ORIGINAL ARTICLE

Evaluation of embryonic toxicology, antimicrobial, anti-inflammatory, and antioxidant activity of the *Equisetum arvense* mediated Magnesium oxide nanoparticles

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ABSTRACT

Introduction: Preparing and stabilising various types of nanoparticles using herbal extract has proven to be an intriguing prospective environmentally beneficial technology. Magnesium oxide nanoparticles (MgONPs) are finding a broad range of applications in the environmental and medical sciences due to their impressive antioxidant, anticancer, and antibacterial activity. The traditional uses of *Equisetum arvense* included wound and ulcer healing, renal issues, tuberculosis treatment, and bleeding control. This work intended to synthesise magnesium oxide nanoparticles in an environmentally friendly manner utilising E. arvense, with potential uses in biomedicine.

Materials And Methods: Using a green fabrication technique, E. arvense extract was used to create magnesium oxide nanoparticles (MgONPs). The antibacterial activity of the resulting MgO NPs against wound infections was evaluated. The ABTS, Nitric Oxide, DPPH, FRAP, and H_2O_2 assays were used to measure antioxidant activity. Utilising zebrafish survivability during MgONPs treatment, cytotoxicity was evaluated.

Results: The green-produced MgONPs showed good antibacterial activities against wound infectious microbes, compared to the E. arvense control. It also demonstrated outstanding biocompatibility and antioxidant activity.

Discussion: The potential application of plants mediated NPs as antibacterial, and an antioxidant agent is the primary conclusion of the research.

KEYWORDS:
Green synthesis, antioxidant, anti-inflammatory, antimicrobial, eco-friendly, biocompatibility

INTRODUCTION

In recent years, the nanoparticles have been synthesised by using the green synthesis process. It is also known as the biosynthesis process; the biosynthesis process was done by using the plant-mediated nanoparticles. The green synthesis process efficacy of applications of natural reducing agents, without using chemicals and with less toxicity, is a perfect and stabilising agent.[1] Magnesium oxide nanoparticles are one of the metal ion-based nanoparticles, a lot of research is going into the metal-based nanoparticles by using plant-based. The metal-based nanoparticles are used to synthesise the green synthesised method and it has maintained the bioefficacy.[2] The *Equisetum arvense* is a perennial herb and native to the plant northern Himalayas. The local name of the plant is Field horsetail or Horsetail, genus *Equisetum*, and the species *arvense*.[3] Horsetail is one of the best treatments for skin disease, to treat pimples and dermatitis, it has present the excellent wound-healing activity of the skin conditions.[4] It has been used for cosmetics like moisturising agents, body shampoos, soaps, cosmetic cleaners, and skin conditioning agents.[5] *E. arvense* contains sterols, a rich source of vitamin C, phenolic acid, and flavonoids.[6] It has also shown efficacy against fungi species.[7] In pharmaceutical studies, *E. arvense* has an additional medicinal therapy, and excellent antioxidant and anti-inflammatory properties.[8]

The plant-based nanoparticles are used, and they reduce toxicity and are eco-friendly. The magnesium oxide nanoparticles have both metal and metal oxide based, they have different biomedical and biochemical applications catalysis, tissue regeneration, and antimicrobial and anticancer agents.[9] Magnesium oxide has excellent stability, bioavailability, and antiviral and antipathogenic activity.[10] The green method is used to develop the magnesium oxide nanoparticle synthesis using cost-reduction, energy efficiency, removal of environmental pollution, and biocompatibility.[11] The green synthesised magnesium oxide nanoparticles are used in biomedical therapy for cancer, inflammation, oxidation, and diabetes.[12] The concept of the research work has been to evaluate the preparation of nanoparticles, antimicrobial activity against wound pathogens, time-kill curve assay, antioxidant, anti-inflammatory activity, thrombolytic activity, cytotoxic effect against brine shrimp lethality assay, and the embryonic toxicology using zebrafish embryos.

MATERIALS AND METHODS

Preparation of plant extract

At an Ayurvedic store in Poonamalli, Chennai, the dried E. arvense was bought. A 150 ml of distilled water were combined with three grams of *E. arvense*. A 150 ml of *E. arvense* extract were thoroughly agitated and then heated to 60°C for 15-20 minutes in the heating mantle. After it was boiled and filtered through muslin material, the extract was

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prepared and used for the manufacture of nanoparticles and additional study.[13]

Preparation of MgO nanoparticles
The magnesium oxide nanoparticles were prepared using 50 mm of magnesium chloride (MgCl), which was precursor to the magnesium oxide nanoparticle. To prepare the magnesium chloride solution, 50 ml of distilled water and 50 mm of MgCl were combined. The 50 mL magnesium chloride solution was combined with the 50 ml *E. arvense* extract. Colour changes were noticed in the solution after it was stirred for 48 hours at 600 rpm using a magnetic stirrer. Up to 250–650 nm, the first stage of UV–visible spectroscopic characterization was seen. After centrifuging the nanoparticle solution for ten minutes at 8000 rpm, the pellets were separated and distributed in various sterile containers.[14]

Antimicrobial activity
E. arvense was used to perform the antibacterial activity for the green process of magnesium oxide nanoparticles utilising the agar well technique. Mueller Hinton Agar medium was added to the sterile Petri plate. Sterile micro tips (9mm) are used to help prepare the agar well diffusion technique in sterile petri plates. Fresh microbiological cultures, including *E. coli, S. aureus, Pseudomonas sp.*, and Candida albicans, were used to generate the various microbial stains using Muller-Hinson broth. In the sterile swab in the agar plates, the microbial broth culture was distributed uniformly. Following the addition of magnesium oxide nanoparticles at three distinct concentrations (25, 50, and 100 µg/mL), the agar plates were allowed to incubate for a full day at room temperature. The inhibitory zones were measured in diameter on the plates after an incubation period. A pure aqueous extract of *E. arvense* was utilised as the control in each microbiological culture.[15]

Time kill curve assay
To create a standardised vaccine, the isolated microbial broth was cultured. Nanoparticle solutions were put into 96-well plates at different concentrations (25, 50, and 100 µg/ml). Additionally, pure aqueous extract of *E. arvense* was used as the positive control, antibiotics as the standard, and simply microbial culture was added as the negative control. At room temperature, the plate was incubated, at regular intervals of 0, 2, 4, 6, 8, and 24 hours, observations for microbial cultures have been collected. It was able to determine the killing rate by plotting the total number of living cells as $\log_{10}$ (CFU/mL) against time.[16]

Anti-inflammatory activity
Bovine serum albumin denaturation assay
Anti-inflammatory efficacy of green production of magnesium oxide nanoparticles using *E. arvense*, the prevention of albumin denaturation approach—which was previously investigated by Mizushima et al., has been somewhat modified. Five different concentrations (10-50 µg/ml) were added to the nanoparticles. Adding a 1% aqueous solution of the fractional representation of bovine albumin together with test extracts to the reaction mixture. To modify the pH of the reaction mixture, a very small amount of 1N HCl was added. The sample extracts were chilled, and the turbidity was measured at 660 nm after 20 minutes of incubation at 37°C and 20 minutes of heating at 51°C.

Egg albumin denaturation assay
A 0.2 ml of egg albumin, PBS (2.8 ml of Phosphate Buffer Saline, keeping a pH range of 6.4), and the five different concentrations of nanoparticle solutions (10-50 µg/ml) were added to the 5 mL reaction mixture. Double-distilled water in an equivalent volume is used as a control. In addition to determining the absorbance of standard values, standard medications like diclofenac sodium were employed. After 15 minutes of incubation at room temperature, the mixed solutions were heated to 50°C for five minutes to bring the water to a boil. Measure the absorbance at 660 nm after the liquids have cooled. The formula was used to determine the percentage of inhibition of protein denaturation.

Membrane stabilization assay
Preparation of Red Blood cell (RBC) suspension.
A euthanized human volunteer was selected based on health, and blood was drawn into centrifuge tubes only after the donor had abstained from NSAID use for two weeks before the experiment. The tubes underwent three rounds of sterilisation using the same volume of regular saline after being centrifuged for ten minutes at 3000 rpm. Following that, using a standard saline solution, the volume of the blood was measured and reconstituted at a 10% v/v concentration.

Heat-induced method
One millilitre of 10 % RBC suspension and one millilitre of magnesium oxide nanoparticles made with *E. arvense* at different concentrations (10-50 µg/ml) made up the reaction mixture (2 ml). Ordinary saline was added to the control test tube in place of magnesium oxide nanoparticles made with *E. arvense*. A popular standard was vitamin C. Each centrifuge tube holding the reaction mixture was incubated for 30 minutes at 56°C in a water bath. Following the incubation time, the tubes were cooled using running tap water. The absorbance of the supernatants was measured at 560 nm after the reaction mixture was centrifuged for five minutes at 2500 rpm. The experiment was performed in duplicate for each test sample.[17]

Antioxidant activity
DPPH assay
A solution of 10 ml methanol and 0.1 mm DPPH was prepared. Five varying amounts of nanoparticle solutions (10-50µg/ml) were combined with 1 mL of DPPH methanol solutions. Following that, the mixed solutions were allowed to sit at room temperature for 30 minutes in a dark incubator. Ascorbic acid served as the standard, while DPPH solution served as the control. A UV-visible spectrophotometer was used to detect the absorbance at 517 nm following the completion of the incubation. The greater activity of scavenging free radicals is indicated by the lower absorbance. Using the DPPH assay, the percentage of inhibition was calculated for each concentration of nanoparticles based on the percentage of antioxidant activity.

H_2O_2 assay
The Halliwell technique[18] has brought about a few changes to the assay's completion. All of the solutions were made from the beginning. In 1.0 ml, the following ingredients make up the reaction's solution: A mixture of 100 µl of 28 mm 2-deoxy-2-ribose (dissolved in phosphate buffer, pH 7.4), 500 µl of various nanoparticle concentration solutions (10-50 µg/ml), 200µl of 1.04 mm EDTA and 200 mM Fecl3, 100µl of H2O2 (1.0 mm), and 100µl of ascorbic acid (1.0 mm) were used. A 60-minute incubation period at 37°C allowed for the determination of the degree of deoxyribose breakdown using the TBA reaction. The absorbance is measured by comparing it to the blank solution at 532 nm. The vitamin E was used as a positive control.

FRAP (Ferric Reducing Antioxidant Power) assay:
This technique works by decreasing the ability of Fe3+ to Fe2+ ions in the sample. Ferric-tripyridyltriazine complex to decrease the ferrous form in the presence of low pH, TPTZ (2,4,6-Tri(2-pyridyl)-s-triazine), and the formulations indicated the blue colour has been absorbed in the UV-Visible spectrophotometer at 593 nm. Aqueous extract (0.7 ml) and 2.3 ml of the FRAP formulation reagents were combined with five different doses of nanoparticle solutions (10-50 µg/ml). The mixed solutions were then left to incubate for 30 minutes at room temperature in the dark. The ascorbic acid used in the standard was substituted with double-distillation water for the control. A UV-visible spectrophotometer was used to detect the absorbance at 593 nm following the incubation period. To demonstrate the increase in the reduction capabilities, the absorbance value was improved.

ABTS Assay
The chemical compound ABTS (2, 2-azinobis (3-ethylbenzothiazoline-6-sulphonic acid)). To create the stock solution, 7 mm of ABTS was combined with distilled water, and 2.45 mM of potassium per sulphate was combined with an equivalent volume of aqueous solution. After the mixed solutions were incubated for 12 to 16 hours at room temperature in the dark and used. The working solution and the measuring absorbance at 734 nm were made by diluting the stock solution in methanol. A working ABTS solution of 2 ml was combined with five different concentrations of nanoparticle solutions (10-50 µg/ml), and the mixture was left to incubate for 10 minutes at room temperature in the dark. Following that, a volume of ABTS working solution equal to one millilitre was added as the control. The standard was butylated hydroxytoluene or BHT. At 734 nm, the absorbance was measured with a spectrophotometer. The percent of inhibition was determined as the percentage of antioxidant activity in each concentration of the nanoparticles on the DPPH assay.

Nitric acid assay
Griess reagent (0.5 ml; 1% sulfanilamide, 2% H_3PO_4, and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) was added) is used to measure nitrite ions, which are produced when sodium nitroprusside spontaneously releases nitric oxide in physiological pH aqueous solution. The creation of nitrite ions is inhibited by nitrite scavengers, which compete with oxygen. Sodium nitroprusside (10 mm) was mixed with different concentrations of dissolved nanoparticle solutions (10-50 µg/ml) in phosphate-buffered saline, and the combination was then allowed to sit at room temperature for 2.30 hours. After that, a control experiment with an identical volume of double-distilled water was conducted. It was determined that the absorbance was 546 nm. Instead of quercetin, a positive control was employed.[19]

Thrombolytic activity
Using a sterile glass slide, one drop of blood was taken and incubated at the ideal temperature for 45 minutes. Add the 20 µg/ml solution of magnesium oxide nanoparticles after the blood has clotted. Afterward, without adding any solution, compare with the control. The ideal temperature for the glass slide was maintained for 90 minutes, and the incubation hours were recorded to track the lysis of the clot. Blood was removed and then re-infused ten times following the incubations.[20]

Cytotoxic effect
Brine shrimp lethality assay
The test organism for the cytotoxic test was *Artemia salina* (nauplii), and the lethality assay of brine shrimp was employed in its execution. Thirty milligrams of *Artemia salina* (brine shrimp egg) were introduced to the saltwater in a sealed black container. The black container with aeration was used to aid in the hatching of the nauplii eggs. *Artemia salina* eggs hatch and transform into larvae after 24 hours. Following that, five distinct doses of nanoparticles were injected into each of the six-well plates that held ten individuals Artemia salina hatchings (5, 10, 20, 40, and 80 µg/mL). The control was added solely to the saltwater and the six well plates were incubated for a full day at room temperature. The number of nauplii in the 6-well plate was counted when the incubation concluded.[21]

Embryonic toxicology
The fish tank had been preserved under excellent environmental conditions, the pH was preserved in the range of 6.8 to 8.4, and the zebrafish were bought from neighbourhood merchants in Tamil Nadu. Fish foods like blood worms or ideal meals, which are both commercially accessible, were fed to the fish twice a day. Where the breading tanks were filled with three male and one female zebrafish. The eggs were carefully removed without causing any damage to the viable embryos that were formed. Three times, the eggs were cleaned in an E3 medium that was devoid of methylene blue. With 10 embryos in 2 ml of solution per well, the eggs were put into three different well plates, measuring 6, 12, and 24. In this experiment, the E3 medium that was diluted from the stock using sterilised water was utilised. Once that was established, the pH was kept between 7.2 and 7.3. In a study conducted 24 to 96 hours after conception, embryos in good health were exposed to different concentrations of nanoparticle solutions (5, 10, 20, 40, and 80 µg/ml). Nanoparticles and the E3 medium solution were combined before the embryos were used. The embryos were counted, and the dead ones were taken out after the incubation period. All the experimental plates were kept at 28°C and covered with foil sheets to avoid light interference.

Evaluation of Zebrafish Embryos
Using a stereo microscope, the developmental stages of the fertilised zebrafish embryos were examined. Following a 24-

78-hour post-fertilization period, the embryos were introduced to different concentrations of nanoparticle solutions (5, 10, 20, 40, and 80 µg/ml). The study's endpoint is the proportion of embryos that die and hatch within the time frame. Under the microscope, any deformity or anomaly was detected for both the treatment group and the control group.[22]

RESULTS

Preparation of Magnesium oxide nanoparticles

The colours of the nanoparticles' solution at the beginning and end stages of the green synthesis of *E. arvense*-mediated magnesium oxide nanoparticles were pale brown and dark brown, respectively. This demonstrates magnesium oxide nanoparticles are present and formed. The most popular method for examining the optical characteristics of nanoparticles is UV-visible spectroscopy. Bio reduction kinetics of the reaction between an aqueous extract of *E. arvense* at varied time intervals and concentrations between 250 and 650 nm. Because surface plasmon vibrations were excited, a maximum absorbance peak was seen at 380 nm for the synthesising of magnesium oxide nanoparticles (Fig.1). Figure 1(B, C, & D) displayed the SEM (Scanning Electron Micrographs comparing the morphology of E. arvense mediated Magnesium oxide nanoparticles. Fig. 1B), shows that the Magnesium oxide nanoparticles had exhibited with more spherical shapes, Similarly Fig. 1C, revealed the morphology of MgO NPs had spherical shape and mild agglomeration, and Fig. 1D while the MgONPs had irregular shapes and more agglomeration.

Antimicrobial activity

Antimicrobial activity against wound infections was demonstrated by the magnesium oxide nanoparticles. Fig. 2 shows the zone of inhibition for magnesium oxide nanoparticles against wound pathogens at varying doses (25, 50, and 100 µg/mL). In 16 mm, *Pseudomonas sp* exhibited the largest zone of inhibition at the higher level (100 µg/mL) and the lowest level (25 µg/mL) in 12 mm. Similarly, *C. albicans* and *S. aureus* observed the inhibitory zone in 14 mm at the higher level (100 µg/mL) and 11 mm at the lower level (25 µg/mL). The *E. coli* was then detected in Fig. 2 at the lowest concentration (25 µg/mL) in 9 mm and at the highest level (100 µg/mL) in 14 mm, respectively. The control observed the same level of zone of inhibition (9 mm) in clinical pathogens.

Time kill curve assay

The time-kill curve assay findings show that the produced MgONPs reduced the number of live cells for the assessed wound pathogens. The time kill curve evaluation graphs in Fig. 2F, 2G, 2H, and 2I, show that the *E. arvense*-mediated MgONPs had a significant bactericidal activity against *Pseudomonas sp.* The produced nanoparticles exhibit ideal bactericidal and fungal activities against *S. aureus* and *C. albicans*. Similarly, the modest bactericidal and bacteriostatic action of MgONPs was observed in *E. coli*. According to the results, the microbial and micro static activities of the E. arvense-mediated MgONPs develop gradually over time. Significantly, as compared to the standard (fungi – fluconazole, and bacteria – amoxyrite) resistance of the assessed organism, the resulting nanoparticles exhibit maximum microbiological activity at a concentration of 100 µg/mL, respectively. MgO NPs mediated by *E. arvense*, work. Metal ions harm the microbial cell wall and can infiltrate it. The microorganism caused mortality and reduced growth. By comparing the results with the control group, it is possible to see how much MgONPs have helped lower bacterial counts and how promising they are as an antibacterial agent for clinical management. It was measured at 600 nm using the ELISA reader. Because the control sample included entirely bacteria, it proliferated at a greater rate. Similar to the standard values, a greater growth reduction was observed in the fourth hour at the higher dose (100 µg/ml). The microbial growth in the fourth hour was also lowered by the lower concentration (25 µg/ml), respectively.

Thrombolytic activity

The clotted drop of blood was added to the 20 µg/ml of magnesium oxide nanoparticles, which have a thrombolytic property. After mixing the clotted blood with the nanoparticle solution for 20 minutes, the clotted blood liquefied once more, as represented in Fig. 3D & E, where Fig 3D as before adding the MgONPs and E) After adding the

MgONPs.*Anti-inflammatory activity*

Bovine serum albumin denaturation assay

The magnesium oxide nanoparticles were found to be effective at all concentrations (10-50 µg/ml) in the anti-inflammatory activity assessments conducted using the bovine serum albumin denaturation assay Fig. 3A. The 50 µg/ml showed the highest percentage of inhibition (78.4%), which was followed by the 40 µg/mL at 72.9 %, 30 µg/ml at 67.7 %, 20 µg/ml at 54.3 %, and 10 µg/ml at 44.6 %. In comparison to typical values, it was shown that magnesium oxide nanoparticles may have an anti-inflammatory effect.

EA assay

Using the egg albumin denaturation assay, Figure 3(B) magnesium oxide nanoparticles were evaluated for their anti-inflammatory action and shown to be effective at all concentrations (10-50 µg/ml). The 50 µg/ml showed the highest percentage of inhibition (78.6%), which was followed by the 40 µg/mL (68.4%), 30 µg/mL (64.1 %), 20 µg/ml (59.3%), and 10 µg/ml (53.7%). When compared to conventional values shown that magnesium oxide nanoparticles may have an anti-inflammatory effect.

MSA assay

Magnesium oxide nanoparticles were found to be effective at all concentrations (10-50 µg/ml) in the anti-inflammatory activity assessments conducted using the membrane stabilization assay. The samples with the highest percentage of inhibition were those containing 50 µg/ml (84.8%), 40 µg/mL (78.7%), 30 µg/mL (73.3%), 20 µg/ml (65.4%), and 10 µg/ml (54.5%). Fig. 3C shows that when compared to typical values, magnesium oxide nanoparticles may have an anti-inflammatory effect.

Antioxidant activity

DPPH assay

Magnesium oxide nanoparticles were found to be effective at all concentrations (10-50 µg/ml) when their antioxidant activity was evaluated using the DPPH test. The 50 µg/ml

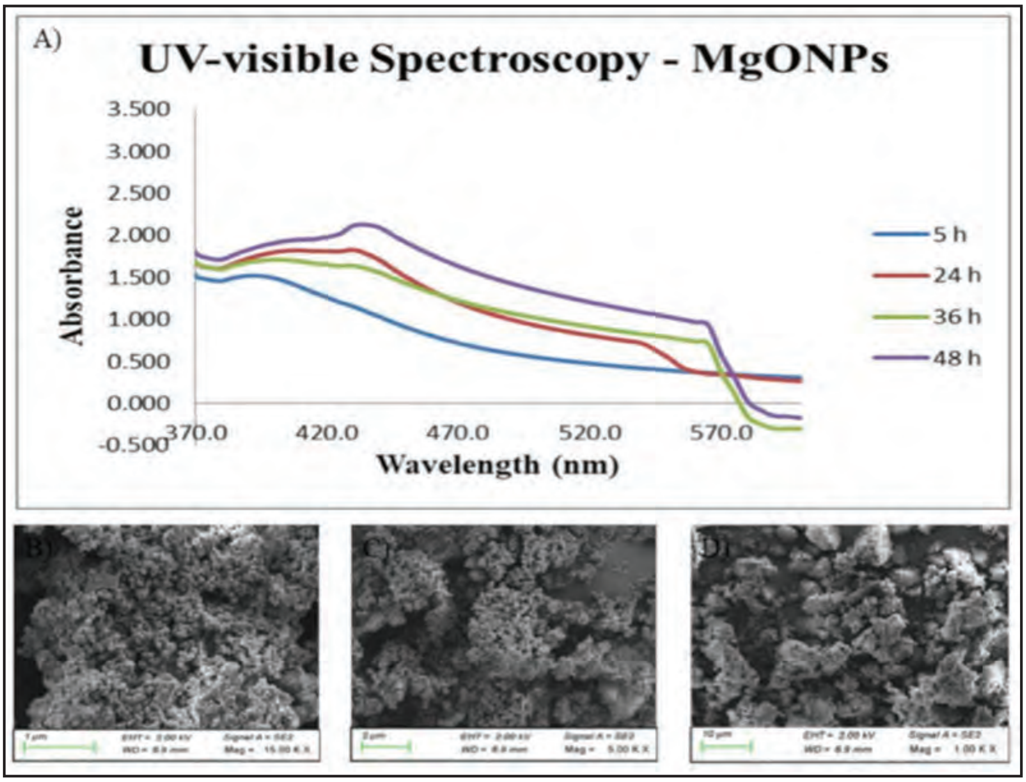

Fig. 1: A) UV-visible absorption spectra of magnesium oxide nanoparticles after bioreduction kinetics of the reaction of E. arvense aqueous extract in the concentration range of 250 - 650 nm with different time intervals. The SEM image of green synthesised magnesium oxide nanoparticles using E. arvense at different micrometre B) 1 µm, C) 2 µm, and D) 10 µm.

showed the largest percentage of inhibition, 89.57%; the 40 µg/mL showed 84.12%; the 30 µg/ml showed 81.09%; the 20 µg/ml showed 74.76%; and the 10 µg/ml showed 62.44%. In comparison to conventional values, Fig. 4A shows that magnesium oxide nanoparticles may have an antioxidant effect.

H_2O_2 assay
The H_2O_2 assay, which measures antioxidant activity, demonstrated the effectiveness of magnesium oxide nanoparticles at all concentrations (10-50 µg/ml). The samples with the highest percentage of inhibition were those with 50 µg/ml (86.9 %), 40 µg/ml (73.16 %), 30 µg/ml (63.5 %), 20 µg/ml (53.7 %), and 10 µg/ml (48.6 %). When compared to conventional values, Fig. 4B shows the possibility antioxidant effect of magnesium oxide nanoparticles.

FRAP assay
The efficacy of magnesium oxide nanoparticles was demonstrated in all concentrations (10-50 µg/ml) by the FRAP assay, which was used to assess antioxidant activity. Following the 50 µg/ml at 86.32%, 40 µg/mL at 80.94%, 30 µg/ml at 77.51%, 20 µg/ml at 73.14%, and 10 µg/ml at 67.86%, the highest percentage of inhibition was documented in this sample. When magnesium oxide nanoparticles are compared to conventional values, Fig. 4C shows that they may have an antioxidant effect.

ABTS assay
The assessment of antioxidant activity using ABTS assay showed the efficacy of magnesium oxide nanoparticles in all various concentrations where (10-50 µg/ml). The highest percentage of inhibition was noted in the 50 µg/ml at 87.58%, followed by the 40 µg/ml at 80.95%, 30 µg/ml at 78.72%, 20 µg/ml at 70.64%, and 10 µg/ml at 65.81%. Fig. 4D, illustrates that magnesium oxide nanoparticles have an antioxidant potential effect compared with standard values.

Nitric oxide assay
Magnesium oxide nanoparticles were found to be effective at all concentrations (10-50 µg/ml) when their antioxidant activity was evaluated utilising a nitric oxide analysis. Eighty-eight percent inhibitions were seen at 50 µg/mL, the maximum percentage of inhibition was found at 40 µg/ml (79.15%), 30 µg/ml (78.01%), 20 µg/ml (72.83%), and 10 µg/ml (67.45 %). When magnesium oxide nanoparticles are compared to conventional values, Fig. 4E shows the antioxidant effect against Nitric oxide assay.

Toxicology studies
Brine shrimp lethality assay
Magnesium oxide nanoparticles' cytotoxic effect is depicted in Fig. 5F, where it is noted that the particles' toxicity was reduced. 80% of live nauplius was found to have the highest concentration (40 & 80 µg/ml), while 100% of live nauplii was found to have the lowest concentration (5 & 10 µg/ml). A reduced percentage of living nauplii has been seen with increasing nanoparticle concentration. It was found that at increasing concentrations, magnesium oxide nanoparticles were less harmful.

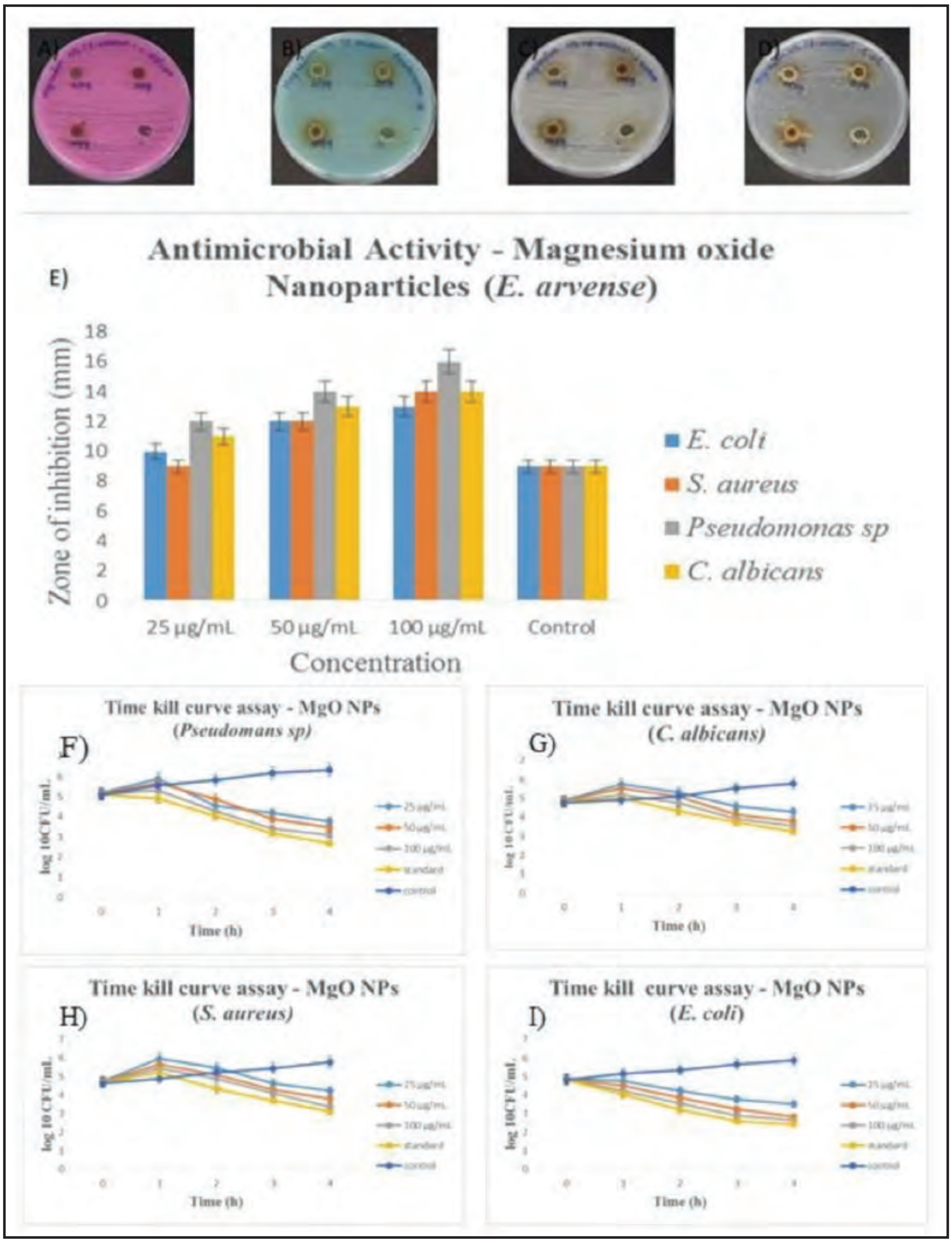

Fig. 2: Image representing Inhibition zone and time-kill curve assay of magnesium oxide nanoparticles against treated Clinical pathogens especially A) *C. albicans* B) *Pseudomonas sp.* C) *S. aureus*, D) *E. coli*, E) graphical representation of antimicrobial activity, F) Time kill curve assay of MgONPs against *Pseudomonas sp.*, G) Time kill curve assay of MgONPs against *C. albicans*, H) Time kill curve assay of MgONPs against *S. aureus*, and I) Time kill curve assay of MgONPs against *E. coli*.

Embryonic toxicology

The hatching rate of zebrafish embryos after being treated with the magnesium oxide nanoparticles.

A range of quantities, including 5, 10, 20, 40, and 80 µg/ml, were introduced to the magnesium oxide nanoparticles mediated by *E. arvense*. The different levels were administered to the zebrafish embryos, which were then examined under a microscope for a duration of 0 to 24 hours. The hatching rate of zebrafish embryos treated with magnesium oxide nanoparticles is depicted in Fig. 5A. 100 % of the zebrafish eggs hatched out at the lowest concentrations (5, 10 & 20 µg/ml). The zebrafish embryos that hatched at a rate of 80% had the highest concentration of nanoparticles. The zebrafish embryos did not hatch later than expected, and the magnesium oxide nanoparticles exhibited reduced toxicity.

The viability rate of zebrafish embryos after being treated the magnesium oxide nanoparticles

A range of quantities, including 5, 10, 20, 40, and 80 µg/ml, has been applied to the magnesium oxide nanoparticles mediated by *E. arvense*. After applying the different levels to the zebrafish embryos, the embryos were examined under a

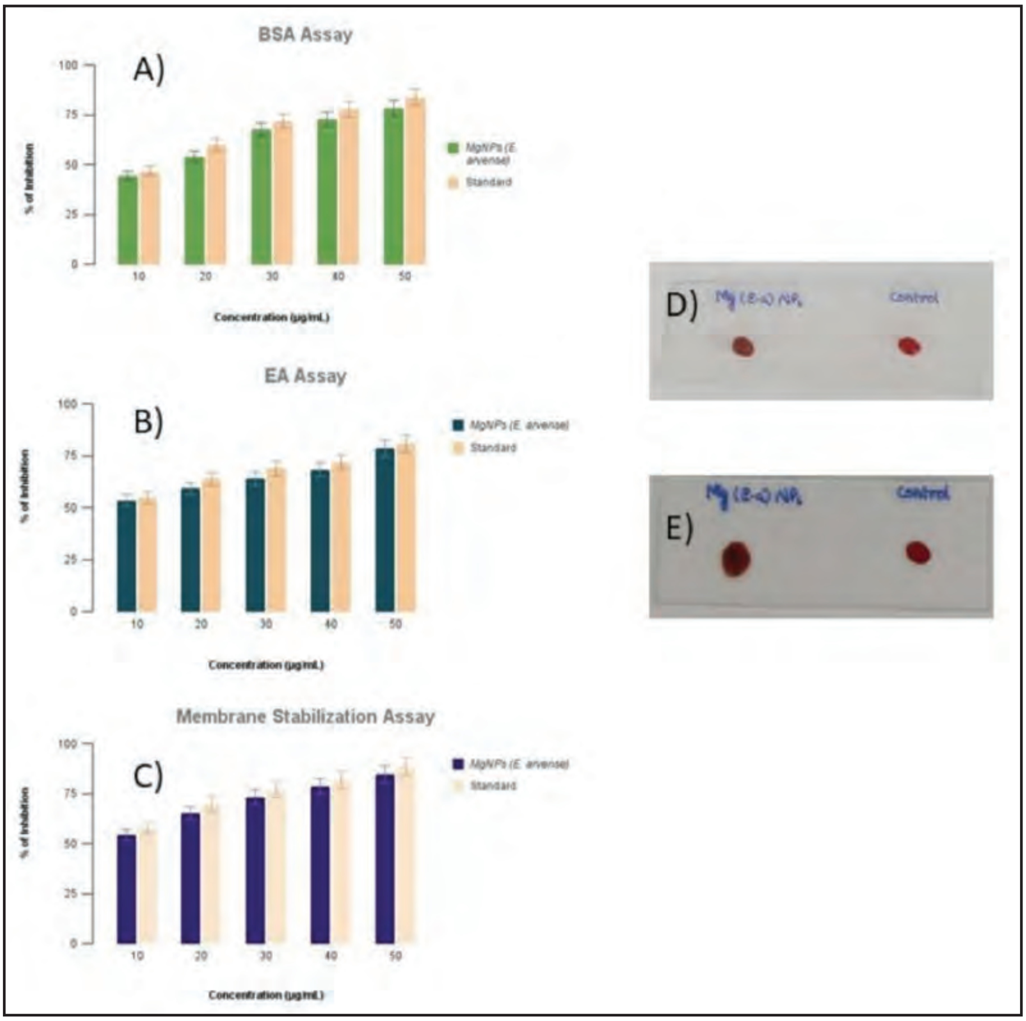

Fig. 3: Image representing the anti-inflammatory activity of Magnesium oxide nanoparticles using A) BSA assay, B) EA assay, C) MSA assay, D) the thrombolytic activity of MgO NPs before adding the sample, and E) after adding the MgO NPs.

microscope for a duration of 0 to 76 hours. Following treatment with magnesium oxide nanoparticles, Fig. 5B displays the viability rate of the zebrafish embryos. The zebrafish embryos exhibit a 100 % survivability rate when seen through the lens of the lowest concentrations (5 & 10 µg/ml). Eighty percent of the zebrafish eggs hatched at nanoparticle doses of 20 and 40 µg/ml. A 60% viability rate has been observed at the highest concentration of magnesium oxide nanoparticles. The zebrafish larvae's delayed growth and the treated embryos' deformity were not seen showed in Fig. 5(C - E), despite the toxicity of the magnesium oxide nanoparticles.

DISCUSSION

The green synthesis of magnesium oxide nanoparticles using E. arvense to produce the biological applications and the synthesis process. The *E. arvense* aqueous extract with the magnesium chloride solution was treated. The magnesium chloride was reacted with water and the plant-based bioreducing compound to release the metal ion. It was reduced to magnesium ions, the magnesium ion reacted with the bioactive of *E. arvense* to make the magnesium oxide nanoparticles. In previous research, the magnesium chloride (MgCl2). H_2O_2 was reacted with the *Moringa oleifera, Vernonia amygadaina, and Occimum gratissimun* extract. Both solutions were placed in the shaker and the 24 hrs magnesium chloride was broken into magnesium ions using the M*oringa oleifera, Vernonia amygadaina, and Occimum gratissimun* bio-reducing agent. The magnesium ion was bonded to the bioactive compound of *Moringa oleifera, Vernonia amygadaina, and Occimum gratissimun* to form the magnesium oxide nanoparticles.[23] The synthesis of magnesium oxide nanoparticles observed the changing of colour from pale brown to dark brownish. Similarly, The phytochemical constituents that appear in the *A. precatorius* bark were identified as a useful substance for obtaining MgO NPs. The aqueous magnesium ions were treated with *A. precatorius* extract and were reduced, and magnesium oxide nanoparticles formed. the *A. precatorius-mediated* magnesium oxide nanoparticles showed a colour change from a brownish colour to a dark brownish colour. The magnesium oxide nanoparticles were observed at the peak of 272 nm using UV characterisation.[24] In similar work, The production of MgO-NPs was confirmed by measuring the maximum surface plasmon resonance (SPR) by UV-Vis spectroscopy. UV-Vis spectroscopy of myco-synthesised MgO-NPs showed maximum SPR at 280 nm.[25]

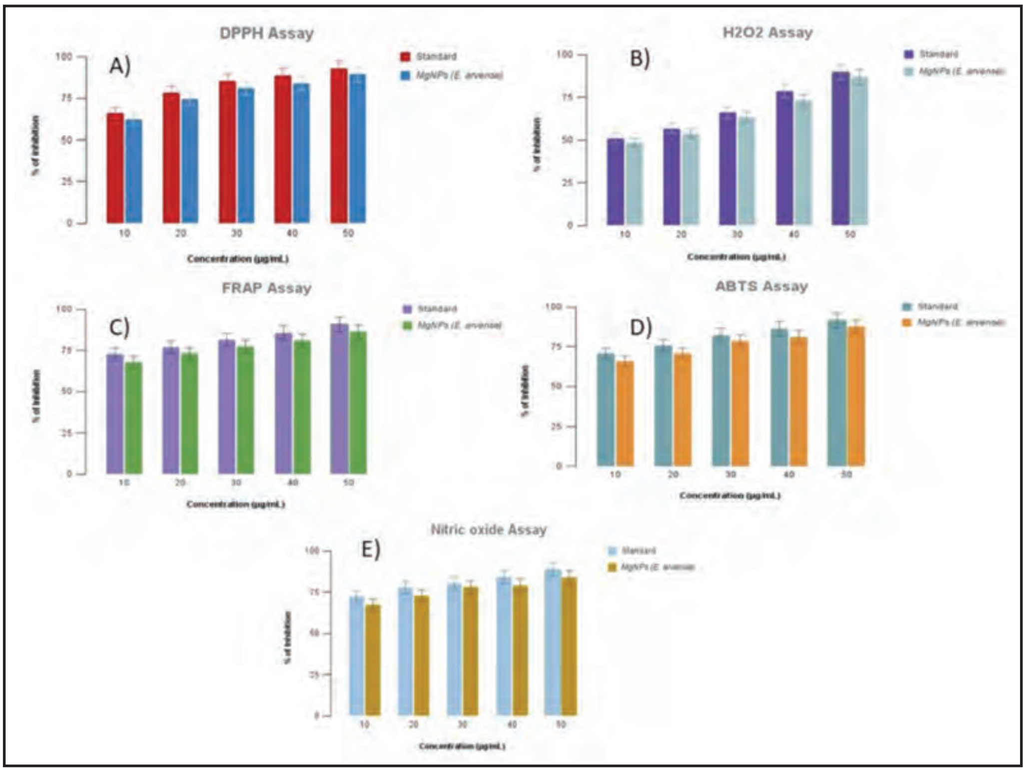

Fig. 4: Image representing the antioxidant activity of Magnesium oxide nanoparticles using A) DPPH assay, B) H2O2 assay, C) FRAP assay, D) ABTS assay, and E) Nitric oxide assay

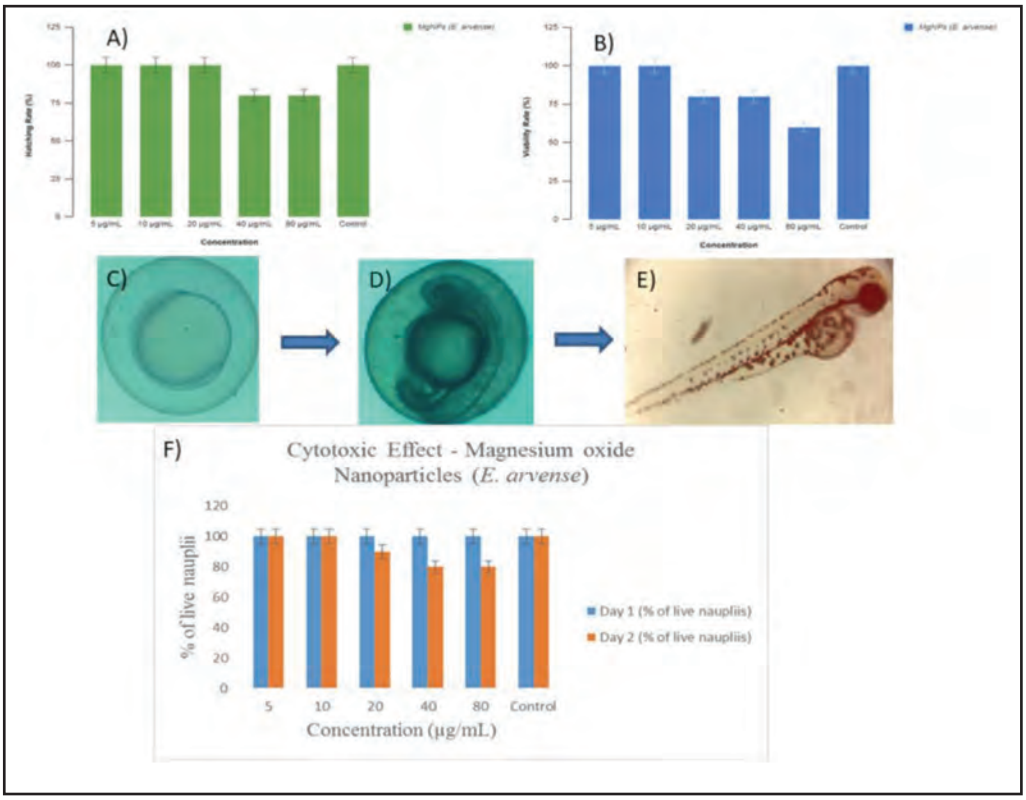

Fig. 5: The graph represents the cytotoxic effect A) Hatching rate of the embryos, B) Viability rate of the embryos, C) Day 1 of embryos, D) Day 2 stage, E) Day 3 stage, and F) Brine shrimp lethality assay

On the previous research study, the magnesium oxide nanoparticles based on rubber leaf shown the more spherical shape and lesser agglomeration.[26] In similar study, the Green synthesis of magnesium oxide nanoparticles using *Hyphaene thebaica* extract showed the SEM micrograph has confirmed the formation of quasi-spherical shape with agglomeration.[27] *Rhizopus oryzae*-Mediated Green Synthesis of Magnesium Oxide Nanoparticles, SEM image showed well-dispersed spherical MgO-NPs without any aggregation.[28] In present research work has shown very good antimicrobial activity against wound pathogens. In similarly, the *Dalbergia sissoo* extract mediated magnesium oxide nanoparticles showed antibacterial efficacy against *E. coli* at 11 mm in 3 mg/ml (higher concentration). The processes of lipid peroxidation and generation of reactive oxidative species in the container were responsible for the antibacterial potential of MgO-NPs.[29]

The previous work, *Plicosepalus curviflorus* extract and green synthesised PC-MgONPs were tested for their antibacterial efficacy against *C. albicans, MRSA, S. aureus, E. coli, and P. aeruginosa*. PC-MgONPs were shown to have a zone of inhibition of 17 mm for *E. coli* and 16 mm for *Candida albicans,* respectively.[30] Similarly, magnesium oxide nanoparticles' potential antibacterial mechanism. However, magnesium oxide nanoparticles have the ability to increase reactive oxygen species (ROS) in bacteria. This process, which results in inflammation, has seriously harmed their proteins, nucleic acids, and membrane lipids.[31] Some of the hazardous radicals that produce ROS in comparatively small amounts are hydrogen peroxide (H_2O_2), a mild oxidising agent, reactive hydroxyl radical (•OH), and superoxide anion radical (O_2^-). H2O2 is produced when the superoxide anion radical (•O_2^-) combines with hydrogen ions to form the •HO_2^- radical. It can interact with proteins, DNA, and cell membranes, ultimately leading to microbial death.[32] In the anti-inflammatory activity, naturally, the *E. arvense-mediated* magnesium oxide nanoparticles have excellent anti-inflammatory properties. In other research work, Green-synthesised MnO2 NPs were tested for their anti-inflammatory activity by Bovine Serum Albumin (BSA) method and Egg Albumin (EA) method. Inflammation is the tissue or organ injurious causes characteristic pain, swelling, temperature, and redness. In the BSA method, the *M. annua* plant extract and MnO2 NPs showed a percentage inhibition of denaturation at 100 µg/mL is 75.42% and 77.49%. In the EA method, showed 59.88% and 71.73% of inhibition denaturation. In the extract showed lesser inhibitory potential compared to MnO2 NPs.[33] Naturally, the *E. arvense-mediated* magnesium oxide nanoparticles have excellent antioxidant properties. Antioxidants are substances that prevent some types of oxidative damage, and plant extracts are numerous in bioactive components with a high antioxidant concentration.[34] In another work, *E. arvense* methanolic extract demonstrated a higher per centage of inhibition was shown in ABTS and FRAP, and the DPPH assay showed the per centage of inhibition. DPPH is a deep purple chemical compound with an odd electron that absorbs light at 517 nm. DPPHH, a yellow material that absorbs at lower wavelengths, is the result of its degradation by antioxidants and free radical scavengers (biomolecules).[35]

The magnesium oxide nanoparticles have a thrombolytic property. Previous research work, the thrombolytic activity was evaluated by measuring the ability of the extracts to lyse blood clots and represented by percent clot lysis (%). The extracts, the highest thrombolytic activity (35.5%) was found for Delonix regia at its 10 mg/ml concentration which was followed by 32.2% thrombolytic activity of *Cassia fistula* at its 10 mg/mL concentration.[36] In which the cytotoxic effect, brine shrimp lethality assay was done. It showed less toxicity of the magnesium oxide nanoparticles. Normally, the brine shrimp cytotoxicity bioassay test can be utilised as an initial assessment for research into the potential for pesticide and anticancer effects.[37] Similarly, Cytotoxicity test was used to measure the degree of toxicity on certain cells. The brine shrimps lethality bioassay was employed here in order to predict its suitability for pharmaceutical applications, magnesium oxide nanoparticles using *Phyllanthus emblica* had the least cytotoxic effects observed in the higher concentrations (80 µl) as only 10% of mortality rate.[38]

In previous research, metal nanoparticles based on the cytotoxic effect using brine shrimp lethality assay. The brine shrimp lethality assay results for AgNPs indicate that, at the concentrations tested, there is no significant cytotoxic effect observed on Day-1. The percentage of live nauplii in all AgNP treatment groups and the control group was 100%. AgNPs, there is a reduction in the percentage of live nauplii compared to the control group, indicating increasing cytotoxicity. This effect becomes more pronounced as the concentration of AgNP increases, with the highest concentration of 80 µg resulting in a noticeable decrease in survival, as reflected by a reduction to 70% live nauplii on Day-2.[39] In the present research work was done by embryonic toxicology using zebrafish embryos, Zebrafish embryo viability was determined to be 50% at 80 µg/ml of *C. bonplandianum* ethanolic extract concentrations, 70% at 40 µg/ml, 80% at 20 µg/ml, 90% at 10 µg/ml, and 100% at 5 µg/ml. the presence of fully developed viable embryos within the egg on the second day, and the emergence of healthy zebrafish on the third day. No somatic malformations, such as a bent tail and bent spine, were observed, and there was no indication of edema.[40] In comparison to the control group, the results exhibited an increasing viability rate at all tested concentrations (100-1000 µg/mL), suggesting minimal liability. At all the concentrations, no abnormalities in development were identified, and the mortality concentration of silver nanoparticles was found to be 750 µg/ml.[41]

LIMITATION

An In-vitro study was assessed on the *E. arvense-mediated* magnesium oxide nanoparticles. In future studies develop characterizations such as TEM, FTIR, XRD, and EDX. The magnesium oxide nanoparticles will develop the gel form, and the gel will be used for the wound healing activity.

CONCLUSION

The current research work was done by using the green synthesis of *E. arvense-mediated* magnesium oxide nanoparticles. The analysis of in-vitro study UV spectroscopy, SEM, antioxidant, anti-inflammatory, antimicrobial, time-kill curve assay, and the toxicology study whereas embryonic toxicology using zebrafish, and brine shrimp lethality assay was also estimated by the *E. arvense* mediated magnesium oxide nanoparticles. In which future studies to develop the characterization and the biomedical applications in in-vivo studies.

CONFLICT OF INTEREST

The authors declare that no conflict of interest would prejudice the impartiality of this scientific work.

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