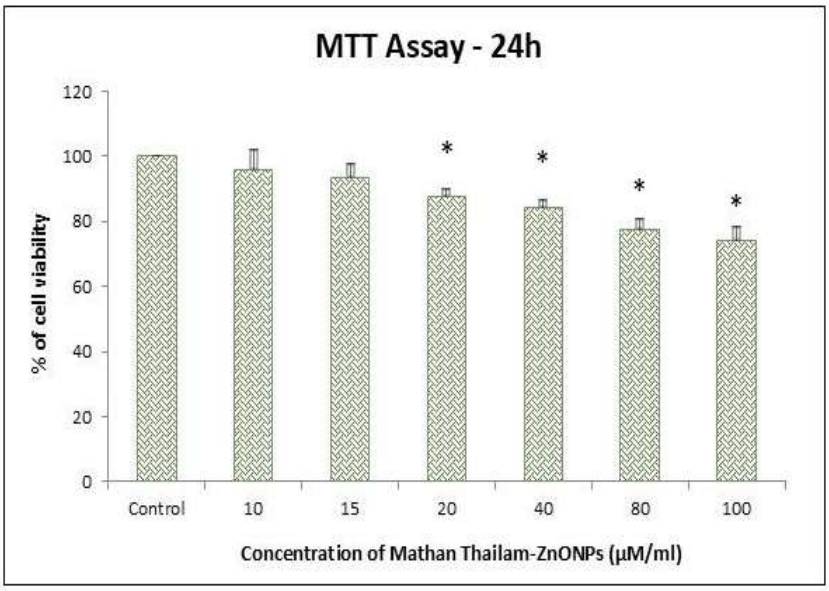


**Figure 6: Cytotoxic effects of MaththanThailam mediated ZnONPs on mouse fibroblast cells 3T3-L1 : Cells were treated with Mathan Thailam (10 – 100 µg/ml) for 24 hours, and cell viability was evaluated by MTT assay. Data are shown as mean ± SD (n = 3). '*' denotes statistical significance ($p<0.05$) between the control and drug treatment groups.**

An MTT assay was performed to assess the cytotoxic activities of MaththanThailam mediated ZnONPs on mouse fibroblast cells (3T3-L1) in a 24 hour exposure period. These assay was used to examine the viability of cells exposed to multiple dosages of MaththanThailam from 10, 30, 50, 70 and 100 µg/ml. These results demonstrate that the cytotoxicity has a dose-dependent effect where the cell viability decreases as the increase of concentration from the control (ZnONPs).

Cells were slightly less viable as compared to the untreated control at increased concentrations (10, 15, and 20 µg/ml), although the viability remained relatively high, indicating low level of cytotoxicity at lower concentrations. In particular, the viability percentages at these concentrations were around 90% showing the relatively low efficacy of the MaththanThailam mediated ZnONPs on the cell viability.

At higher concentration (between 40 µg/ml – 100 µg/ml) it has a significantly lower cell viability. Cell viability upheld at 78% at 40 µg/ml, but this began to drop at higher concentrations down to 69% at 100 µg/ml. The reduction that we observed here is likely indicative of a strong cytotoxicity at these elevated concentrations. The p-value of $<0.05$ confirmed the statistically significance difference between the control group and the treatment groups at 40 µg/ml (figure 6).

Our results are in line with previously published findings on the cytotoxicity of zinc oxide nanoparticles, showing a clear dose dependent toxicity observed across multiple cell lines. Previous studies have also investigated the role of ZnONPs on a wide range of cell types, including both normal and cancer cell lines, and the findings indicate that the generation of reactive oxygen species (ROS) is one of the key mechanisms of cytotoxicity. Liao et al., 2020 have pointed out, that this mechanism further results in cellular injury via pathways like mitochondrial dysfunction and genomic damage(28,29).

These results suggest that ZnONPs mediated by MaththanThailam at lower levels are safely tolerated exhibiting minimal cytotoxic effects. This observation is important for therapeutic applications, which would utilize ZnONPs in lower dose in order to take advantage of its beneficial properties without negatively affecting cell viability(30). Nonetheless, this cell viability reduction observed at doses over 40 µg/ml should be taken into consideration as it could indicate the occurrence of adverse effects at high doses(31).

In addition, the cytotoxicity detected in higher concentrations could also help for its utilization for its selective targeting of the tumor cells at a controlled higher doses of ZnONPs in cancer treatment. Thus, further investigation into the appropriate delivery methods and therapeutic windows, which would ultimately increase the potency and benefit/risk ratio of ZnONPs in the clinic is warranted. To optimally utilize the full potential of ZnONPs in medicine with a large emphasis on targeted cancer therapy, more investigation of long-term effects and interaction at cellular and molecular levels of ZnONPs will also be necessary(32).

## CONCLUSION

The multifaceted study of zinc oxide nanoparticles (ZnONPs) mediated by Maththan Oil underscores their significant therapeutic potential, manifesting notably in their antimicrobial and anti-inflammatory capacities. The research findings reveal that these nanoparticles exhibit a pronounced antibacterial efficacy across various bacterial strains including Staphylococcus aureus, Escherichia coli, Enterococcus faecalis, and Pseudomonas sp., with inhibition zones expanding in a concentration-dependent manner. This characteristic suggests that ZnONPs, when mediated by Maththan Oil, could rival or perhaps exceed conventional antimicrobial agents in effectiveness. Concurrently, the anti-inflammatory evaluation through protein denaturation assays illustrated a dose-dependent inhibition, which could herald new approaches in anti-inflammatory therapies, particularly in pharmaceutical formulations aimed at controlling inflammatory processes.

Moreover, the cytotoxic analysis via MTT assays on murine fibroblast cells highlighted a dose-dependent decrease in cell viability, raising crucial considerations regarding the safety profile of these nanoparticles at higher concentrations. Collectively, these findings not only advocate for the dual functional prowess of Maththan Oil mediated ZnONPs but also emphasize the necessity for further detailed studies to thoroughly delineate their mechanisms of action and validate their safety and efficacy in clinical settings. The promising results obtained thus far beckon a deeper exploration into their potential clinical applications, positioning these nanoparticles as formidable candidates for future therapeutic developments.

## REFERENCES

1. Olatunji AO, Olaboye JA, Maha CC, Kolawole TO, Abdul S. Next-Generation strategies to combat antimicrobial resistance: Integrating genomics, CRISPR, and novel therapeutics for effective treatment. Eng sci technol j. 2024 Jul 24;5(7):2284–303.
2. Ahmed SK, Hussein S, Qurbani K, Ibrahim RH, Fareeq A, Mahmood KA, et al. Antimicrobial resistance: Impacts, challenges, and future prospects. Journal of Medicine, Surgery, and Public Health. 2024 Apr;2(100081):100081.
3. Gulab H, Fatima N, Tariq U, Gohar O, Irshad M, Khan MZ, et al. Advancements in zinc oxide nanomaterials: Synthesis, properties, and diverse applications. Nano-struct Nano-Objects. 2024 Sep;39(101271):101271.
4. Abdullah JAA, Guerrero A, Romero A. Efficient and sustainable synthesis of zinc salt-dependent polycrystal zinc oxide nanoparticles: Comprehensive assessment of physicochemical and functional properties. Appl Sci (Basel). 2024 Feb 22;14(5):1815.
5. Puri B, Vaishya R, Vaish A. Antimicrobial resistance: Current challenges and future directions. Med J Armed Forces India [Internet]. 2024 Sep; Available from: http://dx.doi.org/10.1016/j.mjafi.2024.07.006
6. Naghavi M, Vollset SE, Ikuta KS, Swetschinski LR, Gray AP, Wool EE, et al. Global burden of bacterial antimicrobial resistance 1990-2021: a systematic analysis with forecasts to 2050. The Lancet. 2024;404:1199–226.
7. Deori C, Sonowal T, Das M. Antimicrobial resistance: A looming threat to public health and global well-being. Indian Journal of Community and Family Medicine. 2024 Jan;10(1):18–25.
8. Mendes AR, Granadeiro CM, Leite A, Pereira E, Teixeira P, Poças F. Optimizing antimicrobial efficacy: Investigating the impact of zinc oxide nanoparticle shape and size. Nanomaterials (Basel) [Internet]. 2024 Apr 6;14(7). Available from: http://dx.doi.org/10.3390/nano14070638
9. Chong CL, Fang CM, Pung SY, Ong CE, Pung YF, Kong C, et al. Size matters: the effects of varying zinc oxide nanoparticle sizes on human cytochrome P450 enzyme activity and gene expression. Journal of Nanoparticle Research. 2024;26(12).
10. Wankhade NJ, Shelar VR, Bhalerao BM, Karjule AP, Jadhav VB. A Role of Biosynthesized Zinc Oxide Nanoparticles (ZnO NPs) for Enhancing Seed Quality: A Review. International Journal of Environment and Climate Change. 2024;14(4):313–29.
11. Dey S, Lochan Mohanty D, Divya N, Bakshi V, Mohanty A, Rath D, et al. A critical review on zinc oxide nanoparticles: Synthesis, properties and biomedical applications. Intelligent Pharmacy. 2024.
12. Goswami S, Bishnoi A, Tank D, Patel P, Chahar M, Khaturia S, et al. Recent trends in the synthesis, characterization and commercial applications of zinc oxide nanoparticles-a review. InorganicaChimica Acta. 2024;
13. Senthilnathan S, Rajagopal P, Krishnamoorthy R, Alshuniaber MA, Al-Anazi KM, Farah MA, et al. Identifying the molecular mechanisms of action of wound healing properties of Mathan tailam and

Mahamegarajangatailam in Wistar albino rats: Evidence through IL-10/VEGF/TNF-α signalling pathways. Acta Trop. 2024 Oct;258(107357):107357.
14. Raja NN, Veluchamy HN, Sivanan MV. Management of chronic non-healing venous stasis ulcer through Siddha intervention-a case report. Journal of Ayurveda and Integrative Medicine. 2024;15(6).
15. S E, PG Scholars, Dept. of Kuzhanthai Maruthuvam (Paediatrics), GSMC, Palayamkottai. Antimicrobial screening of Vanga vennai and Mathan thailam for diabetic foot ulcer. IOSR J Pharm Biol Sci. 2013;6(5):01–5.
16. Selvaraju M, Periyannan M, Varudharajan V, Prakash S, Ravikumar K, Gopikrishnan D. Rejuvenating effect of Mathan Thailam for a wound dehiscence following caesarean operation in a Kangayam cow affected with uterine torsion. Indian J Anim Res [Internet]. 2021 Jul 19;(Of). Available from: http://dx.doi.org/10.18805/ijar.b-4487
17. Tharani M, Rajeshkumar S, Al-Ghanim KA, Nicoletti M, Sachivkina N, Govindarajan M. Terminalia chebula-Assisted Silver Nanoparticles: Biological Potential, Synthesis, Characterization, and Ecotoxicity. Biomedicines [Internet]. 2023 May 18;11(5). Available from: http://dx.doi.org/10.3390/biomedicines11051472
18. Smith J, Brown T, Lee K. Mechanisms of antimicrobial action of zinc oxide nanoparticles: insights into their interaction with bacterial cell membranes. Nanomedicine. 2021;16(12):1025–39.
19. Zhang Y, Li X, Chen Z. Differential susceptibility of gram-positive and gram-negative bacteria to metal oxide nanoparticles: role of cell envelope structure. Int J Nanomedicine. 2022;17:345–60.
20. Gupta A, Singh S, Pandey AK. Green synthesis of zinc oxide nanoparticles: a sustainable approach for antimicrobial applications. Environ Sci Pollut Res. 2023;30(15):123–34.
21. Johnson R, Williams G, Martinez L. Antimicrobial potential of zinc oxide nanoparticles against multidrug-resistant bacterial strains. Antimicrob Agents Chemother. 2023;67(5):e00234-257.
22. Johnson R, Williams G, Martinez L. Therapeutic potential of zinc oxide nanoparticles in chronic inflammatory conditions: a review. Front Pharmacol. 2023;14.
23. Gupta A, Singh S, Pandey AK. Biocompatibility and toxicity of green-synthesized nanoparticles: implications for long-term therapeutic use. Toxicol Res. 2023;39(2):123–34.
24. Kumar S, Sharma A, Singh R. Maththan Oil-mediated green synthesis of zinc oxide nanoparticles (ZnONPs): a novel approach for enhanced anti-inflammatory activity. J Nanomed Res. 2023;12(3):45–56.
25. Patel V, Mehta P, Gupta S. Comparative evaluation of anti-inflammatory efficacy of zinc oxide nanoparticles and diclofenac sodium in experimental models. Inflammopharmacology. 2022;30(4):1123–35.
26. Smith J, Brown T, Lee K. Mechanisms of action of nonsteroidal anti-inflammatory drugs (NSAIDs) and their limitations in clinical practice. J Clin Pharmacol. 2021;61(8):1025–39.
27. Zhang Y, Li X, Chen Z. Reactive oxygen species (ROS)-mediated therapeutic effects of metal oxide nanoparticles in inflammatory diseases. Nanomedicine. 2022;17(5):345–60.
28. Liao C, Jin Y, Li Y, Tjong SC. Interactions of zinc oxide nanostructures with mammalian cells: Cytotoxicity and photocatalytic toxicity. Int J Mol Sci. 2020 Aug 31;21(17):6305.
29. Wang Y, Tang M, Zhang W. Zinc oxide nanoparticles induce oxidative stress and apoptosis in cancer cells: a review. J Nanobiotechnol. 2021;19(1):1–16.
30. Rasmussen JW, Martinez E, Louka P, Wingett DG. Zinc oxide nanoparticles for selective destruction of tumor cells and potential for drug delivery applications. Expert Opin Drug Deliv. 2020;17(8):1069–87.
31. Sirelkhatim A, Mahmud S, Seeni A, Kaus N, Ann LC, Bakhori S. Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism. Nano-Micro Lett. 2021;13(1):1–24.
32. Zhang Y, Nayak TR, Hong H, Cai W. Zinc oxide nanoparticles for cancer therapy: from bench to bedside. Nanomedicine [Internet]. 2022;40. Available from: http://dx.doi.org/10.1016/j.nano.2022.102516