Coated Suture To Enhance Self-Healing And Hemocompatibility For Wound Healing Application

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Supervisor: Dr. Muhammad Shoaib Butt

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THESIS ACCEPTANCE CERTIFICATE

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7.	MSE-854	Characterization Of Materials	Elective	3.0	B+
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9.	NSE-961	Surface Coatings and Thin Films	Elective	3.0	B
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My beloved **Parents**

For their believe, trust in me to pursue this degree and achieving this milestone

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All gratitude and praise are to **Allah Almighty**, the Most Gracious and the Most Merciful.He is the entire source of knowledge and wisdom to mankind, who gave us health, thoughts, and capacitated us to achieve this goal. After Almighty Allah, praises are to **Prophet Muhammad (S. A.W.),** the most Perfect and Exalted, and an everlasting sourceof Guidance and Knowledge for humanity.

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Syed Ahmad Hassan

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Abstract

The increasing prevalence of surgical site infections and the demand for advanced wound care solutions have driven the development of innovative biomaterials. This study investigates the quantification of Indica /Ch composite layered silk braided stitches for enhancing tissue repairing in wounds and reducing infections. Sutures were coated with Azadirachta Indica extract in chitosan, and their antibacterial, hemocompatibility, and wound healing properties were evaluated. The coated stitches demonstrated antibacterial activity against Aureus and E. coli, with the satisfactory zone of inhibition observed. Hemolysis assays revealed minimal hemolytic activity (<2%), confirming the safety of the coated sutures for clinical use. In vivo studies using Sprague Dawley rats showed that wounds closed with \ Indica/Ch-coated stitches exhibited reduced inflammation, faster healing, and complete recovery within Two weeks. In contrast, uncoated sutures resulted in persistent inflammation and delayed healing. Scanning Electron Microscopy (SEM) analysis confirmed the uniformity and smoothness of the coating, further supporting its structural integrity. These findings highlight the potential of Azadirachta Indica/chitosan-coated sutures as a safe, effective, and biocompatible solution for surgical wound care. By combining the antimicrobial and antioxidant properties of Azadirachta Indica with the bio adhesive nature of chitosan, this study demonstrates a promising advancement in the development of bioactive suture materials. This research underscores the importance of integrating traditional medicinal knowledge with modern biomedical technologies to address critical healthcare challenges and improve patient outcomes.

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Keywords: Indica, Coated Stitches, Infections, Antibacterial Effect, In vivo tissue Repair.

CHAPTER 1: INTRODUCTION

1.1 Surgical site infections (SSI)

Surgical site infections (SSIs) impose a substantial financial burden on healthcare systems, with studies estimating that SSI management consumes around 0.5% of annual hospital budgets [1]. A critical factor contributing to SSIs is the absence of antimicrobial properties in most commercially available sutures. This deficiency enables pathogens from the skin's natural flora to infiltrate surgical wounds. Once these microorganisms adhere to suture surfaces, they rapidly multiply and form biofilms [2]. Research has identified key bacterial pathogens responsible for SSIs, including Escherichia coli, and Staphylococcus aureus. To address this challenge, antimicrobial-coated sutures have emerged as a promising solution [3]. Chitosan, a biopolymer derived from chitin through alkaline deacetylation, consists of β-(1-4)-2-acetamido-D-glucose and β-(1-4)-2-amino-D-glucose units, with the latter comprising over 60% of its structure [5]. Its antimicrobial efficacy as well as biodegradability and, biocompatibility, and non-toxic, making it an ideal candidate for biomedical applications [6]. Herbal extracts, such as aloe vera, have also been explored for their antimicrobial properties and have been successfully incorporated into suture coatings alongside chitosan [3]. Another promising natural resource is Azadirachta indica, a fast-growing tree belonging to the Meliaceae family, native to regions of Southeast Asia. For centuries, Indica has been utilized in traditional medicine because of its healing properties such as antibacterial nature [7, 8, 9]. A combination of Azadirachta indica ethanolic extract and chitosan presents a viable solution. In this study, silk braided sutures are dip-coated with a chitosan-Azadirachta indica mixture and subjected to comprehensive characterization, including morphological, in vitro, and in vivo analyses. The goal of this research is to advance the development of innovative suture materials that enhance surgical outcomes and minimize the risk of SSIs.

1.1.1 Significance:

Traditional therapies, which employ naturally derived substances such as plant extracts, honey, and larvae, have emerged as viable alternatives to conventional wound care products. These natural remedies offer innovative solutions for managing skin conditions, improving healthcare accessibility, and overcoming the limitations of modern treatments, including high costs, lengthy production processes, and increasing bacterial resistance [10]. This study explores recent advancements in traditional wound healing therapies, emphasizing their therapeutic benefits, mechanisms of action, and clinical trials involving widely used natural compounds. Recent scientific studies have increasingly demonstrated the beneficial effects of traditional therapies in treating a wide range of skin lesions. To widespread the adoption of natural compounds for treatment purposes, thorough study and testing is essential. This includes validating the safety of these products, conducting thorough investigations into potential side effects, and designing well-structured, standardized clinical trials. Furthermore, the implementation of rigorous manufacturing practices and the establishment of comprehensive regulatory frameworks are critical.

1.1.2 Scope:

The skin serves as a barrier between the body's systems and outside. To enhance skin’s healing process, reduce scarring, numerous compounds that have therapeutic nature and other approaches are being studied. These interventions can be broadly categorized into two groups: conventional methods and advanced therapies. Conventional treatments, employed for generations, encompass herbal remedies, animal-based substances, the use of silver-based treatments, and traditional dressings.

Other advanced techniques incorporate cutting-edge techniques such as skin grafts, contemporary wound dressings, bioengineered skin substitutes, and treatments utilizing cells and growth factors. The advancement of these therapeutic options seeks to tackle the diverse challenges associated with wound management. While traditional therapies have been utilized for centuries and offer advantages such as affordability and accessibility, they are often subject to variability influenced by geographic and seasonal conditions, as well as inconsistencies between batches. In contrast, modern therapies tend to provide greater reliability and scientific validation but may come with higher costs and limited availability in resource-constrained regions. The ongoing refinement of both traditional and contemporary wound care strategies highlights the significance of adopting a

comprehensive approach to skin repair. By combining the strengths of time-tested practices with the innovations of modern science, it becomes possible to create more effective, dependable, and accessible solutions for wound management. This integrative strategy not only improves the healing trajectory but also addresses the economic and logistical barriers associated with wound care, ultimately enhancing patient outcomes across varied settings.

1.2 Relevance

1.2.1 Translational relevance

The rising interest for traditional therapies in skin wound care has led to a significant surge in scientific research aimed at evaluating their clinical efficacy, safety, and potential side effects. This expanding field of study has driven the creation of innovative products and clinical practices, which are increasingly being adopted by healthcare professionals to treat various skin injuries. Despite these advancements, more work is needed to obtain regulatory approval for traditional therapies and natural healing compounds, enabling their integration into national healthcare systems.

This growing interest is not just limited to isolated studies; it has fostered a collaborative effort among researchers, clinicians, and policymakers. These collaborations aim to validate the effectiveness of traditional remedies through rigorous scientific methods, ensuring they meet the high standards required for modern medical practices. For instance, herbal compounds that have been used for centuries in traditional medicine are now being subjected to clinical trials to determine their therapeutic potential and safety profiles [18]. Similarly, animal-derived products and other natural substances are being meticulously studied to understand their mechanisms of action and possible side effects.

Moreover, the increasing acceptance of traditional therapies in mainstream medicine has led to the development of hybrid treatment approaches that combine the best aspects of both traditional and modern therapies. These integrative methods often enhance healing outcomes by leveraging the natural healing properties of traditional remedies while also benefiting from the precision and efficiency of modern medical technologies. For example, a traditional herbal ointment might be

used alongside bioengineered skin substitutes, providing a dual approach that maximizes healing and minimizes scarring.

Regulatory challenges remain a significant hurdle in the broader adoption of traditional therapies. Many natural healing compounds have not yet received the necessary approvals from health authorities, which limits their availability and use in formal healthcare settings. To address this, ongoing efforts are focused on standardizing the production and quality control of these compounds, as well as conducting comprehensive clinical trials to provide robust evidence of their efficacy and safety. Achieving regulatory approval would not only validate these therapies but also ensure that they can be safely and effectively used within national healthcare systems.

The renewed interest in traditional therapies for skin wound care is driving significant advancements in both research and clinical practice. By bridging the gap between traditional wisdom and modern science, there is great potential to enhance patient outcomes and expand the arsenal of effective treatments available to clinicians. However, continued efforts to secure regulatory approval and integrate these therapies into mainstream healthcare are essential for their widespread adoption and sustained impact.

1.2.2 Clinical relevance

Traditional healing agents occupy a pivotal position in wound care due to their proven clinical efficacy, simplicity of use, and cost-effectiveness. These. therapies offer an affordable and accessible alternative for treating a variety of challenging wounds, including ulcers, burns, and infected lesions [20, 21]. The therapeutic effects of traditional remedies are broad and multifaceted, promoting healing and enhancing the quality of newly formed skin. Their ability to stimulate natural healing processes makes them particularly valuable in healthcare settings where resources are limited.

Moreover, the versatility of traditional therapies allows them to be integrated with modern. clinical. practices, advanced biomaterials, and pharmacological agents. This integration facilitates the development of-innovative treatment protocols that address critical medical needs, such as reducing bacterial resistance and shortening the healing duration. By harnessing the strengths of

both traditional and contemporary approaches, healthcare providers can create synergistic treatments that optimize patient outcomes.

The potential for combining traditional therapies with modern treatments is especially promising in the context of surgical site infections (SSIs) and skin regeneration. For instance, the antimicrobial properties of natural compounds like Neem and chitosan can be leveraged through modern biomedical techniques to develop antimicrobial sutures that are both effective and biocompatible. These sutures can significantly reduce the risk of post-operative infections, thus addressing a major clinical concern. Additionally, the use of such biocompatible and non-hazardous materials aligns with current trends in sustainable and safe healthcare practices, providing an economically viable solution that benefits both patients and healthcare systems.

The integration of traditional healing agents with modern medical technologies not only holds the potential to revolutionize wound care but also to address broader clinical and economic challenges. By combining the time-tested benefits of natural compounds with the precision and efficacy of modern biomedical innovations, it is possible to create advanced therapeutic solutions that are both effective and accessible. This holistic approach can enhance the standard of care, particularly in the management of SSIs, and support the ongoing efforts to improve skin regeneration techniques. Through continued research and development, the synergistic use of traditional and modern therapies promises to deliver significant advancements in wound care, ultimately leading to better health. outcomes and improved quality of life for patients [19].

Current healing therapies provide an economical and accessible option for addressing a wide range of complex wounds, including ulcers, burns, and infected lesions [10, 11]. The therapeutic benefits of traditional remedies are diverse and comprehensive, promoting tissue repair and improving the quality of regenerated skin. Their capacity to activate natural healing mechanisms makes them especially valuable in healthcare environments with constrained resources. The potential for merging traditional therapies with modern medical techniques is particularly promising in the context of surgical site infections (SSIs) and skin regeneration. For example, the antimicrobial properties of natural substances like Azadirachta indica and chitosan can be harnessed through cutting-edge biomedical methods to develop antimicrobial sutures that are both highly effective and biocompatible. These sutures can significantly lower the risk of post-surgical infections,

addressing a critical concern in clinical practice offering an economically feasible option that benefits both patients and healthcare systems. The fusion of traditional healing agents with modern medical technologies not only has the potential to transform wound care but also to tackle broader clinical and economic challenges. This integrated approach can elevate the standard of care, particularly in managing SSIs, and support ongoing efforts to refine skin regeneration techniques. Through sustained research and development, modified modern therapies holds the promise of delivering significant advancements in wound care, ultimately leading to improved health outcomes and enhanced quality of life for patients [12]

CHAPTER 2: LITERATURE REVIEW

Surgical sutures are a fundamental component in the process of wound closure, playing a vital role in ensuring the integrity and stability of the surgical site. The choice of suture material and technique can significantly impact the healing process, patient comfort, and overall clinical outcomes. With the advent of a variety of suture types and materials, it is imperative to understand the characteristics, advantages, and limitations of each to optimize wound closure. This research aims to provide a comprehensive overview of the different types of surgical sutures and their applications across various wound types. By examining the efficacy and suitability of each suture material for specific wound conditions, this study seeks to contribute to the development of evidence-based guidelines for the selection and use of sutures in diverse clinical settings. Ultimately, the goal is to enhance patient outcomes and improve the quality of wound care through informed decision-making regarding suture materials and techniques.

2.1 Overview of the wound-healing process

Wound healing is a complex biological process that involves multiple tissues working together to repair damage and regenerate lost cells. The process begins with hemostasis, which occurs immediately after an injury and focuses on stopping bleeding and preventing the infiltration of harmful microorganisms into the body as shown in Figure 2.1 below. During hemostasis, blood vessels constrict, platelets aggregate, and fibrin clot forms, eventually leading to the creation of a protective scab. This scab acts as a shield for the damaged tissue while also providing structural support.

Growth factors, for example TGF-β, PDGF, (FGF), and EGF are vital in facilitating cellular communication and interaction with the extracellular matrix (ECM). They stimulate cell recruitment, proliferation, morphogenesis, and differentiation. Following hemostasis, the wound repairing requires such as neutrophils, macrophages, and lymphocytes, into the wound site to promote and facilitate localized tissue repair.

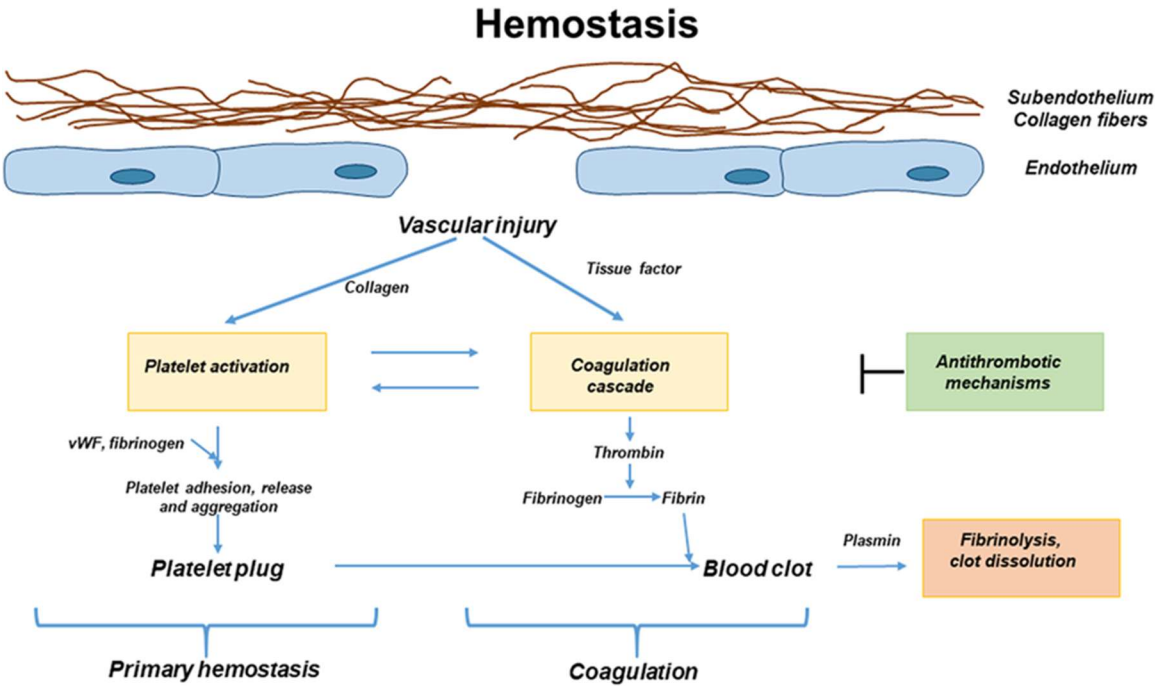

Figure 2.0.1: Overview of Hemostasis [12]

These cells perform multiple functions, such as promoting inflammation, preventing microbial invasion, and eliminating pathogens. Epithelial cells replace dead cells, while fibroblasts produce key components of the extracellular matrix, fibronectin, hyaluronan, glycosaminoglycans, and proteoglycans. These substances provide the structural integrity necessary for skin repair. In the final stage as shown below in Figure 2.2, known as the maturation or remodeling phase, the newly formed tissue undergoes continuous reorganization to closely resemble the composition and properties of healthy skin. The primary goal of this phase is to restore the injured skin to its original state, ideally without leaving scars. This intricate process underscores the importance of selecting appropriate suture materials and techniques to support each phase of healing, ensuring optimal recovery and restoration of tissue integrity.

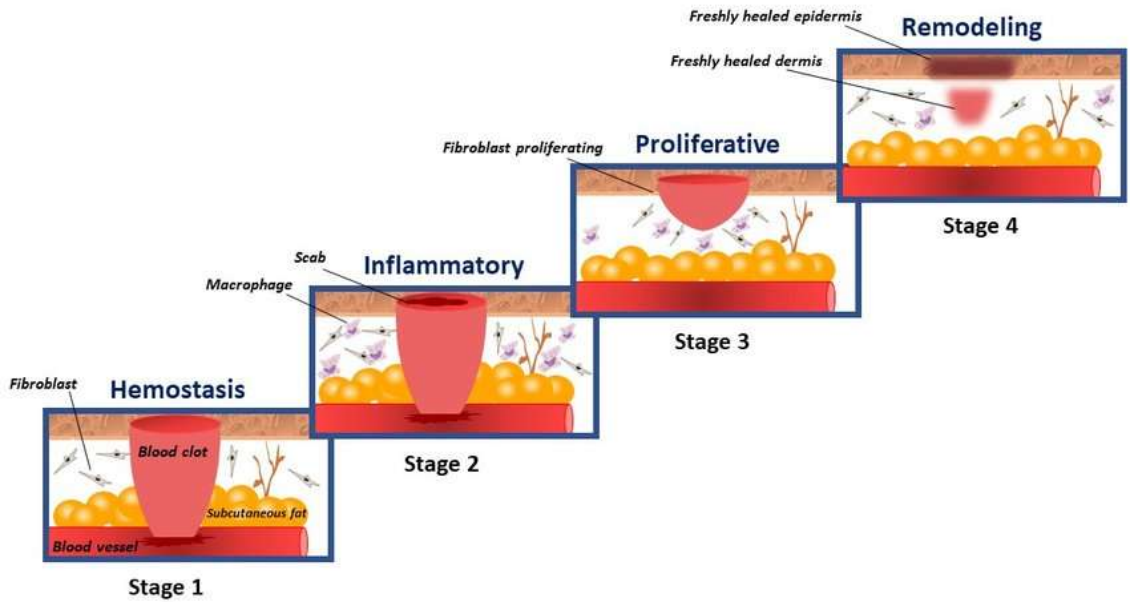

Figure 2.0.2: Stages of wound healing [14]

2.1.1 Conventional Methods for Wound Healing

After hemostasis and inflammation are managed, the healing process progresses with the migration of epithelial cells and fibroblasts to the wound site. These cells facilitate the growth of new capillaries, synthesize collagen, and contribute to the formation of new tissue. Epithelial cells replace dead cells, while fibroblasts generate essential components of the extracellular matrix (ECM). Released elements are critical for maintaining skin's structural integrity. The process of neovascularization, where new blood vessels and lymphatic structures sprout from existing ones, results in the formation of granulation tissue. In the maturation phase, the newly formed tissue undergoes constant reorganization to approximate the composition and properties of normal skin. The ultimate objective of wound healing is to regenerate the damaged skin as closely as possible to its original condition, ideally without scarring.

2.1.2 Revitalizing Traditional Medicine for Chronic Wound Care

The skin’s natural ability to heal can be severely compromised under certain conditions, such as extensive skin damage, severe burns, chronic wounds, non-healing ulcers, and diabetes. When the healing process is disrupted, wounds may become chronic. Chronic wounds are among the most challenging and painful conditions to treat, imposing significant medical and economic burdens

on patients and healthcare systems globally. Chronic wounds frequently require extended hospital stays and the use of costly advanced wound care products, such as bioengineered skin substitutes and medicated dressings, thereby escalating healthcare expenses. Despite numerous clinical interventions aimed at improving healing outcomes, effective treatments for chronic wounds remain limited.

To address this critical need, substantial research has been directed toward exploring traditional therapies as viable alternatives for managing chronic wounds. Traditional medicine practices and natural compounds have been utilized to create optimal healing environments and prevent wound healing failure. These therapies are valued for their therapeutic benefits, accessibility, and being relatively cheaper. These therapies encompass a wide range of practices, products, and knowledge derived from different cultures, utilizing natural compounds.

In many regions of Africa, Asia, and Latin America, traditional medicine serves as a cornerstone of healthcare, significantly enhancing medical accessibility for countless communities. Remarkably, up to 80% of people in Asia and Africa rely on traditional remedies as their primary form of healthcare. In China, these practices account for 40% of all medical services. Even in developed nations, the appeal of traditional medicine is growing. Advances in extraction methods, purification techniques, and clinical applications have significantly improved the quality, efficacy, and safety of traditional treatments. Despite this, recent years have seen a surge in research aimed at uncovering the mechanisms behind the healing properties of traditional compounds, thereby deepening our understanding of their actions and benefits.

2.1.3 Plant-Based Healing Agents

Plant-based healing agents are among the most widely used traditional remedies for managing skin injuries. These include herbs, herbal formulations, and finished products enriched with bioactive materials that help in the repair. A diverse range of natural extracts from various parts of the planet are used for treating skin injuries. These herbal products are available in multiple forms and are typically applied topically, administered systemically, or taken orally [15].

Herbal compounds have long been valued for their medicinal properties, which can accelerate wound healing through various mechanisms. For example, aloe vera, a common herbal remedy, is known for its soothing and anti-inflammatory properties, making it effective for treating burns and

minor cuts. Similarly, turmeric, which contains curcumin, has potent anti-inflammatory and antimicrobial effects, aiding in the prevention of infection and promoting tissue regeneration. Other notable herbs include chamomile, which has been used for its anti-inflammatory and antioxidant properties, and calendula, known for its ability to stimulate tissue and wound healing.

Herbal-based treatments are not only diverse in their sources but also in their applications. For instance, they can be found in modern formulations such as bioactive wound dressings, which incorporate herbal extracts to enhance their healing capabilities. These dressings can provide a moist wound environment, protect against infections, and deliver herbal compounds directly to the wound site. Additionally, herbal emulsions and creams can be applied to the skin to provide continuous exposure to the active compounds, promoting faster and more effective healing.

The global interest in herbal medicine continues to grow, driven by the increasing demand for natural and less invasive treatments. Scientific research has been focused on identifying the active components of these herbs and understanding their mechanisms of action. This research has led to the development of standardized herbal products with known concentrations of active ingredients, ensuring consistency and efficacy in treatment. As more clinical studies validate the benefits of these herbal compounds, their integration into conventional medical practices is likely to expand, providing more options for effective wound care.

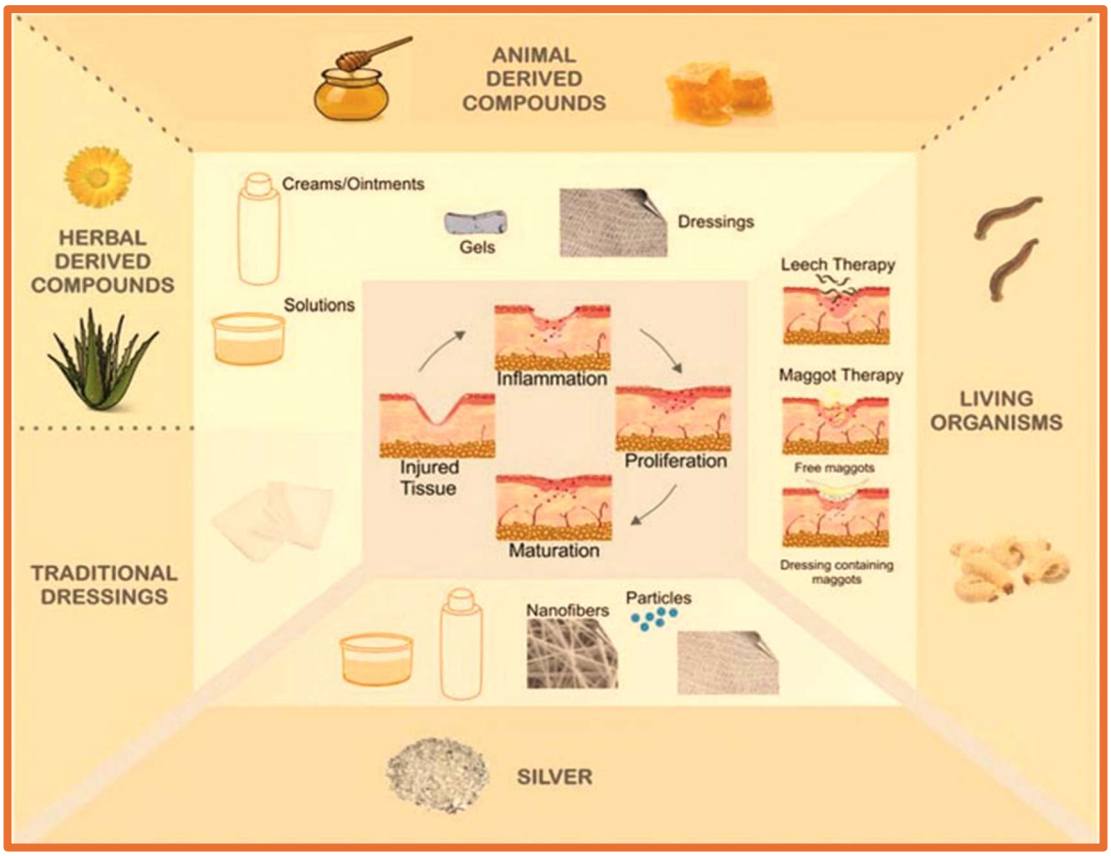

Figure 2.0.3: *Various traditional therapies and compounds are employed at different stages of the healing process, available in numerous physical forms, both on the market and under research, to stimulate healing. [34]*

2.2 Incorporating Active Pharmaceutical Ingredients (APIs)

The production of suture threads from raw polymers typically involves techniques such as melt spinning or wet spinning. Another approach is the electrospinning method, for producing ultra-thin fibers for sutures. This technique uses an electric field to draw fibers from a liquid solution, offering a simple and cost-effective approach. Electrospinning is particularly advantageous for manufacturing drug-eluting sutures due to its ability to operate continuously and minimize processing steps, making it an attractive option for producing low-cost, high-efficiency sutures [17, 18].

The transformation of raw polymers into suture threads primarily relies on melt spinning or wet spinning techniques. These processes involve either melting the polymer or dissolving it in a suitable solvent, followed by extrusion through a spinneret to form fibers. To create drug-eluting

sutures, these methods have been modified to incorporate medications into the polymer matrix before spinning. Typically, both the drug and polymer are dissolved in an organic solvent and extruded either by gravity or under pressure from a syringe. The resulting fibers are then dried or precipitated in a designated medium to finalize the product. In recent years, electrospinning has gained popularity as a method for producing ultra-thin fibers for sutures. This technique employs an electric field to draw fibers from a liquid solution, offering a straightforward and cost-effective approach. Electrospinning is particularly advantageous for manufacturing drug-eluting sutures due to its ability to operate continuously and minimize the number of processing steps. This makes it an attractive option for producing low-cost, high-efficiency drug-eluting sutures [28,29].

2.2.1 Advanced Electrospinning Technology

Electrospinning has garnered significant attention in tissue engineering and drug delivery, with numerous studies focusing on drug-loaded electrospun fibers [19, 20] and the influence of processing parameters on their fabrication [21]. The process involves loading a drug-polymer solution mixture into a syringe, which is then passed through a metallic capillary. A high voltage is applied, creating a charged jet that undergoes rapid solvent evaporation while stretching the fiber. The resulting fibers are collected as nonwoven mats. Electrospinning is performed at low temperatures, making it suitable for processing thermolabile APIs. A wide range of API-polymer systems have been developed, with some fibers capable of delivering multiple APIs for combined therapeutic effects [22].

Regardless of the API's physicochemical properties, its presence can affect the characteristics of the electrospun fibers. Increased drug loading often reduces the strength of the stitches, necessitating optimization of the process for each API-polymer system [24]. While drug concentrations up to 22 wt% have been reported, higher drug loads can significantly weaken tensile strength. For instance, a suture containing 22 wt% bupivacaine HCl in poly(lactic-co-glycolic acid) (PLGA) experienced a 70% reduction in tensile strength compared to drug-free sutures [25].

During uniaxial electrospinning, APIs may concentrate near surface, particularly with small hydrophilic molecules and thinner sutures. This phenomenon can lead to burst release, which does not align with the goal of controlled, sustained drug delivery. To address this, modifications such as coaxial electrospinning have been developed to achieve different distributions [26]. Coaxial

electrospinning produces composite fibers with core-shell structures, where the API is encapsulated in the core and surrounded by a polymer shell [27, 28, 29]. The drug release from these systems tends to be slower due to diffusion because of the polymeric shell, and the fiber structure may collapse after drug release, affecting tensile properties [30].

2.2.2 Melt Extrusion for Suture Production

Melt extrusion addresses the issue of solvent removal by melting the polymer instead of dissolving it. This method produces consistent materials with homogeneous drug distribution across the suture's cross-section [33]. To slow drug release, the drug can be modified before mixing with the polymer. For example, intercalating diclofenac into synthetic hydrotalcite and then mixing with poly(ε-caprolactone) (PCL) before extrusion prolonged drug release. The intercalated drug was fully released from PCL sutures after 55 days, compared to 14 days for raw drug embedded in PCL, with both sutures containing similar drug amounts [18]. Despite its advantages, melt extrusion for drug-eluting sutures is less common, possibly due to the high processing temperatures, which are unsuitable for thermolabile APIs. Melt-extruded threads typically have diameters ranging from 50 to 300 μm, while electrospun fibers range from nanometers to 350 μm. Consequently, multifilament sutures are created by weaving electrospun monofilaments.

The innovation in suture production techniques is pivotal for advancing medical treatments and patient outcomes. Both electrospinning and melt extrusion offer unique advantages, allowing for the customization of sutures to meet specific medical needs. As research progresses, these methods are expected to become more refined, offering even greater control over drug delivery and material properties. This ongoing development highlights the importance of interdisciplinary collaboration in the fields of materials science, pharmacology, and medical engineering, ensuring that new technologies can be effectively translated into clinical practice.

2.2.3 Post-addition of API

One of the simplest methods for incorporating active pharmaceutical ingredients (APIs) into sutures involves adsorbing the drug directly onto the suture's surface. However, this approach often results in an initial burst release of the drug once the suture is implanted [33]. This technique is particularly suitable for non-bioabsorbable polymers. To achieve more controlled drug release, a polymer coating containing the API can be applied to the surface of the suture. By adjusting the

coating's properties, it is possible to fine-tune the drug release kinetics. Absorbable sutures incorporate triclosan, a broad-spectrum antimicrobial agent, within a PLGA (polylactic-co-glycolic acid) coating. Constructed from copolymers of glycolide and ε-caprolactone, poly(p-dioxanone), and poly(glycolic-co-lactic) acid, respectively, these sutures have demonstrated the ability to inhibit bacterial growth for up to 11 days [34].

2.2.4 Methods for Post-Adding APIs to Sutures

Another advanced coating technology, particularly suited for sutures with charged surfaces, is layer-by-layer (LbL) coating. This method involves the sequential deposition of positive and negative polymers, with the drug either being simultaneously loaded or post-impregnated into the multilayer structure [35]. For instance, a commercial silk fiber was successfully coated with alternating layers of poly(allylamine hydrochloride) and dextran/hyaluronic acid. After soaking in an aqueous ibuprofen solution, the final product released 76% of the drug over 24 hours. While LbL deposition involves multiple steps, alternative techniques such as dip-coating are faster and more straightforward [36]. By manipulating factors such as the molecular weight of PLGA and the ratio of organic to aqueous phases, the microstructure of the shell and the drug diffusion rate could be precisely adjusted [37]. This process demonstrated that reducing pore size by 70% resulted in a 30% decrease in burst release, extending the release of drug up to 50 days. By varying the polymer composition, we can further refine the drug content and release profile [38]. Braided sutures benefit significantly from such coatings. The PCL coating provided protection against suture hairiness, although friction tests between sutures led to the formation of coating clusters. Commercial sutures often feature coatings containing a dye for visibility or a lubricant to facilitate implantation. To ensure proper adhesion of new coatings, any existing coatings must first be removed [39] introduced a novel technique involving the physical assembly of a commercial suture and a drug-containing sheet manually braided around the suture. This approach aimed to maintain the suture's mechanical properties while increasing drug content. This method involves producing a coating around a pre-manufactured suture, which increases its diameter and alters its tensile strength. While coatings with poor mechanical resistance do not affect the suture's tensile strength, forming a load-bearing coating can enhance the suture's overall mechanical properties [40]. In general, the post-loading of APIs onto manufactured sutures through various coating techniques provides a versatile approach to enhancing their antimicrobial properties and controlling drug

release. These advanced methods address critical needs in surgical wound care, offering promising solutions for improving the efficacy and safety of sutures used in medical procedures.

2.3 Biodegradable Sutures

Biodegradable sutures, made from polymers like polyglycolic acid (PGA), polylactic acid (PLA), and polydioxanone (PDO), offer significant advantages in wound healing by being absorbed naturally over time. This absorption process eliminate s the need for suture removal, thereby reducing the risk of infection and enhancing patient comfort. The degradation occurs through hydrolysis, a mechanism where water molecules break down the polymer chains, resulting in a gradual loss of mechanical strength until the suture is completely absorbed [7]. These sutures provide critical temporary support during the initial phases of wound healing. The rate at which they degrade can be tailored by adjusting the polymer composition, making them versatile for various tissue types and healing timelines. For instance, fast-absorbing sutures are suitable for tissues that heal quickly, such as those in pediatric patients, while slower-degrading sutures are preferred for tissues that require prolonged support, such as tendons and ligaments. Additionally, biodegradable sutures reduce the risk of chronic inflammation since they are naturally absorbed by the body, minimizing the presence of foreign material that can provoke an immune response.

2.3.1 Antimicrobial Sutures

The integration of antimicrobial agents into suture materials has revolutionized infection control in surgical procedures. These sutures, often coated with substances like triclosan or chlorhexidine, aim to reduce the risk of surgical site infections (SSIs), which are significant complications that can delay healing and increase healthcare costs. Triclosan, a broad-spectrum antimicrobial agent, is commonly used for this purpose due to its effectiveness against a wide range of bacteria [7].

Antimicrobial sutures work by continuously releasing the antimicrobial agent at the wound site, thereby inhibiting the growth of pathogenic microorganisms. This controlled release ensures a sustained antimicrobial effect throughout the critical period of wound healing. Studies have demonstrated that antimicrobial sutures can significantly reduce the incidence of SSIs, leading to better patient outcomes and reduced hospital stays.

2.3.2 Advanced Sutures with Drug Delivery Capabilities

Recent advancements in suture technology have led to the development of sutures capable of delivering therapeutic agents directly to the wound site. These drug-eluting sutures are designed to release drugs in a controlled manner, enhancing the healing process and providing targeted treatment. The incorporation of drugs into sutures can be achieved through various methods, including coating the suture surface with drug-loaded polymers, embedding the drug within the suture material, or using advanced techniques like electrospinning and melt extrusion. Electrospinning is a particularly promising technique for creating drug-eluting sutures. It involves using an electric field to draw fibers from a polymer solution, forming ultra-thin fibers that can incorporate drugs within their matrix. This method allows for precise control over the drug release profile, making it possible to achieve sustained release over extended periods [18]. For instance, electrospun sutures containing anti-inflammatory drugs or growth factors can significantly enhance wound healing by reducing inflammation and promoting tissue regeneration.

Melt extrusion, on the other hand, involves melting the polymer and drug mixture to form fibers. This technique ensures a homogeneous distribution of the drug within the suture, leading to consistent drug release. Melt-extruded sutures are particularly beneficial for incorporating thermally stable drugs that can withstand the high temperatures involved in the process [33].

2.4 Emerging Trends in Suture Technology

2.4.1 Smart Sutures

Smart sutures represent a cutting-edge development in wound care, incorporating sensors and electronic components to monitor the healing process in real-time. These sutures can detect changes in wound conditions, such as pH, temperature, and moisture levels, which are critical indicators of infection or improper healing. By providing continuous feedback, smart sutures enable healthcare providers to intervene promptly and adjust treatment plans as needed.

The integration of sensors into sutures involves embedding tiny electronic devices within the suture material. These devices can transmit data wirelessly to external monitors or smartphones,

allowing for remote monitoring of the wound. The information gathered can be used to track healing progress, detect early signs of complications, and ensure optimal wound care. Smart sutures hold great potential for improving patient outcomes, particularly in cases where frequent wound assessments are necessary.

2.4.2 Biodegradable Electronic Sutures

Building on the concept of smart sutures, biodegradable electronic sutures combine the benefits of electronic monitoring with the advantages of biodegradability. These sutures are made from materials that naturally dissolve in the body over time, eliminating the need for removal. The electronic components are designed to degrade harmlessly alongside the suture material, ensuring that no foreign objects remain in the body after healing is complete [33]. The development of biodegradable electronic sutures involves significant challenges, including ensuring the stability and functionality of electronic components within a biodegradable matrix. However, advances in materials science and bioengineering are paving the way for these innovative sutures to become a reality. Biodegradable electronic sutures offer a promising solution for continuous wound monitoring without the drawbacks of permanent electronic implants.

2.4.3 Nanotechnology in Sutures

Nanotechnology is playing an increasingly important role in the development of advanced suture materials. By incorporating nanoparticles into suture threads, researchers can enhance their properties and introduce new functionalities. For example, silver nanoparticles are known for their potent antimicrobial activity and can be used to create sutures with enhanced infection-fighting capabilities.

Nanoparticles can also be used to improve the mechanical properties of sutures, such as tensile strength and elasticity. This is particularly important for sutures used in areas subject to significant mechanical stress, such as joints and tendons. Additionally, nanoparticles can be engineered to

deliver drugs in a controlled manner, further enhancing the therapeutic potential of sutures. The application of nanotechnology in sutures is a rapidly evolving field, with ongoing research focused on optimizing nanoparticle formulations and ensuring their safety and efficacy. As these technologies mature, they are expected to lead to the development of next-generation sutures that offer unprecedented levels of performance and functionality [18].

CHAPTER 3: MATERIALS AND METHODS

3.1 Preparation of Indica extract

Fresh leaves of *Azadirachta indica* (Neem) were procured from a local plant nursery situated in Rawalpindi, Pakistan. Immediately after collection, the leaves underwent a rigorous cleaning process to eliminate any adhering dust, soil particles, or other impurities. This was achieved by thoroughly washing the leaves with distilled water to prevent potential contamination that could interfere with subsequent extraction procedures. After cleansing, the leaves were allowed to dry completely under ambient room temperature conditions, ensuring the gradual removal of moisture without exposure to direct sunlight, which could potentially degrade heat-sensitive bioactive compounds.

Once the leaves had completely dried, they were subjected to mechanical grinding using an electric blender. This process was carefully executed to obtain a uniform fine powder, which not only facilitated efficient extraction but also increased the surface area available for solvent interaction. The resulting powdered material was stored in an airtight container at room temperature to prevent any moisture absorption or degradation prior to extraction.

For the extraction of bioactive constituents, absolute ethanol was selected as the solvent due to its high efficiency in isolating a broad spectrum of phytochemicals, including polyphenols, flavonoids, and alkaloids. A precise quantity of 4 grams of the finely powdered *A. indica* leaves was weighed and subsequently immersed in 100 mL of absolute ethanol. The prepared suspension was then placed in a shaking water bath, where it was incubated at a controlled temperature of 65°C for approximately 30 minutes. Continuous agitation during this period facilitated enhanced mass transfer, thereby promoting the dissolution of bioactive compounds into the solvent matrix.

Following the extraction process, the mixture underwent centrifugation at 5000 revolutions per minute (rpm) for a duration of 10 minutes. This step effectively separated the solid plant residues from the liquid extract by allowing heavier particulates to sediment at the bottom. The resulting supernatant, containing the enriched ethanolic extract, was carefully decanted to prevent any disturbance of the settled residues. To ensure complete removal of any residual particulate matter, the extract was further purified by filtration using Whatman No. 1 filter paper. The filtrate, now

a clear solution, was collected in sterile glass vials and subsequently stored at a temperature of 4°C to preserve its chemical stability and prevent any microbial contamination until further utilization in experimental procedures. The schematic representation of the extraction process is illustrated in Figure 3.1.

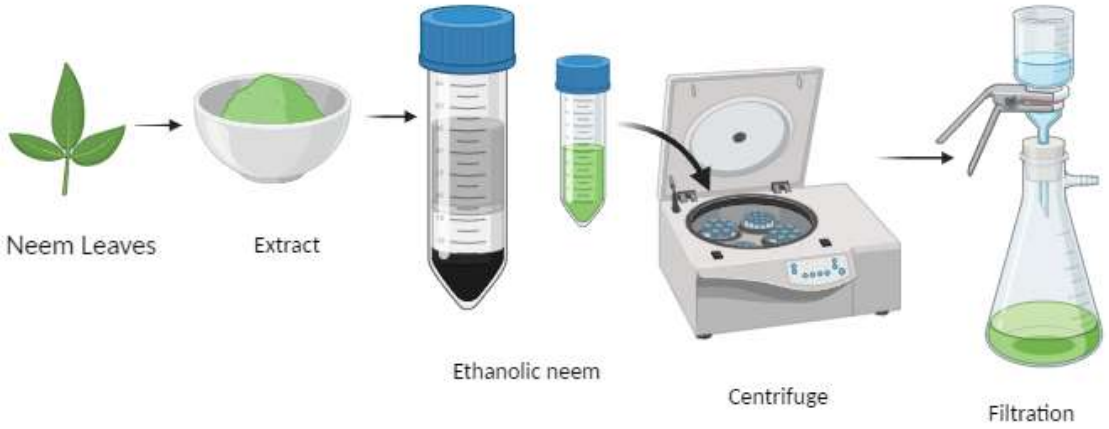

Figure 3.0.1: Preparation of Neem Extract

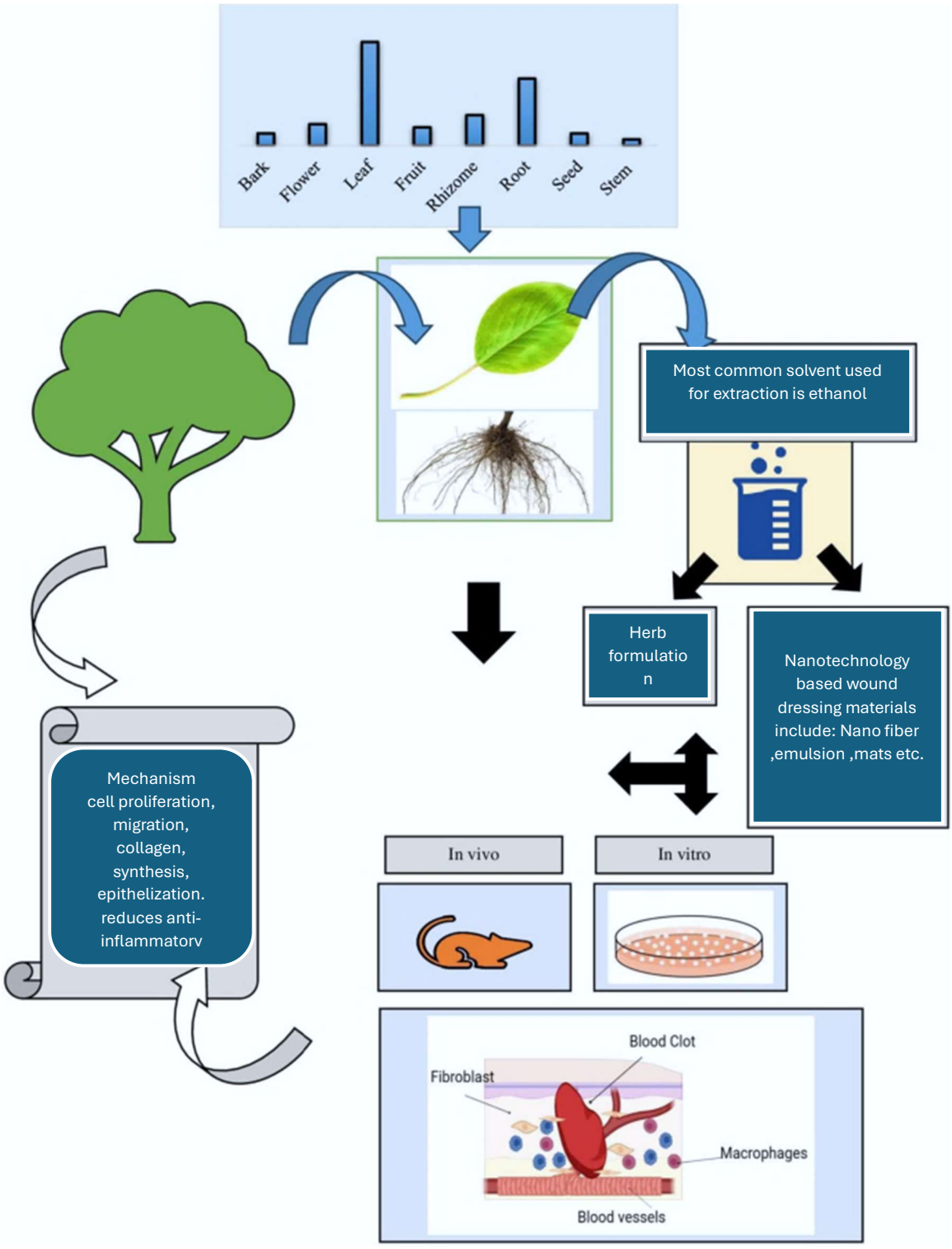

Figure 3.0.2: Steps Summary Involved in experimentation. [30]

3.1.1 Preparation of coating material

For the formulation of a chitosan-based suspension, high-purity chitosan was sourced from Shanghai Macklin Biochem (China). To ensure complete dissolution and uniform dispersion, a precisely weighed quantity of 1 gram of chitosan was gradually introduced into an acetic acid solution under continuous stirring. The acetic acid served as a solvent, facilitating the protonation of chitosan's amino groups, thereby enhancing its solubility and stability in the solution. The dissolution process was carried out under controlled conditions to prevent aggregation and ensure a homogeneous mixture.

Following the preparation of the chitosan solution, a predefined concentration of the plant extract was carefully introduced into the medium. The mixture was subjected to continuous magnetic stirring at a controlled temperature of 37°C under ambient environmental conditions. The stirring process was maintained to promote uniform dispersion of the bioactive compounds within the chitosan matrix, facilitating potential interactions between the chitosan and the phytochemicals present in the plant extract. This step was crucial in ensuring the stability and consistency of the final formulation, which would later be used for coating applications.

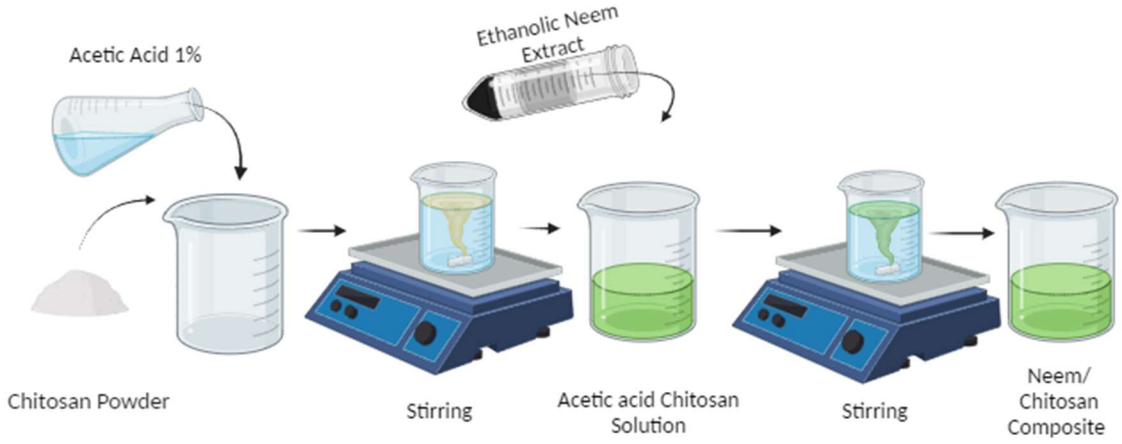

Figure 3.0.3: Preparation of Neem/chitosan composite

3.1.2 Dip coating of silk braided sutures

The dip coating technique was employed to coat non-absorbable silk braided sutures, which were procured from Yancheng Huida (China). The Composite coating solution was poured into a beaker, which was then placed in the designated area inside the dip coating machine. The suture material was carefully wound around a specially designed frame to hold it securely during the coating process.

The frame, with the sutures attached, was immersed into the beaker containing the coating solution. The immersion was conducted at an optimal speed to ensure uniform coating of the sutures. The sutures remained submerged in solution for half an hour and this time is adequate enough for adhesion of the indica /Ch composite to the suture material. The sutures were dried to ensure the coating was firmly set and stable.

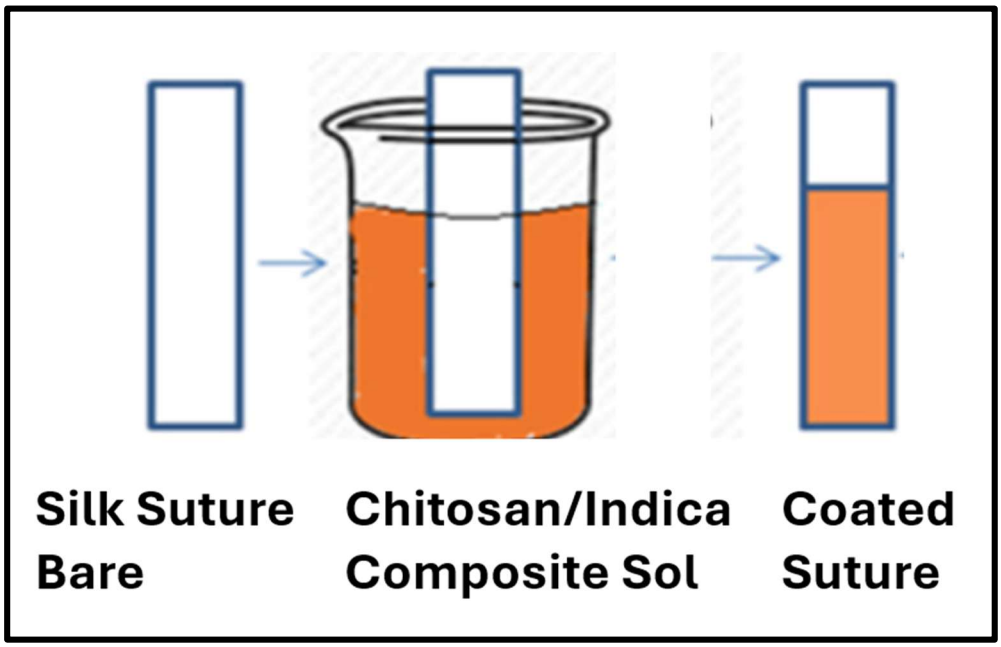

Figure 3.0.4: Dip Coating Methodology

3.2 Characterizations of Neem/chitosan coated sutures:

3.2.1 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR of *indica* /chitosan-coated stitches revealed the presence of functional groups and the nature of interactions between *indica* and Ch solution.

The FTIR spectra of the indica /Ch composites were recorded at range spanning from 4000 cm^{-1} to 400 cm^{-1}. This spectral range allowed for the identification of key functional groups. The resulting spectra provided detailed information about the molecular interactions and chemical bonding between the components of the composite, confirming the successful incorporation of Azadirachta indica extract into the chitosan matrix.

3.2.2 Scanning Electron Microscopy (SEM)

To evaluate the morphological characteristics of the sutures coated with Azadirachta indica and chitosan, a Scanning Electron Microscope (SEM) analysis was conducted. This technique was employed to examine the surface structure of the coated sutures in detail. The specific equipment used for this analysis was the JSM-6490A Scanning Electron Microscope from JEOL, Japan. The SEM analysis provided high-resolution images that revealed the detailed surface topography and composition of the coated sutures. These images allowed researchers to assess the uniformity and coverage of the Azadirachta indica /chitosan coating, identify any surface irregularities or defects, and evaluate the overall quality of the coating application. Such information is critical for understanding how the coating might influence the suture's performance, including its mechanical properties, biocompatibility, and drug-release characteristics. By analyzing the SEM images, researchers could confirm whether the coating adhered evenly to the suture surface and whether the composite material maintained structural integrity. This step is essential for ensuring that the coated sutures meet the necessary standards for use in medical applications, particularly in wound healing and infection prevention.

3.2.3 Tensile strength

The tensile strength of the suture is a crucial characteristic that aids the practitioner in forming a knot. In the event that the suture is exceedingly weak and subjected to significant knotting force, it is prone to easy breakage. Consequently, it is imperative to ascertain the tensile strength of the suture that has been coated. The mechanical attribute such as Tensile Strength (TS) was assessed using Shimadzu AUTOGRAPH AG-X plus equipped with a load cell and minor adjustments. A coated suture measuring 75 cm in length was chosen and fastened between two clamps placed at a separation of 15 cm. These characteristics were assessed in order to confirm the physical integrity of the coated sutures in relation to their physical strength.

3.3 Extract release of indica coated sutures

The drug release profile of the Azadirachta indica /chitosan-coated sutures was evaluated in phosphate-buffered saline (PBS) to assess the release kinetics of the active compounds during application. The coated sutures were dipped in PBS solution (pH 7.4) at a temperature of 37°C, simulating physiological conditions. At regular intervals, the residual PBS was removed, and the solution was replaced with 3 mL of fresh PBS every 10 minutes. Prior to immersion in PBS, the sutures were weighed in their dry state. Additionally, the drained PBS solutions were analyzed to determine the indica release profile by using a spectrophotometer. The absorbance was measured in fixed wavelength of 500 nm. This method allowed for the quantification of released drug concentration in the PBS medium. These measurements enabled the construction of a detailed drug release profile, offering insights into the release kinetics and the potential sustained-release behavior of the coated sutures. This analysis is critical for understanding the therapeutic efficacy and performance of the sutures in clinical applications, particularly in wound healing and infection prevention.

3.4 Antimicrobial Susceptibility test for Neem/chitosan coated sutures

The Zone of inhibition was calculated for the indica /Ch composite surgical stitches was assessed using the disc diffusion.

Preparation of Mueller-Hinton Agar (MHA) Plates by Sterile MHA agar was prepared by dissolving 38.2 g of MHA in 1000 mL of distilled water. The mixture was then autoclaved to ensure sterility. After autoclaving, the molten agar was carefully poured into sterile Petri plates and allowed to solidify. The plates were incubated at 37°C for 24 hours to check for any undesirable bacterial growth. Plates free from contamination were used for subsequent experiments.

Inoculation and Suture Placement, Bacterial cultures were added to the petri plates and were spread evenly. The stitches were placed on top of the bacterial petri plates filled with MHA Ahar. One section served as the negative control, containing a sterile Whatman filter paper No. 1. Another section served as positive control, containing a disc impregnated with the antibiotic ciprofloxacin.

The inoculated plates were incubated at 37°C for 24 hours. After incubation, the zone of inhibition around each suture piece was measured in millimeters (mm) using a ruler. The zone of inhibition represents the area where bacterial growth was inhibited, indicating the antibacterial efficacy of the coated sutures.

3.5 Hemolysis assay

To assess the hemocompatibility of the Azadirachta Indica /chitosan-coated sutures, a hemolysis assay was conducted. This assay evaluates the potential of the coated sutures to cause red blood cell (RBC) lysis, which is critical for determining their biocompatibility and safety for clinical use.

3.5.1 Preparation of Red Blood Cells (RBCs)

Fresh human blood (3 mL) was collected in an EDTA tube to prevent clotting. The blood was divided into two microcentrifuge tubes, each containing 1.5 mL, and centrifuged at 6000 rpm for 10 minutes to separate the components. After centrifugation, the supernatant (plasma) was discarded, leaving behind the RBC pellet. To isolate and purify the RBCs, 1 mL of phosphate-buffered saline (PBS) was added to a 500 µL sample of blood, and the mixture was centrifuged again at 6000 rpm for 10 minutes. This washing process was repeated five times with PBS to ensure the removal of any residual plasma or other contaminants, resulting in a pure RBC suspension.

3.5.2 Preparation for Test Suspensions

The purified RBC suspension was mixed with PBS solutions containing Azadirachta Indica /chitosan-coated sutures. These suspensions were prepared to evaluate the hemolytic potential of the coated sutures at different coating concentrations. Positive control was prepared using a 0.5% Triton X-100 solution, which induces complete hemolysis of RBCs.

Negative control was prepared using PBS alone, which does not cause hemolysis. The suspensions were incubated at room temperature for 2 hours to allow interaction between the RBCs and the coated sutures. During this time, any potential hemolysis caused by the sutures could occur.

3.5.3 Post-Incubation Processing

After incubation, the samples were centrifuged at 6000 rpm for 10 minutes to separate the intact RBCs from the supernatant. The supernatant, which contained any released hemoglobin due to hemolysis, was carefully collected. This supernatant was then transferred into a microtiter plate in triplicates to ensure reproducibility of the results.

3.5.4 Measurement of Hemolysis

The optical density (OD) of the supernatant was measured using a spectrophotometer at a wavelength of 540 nm, which corresponds to the absorption peak of hemoglobin. The degree of hemolysis was calculated as a percentage relative to the positive and negative controls using the following formula

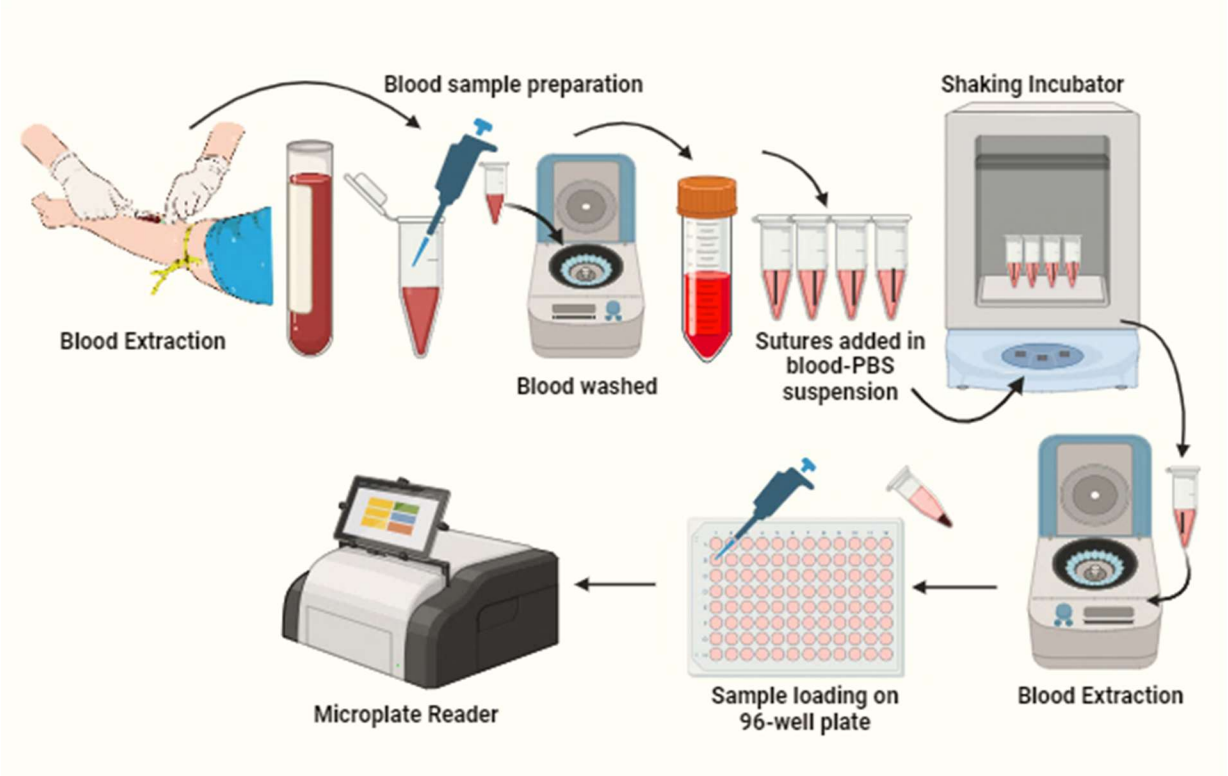

Figure 3.0.5: Hemolytic assay process

The absorbance of each sample was measured at 540 nm using a UV microplate reader, a crucial step to quantify the extent of hemolysis. The hemolytic rate for each sample was then calculated using the appropriate formula:

$$\text{Hemolysis (\%)} = \frac{As - An}{Ap - An} \times 100$$

As = Absorbance. of sample

An= Absorbance. of negative-control **Ap**= Absorbance. of positive-control

This formula allows for a precise calculation of the hemolytic activity, taking into account both the baseline (negative control) and the maximum hemolytic activity (positive control). The detailed steps and methodology are illustrated in Figure 3.6, ensuring clarity and reproducibility of the process

3.6 In vivo investigation of Neem/chitosan coated sutures

To systematically assess the antimicrobial efficacy and wound healing potential of Neem/chitosan-coated sutures, an in vivo study was conducted using Sprague Dawley rats. The selected subjects, each weighing between 100 and 150 grams, were procured from the Atta-ur-Rahman School of Applied Biosciences at the National University of Science and Technology (NUST) in Islamabad, Pakistan. Upon arrival, the animals were housed in a controlled environment within the institutional animal facility and allowed a one-week acclimatization period. During this time, they were maintained under standard laboratory conditions, including a regulated 12-hour light/dark cycle, with unrestricted access to food and water.

Prior to surgical intervention, the rats underwent an eight-hour fasting period to ensure procedural safety and minimize potential anesthetic complications. General anesthesia was induced through intraperitoneal administration of ketamine (80 mg/kg) and xylazine (10 mg/kg) to facilitate a pain-free surgical procedure. The dorsal fur of each rat was carefully shaved to expose the surgical site, after which two symmetrical incisions, each measuring approximately 1 cm in

length, were made on either side of the spinal midline. One of these incisions was closed using Neem/chitosan-coated sutures, while the other was sutured with uncoated sutures, serving as the control.

To replicate a clinically relevant infection model, bacterial inoculation was performed 24 hours post-surgery. The wound sites were deliberately exposed to Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus) to evaluate the antimicrobial properties of the coated sutures. Wound progression was meticulously documented through daily photographic records over a 14-day period. This longitudinal assessment facilitated a comparative analysis of healing kinetics and infection control efficacy between the experimental and control groups. The experimental methodology and wound evaluation process are illustrated in Figure 3.7.

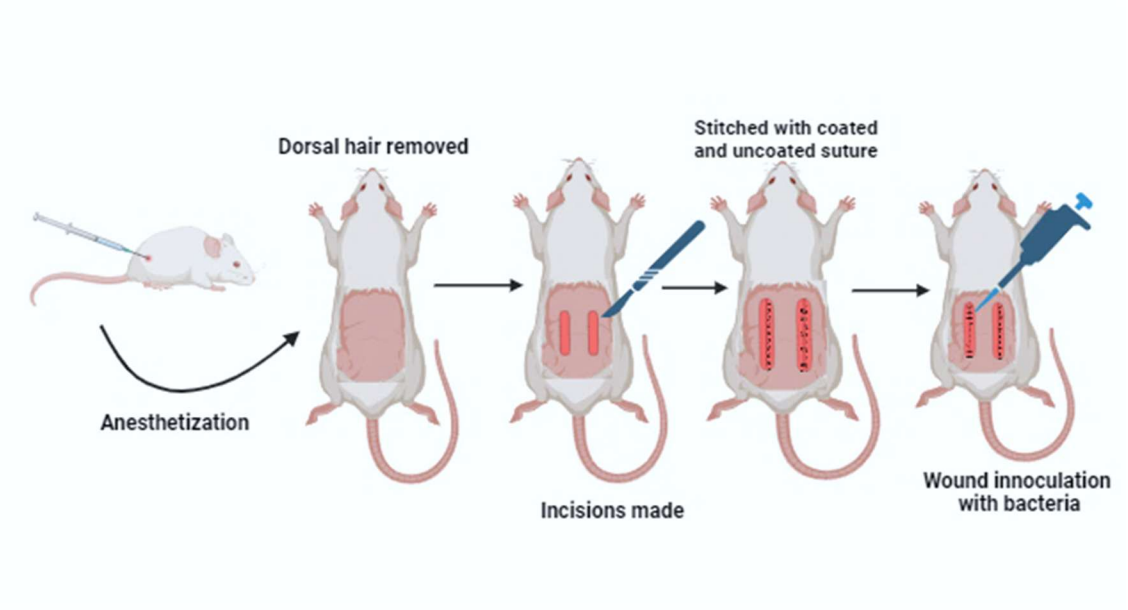

Figure 3.6: Schematic representation of vivo testing for wound closure

CHAPTER 4: RESULTS AND DISCUSSION

4.1 FTIR

The infrared spectra of chitosan, as shown in Figure 4.1, reveal a prominent peak at approximately 3339 cm^{-1}, which corresponds to the stretching vibrations of the -OH bond overlapping with N–H stretching vibrations in chitosan [20]. Peaks observed around 2876 cm^{-1} are attributed to the stretching vibrations of the C–H bond. Additional absorption peaks at 1658 cm^{-1}, 1598 cm^{-1}, 1424 cm^{-1}, and 1382 cm^{-1} represent specific molecular vibrations. These peaks are associated with the stretching of the C=O bond in the amide I region, the bending vibrations of the N–H bond in N-acetylated residues (amide II region), the bending of the C-H bond, and the bending of the OH bond, respectively. Furthermore, the peak at 1155 cm^{-1} is assigned to the anti-symmetric stretching of the (C–O–C) bridge, while peaks at 1088 cm^{-1} and 1034 cm^{-1} are linked to skeletal vibrations involving C–O stretching [21].

Figure 4.1 also illustrates the FTIR spectra of the prepared Azadirachta indica extract sample. The spectra, recorded in transmittance mode within the range of 500 cm^{-1} to 4000 cm^{-1}, display a peak around 3399 cm^{-1}, confirming the presence of a hydroxyl (-OH) functional group involved in intermolecular hydrogen bonding in Azadirachta indica leaves. A strong stretching band indicates N-H stretching and bending vibrations of the amine group ($-NH_2$). The peak at 2923 cm^{-1} corresponds to the stretching of the C–H bond, while the peak at 1731 cm^{-1} is attributed to the C=O bond stretching of functional groups in the ketone and aldehyde families. Aliphatic C–C bonds were identified at 1252 cm^{-1}, and the band observed at 1034 cm^{-1} in powdered Azadirachta indica leaves was assigned to the stretching vibration of C–O bonds [22, 23].

In the Azadirachta indica-chitosan composite, the absorption band around 1400 to 1420 cm^{-1} appears across all concentrations, indicating symmetric stretching of the functional group. A broad peak around 1600 cm^{-1} corresponds to C=C double bond stretching, while the peak around 1700 cm^{-1} signifies the presence of the C=O bond. These spectral features can be correlated with the antimicrobial properties of the nanocomposite. The Azadirachta indica-chitosan composite demonstrates optimal antimicrobial efficacy, confirming that the active components remain functionally effective [24].

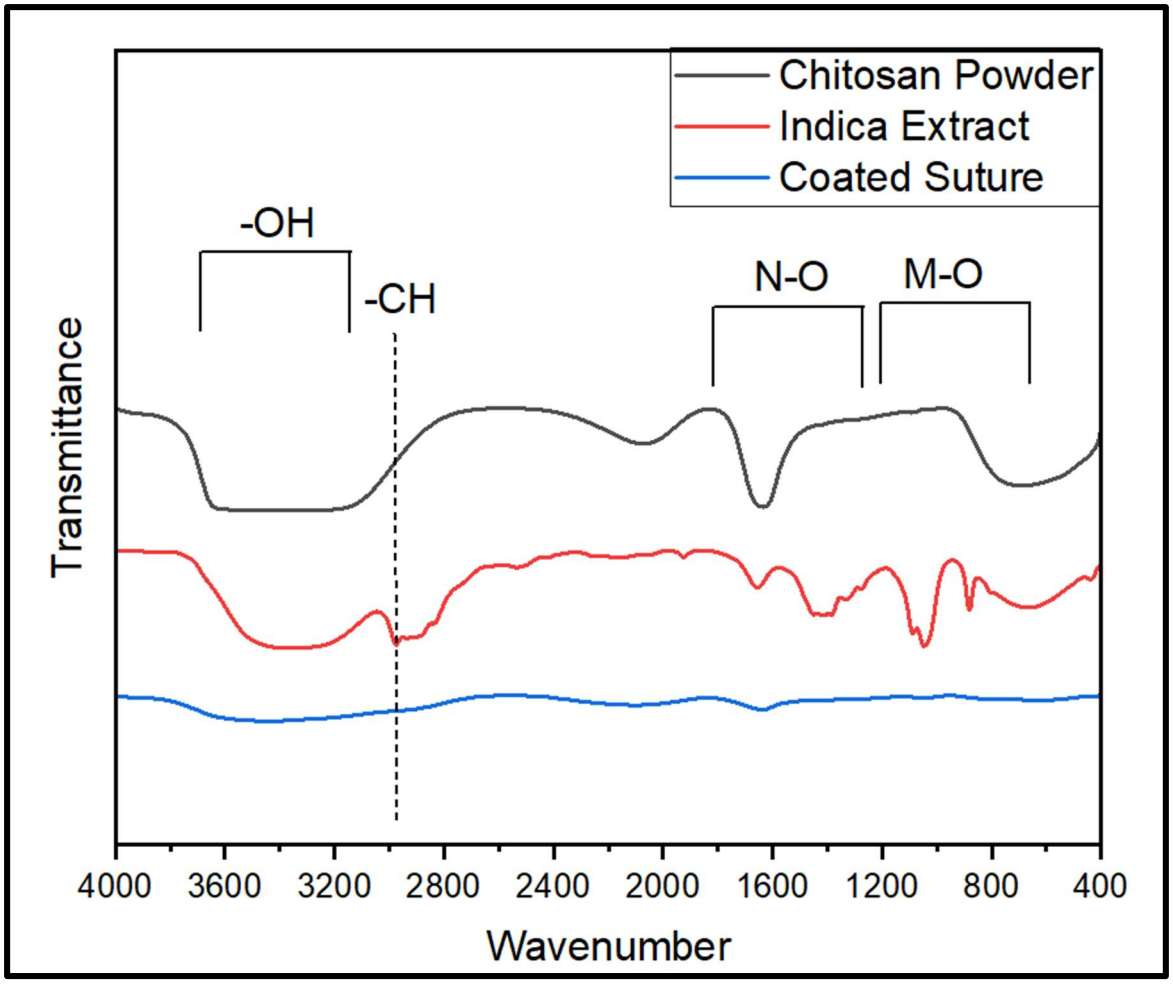

Figure 4.0.1: FTIR spectra. The graph shows the FTIR spectra of Chitosan, ethanolic Neem extract and Neem/chitosan composite with varying concentrations.

4.2 Scanning Electron Microscope (SEM)

The Scanning Electron Microscope (SEM) analysis provided critical insights into the surface morphology of both Azadirachta Indica/Chitosan-coated sutures and their uncoated counterparts. As illustrated in Figure 4.2, the SEM images revealed a striking contrast between the two types of sutures, underscoring the impact of the coating on surface characteristics.

The SEM images of the Azadirachta Indica/Chitosan-coated sutures demonstrated a smooth and coated texture, indicative of consistent and even application of the coating material. This homogeneity highlights the effectiveness of the coating process in achieving complete coverage

of the suture fibers. A smooth and uniform surface is essential not only for enhancing the suture's functionality but also for ensuring its antimicrobial properties. Such a surface minimizes the risk of bacterial adhesion and proliferation, which are common precursors to surgical site infections. In contrast, the SEM images of the uncoated sutures revealed a markedly different surface morphology. The uncoated sutures exhibited an irregular and rough texture, with noticeable inconsistencies along the suture fibers. These irregularities create potential niches where bacteria can adhere and proliferate, thereby increasing the risk of infection. The lack of a smooth surface in uncoated sutures underscores the importance of the Azadirachta Indica/Chitosan coating in improving both the structural integrity and antimicrobial efficacy of the sutures. The smooth and uniform surface achieved through the Azadirachta Indica/Chitosan coating enhances the suture's ability to resist bacterial colonization, making it more suitable for biomedical applications. The rough and inconsistent surface of uncoated sutures highlights their limitations in terms of infection control and overall performance.

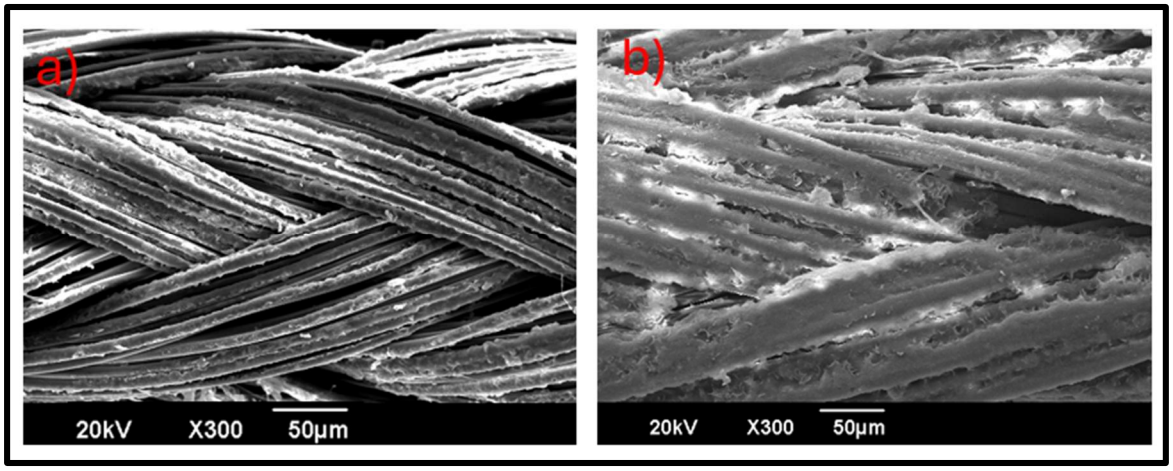

Figure 4.0.2: SEM images of coated (a) and uncoated (b) silk braided sutures.

4.3 Drug release profile

The drug release profile of the *Indica* /Ch composite stitches is shown below in Figure 4.3. The stitches exhibited high drug release in the first few hours of being submerged. This was followed by a slower release rate between 25 and 90 hours, The drug release was extended till 160 hours passed [25]. This phenomenon is typical in drug delivery systems where surface-bound compounds

are quickly released upon contact with the surrounding medium. The sustained and controlled release observed after the initial burst phase is likely due to the incorporation of chitosan, a bioadhesive polymer. Chitosan possesses an overall positive charge due to the presence of amine groups, enabling it to adhere strongly to negatively charged biological surfaces.

This bioadhesive property enhances the retention of the drug-loaded system at the target site, facilitating prolonged and targeted drug delivery. The interaction between chitosan and the biological environment ensures a more consistent and extended release of the active compounds, as evidenced by the linear release pattern observed beyond 90 hours. These findings highlight the effectiveness of the *Azadirachta Indica* /chitosan composite coating in achieving a controlled and extended drug release profile, making the coated sutures a promising candidate for advanced wound care applications.

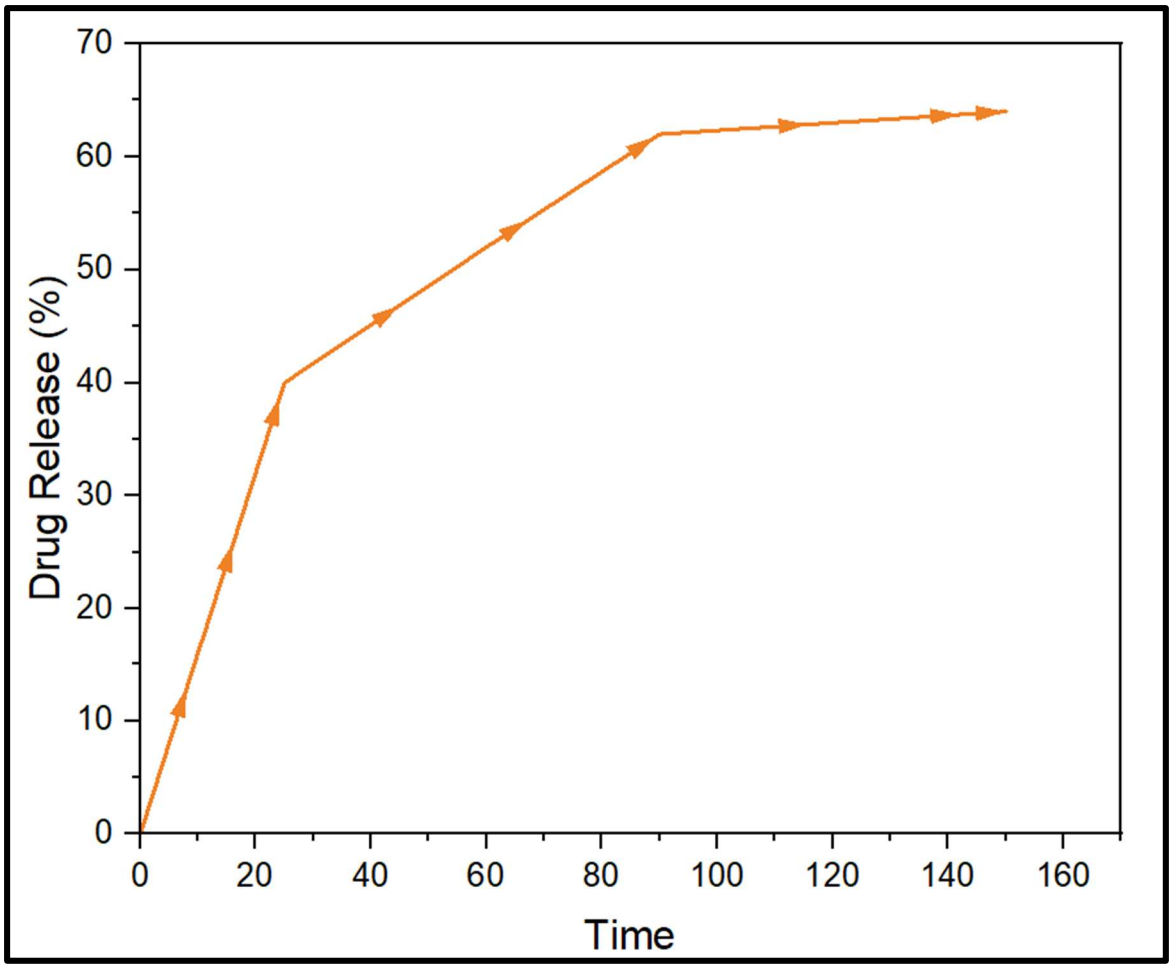

Figure 4.0.3: Drug release profile of Neem/chitosan coated suture.

4.4 Antimicrobial activity

The antibacterial efficacy of Azadirachta Indica /chitosan-coated sutures was evaluated by measuring the zone of inhibition against Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus) in millimeters (mm). The results, as depicted in Figure 4.4, demonstrate that the coated sutures exhibit significant antibacterial activity against both bacterial strains.Antimicrobial Activity Against S. aureus The sutures coated with Azadirachta Indica displayed differing good levels of antibacterial activity. As illustrated in Figure 4.3, ZOI against the bacterial strain S. aureus reached 16 mm. This indicates that S. aureus is highly susceptible to the antimicrobial effects of Azadirachta Indica and chitosan.Antimicrobial Activity Against E. coli In comparison, E. coli was less susceptible to the antimicrobial effects of Azadirachta Indica than S. aureus. However, the coated sutures still displayed a considerable ZOI, of approximately 14 mm. Previous studies on Azadirachta Indica have also demonstrated its notable antibacterial properties. For instance: One study reported a zone of inhibition of 11 mm against E. coli and 12 mm against S. aureus [17]. Another study assessed the combined antimicrobial activity of Azadirachta Indica and chitosan, reporting zones of inhibition of 17 mm against S. aureus and around 10 mm against E. coli [18]. These findings align with the results of the current study. The incorporation of chitosan likely enhances the antimicrobial efficacy due to its inherent bio adhesive and antimicrobial properties, which contribute to prolonged drug release and sustained activity at the wound site.

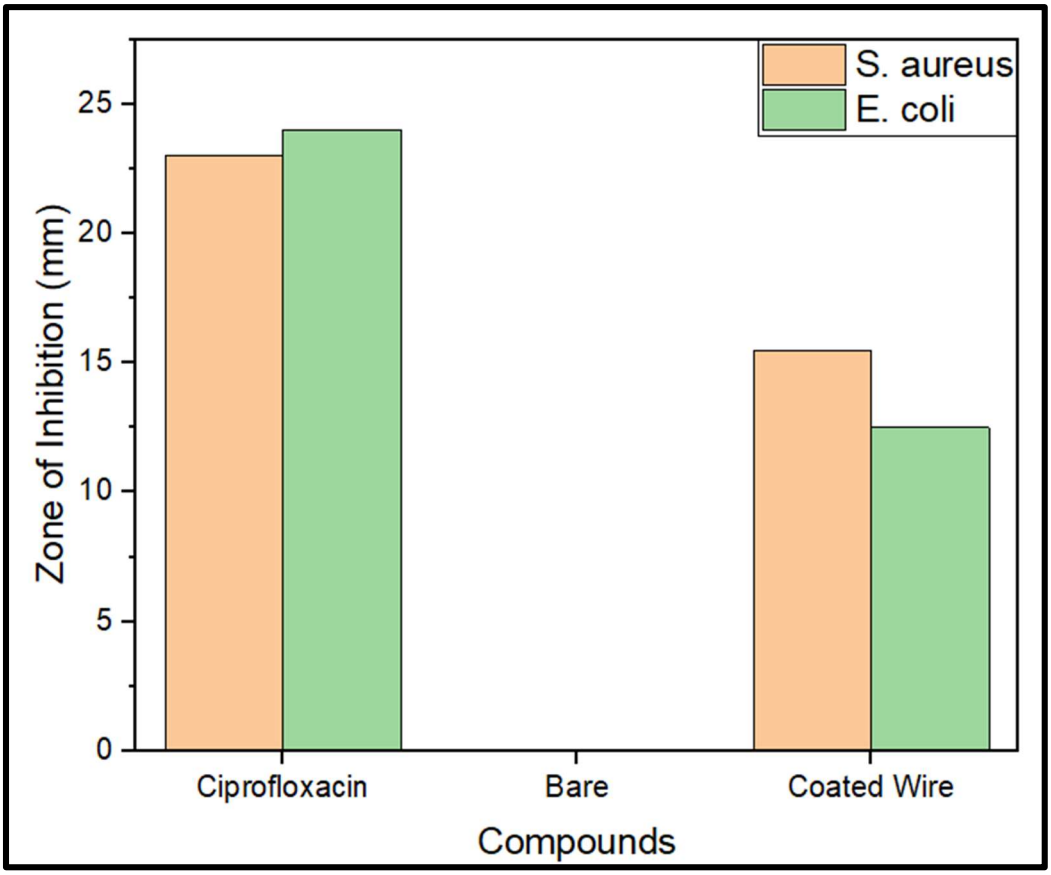

Figure 4.0.4: Anti-bacterial activity of Neem/chitosan sutures

The significant antibacterial activity of the Azadirachta Indica /chitosan-coated sutures highlights their potential for preventing surgical site infections caused by pathogens such as E. coli and S. aureus. The ability to tailor the coating concentration allows for optimization of the antimicrobial effect, ensuring effective infection control while supporting the wound healing process.

4.5 Hemolytic activity

Hemolytic activity assay was conducted using human red blood cells (RBCs) exposed to ethanolic indica extract coated on silk braided sutures. In this study, 0.01% Triton X-100 was used as the positive control, achieving 12% hemolysis, while phosphate-buffered saline (PBS) served as the negative control, showing 0% hemolysis. The chitosan and indica as well as their composite Azadirachta indica/chitosan-coated sutures exhibited minimal hemolytic activity toward RBCs.

The findings revealed a concentration-dependent increase in hemolysis, with higher concentrations of ethanolic Azadirachta indica extract in chitosan resulting in elevated hemolytic rates. The maximum hemolysis observed was 1.3% of the composite coating, as depicted in Figure 4.5. [26]. The observed hemolysis of less than 2% indicates that Azadirachta indica/polymer-coated sutures exhibit excellent hemocompatibility and are safe for use as biomaterials in surgical wound closure.

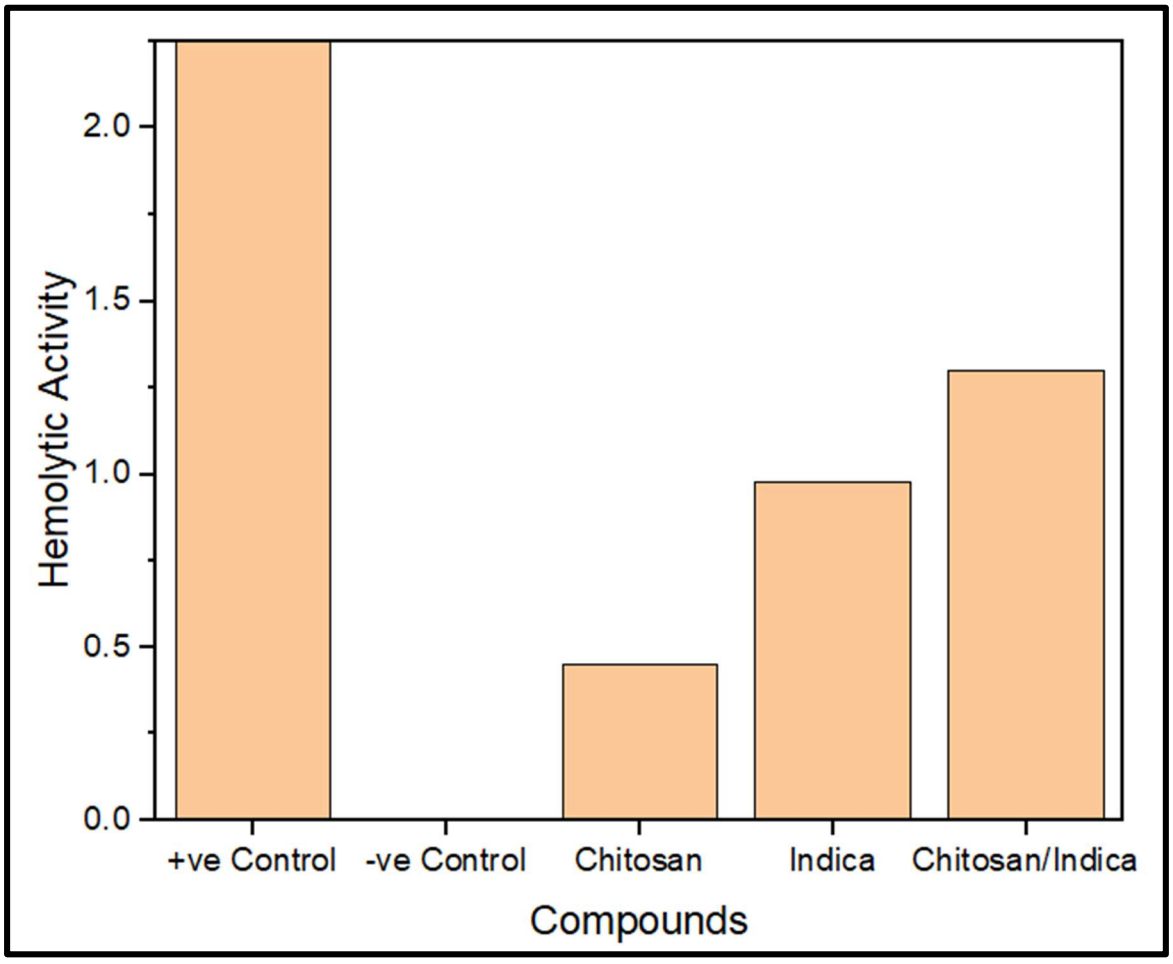

Figure 4.0.5: Hemolytic activity Indica/polymer coated sutures

4.6 In vivo evaluation of Extract/Polymer coated sutures

The study evaluated the tissue repair stages in live white rats that were stitched. The cut made on the left abdomen of the rat was closed using the commercially available unmodified stitch while

in contrast the right cut was closed using our Indica/Ch composite loaded stitch. After 1 day, both cuts were sprayed with live bacterial cultures on each side of the cut. Initial observations revealed inflammation on both sides. The wounds were monitored over 14 days, with images captured on the 7th and 14th days, as shown in Figure 4.6. By the 7th day, the side treated with the coated suture showed significantly reduced inflammation. After 2 weeks, the stitches cut from the body. The area around the bare stitch still had infections and not properly headed, as shown in Figure 4.6, while the wound treated with the coated suture was fully healed, with visible hair growth and no signs of inflammation.

By the 14th day, the differences in healing were striking. The uncoated suture still displayed inflammation, characterized by redness and swelling. In contrast, the wound treated with the coated suture was completely healed, with no inflammation and visible hair growth at the site. The Azadirachta indica/chitosan-coated sutures not only promoted faster healing but also provided a protective barrier against bacterial infection, reducing the risk of complications. These findings highlight the potential of Azadirachta indica and chitosan as bioactive coatings for sutures, offering a promising approach to improving post-surgical outcomes by enhancing healing and minimizing infection risks.

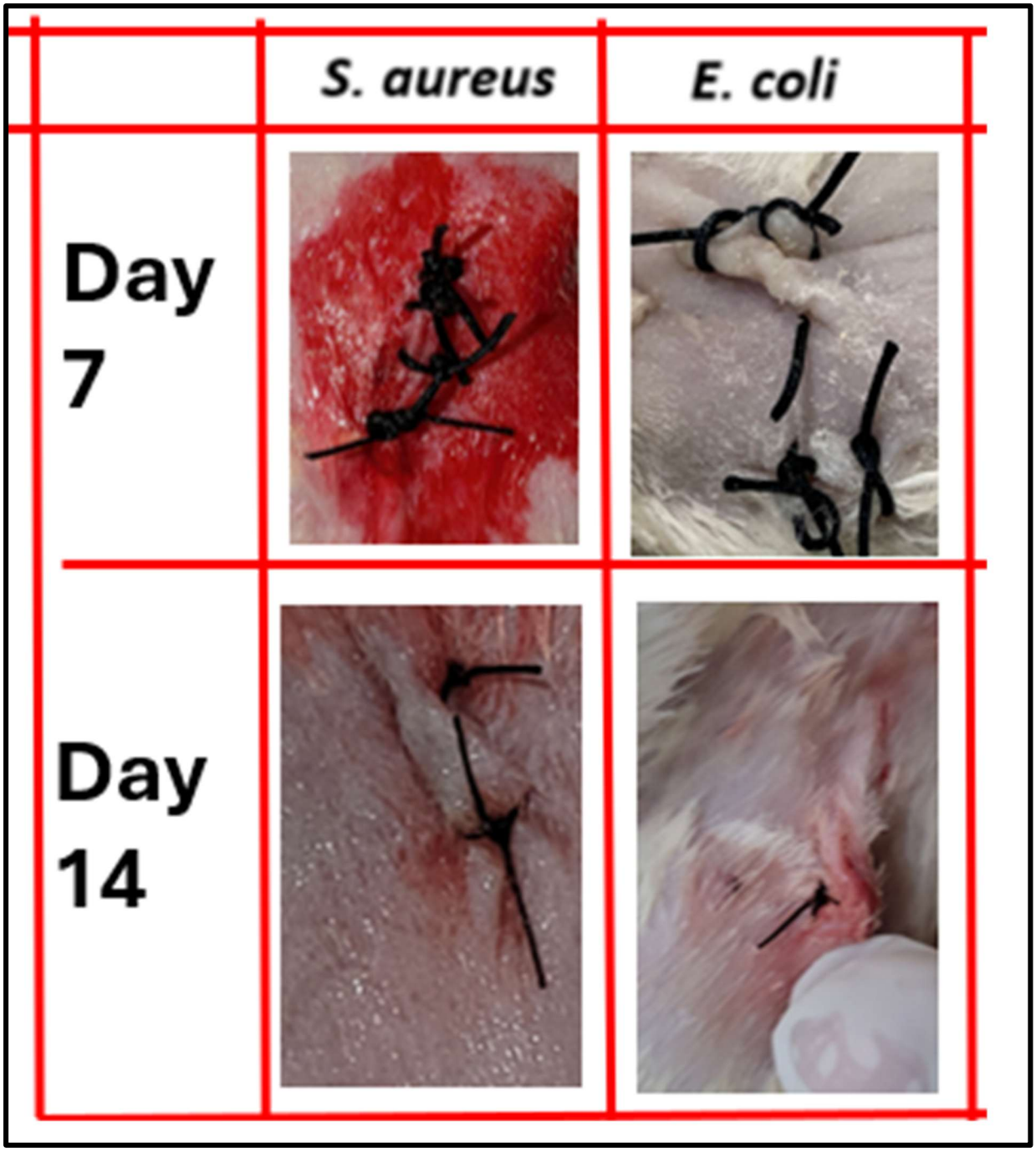

Figure 4.0.6: In vivo evaluation of Indica/Polymer coated sutures.

CHAPTER 5. CONCLUSION

The integration of Azadirachta Indica and chitosan into surgical sutures represents a significant advancement in the field of wound care and infection prevention. This study demonstrates the successful development of Azadirachta Indica/chitosan-coated sutures, which exhibit remarkable antibacterial activity, controlled drug release, and excellent hemocompatibility. The findings highlight the potential of these coated sutures to address critical challenges in surgical wound management, including bacterial infections and reduced healing times. Through techniques such as Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), and drug release studies, we confirmed the uniformity of the coating, the sustained release of active compounds, and the structural integrity of the sutures. The antibacterial assays revealed significant inhibition zones against both Escherichia coli and Staphylococcus aureus. Furthermore, the hemolysis assay demonstrated minimal hemolytic activity (<2%), confirming the safety of the coated sutures for clinical use. The in vivo wound healing experiments using Sprague Dawley rats further validated our findings. These results underscore the dual functionality of the sutures: providing mechanical support while delivering therapeutic agents directly to the wound site. The combination of Azadirachta Indica's antimicrobial properties and chitosan's bio adhesive and biocompatible nature offers a synergistic solution that enhances the overall performance of the sutures. This study not only highlights the potential of natural compounds like Azadirachta Indica in biomedical applications but also emphasizes the importance of advanced coating technologies in developing next-generation biomaterials. The successful development of these sutures paves the way for their use in a wide range of surgical procedures, particularly in environments prone to microbial contamination.

In conclusion, Azadirachta Indica/chitosan-coated sutures represent a promising innovation in surgical wound care. Their ability to combine antimicrobial efficacy, controlled drug delivery, and biocompatibility makes them a valuable addition to the arsenal of tools available for improving patient outcomes. Further research and clinical trials are warranted to optimize their performance and facilitate their translation into mainstream medical practice. By bridging traditional knowledge with modern scientific advancements, this work exemplifies the potential of interdisciplinary approaches to revolutionize healthcare solutions.

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