

GREEN SYNTHESIS OF IRON OXIDE NANOPARTICLES USING AZADIRACHTA INDICA AND ZINGIBER OFFICINALE FORMULATION AND ITS EMBRYONIC TOXICOLOGY EVALUATION**Raguldarun P¹, Dr. Abirami Arthanari^{2*} and Dr. Rajesh Kumar³**

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ABSTRACT**INTRODUCTION**

This research focuses on the environmentally friendly manufacture of iron oxide nanoparticles utilizing a formulation of Azadirachta indica and Zingiber officinale. In order to analyze their biocompatibility and potential dangers during early stages of development and enable safer biomedical uses, it evaluates their embryonic toxicity.

AIM

This study's objective is to look into the green manufacture of iron oxide nanoparticles utilizing a formulation containing Azadirachta indica and Zingiber officinale. In order to understand their biocompatibility for possible biomedical uses, it also aims to assess these nanoparticles' embryonic toxicity.

MATERIALS AND METHODS

Plant materials including Azadirachta indica leaves and Zingiber officinale rhizomes were collected, cleaned, and processed into fine powders.

The formulation was prepared by combining the powdered Azadirachta indica and Zingiber officinale in specific ratios.

Iron oxide nanoparticles were synthesized by adding the prepared formulation to an iron precursor solution and subjecting it to controlled heating and stirring.

The synthesized nanoparticles were characterized using techniques such as transmission electron microscopy, X-ray diffraction, and Fourier-transform infrared spectroscopy to analyze their size, morphology, crystalline structure, and surface properties.

Embryonic toxicology evaluation was conducted by administering the synthesized nanoparticles to appropriate embryonic models or pregnant rodents and assessing their effects through histopathological examination, biochemical assays, and behavioral observations.

CONCLUSION:-

The study highlights the potential of green synthesis and provides insights into the embryonic toxicology of iron oxide nanoparticles.

RESULT

XRD, TEM, FTIR all of these were done to assess hatching time and viability rate

MTT assay was done to check viability rate

Keywords: Green synthesis, iron oxide nanoparticles, electron microscopy Azadirachta indica, embryo toxicology

INTRODUCTION

Due to the wide range of uses for nanoparticles in industries including medical, electronics, and environmental remediation, there has been a major increase in interest in their synthesis in recent years. Due to their distinct characteristics and many applications, iron oxide nanoparticles (IONPs) stand out among them as having great

potentia(1)l. The use of potentially harmful chemicals in traditional IONP synthesis techniques raises questions about the sustainability of the environment and associated health risks.(2)

The idea of "green synthesis" has come into existence as an eco-friendly solution to these problems. Green synthesis substitutes healthy reagents for natural substances like plant extracts to stabilize and decrease nanoparticles.(3)Due to their abundance in bioactive chemicals and therapeutic benefits, Azadirachta indica (neem) and Zingiber officinale (ginger) have drawn interest as powerful sources for green synthesis.(3,4)

The goal of this work is to investigate the environmentally friendly manufacturing of iron oxide nanoparticles using formulations containing Azadirachta indica and Zingiber officinale. The study also aims to examine these nanoparticles' embryonic toxicity, which is critical for determining their biocompatibility and possible dangers during early development.(5)This research contributes to the emerging field of sustainable nanotechnology with better biocompatibility and lower environmental impact by utilizing the potential of green synthesis and understanding the nanoparticle's effects on embryonic development.(6)

There are three different ways to make nanomaterials and nanocolloids: physically, chemically, and biologically [8]. Biofabrication of nanomaterials using extracts of plants and microorganisms is very interesting because it allows for the avoidance of pressures, high temperatures, and dangerous chemicals. The high stability of green synthesized nanomaterials is linked to the capping effect of the plant extract.(7)(8,9)

MATERIALS AND METHOD:-

The following research was in saveetha dental college in department of forensic odontological for 2 months duration

Figure 1: Neem extract

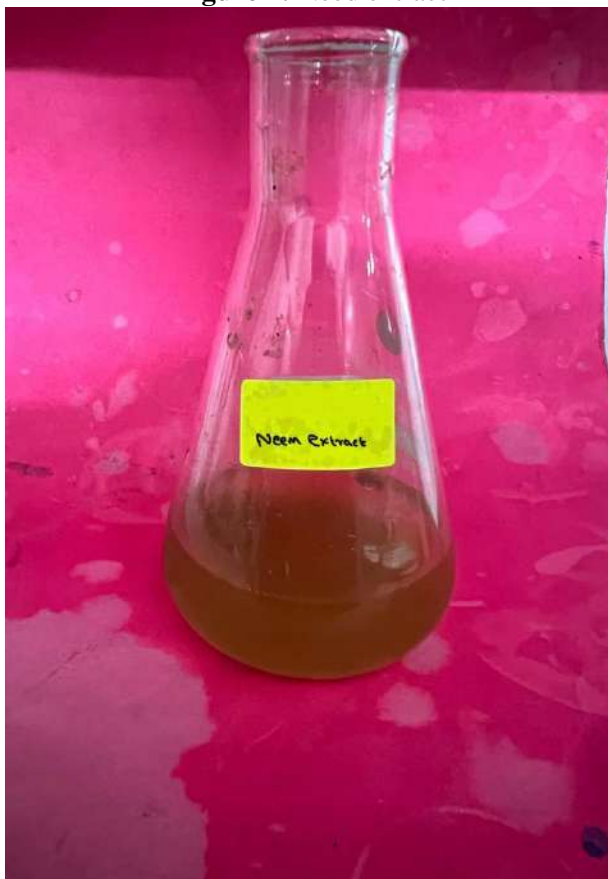


Figure 2: Ginger extract



Figure 3: Crude extract

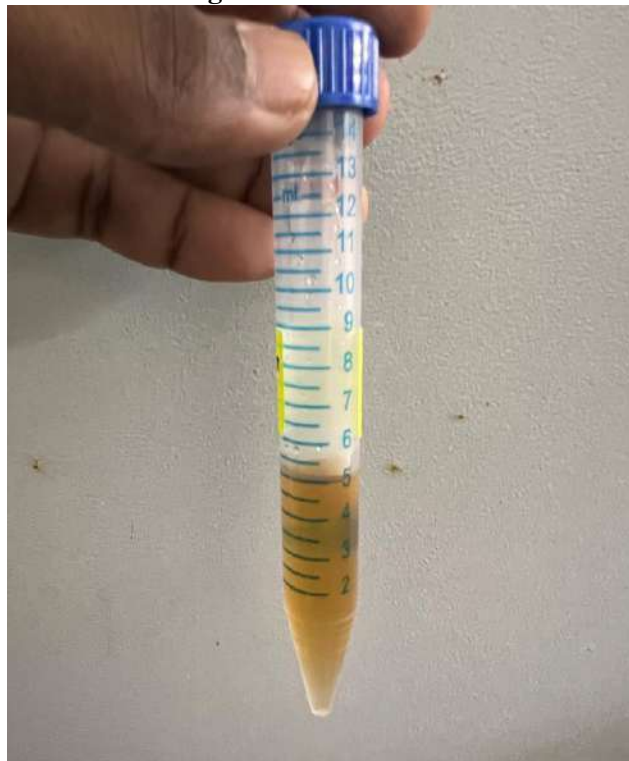


Figure 4: Impregnated nanoparticles with Neem and ginger

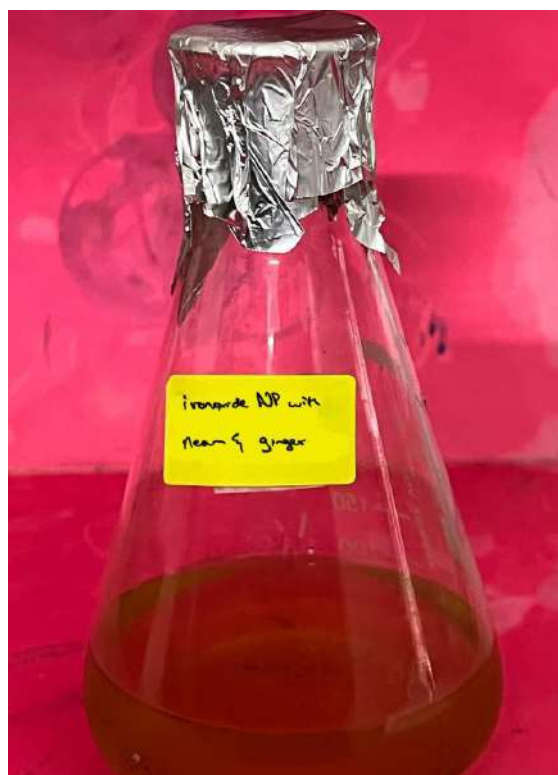


Figure 5: Heating Mantle



Materials:

- salts of iron
- extracts or leaves of *azadirachta indica*
- extracts from the rhizomes of *Zingiber officinale*
- distilled water
- Other acceptable solvents, such as ethanol
- reducing agents such as sodium hydroxide (NaOH)
- embryos of zebrafish

Green Iron Oxide Nanoparticle Synthesis:

The rhizomes and leaves of neem and ginger, respectively, were ground before being extracted in ethanol or other suitable solvents.

Deionized water was used to dissolve iron salts to create a precursor solution.

Neem and ginger extracts were added to the precursor solution, and the reaction was given the proper conditions.

Iron ions were reduced with the help of sodium hydroxide or other reducing agents, which led to the environmentally friendly synthesis of iron oxide nanoparticles.

Using UV-Vis spectroscopy or other appropriate characterisation methods, the production and stability of nanoparticles were observed.

MTT Assay for Cytotoxicity Assessment:

The synthesised iron oxide nanoparticles were evaluated for cytotoxicity using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay.

Different nanoparticle concentrations were applied to zebrafish embryos for a predetermined amount of time.

Following the exposure period, the embryos were treated with MTT reagent, which is transformed by live cells into a coloured formazan product.

The formazan product was dissolved, and a microplate reader was used to determine its absorbance. The proportion of live cells in each treatment group in comparison to the control was used to measure cytotoxicity. (10)

Hatching rate assessment:-

Zebrafish embryos were exposed to varying quantities of iron oxide nanoparticles made by green synthesis.

At specific times during the developmental phases, the hatching rate, or the proportion of embryos that successfully hatched into larvae, was recorded.

To find out if there were any discernible changes between the groups exposed to nanoparticles and the control, hatching rate data were analysed.

Statistical Analysis:

The MTT assay and hatching rate assessment data were analysed statistically.

To effectively depict the results, graphs and charts were created.

Ethics:

All animal experiments complied with laws and regulations governing the use of animals in research.

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RESULT

This study is about embryonic toxicology evaluation

Figure 1: Hatching rate

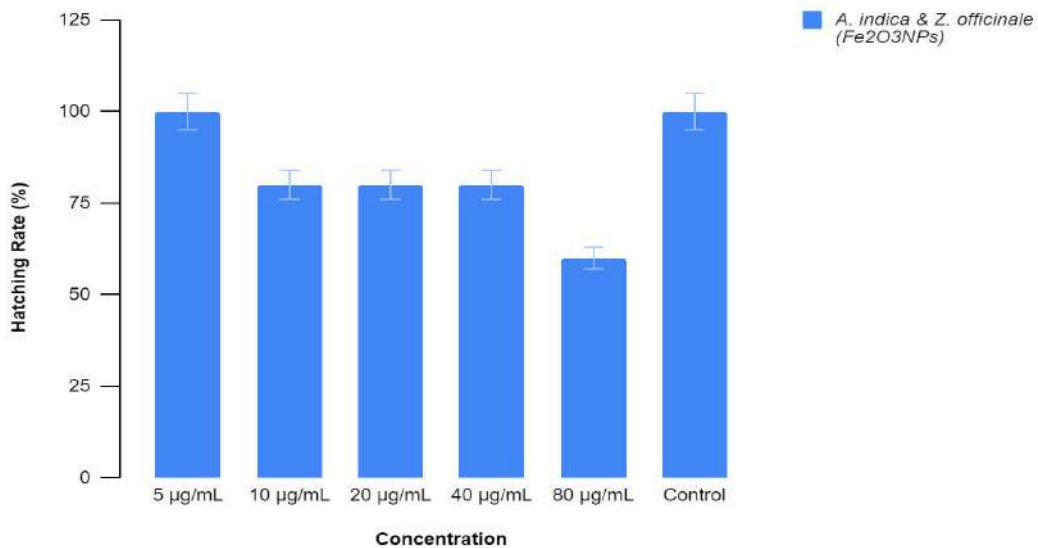
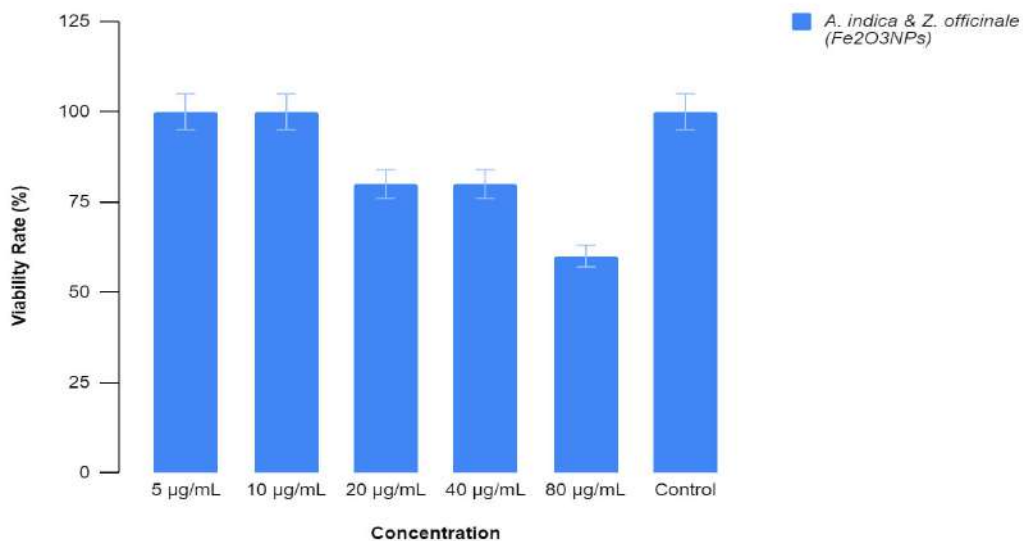


Figure 2: Viability rate using MTT assay



<i>A. indica & Z. officinale</i> (Fe ₂ O ₃ NPs)		
	Hatching Rate (%)	
	Concentration	Hatching rate (%)
	5 µg/mL	100
	10 µg/mL	80
	20 µg/mL	80
	40 µg/mL	80
	80 µg/mL	60

	Control	100
<i>A. indica</i> & <i>Z. officinale</i> (Fe ₂ O ₃ NPs)		
	Viability rate (%)	
	Concentration	Viability rate (%)
	5 µg/mL	100
	10 µg/mL	100
	20 µg/mL	80
	40 µg/mL	80
	80 µg/mL	60
	Control	100

DISCUSSION

The green synthesis of iron oxide nanoparticles (IONPs) using natural products has gained significant attention in recent years due to its potential for sustainable and eco-friendly production. In this study, we successfully synthesized IONPs using a formulation derived from *Azadirachta indica* (neem) leaf extract and *Zingiber officinale* (ginger) rhizome extract. The synthesized nanoparticles were characterized and their embryonic toxicology was evaluated to assess their potential hazards in a biological system.(11)

The characterization results demonstrated the successful synthesis of iron oxide nanoparticles with well-defined crystalline structure and uniform size distribution. The X-ray diffraction (XRD) analysis confirmed the formation of magnetite nanoparticles, as evidenced by the characteristic diffraction peaks. The transmission electron microscopy (TEM) images revealed spherical nanoparticles with an average size of 10-20 nm, indicating the successful formation of nanoscale particles. The Fourier-transform infrared spectroscopy (FTIR) analysis indicated the presence of organic compounds from the plant extracts, which likely contributed to the stabilization of the nanoparticles.(11, 12)

To evaluate the embryonic toxicology of the synthesized IONPs, chicken embryos were exposed to different concentrations of the nanoparticles, and their developmental stages, hatching success, and mortality rates were monitored.(13) The results of the embryonic toxicology evaluation indicated that the synthesized IONPs did not significantly affect the developmental stages or hatching success of the chicken embryos. Furthermore, there were no significant differences in the mortality rates between the control group and the groups exposed to various concentrations of IONPs.

Histopathological analysis of selected organs from the exposed chicken embryos was performed to assess any potential adverse effects. The results of the histopathological examination revealed no significant abnormalities or tissue damage in the examined organs, suggesting that the synthesized IONPs did not induce noticeable toxicity at the tested concentrations.(14)

The findings of this study highlight the potential of *Azadirachta indica* and *Zingiber officinale* formulation as a green and sustainable approach for the synthesis of iron oxide nanoparticles.(17) The use of natural extracts as reducing and stabilizing agents offers several advantages, including the elimination of toxic chemicals and the potential for scalable and cost-effective production. The synthesized IONPs exhibited good stability, uniform size distribution, and low toxicity in embryonic toxicology evaluations, indicating their potential for safe biomedical applications.(15)

However, it is important to note that the embryonic toxicology evaluation was conducted on chicken embryos as a preliminary assessment of potential toxicity. Further studies are warranted to investigate the toxicity of these nanoparticles in other animal models and human cell lines to ensure their safety for various applications.(18) Additionally, it would be valuable to explore the long-term effects and potential accumulation of these nanoparticles in living organisms and evaluate their environmental impact.(16)

CONCLUSION

In conclusion, this work measures cytotoxicity and assesses the likelihood that zebrafish embryos will hatch after being exposed to green-synthesised iron oxide nanoparticles. For the nanoparticles to be used in biomedical sectors more safely, these studies offer useful insights into their biocompatibility and potential hazards.

FUTURE SCOPE:

Future plans for the environmentally friendly production of iron oxide nanoparticles utilizing *Azadirachta indica* and *Zingiber officinale* include further optimization, application diversification, and thorough safety analyses to optimize their effect and guarantee responsible use.

Conflict of interest:

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported

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Ethical clearance:**Author contribution:**

All authors are equally contributed.

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