Superparamagnetic iron oxide nanoparticles as a novel therapeutic for Atherosclerosis plaque reduction

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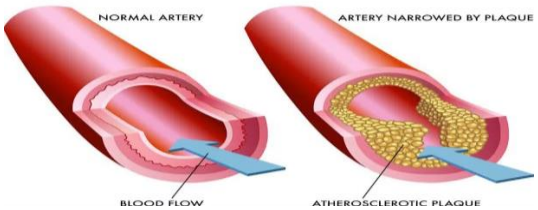
Abstract: Artery blockage, also known as atherosclerosis, is among the deadliest diseases that threatens our world. It is responsible for the death of almost 17.8 million lives annually making it the leading killer worldwide (Di Cesare et al., 2024). It is caused by the buildup of plaques due to the accumulation of a mixture of fats, cholesterol, and other substances on the inner walls of arteries, limiting blood flow due to an unhealthy diet or genetic disorders like the Familial hypercholesterolemia. Traditional treatments for Atherosclerosis involve invasive surgical procedures, such as angioplasty. Although these treatments are effective in removing the plaques blocking the arteries, they are expensive and have significant risks, including infection, damage to blood vessels, and bleeding. Our solution to this issue takes advantage of the paramagnetic properties of the iron oxide nanoparticles (IONPs) for a non-invasive and safer approach to treating artery blockages. Strong magnetic responsiveness is one of the special qualities of iron oxide nanoparticles that allow for accurate drug delivery (Hossaini Nasr & Huang, 2021). These nanoparticles will be coated with polymers like polyethylene glycol (PEG) and chitosan and loaded with enzymes like lipase that can degrading lipids like triglycerides and converting the LDL cholesterol to HDL cholesterol in the arteries. After the preparation of the treatment material, an in vivo study was performed to check the efficiency of the nanoparticles. The results of the in vivo study showed that the treatment managed to reduce 50% of the triglycerides in the bloodstream and 23% of the LDL cholesterol. These results show that using IONPS as a novel therapeutic for Atherosclerosis plaque reduction can effectively target and degrade plaques without the need for surgery, significantly improving the safety and efficiency of the treatment.

Keywords: Atherosclerosis, plaques, iron oxide nanoparticles, chitosan, polyethylene glycol (PEG), HDL and LDL cholesterol, triglycerides and lipase.

1. Introduction

Cardiovascular diseases are the leading cause of death all over the world. Among them, atherosclerosis in particular is regarded as one of the most deadly conditions. Atherosclerosis is characterized by the narrowing and hardening of the blood vessels due to the precipitation of the lipids and fats on the walls of the arties. This plaque is formed from the deposition of low-density lipoproteins (LDL), cholesterol, and fats obstructing normal blood flow as shown in fig[1]. Atherosclerosis accounts for approximately 17.8 million deaths every year, solidifying its position as the leading global cause of mortality (Di Cesare et al., 2024). There are many causes for the atherosclerosis, including unhealthy lifestyle, poor dietary choices, smoking, hypertension, and genetic disorders such as Fa-

milial Hypercholesterolemia (FH) which is a condition where the body is unable to remove the LDL cholesterol from the bloodstream. If left untreated, atherosclerosis can lead to severe problems, including myocardial infarction, stroke, and peripheral artery disease. Prior treatments for Atherosclerosis include pharmacological therapies, and invasive surgical operations such as angioplasty, stenting, and coronary artery bypass grafting (CABG). While these treatments are effective in restoring blood flow, they have some disadvantages that insists finding better alternatives. These disadvantage include high costs, and the risk for vascular injury, bleeding, infection, and re-narrowing of arteries. There is therefore an urgent need for less invasive, safer, and more efficient therapeutic alternatives. This research offers a solution that uses the superparamagnetic properties of the Iron Oxide Nanoparticles (SPIONs) to treat atherosclerosis in a targeted, non-invasive manner. SPIONs exhibit strong paramagnetic responsiveness, enabling them to be guided by external magnetic fields to specific sites within the body. This property makes them highly suitable for drug delivery, reducing errors and improving the efficiency of the treatment. In our solution, iron oxide nanoparticles are coated with biocompatible polymers such as Polyethylene Glycol (PEG) and chitosan, to improve their stability, circulation time, and biocompatibility. These nanoparticles are then loaded with enzymes, such as lipase, which have the ability to degrade lipids and aid in the conversion of harmful LDL cholesterol into HDL cholesterol within the arterial plaque. When the nanoparticles reach the site of plaque accumulation, the loaded enzymes can initiate degradation of plaques, effectively reducing the plaque without the need for surgery. By integrating nanotechnology with cardiovascular medicine, this study explores a transformative strategy that has the potential to revolutionize atherosclerosis treatment. The goal is to develop a non-invasive, efficient, and cost-effective treatment for arterial plaque reduction that minimizes side effects.

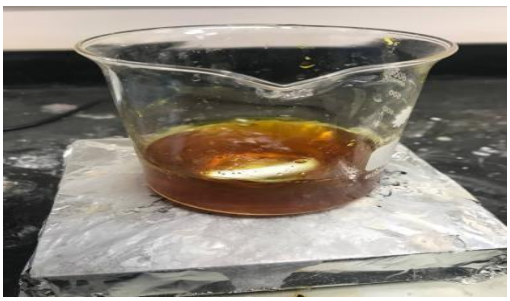


Fig[1] comparison between the healthy and and the blocked artery

2. Materials and Methods

1. Synthesis of Superparamagnetic Iron Oxide Nanoparticles (SPIONs)

Co-precipitation method was used to prepare SPIONs suitable for biomedical applications. A mixture of ferric and ferrous ions was be used. Ferrous sulfate ($FeSO_4$) and ferric chloride ($FeCl_3$) were dissolved in water in a 1:2 molar ratio as shown in fig[3]. While stirring continuously at 60°C, 25% ammonium hydroxide (NH_4OH) was added dropwise to the solution to precipitate iron oxide nanoparticles. The reaction mixture was maintained under stirring for one and a half hours until a black precipitate formed, indicating the formation of the iron oxide nanoparticles (Fe_3O_4) as shown in fig[2]. The precipitate was separated using a magnet, repeatedly washed with ethanol and deionized water to remove unreacted ions, and then dried in a vacuum oven at 65°C for 24 hours. The process


Fig[2] The coperciptation process of the Ferrous sulfate and ferric chloride



Fig[3] The solutions of the ferrous sulfate and ferric chloride

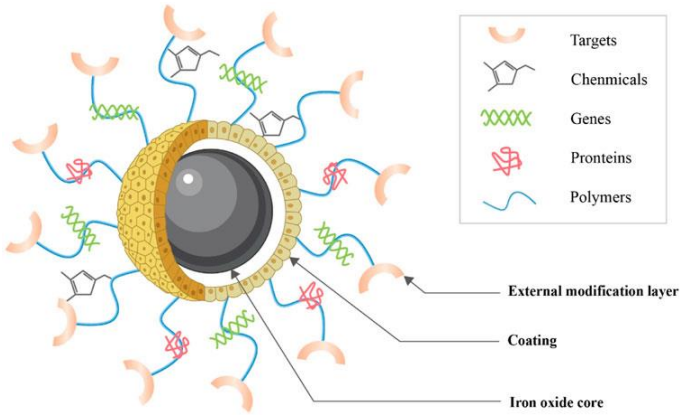
of the characterization of the iron oxide nanoparticles was made to determine the size of the nanoparticles. They were observed by using a Zeta potential device.

2- Coating the nanoparticles with Polymers

To improve the stability and biocompatibility of SPIONs and carry the enzymes used for lipid degradation as shown in fig[4], they were coated with Polyethylene Glycol (PEG) and chitosan.

PEGylating: Dried iron oxide nanoparticles were dispersed in phosphate-buffered saline (PBS), and PEG-1000 was added. The mixture was stirred overnight at room temperature. PEG chains form a hydrophilic corona around the particles, preventing aggregation and prolonging circulation time in the bloodstream.

Chitosan Coating: Separately, low molecular weight chitosan was dissolved in acetic acid (1% v/v) and added to the PEG-coated SPIONs. This dual coating enhances biocompatibility, and drug-loading capacity. The coated nanoparticles were washed and stored at 4°C for subsequent use.

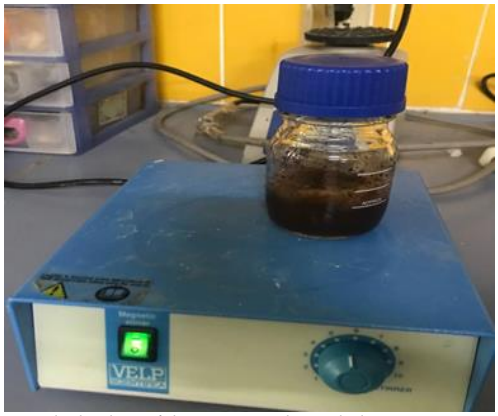


Fig[4] A diagram showing the coatings of the iron oxide nanoparticles

3- Nanoparticles Loading with Enzyme

To make the iron oxide nanoparticles capable of degrading fats like the triglycerides in the arteries, they were loaded with lipase enzyme. Lipase was chosen due to its biological activity against cholesterol and triglyceride accumulation, as it is capable of breaking down lipid components in atherosclerotic plaques. A solution of lipase was placed in the lyophilizer for four days to get the lipase powder and ensure its effectiveness as shown in fig[6].

Drug loading was performed via electrostatic adsorption. A solution of lipase powder in PBS was prepared then the nanoparticles were soaked in this solution where the ratio between the nanoparticles and the lipase powder 3:1. SPIONs were incubated with lipase in PBS (pH 7.4) under gentle agitation for 5 hours as shown in fig[5].



Fig[5] The loading of the nanoparticles with the Lipase enzyme



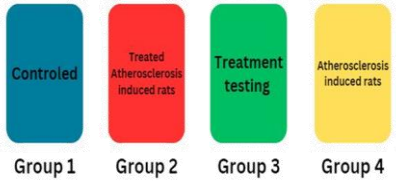
Fig[6] The Lipase enzyme after the lyophilization

4- In Vivo experimentation for testing of the treatment materials

The efficiency of the treatment and its safety was evaluated by in vivo experimentation. 4 groups of rats were prepared where each group contains 4 female rats as shown in fig[8]. To test the treatment materials, Atherosclerosis was induced in the rats. Atherosclerosis was induced in the rats by feeding the rats on a high cholesterol diet and sodium cholate. Two groups of Rats were injected with 0.3 ml of triton x-100 with concentration of 400 mg/kg of rat to accelerate the accumulation of lipids in the arteries and the development of atherosclerosis (every 2 days for two weeks). The controlled group represent the healthy normal rats without any injection.

- Heavy cholesterol diet:

Diet Composition (per 100g food):
2% cholesterol (Egg yolk powder)
0.5% cholic acid (Enhances cholesterol absorption)
20% lard/butter



Fig[7] A diagram representing the groups of rats

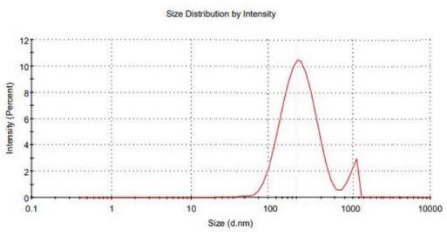
This process continued for two weeks were one group of rats was feeding on the high cholesterol diet only while another group was injected with the treatment material along the cholesterol with concentration 15 mg/ml as shown in fig[9]. To test the safety of the materials used one group of rats was injected with the treatment material only. Blood samples were extracted from the rats. Then CBC, lipid profile, liver function and Kidney function tests was made to measure the efficiency of the treatment and the effect of the treatment materials on the blood.


Fig[8] the four groups of rats.


Fig[9] The nanoparticles used for the treatment of the rats

3. Results

The process of co-precipitation of ferric and ferrous ions formed 9.4 grams of iron oxide nanoparticles with a diameter of typically in the range of 100-300 nanometers according to the zeta potential device measurement as shown in fig[10]. After the coating of the iron oxide nanoparticles the iron oxide was able to stick to fats and degrade them.

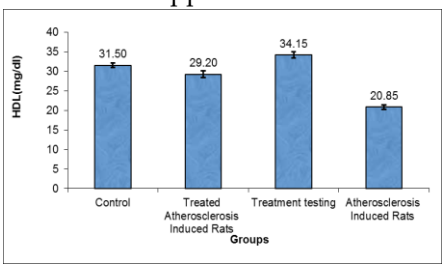


Fig[10] The Zeta potential reading of the nanoparticles diameter

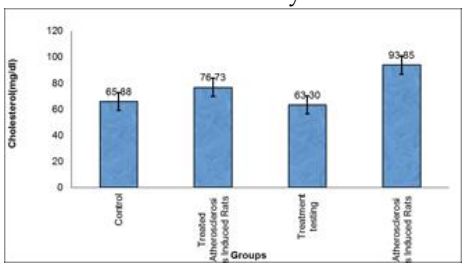
To evaluate the efficiency and biosafety of the synthesized nanoparticles, an in vivo study was conducted. The tests of the treatment was made on 4 groups of rats each group contain 4 rats where CBC, lipid profile, kidney and liver function tests were made after the treatment.

1- Lipid profile results.

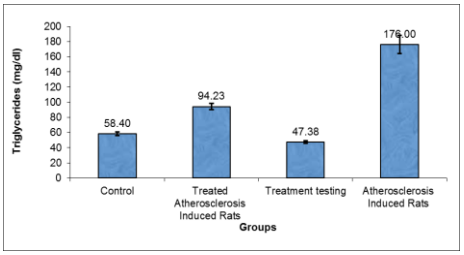
The results revealed that the enzyme-loaded SPIONs has successfully reduced the lipid levels in the bloodstream and the plaques. As it managed to decrease the triglycerides in the blood to about 50% as shown in fig[13].It reduced 23% of the LDL cholesterol in the blood compared to the rats with the atherosclerosis only as shown in fig[14]. These results support the effectiveness of the treatment and its reliability.



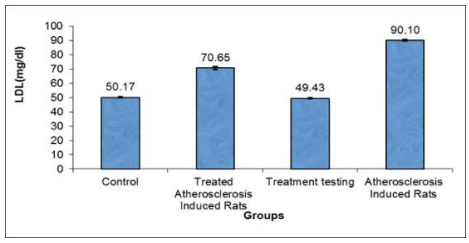
Fig[11] The HDL test results of the



Fig[12] The cholesterol test results of the



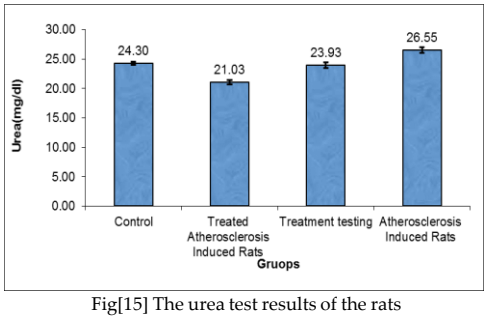
Fig[13] The Triglyceriders test results of the rats



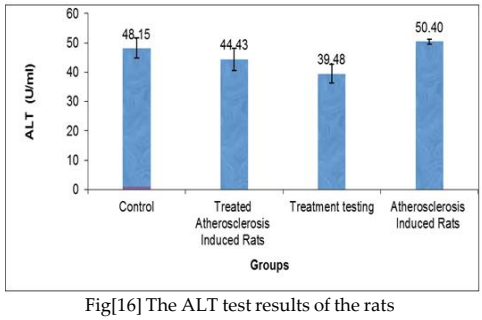
Fig[14] The LDL test results of the rats

2- the liver and kidney function tests

The analysis of liver and kidney function markers — including urea and alanine aminotransferase (ALT) — showed no significant differences from normal levels which indicates that our material doesn't have an toxic effect on the liver or kidney as shown in fig[15] and fig[16].



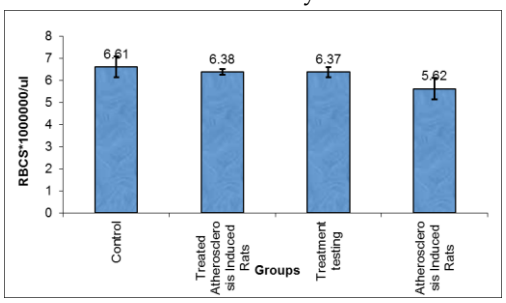
Fig[15] The urea test results of the rats



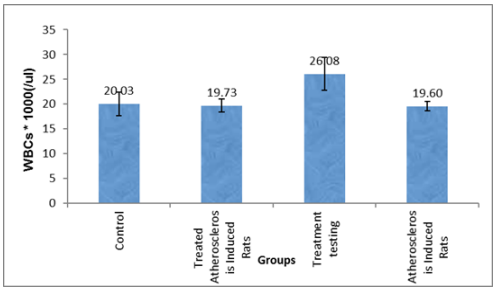
Fig[16] The ALT test results of the rats

3- the CBC tests

Similarly, the CBC test results demonstrated stable red and white blood cell counts, further showing that our material doesn't have an effect on the blood components ensuring the safety of our materials as shown in fig[17] and fig[18].



Fig[17] The RBCs test results of the rats



Fig[18] The WBCs test results of the rats

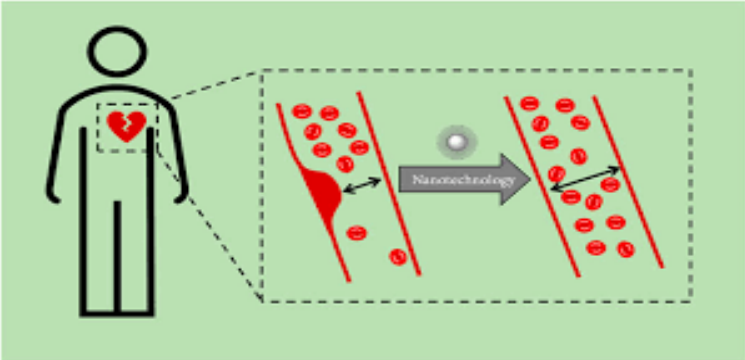
Overall, these results confirm that the coated iron oxide nanoparticles not only exhibit potent lipid-degrading properties but also maintain a favorable biosafety profile, making them a promising candidate for non-invasive atherosclerosis treatment.

4. Discussion

The results of the study demonstrate the effectiveness and biocompatibility of iron oxide nanoparticles (IONPs) for the non-invasive treatment of atherosclerosis disease. They ensure that the nanoparticles significantly reduce lipid and fats concentrations in the blood without causing any harm or inducing toxicity in major organs or altering blood composition.

The IONPs prepared through co-precipitation of ferric and ferrous ions, yielded 9.4 grams of nanoparticles within the range of 100–300 nanometers. This size is suitable for biomedical applications and effective interaction with target sites such as arteries' plaques.

One of the most notable findings is the nanoparticles' ability to reduce blood triglycerides by approximately 50% and LDL cholesterol by 23%. These reductions show the lipid-degrading potential of the enzyme-loaded nanoparticles and suggest their promising role in managing cardiovascular risk factors. Additionally, this non-invasive approach offers a safer alternative to conventional treatments like angioplasty, which may be invasive or produce systemic side effects.



Fig[19] A diagram showing the usage of nanotechnology to treat Atherosclerosis

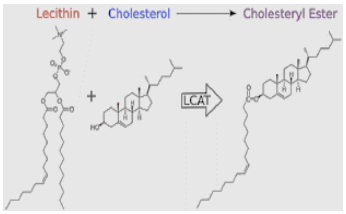
The study also confirmed the biosafety of the developed nanoparticles. Liver and kidney function tests remained within normal physiological ranges, indicating no toxicity following treatment. Complete blood count (CBC) results, including red and white blood cell counts, further confirmed that the nanoparticles did not negatively impact hematological health. This suggests that the treatment can be administered systemically without compromising essential bodily functions, which is a critical advantage for this treatment.

Limitations and Future Directions

However the solution managed to decrease the plaques size inside the arteries, there are several limitations that face it. First, the sample size used in the in vivo experiment was relatively small, which may limit the statistical power and generalizability of the findings. A larger sample will be necessary to confirm these results and account for individual variability. Secondly, the controlling of the nanoparticles inside the body needs accurate devises that can make larger magnetic fields to control the nanoparticles more efficiently inside the arteries. Thirdly, while the short-term results are promising, long-term safety need to be studied to fully assess the reliability of this treatment. Additionally, scaling up the nanoparticle synthesis and ensuring batch-to-batch consistency are essential challenges before human trials can be considered.

Future Work

- Green synthesis of iron oxide nanoparticles approaches to minimize environmental impact and cost.
- Experiment with different coating materials to enhance stability, efficiency and biocompatibility.
- Create nanoparticles that combine imaging with therapy.
- Improve external magnet systems for better precision in targeting nanoparticles.
- Lipase enzyme degrades the triglycerides in the blood however to degrade other types of fats that cause the build-up of plaques inside the arteries, we should introduce more enzymes that can increase the efficiency and the speed of the treatment like the LCAT enzyme. The LCAT enzyme can convert the bad LDL cholesterol to HDL cholesterol breaking down the plaques inside the arteries and hence increasing the efficiency of the treatment as shown in fig[20].



Fig[20] A diagram showing the function of the LCAT enzyme

This research provides evidence that enzyme-loaded iron oxide nanoparticles offer a safe, effective, and non-invasive method to treat atherosclerotic plaque buildup. Their ability to target and degrade lipids without harming vital organs or affecting blood represents a significant advancement in the development of next-generation Nano therapeutics for cardiovascular disease.

5. Conclusions

Using iron oxide nanoparticles (IONPs) coated with biocompatible materials, such as PEG, chitosan and enzymes, will provide a safer, non- invasive alternative for treating artery blockages according to the results of the blood tests. As the treatment showed 50% reduction in the triglycerides in the blood and a 23% reduction in the LDL cholesterol. Unlike traditional interventions such as angioplasty or bypass surgery, which are highly invasive, expensive, and associated with significant risks, our nanoparticle-based therapy will leverage external magnetic fields to precisely guide and localize IONPs at the site of arterial plaque accumulation. Upon reaching the target, these nanoparticles will bind to and enzymatically degrade the arteries' plaques, restoring normal blood flow while minimizing complications like blood clots and restenosis. The Number of deaths due to atherosclerosis and cardiovascular disorders will decrease significantly. The cure will be more accessible for poor people. So our solution will help achieve SDGs 1 and 3, which are improving healthcare and fighting poverty.

6. Acknowledgment

We would like to express our gratitude to everyone who has helped us through this project. We would like to thank the researchers and organizations who made previous efforts in Nanotechnology and its application in cardiovascular diseases, which was a foundation for our work. We acknowledge the assistance of the institutions which supervised our research and give us the access to the materials needed for the study including the City of Scientific Research and Technological Applications (SRTA-City) and the Alexandria University of Sciences, enabling us to strengthen the scientific foundation of our work. This project would have failed if the efforts of everyone involved had not been collective, and we are truly grateful to have been afforded the opportunity to present our contribution to a safer approach for the treatment of cardiovascular diseases.

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