

**EFFECT OF CHITOSAN HYDROGEL-LOADED TURMERIC EXTRACT ON
OXIDATIVE STRESS MARKERS IN DIABETIC WOUNDS IN RATS**

Project Report

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BY

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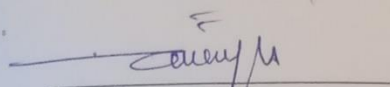
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December, 2025

CERTIFICATION

This is to certify that the research project have been conducted by AHMED, Mariam Wuraola (2010304003) and has been approved as meeting part of the requirements for the award of Bachelor of Science (B.Sc) degree in Biochemistry of Usmanu Danfodiyo University, Sokoto.

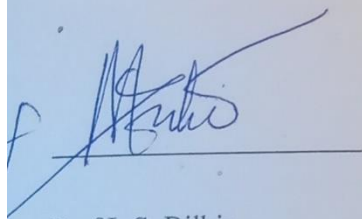


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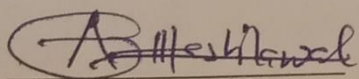


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DEDICATION

This research project is dedicated to Almighty Allah for His unwavering protection and to my beloved father and mother, Mr. and Mrs. Moshood.

ACKNOWLEDGEMENTS

All praise and gratitude are due to Allah (S.W.T), the Exalted, the Lord of heaven and the earth, for His guidance offered during the entire course of this work and my life in general. May His peace and blessings be upon our beloved Prophet Muhammad (S.A.W), his entire companions, and those who follow him till the last day. I extend my heartfelt appreciation to my supervisor, Prof. L.S. Bilbis, for his invaluable guidance and unwavering commitment to ensuring the successful completion of this work. I would love to express my profound appreciation to my co – supervisor Prof S. A. Muhammed, who has been a source of guidance and inspiration towards achieving this milestone, and also for impacting in me the knowledge of data analysis. I pray Allah reward you abundantly. Also, special thanks to Sister Aminah Jiyah for her support and guidance in the project's practical. I also appreciate the efforts of Mr. Gimba Abubakar Yaya for his advice and guidance over the execution of my project and; the same gratitude is extend to Mr. Nasiru Ahmad Alkanchi for his continuous guidance and unwavering support in the process of this project, you are deeply appreciated, sir.

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ABSTRACT

Delayed wound healing is one of the most severe complications of diabetes mellitus with significant resultant morbidity and mortality. It is currently believed that oxidative stress plays a pivotal role in diabetic wound healing. An imbalance of free radicals leads to cell damage, tissue damage and delayed wound healing. This study investigated the effect of chitosan hydrogel loaded with turmeric extract on oxidative stress markers in diabetic wounds in rats. Diabetes was induced in the rats using alloxan, and after confirmation of diabetes by their high blood glucose level of more than 200mg/dl, the wound was created on the dorsal parts of their body. Diabetic rats were grouped into three, each group containing 5 rats. Two other groups served as the controls (not induced with diabetes) and the negative control (untreated). The three groups were treated with turmeric extract, naïve and the loaded hydrogel, respectively. At the end of the treatment, wound tissue was collected for analyses of malondialdehyde (MDA), catalase (CAT), glutathione (GSH), and superoxide dismutase (SOD). Results showed that the chitosan hydrogel loaded turmeric extracts significantly mitigated oxidative stress, evidenced by a marked decrease in MDA levels ($P<0.05$) and an increase in the activities of antioxidant enzymes (CAT, GSH, SOD) compared to the untreated diabetic group ($P<0.05$). Overall, the results demonstrated that chitosan hydrogel loaded with turmeric improved antioxidant status in the wound tissue of diabetic rats, confirming its therapeutic efficacy for wound management.

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CHAPTER ONE

1.0 Introduction

1.1 Background of study

Diabetes mellitus is a chronic disorder defined by impaired insulin secretion or insulin resistance of target tissues, resulting in high blood glucose levels. The prevalence of diabetes mellitus has been steadily rising over the past few decades, and it is estimated that by 2045, more than 700 million individuals will be affected by this metabolic disorder(Armstrong *et al.*, 2023; Matoori *et al.*, 2021). In diabetes mellitus, impaired diabetic wound healing is one of major complications and can have a long-term negative effect on quality of life, morbidity and mortality (Nakamura - Garcia *et al.*, 2022), Impaired diabetic wound healing can lead to distressing consequence or severe complications such as infections, amputations, and substantial reductions in the quality of life for those afflicted (Wang *et al.*, 2023). The primary characteristic of these wounds is increased levels of reactive oxygen species (ROS) due to persistent hyperglycemia, which induce excessive oxidative stress and impair antioxidant capacity (Deng *et al.*, 2021). Oxidative stress occurs when there is an imbalance between antioxidants and pro-oxidants, influenced by factors like disease, drug effects, toxicity, and inflammation. It is thought to play a key role in vascular complications associated with type 1 diabetes (Pham-Huy, 2008). At the cellular level, ROS inhibit fibroblast proliferation, collagen synthesis, and keratinocyte migration processes essential for extracellular matrix formation and re-epithelialization. These disruptions, combined with hyperglycemia-induced immune dysfunction and increased susceptibility to infection, create a cycle of inflammation and tissue damage that significantly delays wound closure in individuals with diabetes.

Oxidative stress markers, such as Superoxide Dismutase (SOD), serve a critical role in cellular defense by converting the highly reactive superoxide radical into hydrogen peroxide and molecular oxygen. Following this reaction, Catalase (CAT) further detoxifies hydrogen peroxide, transforming it into harmless water and oxygen, completing the detoxification pathway. Furthermore, the tripeptide Glutathione (GSH) is a crucial non-enzymatic scavenger, protecting cells from direct ROS attack and regenerating other antioxidants. The resulting oxidative damage to lipids, particularly in cell membranes, is quantified by elevated levels of Malondialdehyde (MDA), a key biomarker of lipid peroxidation. Therefore, the imbalance evidenced by depressed SOD, CAT, and GSH activity alongside increased MDA creates a cytotoxic environment that compromises cellular viability, delays re-epithelialization, and ultimately frustrates effective diabetic wound (Patel *et al.*, 2013). Additionally, the presence of advanced glycation end products (AGEs) and altered extracellular matrix (ECM) components further complicates the healing process. Therefore, despite substantial efforts to develop effective treatments, diabetic wound treatment remains a formidable task for healthcare providers and researchers alike.

Turmeric (*Curcuma longa*) is a widely known plant extract that contains curcumin, which is known as an anti-inflammatory and antioxidant agent (Lestari *et al.*, 2014). The wound healing activity of turmeric can be attributed to its biochemical effects that include its anti-infectious, anti-inflammatory, and anti-oxidant activities. It functions as a potent scavenger, directly neutralizing harmful free radicals and promoting the production of antioxidant enzymes in the wound tissues, thereby protecting essential cells like fibroblasts and keratinocytes from oxidative damage and enhancing the healing of diabetic wounds. Hence, the use of turmeric can be a significant approach in enhancing the impaired healing of diabetic wounds. However, regardless of such distinct

biological activities of curcumin, it suffers from poor bioavailability and stability (Sohn *et al.*, 2021).

Chitosan is a derivative of chitin, a polysaccharide found abundantly in crustacean exoskeletons of crab, shrimps, and lobsters. This naturally occurring polysaccharide, which can form hydrogels are widely exploited for its use in tissue engineering, drug delivery and cell encapsulation (Rajitha *et al.*, 2016), it has been prepared as a biomaterial with a wide range of biomedical and industrial applications because of its biological activities such as biodegradability, biocompatibility, antimicrobial activity, and stimulation of healing (Matica *et al.*, 2019). Chitosan is positively charged and is soluble in aqueous solutions of acids, forming a viscous homogenate. In modern medicine, chitosan attracts researchers due to its film-forming characteristics with unique functional and biomedical properties. Furthermore, it enhances wound healing by averting the accumulation of exudate, promoting gas exchange and drainage of the wound, and improving re-epithelization. In this work, turmeric was incorporated into chitosan to form a hydrogel for the treatment of diabetic wounds in rats, which enables better stability and controlled release. Chitosan nanoparticles prevent the rapid clearance of the drug from the site of inflammation to the systemic circulation due to the vascularity and tissue permeability associated with inflammation. Therefore, this study aims to assess the effect of chitosan hydrogel-loaded turmeric on oxidative stress markers on diabetic wounds in rats.

1.2 Statement of the problem

Diabetic mellitus has reached pandemic proportions globally. As of 2024, about 589 million adults worldwide (aged 20–79) are living with diabetes, according to the 11th edition of the IDF Diabetes Atlas (IDF, 2025). In the African region alone, 24.6 million adults have diabetes, with 3.0 million of them in Nigeria (IDF, 2025). It is a global health challenge with rising prevalence and significant

economic burdens, especially in low-resource settings such as sub-Saharan Africa. Nigeria remains heavily affected, with millions living with diabetes and its associated complications (Adewale *et al.*, 2024). One of the most severe and challenging complications is the development of chronic, non-healing cutaneous ulcer, often known as diabetic wounds or foot ulcers, which is due to delayed healing, infections, and, in most cases, leads to amputations and high mortality (Shi *et al.*, 2024). A major contributor to this is oxidative stress. Chronic hyperglycemia promotes excessive production of reactive oxygen species (ROS), leading to depletion of key antioxidants such as superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), alongside increased malondialdehyde (MDA), a marker of lipid peroxidation and tissue damage (Zhang *et al.*, 2021; Patel *et al.*, 2022). This imbalance disrupts cellular signaling, impairs angiogenesis, prolongs inflammation, and ultimately delays tissue repair. Although various strategies such as microbial infection control, wound offloading, endovascular treatment, moist dressings, and surgical repair are effective for wound healing, slower wound healing has been reported in diabetic wounds (Mehrabani *et al.*, 2017). Turmeric extract has been found to contain antioxidants that can scavenge (ROS) and accelerate wound healing but it is limited by bioavailability and rapid elimination, therefore this study aims to combine chitosan, a natural polymer with turmeric and solve all these limitations.

1.3 Justification of the study

Standard diabetic wound care, including infection control, debridement, pressure off-loading, and maintenance of a moist wound environment, remains the cornerstone of therapy but often fails to achieve complete and lasting healing. Consequently, the urgent need for novel and effective therapeutic strategies has focused attention on natural products, especially plant-derived compounds, which offer immense potential due to their diverse array of bioactive properties.

Among these, curcumin, the active component of turmeric, demonstrates antioxidant, anti-inflammatory, and antimicrobial effects, making it a promising candidate for managing diabetic wound complications (Sideek *et al.*, 2023). However, turmeric's clinical application is limited by poor bioavailability, resulting from low solubility, rapid metabolism, and elimination. Chitosan-based delivery systems have emerged as an effective solution; chitosan hydrogels protect turmeric from degradation, enhance solubility, improve absorption, and provide sustained release at the wound site (Emiroglu *et al.*, 2017; Ibrahim *et al.*, 2018). Beyond improving bioavailability, chitosan itself exhibits wound-healing properties, including biocompatibility, antimicrobial activity, and the ability to stimulate tissue regeneration and angiogenesis (Riaz *et al.*, 2022). These multifunctional systems offer a promising strategy to overcome the limitations of standard care, combining the antioxidant and anti-inflammatory effects of turmeric with the unique wound-healing advantages of chitosan.

1.4 Aim and objectives

1.4.1 Aim

To assess the effect of chitosan hydrogel loaded turmeric extract on markers of oxidative stress in diabetic wounds in rats.

1.4.2 Objectives

1. To determine the antioxidants status of diabetic wounds in rats treated with chitosan hydrogel loaded turmeric extracts.
2. To assess the level of MDA in diabetic wounds in rats treated with chitosan hydrogel loaded turmeric extracts.

CHAPTER TWO

2.0 Literature Review

2.1 Overview of Diabetes Mellitus

Diabetes mellitus (DM) is a chronic metabolic disease characterized by elevated blood glucose caused by a deficiency or resistance to insulin (Atkinson *et al.*, 2014). Chronic hyperglycemia can lead to multiple organ injury, thereby causing various complications, such as diabetic retinopathy (DR), diabetic kidney disease (DKD), and diabetic cardiovascular disorders (Zheng *et al.* 2018). Epidemiological studies have revealed that DM has emerged as a significant threat to human mortality. At present, the International Diabetes Federation (IDF) estimates that DM affects approximately 536.6 million adults worldwide, and that number is expected to increase to 783.2 million by 2045 (Sun *et al.*, 2022). In addition to its high incidence, the pathogenesis of diabetic complications is also very complex. In the early stages of DM, hyperglycemia induces oxidative stress and excessive advanced glycation end product (AGE) formation (Domingueti *et al.* 2016). As the disease progresses, protein glycation and mitochondrial DNA (mtDNA) damage to respiratory chain components can, in turn, exacerbate oxidative stress injury (Bhatti *et al.*, 2022). Hyperglycemia and its associated carbohydrate, fat, and protein metabolic dysfunctions affect multiple organs of the body and disrupt their normal functioning. These disruptions progress gradually and arise mostly due to the adverse effects of hyperglycemia and its associated metabolic anomalies on the normal structure and functioning of micro- and macrovasculature, which lie at the core of organ structure and function throughout the body. The structural and functional disruptions in the organ system vasculature led to micro- and macrovascular complications. Organ damage, dysfunction, and, ultimately, organ failure characterize these complications and affect body organs, which include, in particular, the skin, eyes, kidneys, heart, and nerves. Eye-related

complications result in retinopathy with progression to blindness. Kidney-associated complications lead to nephropathy and potential renal failure. Heart-related complications include hypertension and coronary heart disease. Nerve-associated complications lead to neuropathy, which can be autonomic and/or peripheral. Cardiovascular, gastrointestinal, and genitourinary (including sexual) dysfunctions are characteristic manifestations of autonomic neuropathy, whereas foot infections, including ulcers requiring amputation and in most cases lead to mortality.

2.2 Wound Healing

Wound healing is an intricate process of tissue repair or remodeling that occurs in response to injury (Kumari *et al.*, 2022). The primary requirement of wound treatment is to achieve healing quickly and completely without diffuse infection and sepsis. According to the situation of wound healing, wound healing can be divided into chronic and acute. Acute wounds heal as expected and undergo a normal, orderly, and timely repair process, thereby continuing to restore the integrity of anatomical and functional (Singh *et al.*, 2013). The healing process can involve various cells and is divided into four overlapping stages, including hemostasis, inflammation, proliferation, and remodeling as shown in figure 1. The acute wound healing process starts with hemostasis to prevent blood loss and microbial invasion of the wound, and then enters the inflammation stage. At this stage, proinflammatory cells and neutrophils are upregulated, and pathogens, debris, growth factors, and other cytokines are eliminated by macrophages. The proliferative phase overlaps with the inflammation phase. In the inflammatory phase, the damaged area is activated and filled with new tissues, new blood vessels, and matrix structures. In the final remodeling phase, the extracellular matrix's tensile strength increases, and the blood supply in the damaged area decreases (Singh *et al.*, 2013; Dolp *et al.*, 2019). Chronic wound trauma can be defined as continuous intervention on any tissue, but it takes a long time to heal or may even be non-healing

or recurrent (Singh *et al.*, 2013). Many factors, such as chronic diseases, vascular insufficiency (Zhao *et al.*, 2016), diabetes, malnutrition, aging, and other local factors such as stress, infection, and edema (Zhao *et al.*, 2016), can lead to delayed wound healing. Diabetes-related peripheral neuropathy coupled with interruption of perfusion can easily lead to poor structure of the foot, loss of consciousness, and an increased risk of ulcers due to repeated mechanical stress. Moreover, patients with diabetic ulcers are prone to complications, including functional limitations, difficulty walking, and infections such as cellulitis, abscesses, osteomyelitis, gangrene, and sepsis.

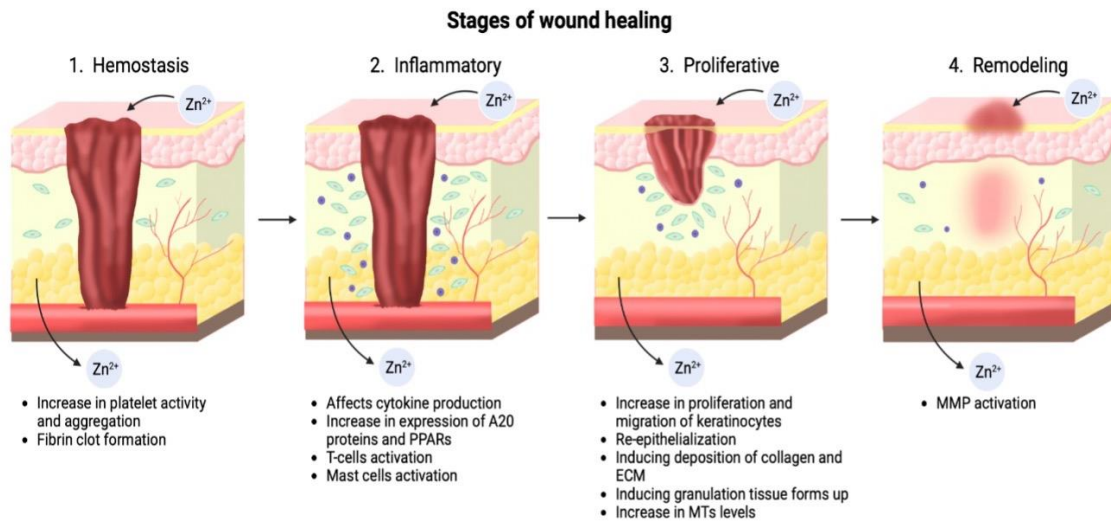


Figure 1: Stages of wound healing (Lebedeva *et al.*, 2023)

Individuals with diabetes demonstrate disturbances in all four individual healing stages that collectively lead to an overall impaired wound healing predisposing to chronic non-healing wounds such as diabetic foot ulcers (Avishai *et al.*, 2017).

2.3 Oxidative stress

The term oxidative stress in relation to biological systems appeared for the first time in the scientific literature in the early 70's (Azzi, 2022). The concept of oxidative stress was first defined by Prof. Sies in 1985 (Sies 1985, 2018, 2020). Oxidative stress is an imbalance between reactive oxygen species (ROS) and antioxidant defense mechanisms in the body. Chemically, ROS is a byproduct formed from hydrogen peroxide (H_2O_2), oxygen (O_2), and water (H_2O) (Marrocco *et al.*, 2017; Zhong *et al.*, 2018). In the human body, endogenous ROS are produced mainly from mitochondria as a byproduct of mitochondrial respiration (Sarniak *et al.*, 2016). Excessive ROS in the body depletes cellular antioxidant defenses, leading to oxidative stress. This dysregulation of redox homeostasis has been linked to the pathogenesis of aging and various diseases, including diabetes (Liguori *et al.*, 2018). diabetes mellitus is a significant global health challenge, and its prevalence is increasing worldwide (Standl *et al.*, 2017; Zheng *et al.*, 2018). Studies have shown that oxidative stress is a key contributor to the pathogenesis and progression of diabetes and its associated complications, such as impaired wound healing (Jung *et al.*, 2020).

2.4 Oxidative stress impairs diabetic wound healing

Several pathological mechanisms contribute to the accumulation of ROS in the body, leading to excess oxidative stress. These include increased mitochondrial superoxide production, formation of advanced glycation end-products, increased activity of ROS-generating enzymes such as NADPH oxidase, and decreased expression of antioxidant enzymes such as CAT, catalase, GSH, glutathione, and SOD, superoxide dismutase. Although all of them are secondary to hyperglycemia. Hyperglycemia induces a marked increase in intracellular ROS generation, which in turn activates metabolic pathways such as the polyol pathway and PKC signaling, leading to the depletion of reducing equivalents and suppression of endogenous antioxidant enzymes and compounds (e.g., glutathione). In addition, acute elevations in serum glucose and chronic

accumulation of advanced glycation end products (AGEs) further intensify oxidative stress via the AGE–RAGE axis, fueling ROS production (Liu *et al.*, 2023; Chen *et al.*, 2024). AGEs act as potent pro-oxidants and pro-inflammatory mediators, contributing to cellular dysfunction, collagen cross-linking, and impairments in fibroblast survival, all of which are risk factors for tissue injury and the persistence of chronic ulcers (Liu *et al.*, 2023; Jin *et al.*, 2024). In diabetic wounds, redox homeostasis is compromised by chronic ROS overproduction leading to redox imbalance as shown in figure 2, this exhausts antioxidant defenses and induces widespread oxidative damage in cells, ultimately impairing wound-healing processes (Wan *et al.*, 2022).

The impacts of oxidative stress on the individual phase of wound healing are summarized as follows.

In the inflammation phase, oxidative stress in wounds influences the normal function of macrophages and neutrophils, resulting in prolonged inflammation. The increased number of neutrophils results in the production of ROS and excessive damage to the surrounding cells, tissues, and fibroblasts (Park *et al.*, 2018). It also influences macrophage differentiation and polarization, impairing wound healing. Diabetes induces pathological hematopoietic stem cells (HSCs), oxidant stress that can reduce the number and function of terminally differentiated inflammatory cells. Yan *et al.*, (2018) reported that diabetes induces oxidant stress in HSC through a Nox-2-dependent mechanism and decreases microRNA let-7d-3p, which, in turn, upregulates the expression of Dnmt1. Increased Dnmt1 expression results in the downregulation of the genes responsible for HSC differentiation to monocytes/macrophages, and consequently reduces macrophage infiltration, driving polarization toward M1 macrophages (pro-inflammatory function), which causes excessive and prolonged inflammation in murine diabetic wounds

(Khanna *et al.*, 2010; Gallagher *et al.*, 2015). Moreover, diabetic fibrocytes show a pro-inflammatory phenotype, which also contributes to arresting the process at the inflammatory.

In the migration-proliferation phase, oxidative stress exerts its effects on endothelial cells, keratinocytes, and fibroblasts, resulting in endothelial dysfunction, abnormal keratinocyte migration and proliferation, as well as impaired fibroblast proliferation, migration, and differentiation. Oxidative stress is commonly implicated as an important unifying mechanism in endothelial dysfunction, which underlies both the micro- and macrovascular complications of diabetes (Rochette *et al.*, 2014). It is a cell damage-related factor that causes damage to proteins, lipids, and DNA in the cells, leading to cell death and subsequent tissue dysfunction (Long *et al.*, 2016). Oxidative stress alters the functional capacity of endothelial nitric oxide synthase (eNOS) and directly degrades vasoprotective nitric oxide (NO) by ROS, resulting in diminished bioavailability of NO and endothelial dysfunction. Oxidative stress also impairs keratinocytes. High-glucose environments disturb keratinocyte function, including migration and proliferation. Gap junction abnormalities increase oxidative stress (Hu and Lan, 2016). Higher oxidative stress is associated with increased production of Interleukin (IL)-8 in high glucose-treated keratinocytes, which is responsible for recruiting neutrophils and impaired wound healing. It contributes greatly to antiangiogenic molecule thrombospondin-1 (TSP1) DNA hypomethylation in keratinocytes exposed to hyperglycemia, resulting in overexpression of TSP1 that impairs proper wound healing (Lan *et al.*, 2016).

In the remodeling phase, oxidative stress impairs collagen deposition and ECM remodeling. Delayed diabetic wound healing is characterized by an increase in matrix metalloproteinase (MMPs) and a reduction in some growth factors, such as TGF- β 1 (Long *et al.*, 2016). TGF- β 1 signaling works as the regulator in a series of biological processes of skin regeneration and wound

healing, such as angiogenesis and re-epithelialization. High levels of ROS impair collagen production and tissue growth, resulting in halted wound closure (Zhang *et al.*, 2021). Interference with ECM production is highlighted through the disruption of TGF- β 1 signaling (He *et al.*, 2014). High ROS levels affect ECM remodeling by regulating MMP expression, which remodels tissue through proteolytic degradation (Caley *et al.*, 2015). Altogether, the impact of oxidative stress induced by hyperglycemia is widespread, impairing the majority of cells and mechanisms involved in wound healing.

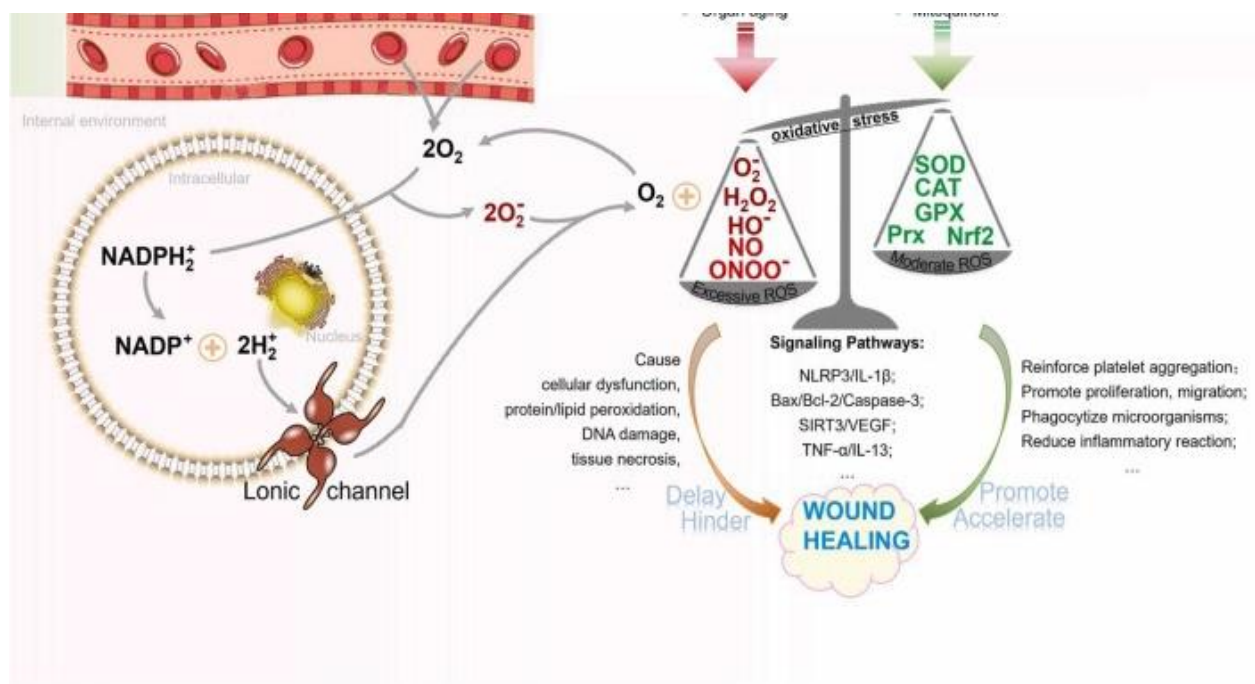


Figure 2: Detrimental effect of oxidative stress on wound healing (Wang *et al.*, 2023)

2.5 Oxidative stress markers

2.5.1 MDA

Malondialdehyde is a marker for oxidative stress that is significantly elevated in diabetic wound healing, indicating tissue damage resulting from an imbalance between free radicals and antioxidants (Bhatti *et al.*, 2022). The chronic hyperglycemia and dyslipidemia characteristic of Diabetes Mellitus (DM) create a state of heightened oxidative stress, making cellular polyunsaturated fatty acids (PUFAs) in membranes highly susceptible to lipid peroxidation, a critical biomarker of oxidative stress due to excessive Reactive Oxygen Species (ROS). This chain reaction of membrane damage is quantified through the primary breakdown product, Malondialdehyde (MDA), a highly reactive dialdehyde that is the most widely studied and utilized biomarker of lipid peroxidation. Increased MDA levels are consistently reported in diabetic patients compared to healthy controls, showing a significant positive correlation with indicators of poor glycemic control (Nyamadzawo *et al.*, 2025). MDA is measured using the Thiobarbituric Acid Reactive Substances (TBARS) assay, where MDA reacts with two molecules of Thiobarbituric acid (TBA) under acidic conditions and heat to form a pink-colored chromogen (MDA-TBA adduct) that is subsequently quantified spectrophotometrically at a wavelength of 532nm (Ayala *et al.*, 2014). Crucially, as a toxic aldehyde, MDA covalently modifies critical macromolecules, forming adducts that disrupt protein and DNA function, thus playing a key pathological role that contributes directly to the development and progression of microvascular complications, particularly diabetic retinopathy, and macrovascular diseases like atherosclerosis (Sunita *et al.*, 2020).

2.5.2 GSH

Glutathione (GSH) is a ubiquitous tripeptide that is biosynthesized *in situ* at high concentrations (1–5 mM) and is involved in the regulation of cellular homeostasis via multiple mechanisms. The main known action of GSH is its antioxidant capacity, which aids in maintaining the redox cycle of cells (Georgiou-Siafis & Tsiftoglou, 2023). The ratio of reduced GSH to GSSG is an indicator of cellular oxidative stress levels (S *et al.*, 2024). Glutathione disulfide is an important redox couple in cells, and its deficiency causes oxidative stress in the body. Hence, GSH is involved in the pathogenesis of various diseases (Wu *et al.*, 2004). GSH peroxidases and GSH reductases contribute to the scavenging of various forms of ROS. In diabetes, chronic hyperglycemia increases reactive oxygen species (ROS), leading to oxidative stress and disrupting the activities of GPx and GR (Caturano *et al.*, 2023). This impaired glutathione system is directly relevant to delayed diabetic wound healing, as excess ROS damages fibroblasts and keratinocytes, interferes with collagen synthesis, and suppresses angiogenesis processes essential for wound closure (Zhang *et al.*, 2021). Consequently, diminished GPx/GR activity and lower GSH availability create a sustained oxidative environment in diabetic wounds, slowing tissue repair and promoting chronic, non-healing ulcers (Wang *et al.*, 2025).

2.5.3 Catalase

Catalase is an antioxidant enzyme present in nearly all living organisms. It plays a vital role in cellular defense mechanisms by mitigating oxidative stress caused by reactive oxygen species (ROS). It exhibits one of the highest catalytic efficiencies among enzymes, with a single catalase molecule converting millions of H₂O₂ molecules into water and oxygen every second. In mammals, it is predominantly localized in peroxisomes, but it is also found in cytosolic and mitochondrial compartments, where it functions in concert with superoxide dismutase (SOD) and glutathione peroxidase (GPx) to ensure the efficient clearance of reactive oxygen species (ROS).

(Alhumaydhi *et al.*, 2025) In case of catalase deficiency, β -cells of the pancreas that contain a large number of mitochondria, undergo oxidative stress by producing excess ROS, which leads to β -cells dysfunction and ultimately diabetes. While investigating hyperglycemia-induced functional changes, hydrogen peroxide production, superoxide, mitochondrial membrane polarization, and gene expression fingerprints of related enzymes in endothelial cells suggest that hyperglycemia increased hydrogen peroxide production and down-regulated CAT gene expression (Patel *et al.*, 2013)

2.5.4 SOD

Superoxide dismutase is an antioxidant enzyme involved in oxidative stress. superoxide dismutase provides the first line of defense against ROS-mediated cell injury by catalyzing the disproportionation of superoxide, the primary ROS in oxygen metabolism, to molecular oxygen and peroxide. We can say that superoxide is dismutated to other compounds that are less toxic by SODs (Tiwari *et al.*, 2013). A decline in the level of superoxide dismutase (SOD) in both diabetic tissues and blood has been documented in recent studies. For example, a cross-sectional study found significantly lower serum SOD activity in patients with type 2 diabetes and acute coronary syndrome, suggesting that reduced SOD is associated with cardiovascular dysfunction in diabetes (Zhang *et al.*, 2024). Moreover, in diabetic nephropathy, researchers reported decreased serum SOD and catalase levels compared with diabetic controls, highlighting oxidative stress as a key contributor to microvascular complications (Ahmad *et al.*, 2023). Specifically, extracellular SOD (SOD3, also called EC-SOD) is reduced: patients with recent-onset diabetes who developed sensorimotor polyneuropathy had lower circulating SOD3, indicating impaired extracellular antioxidant defense (Strom *et al.*, 2017).

2.6 Natural plants

Natural products such as plants have been used in traditional medicine across various cultures since ancient times. These naturally derived therapies have long served in the prevention and treatment of many diseases and have been shown to regulate different metabolic pathways (Kant *et al.*, 2015). The use of plant medicines in Africa represents a long history of human interactions with the environment (Kunnumakkara *et al.*, 2017)

2.6.1 Turmeric

Turmeric or *Curcuma longa* is an herbaceous plant of the family Zingiberaceae that has been considered an important therapeutic agent in Indian and Chinese traditional medicine; it is mainly cultivated in tropical and subtropical regions (Prasad *et al.*, 2014). Turmeric contains 69.4% carbohydrates, 6.3% protein, 5.1% fat, 5.8% essential oils, and 3–6% of curcuminoids (Amalraj *et al.*, 2016), fig 3 shows the main curcuminoids in turmeric which are $\approx 77\%$ curcumin (curcumin I), $\approx 17\%$ demethoxycurcumin (curcumin II), $\approx 3\%$ bis-demethoxycurcumin (curcumin III), and cyclocurcumin (curcumin IV). Curcumin, the bioactive compound of turmeric, has been reported to have several properties, such as being an antioxidant, anti-inflammatory, anti-microbial, and safe in its pharmacological profile (Kunnumakkara *et al.*, 2017), which may have utility for treating diabetes complications. In its application to diabetic wound treatment, the study shows curcumin stimulates angiogenesis, leading to the formation of new blood vessels and facilitating the transport of vital nutrients and oxygen to the wound site. Additionally, curcumin fostered cell proliferation, generating new cells and boosting collagen production, the chief protein component of the skin, at the wound site

Despite its biocompatibility and non-toxicity, the biomedical application of curcumin is limited by its poor water solubility, and its absorption is limited in the gastrointestinal tract. To overcome this

limitation, curcumin has been incorporated into polymer-based materials (Alvens *et al.*, 2021). The in vivo findings on diabetic mice demonstrated that using the curcumin hydrogel was more effective than the control hydrogel. The curcumin hydrogel notably reduced wound size, inducing new blood vessel formation and augmented collagen formation at the wound site (Liu *et al.* , 2018).

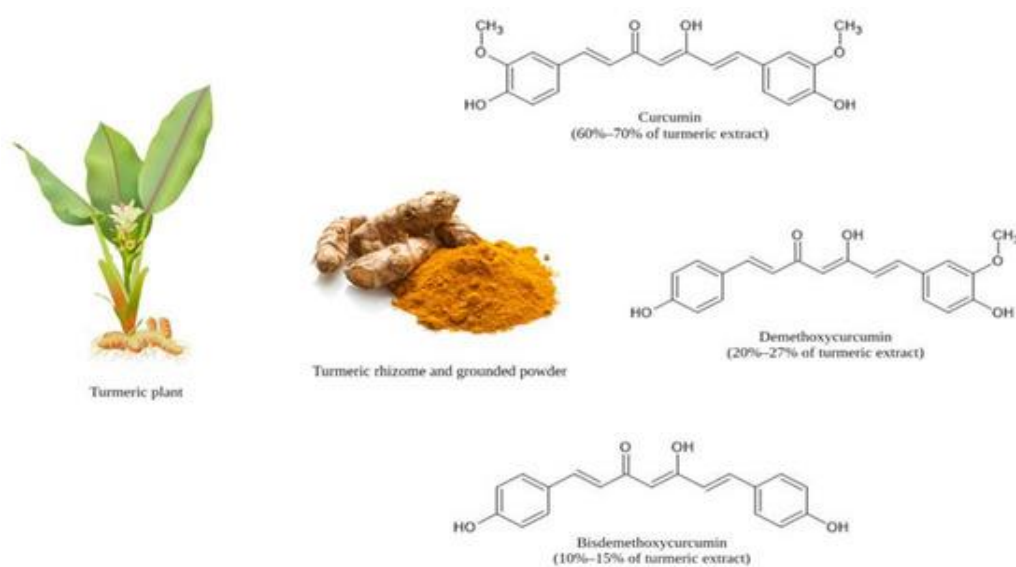


Figure 3. The turmeric (*Curcuma longa*) plant and rhizome, and the chemical structures of the curcuminoids, including curcumin, demethoxycurcumin, and bisdemethoxycurcumin. Figure adapted and modified from (Nelson *et al.*, 2017).

2.7.1 Chemical modification of chitosan

The Schiff base reaction of chitosan involves a condensation reaction of the amino groups on chitosan with carbonyl compounds (such as fatty aldehydes, aromatic aldehydes, and ketones) in a neutral medium to remove water molecules and synthesize the Schiff base with imine group characteristics (Wei *et al.*, 2019). Commonly used solvent media for the chitosan Schiff base

reaction include acetic acid, ethanol, and methanol, or their mixtures. On the one hand, Schiff base modification of chitosan alters the molecular structure of the chitosan molecular chain and increases the number of positively charged ions, thereby significantly enhancing the water solubility and antibacterial activity of chitosan. Schiff base modification of chitosan is, therefore, widely used to enhance the antibacterial properties of chitosan. On the other hand, the Schiff base reaction of chitosan is often used to protect amino groups on chitosan, so modification only occurs on the hydroxyl groups. The chemically modified chitosan has extensive biological activities, such as antibacterial, antitumor, and antioxidation properties, and can be used as a catalyst (Tamer *et al.*, 2016).

2.6.2 Antioxidant properties of Turmeric

The impairment of wound healing in diabetes is critically linked to an environment of intense oxidative stress, where excessive Reactive Oxygen Species (ROS) production overwhelms the tissue's natural defenses, causing cellular damage and arresting the normal repair process (Akbarzadeh *et al.*, 2018). The active compound in turmeric, curcumin, possesses notable antioxidant properties and provides a powerful therapeutic intervention by acting as a multifaceted antioxidant. Firstly, it functions as a potent scavenger, directly neutralizing harmful free radicals in the wound bed, thereby protecting essential cells like fibroblasts and keratinocytes from oxidative damage (Kebede *et al.*, 2021). Secondly, curcumin enhances the endogenous antioxidant system by activating the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway (Ashrafizadeh *et al.*, 2020). This activation promotes the transcription of genes for key antioxidant enzymes, including superoxide dismutase (SOD) and glutathione peroxidase (GPx), reinforcing the tissue's long-term defense capacity. By mitigating this oxidative burden, curcumin simultaneously suppresses the closely linked chronic inflammation, creating a more favorable microenvironment

that ultimately supports accelerated wound closure, collagen synthesis, and effective tissue regeneration (Liu *et al.*, 2018).

2.6.3 Anti-Inflammatory Activity

Inflammation is a complex response in the body that is necessary for wound healing. It is the natural way for the body to respond to an injury or infection, and it plays a fundamental role in the early stages of tissue repair. However, excessive inflammation can cause tissue damage and chronic diseases, which makes it crucial to control inflammation (Deng *et al.*, 2021). Curcumin possesses potent anti-inflammatory properties. One of the mechanisms by which curcumin exerts its anti-inflammatory effects is by reducing the production of several inflammatory cytokines, such as interleukin (IL)-1, IL-6, IL-8, and tumor necrosis factor-alpha (TNF- α), which are small proteins that play a vital role in the inflammatory response. Specifically, curcumin reduces the production of two critical cytokines, TNF- α and IL-1, which are generated by monocytes and macrophages and are known to trigger inflammation (Peng *et al.*, 2021). Curcumin inhibits the activity of NF-(κ)B, a transcription factor that plays a critical role in initiating inflammatory responses. Normally, NF-(κ)B is activated by a variety of kinases, including AKT, PI3K, and IKK. By inhibiting NF-(κ)B, curcumin may help reduce the inflammatory processes in the body (Merrell *et al.*, 2009). Besides its effects on NF-(κ)B, curcumin also acts through other signaling pathways to combat inflammation. For example, curcumin can enhance PPAR- γ activity by hindering vascular smooth muscle cell proliferation to decrease angiotensin II-induced inflammation (Antoine *et al.*, 2015; Wang *et al.*, 2015). In addition, it has also been demonstrated to impede the TLR4-MD2 signaling complex by taking over LPS for MD2 binding to reduce inflammation (Zhang *et al.*, 2018).

2.7 Natural polymers

Natural polymers, also known as biopolymers, are large molecules that occur in nature and are produced by living organisms. When these polymers are crosslinked in the presence of water, they form hydrogels. Hydrogels possess network structures in three dimensions (3D) composed of cross-linked polymer chains, enabling them to absorb substantial amounts of wound exudate. These hydrogels are distinguished by their elevated water content, soft composition, and porosity (Ho *et al.*, 2022; Brumberg *et al.*, 2020), exhibiting considerable potential as dressings for skin repair in recent years. Chitosan as seen in fig 5 is the product of the chemical treatment of the deacetylation of chitin. It offers a variety of physiological functions, including biodegradability, biocompatibility, non-toxicity, bacteriostatic properties, anticancer potential, lipid-lowering capabilities, immune-enhancement effects, etc. Additionally, chitosan's exceptional properties, such as its anticoagulant features and wound healing promotion, have led to its extensive employment in medical dressings (Ogawa *et al.*, 2004; Hamed *et al.*, 2018). Chitosan hydrogels are considered excellent materials for wound dressing due to their biodegradable, biocompatible, and antimicrobial properties, and they hold great potential for clinical application in the treatment of skin wounds (Desai *et al.*, 2023).

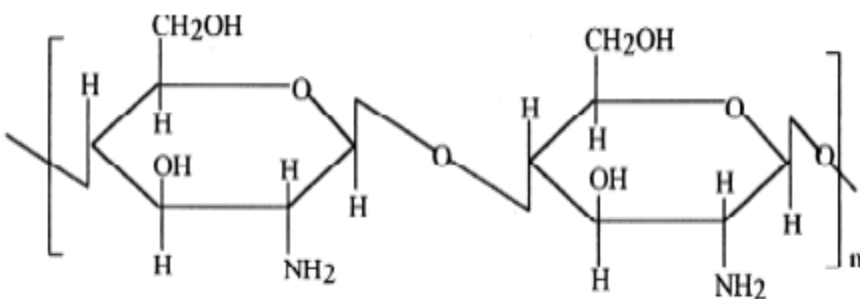


Figure 4: Chemical structure of chitosan (Sharma *et al.*, 2022)

2.7.2 Antioxidants activity of chitosan

Free radicals, which have extremely high oxidative activity and are extremely unstable, can attack cell membranes, mitochondrial membranes, and unsaturated fatty acids in membranes, thus resulting in enhanced lipid peroxidation. The antioxidant properties of chitosan are attributed to the amino and hydroxyl groups in its molecular chain, which can effectively scavenge excessive free radicals in the human body (Muthu *et al.*, 2021). In addition, it can significantly reduce serum-free fatty acid and malondialdehyde concentrations and increase antioxidant enzyme activities, such as superoxide dismutase, catalase enzyme activities, etc., thereby scavenging free radicals. Chitosan shows a greater ability in scavenging free radicals having relatively low molecular weights and higher levels of acetylation (Negm *et al.*, 2020). Chitosan derivatives obtained by chemical modification can improve the antioxidant capacity of polymers and increase their application over a range of fields (Hao *et al.*, 2021).

2.8 Chitosan-loaded turmeric hydrogel

Topical application of curcumin-loaded hydrogels has been identified as an effective approach for treating chronic wounds. It solves the problem of bioavailability and rapid elimination of the turmeric drug by ensuring sustained release and delivery to the wound site. Li *et al.* loaded curcumin in a nanocarrier and incorporated it into the N, O-carboxymethyl chitosan/oxidized alginate hydrogel. The hydrogel was subsequently applied in the full-thickness animal model, and the results showed significantly accelerated wound healing with complete wound closure within 14 days (Li *et al.*, 2012). In another study, Gong *et al.* incorporated curcumin-loaded micelles into a hydrogel system, which showed strong tissue adhesion and prolonged curcumin release (Gong *et al.*, 2013). An *in vivo* animal study with the application of the hydrogel demonstrated accelerated wound healing by stimulating angiogenesis, improving collagen organization, and promoting granulation tissue formation (Gong *et al.*, 2013). A recent study developed a hydrogel by loading

chitosan, Pluronic, and curcumin to create a single-loaded formulation. These findings indicated that the curcumin-loaded hydrogel exhibited enhanced water absorption capacity. Additionally, the curcumin release profile demonstrated a more sustained release from the chitosan-loaded hydrogel. In vivo studies further revealed that the application of the curcumin-loaded hydrogel significantly accelerated wound healing compared to untreated wounds (Pham *et al.*, 2019). Furthermore, Mahmood *et al.*, (2022) developed a curcumin-loaded self-emulsifying drug delivery system and found that it dramatically accelerated wound healing and re-epithelialization compared to the untreated and purely drug-treated groups in an in vivo diabetic wound model (Wu *et al.*, 2023). Chitosan nanoparticles act as penetration enhancers of curcumin by opening the narrow boundaries in the epithelium. The transport of chitosan nanoparticles is through the transcellular transport (inside cells) and the paracellular transport (in the gap between cells). This causes curcumin to work according to the desired location (Francis *et al.*, 2019). Chitosan has mucoadhesive properties that able to do adhesion with mucus and release curcumin from time to time. These properties are obtained because chitosan is cationic, and the mucous layer is negatively charged, which leads to electrostatic attraction. This sensitivity controls the release of curcumin in nanoparticles, which increases the bioavailability of curcumin (Matalqah *et al.*, 2020).

CHAPTER THREE

3.0 Materials and Methods

3.1 Apparatus

Table 1: List of Apparatus

Equipment	Specification	Manufacturer
Test tube	Glass	Pyrex England
Weighing balance	Mettler P 163	Gallen Kamp, UK
Round-bottom flask	250/500ml	Pyrex glass, UK
Measuring cylinder		Pyrex glass, UK
Filter paper		Whatman
Spectrophotometer	721A/ AE350	ERMA INC
Vacuum drying oven	DZF-6021	JINGHONG
Micro pipette		H. Chandan mallard and Co.
Funnel	Plastic	Yuyaoshouhong Co. Ltd
Spatula	Metal	Bausch and lomp
Foil paper		Superman foil
Conical flask	Glass ware	Pyres glass England
Water bath	SB-1100	EYELA
PH meter	Hanna HI 2211	Hanna Instruments, Romania
Magnetic stirrer		B&T
Rotary evaporator		EYELA
Musin cloth		BCH LAB
Beaker		BCH LAB
Centrifuge machine		HANIL

3.1.1 Reagents

Table 2: List of Reagent

Reagents	Specification	Manufacturer
Potassium phosphate buffer	Analytical grade	LOBA CHEMIE
Hydrogen peroxide		MOLYCHEM
Sodium chloride		LAB TECH CHEMICAL
Acetic acid		LOBA CHEMIE
Thiobarbituric acid		KERMEL
Trichloroacetic acid (20%)		KERMEL
Glutathione		ELISA KIT
Distilled water		BCH LAB
Buffer		EAST ANGLIA CHEM
Methanol (95% v/v)		BCH LAB
Pyrogallol		LOBA CHEMIE
Alloxan		GANGDONG SCI. TECH
Metaphosphoric acid		SIGMA Aldrich
Sodium hydroxide		MOLYCHEM
Sodium dihydrogen		MOLYCHEM
orthophosphate		MOLYCHEM
Disodium hydrogen		Merck Chemicals, Germany
orthophosphate		BDH Laboratory Supplies, UK
Phosphate buffer saline		
Ketamine		

3.2 Sample collection and preparation of extracts

Fresh turmeric was bought from Maggi market, Sokoto state, Nigeria. It was cleaned by washing, and then peeled to remove the skin, removing. After peeling it was dried in the shade for two days, after which it was ground to semi-powder. A quantity (220g) of the semi powdered turmeric was dissolved in 1000 ml 95% v/v methanol and stored for 48 hours. After the time elapsed it was filtered with muslin cloth and Whatman filter papers, and transferred to a rotary evaporator for concentration under reduced pressure 70-75°C to remove excess methanol and allowed to dry in a drying oven at 104°C. The % of extract yield was calculated using the formula:

$$\% \text{yield} = \frac{W_3 - W_1}{W_2} \times 100$$

Where

W_3 = weight of sample + beaker

W_2 = weight of sample

W_1 = weight of beaker after drying

3.3 Preparation of hydrogel

The chitosan hydrogel is prepared by dissolving 0.3g of chitosan in 250 mL of acetic acid and allowing the mixture to stir on a magnetic plate for 12 hours to ensure complete dissolution and form a uniform, acidic polymer and a pale-yellow solution. The cross-linking process is then initiated by the controlled addition of 0.5 mL of a glutaraldehyde cross-linker to this solution and allowed to stir for 30 minutes at room temperature. Finally, the entire system is neutralized to a pH of 7 using NaOH, which stabilizes the hydrogel structure by making the chitosan insoluble and

removing the residual acidity. 30 mg of turmeric extract was dissolved in 2ml of methanol and added to the crosslinked gel to form the chitosan-loaded turmeric hydrogel.

3.4 Animal study

3.4.1 Methodology

Experimental Animals

Following acceptance from the Animal Ethical Committee at Usmanu Danfodiyo University, thirty healthy rats were purchased from the faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria, and were fed to maturity state. Weighing between 100 and 190g was used for the Study. They were housed at the animal house of the department of Biochemistry, Usmanu Danfodiyo University, Sokoto, for six weeks before the commencement of the experiment. During this period, they were fed on grower mash, a product of GCOM Bukura Jos, Nigeria, and allowed access to clean water ad libitum

The rats were divided into six groups and six per group as follows.

Experimental Design and Grouping

Group A: Control + normal saline

Group B: Diabetic untreated + normal saline

Group C: Diabetic + Naïve Chitosan

Group D: Diabetic + turmeric extract

Group E: Diabetic + Chitosan-Turmeric Hydrogel

3.4.2 Induction of diabetes with alloxan

All rats were put to fast for 12 hours before injection, maintaining access to drinking water. Diabetes mellitus was induced in overnight fasted rats by administering 130mg/kg of Alloxan Monohydrate dissolved in cold sterile saline/buffer via a single Intraperitoneal (IP) injection (Masiello *et al.*, 1998). The rats' blood glucose levels were measured at 24, 48, and 72 hours, respectively, after induction (Furman, 2015). Only rats exhibiting a Fasting Blood Glucose of 200 mg/dL were selected for the wound healing study. (Altan *et al.*, 2018).

3.4.3 Measure of blood glucose levels

Blood samples were withdrawn from the tail vein of rats by cutting the tip of the tail using scissors in all rats. To prevent infection at the tip of the tail, 70% ethanol using cotton will be applied during each measurement. BGL was measured using a glucometer and glucose standard strip/kits (fine test strip, Germany).

3.4.4 Wound Creation

Rats were anesthetized with an intraperitoneal injection of 25 mg/kg of ketamine (Ketanest; Pfizer Inc, New York, NY) before creation of the skin injury. The rats under anesthesia were placed face down on the dissection pad, with their backs exposed. Before commencing the surgery, the dorsal region of the anesthetized animals was shaved and cleaned using 70% ethanol. An excision-type wounds were created on the dorsal interscapular region of the rats in a circular manner (1.5 cm in diameter) by removing the skin using scissors and a scalpel, then the wounds were left open. After which , each rat group will be kept in separate cages to prevent cannibalism and disturbance.

3.4.5 Treatment of Wounds

Based on the groupings, each rat received treatment for 14 days. The application of the treatment on the wounds commenced from the day at which the wound was created (day 0) and continued daily for fourteen consecutive days at the same time (12 pm). The wounds were cleansed with sterile gauze soaked with saline solution before placing the treatments (0.1 gm) above the wound. Throughout the experiment, the wounds were left uncovered (Panda *et al.*, 2021)

3.5 Preparation of Tissue Homogenate

Wound samples from rats were separately put on ice and rinsed with ice-cold isotonic saline. A (10% w/v) homogenate was prepared in 0.1M phosphate buffer (pH 7.4). The homogenate was centrifuged at 3000 rpm for 30 minutes. The aliquots of supernatant were separated and used for biochemical assay.

3.6 Biochemical assay for oxidative stress markers

3.6.1 Malondialdehyde

Principle

One molecule of MDA in the tissue homogenate reacts with two molecules of Thiobarbituric acid in an acidic medium and gives rise to a pink colored complex, which is measured at 532nm with the aid of a spectrophotometer.

Table 3:Procedure for MDA

REAGENTS	Test (mL)	Blank (mL)
TCA 20%	1.8	2
Plasma	0.2	
TBA 0.67%	0.5	

The sample was kept in boiling water for 10 minutes, then cool and centrifuge at 2000 rpm for 30 minutes after which the absorbance of the supernatant was measured at 532nm against the blank reagent. MDA was calculated from the molar extinction coefficient, concentration of MDA, and expressed in g/tissue.

$$\text{.nmole/g} = \frac{\text{Absorbance} \times 10^5}{1.56}$$

3.6.2 Determination of superoxide dismutase

Principle

The principle of superoxide dismutase is based on the competition between pyrogallol autoxidation and by superoxide dismutation of these radicals by SOD.

Table 4: Procedure for SOD

REAGENTS	Test (mL)	Blank (mL)
Buffer	0.10	0.15
Distilled water	0.83	0.83
Tissue homogenate	0.05	

The sample was incubated at room temperature for 10 mins, after the time elapsed, the sample was then transferred in to cuvette with addition of 0.02ml of pyrogallon, mixed immediately by inversion and the increase in absorbance at 420nm for approximately 3 minutes was recorded. The change in absorbance at 420/minutes was obtained using the maximum linear rate for both the test and the samples. The activity of superoxide dismutase was be calculated according to the standard curve of superoxide dismutase and expressed as U/g protein.

3.6.3 Determination of glutathione

Principle

Glutathione reacts with an excess of alloxan to produce a substance with an absorption peak at 340nm.

Table 5: Procedure for GSH

REAGENTS	Test (mL)	Blank (mL)
Tissue homogenate	1	
5% metaphosphoric acid	1	1
Acetic acid	1	1
Buffer	1	1
Glutathione	1	1
NaoH	1	

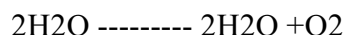
The samples were mixed and incubated for 3 minutes and absorbance was taken at 340nm.

%GSH was obtained using the formula

$$\% GSH = \frac{\text{Abs of sample}}{\text{Abs of standard}} \times 100$$

3.6.4 Determination of Catalase

Principle



In which the disappearance of peroxide was followed spectrophotometrically at 240nm, one unit is equal to one microlitre of hydrogen peroxide decomposed per minute under a specific condition at 25 degrees Celsius.

Table 6: Procedure for CAT

A quantity of 0.9g hydrogen peroxide was dissolved in 25ml of potassium phosphate buffer, 50Mm to form the catalase solution and the above procedure followed.

REAGENTS	Test (mL)	Blank (mL)
Catalase solution	2.9	2.9
Tissue homogenate	1	

A volume of 2.9mL of buffer at 25 degrees Celsius and PH of 7.0 was used as blank. The solution was then mixed immediately by inversion, and the time required for absorbance at 240nm to decrease from 0.45 to 0.40 absorbance units

The results were calculated using the formula and expressed in g/tissue

$$\frac{3.45 \times dF}{mins} \times 0.1$$

df = dilution factor

0.1 = volume in milliliters of enzyme used

Mins = time in minutes required for the Absorbance at 240nm to decrease from 0.45 to 0.40 absorbance units

Statistical analysis

The experimental data were expressed as Mean $\pm$ SD. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test to compare the means between multiple groups and the statistical significance was recorded was recorded at $P < 0.05$

CHAPTER FOUR

RESULTS, DISCUSSION, CONCLUSION, AND RECOMMENDATIONS

4.1 Results

4.1.1 Malondialdehyde Levels

The results of the biochemical assay for the oxidative markers are presented in chart below, the result of the lipid peroxide damage of the treatment is compared to that of diabetic untreated is shown in the figure below, the amount of malondialdehyde was remarkably higher than that of chitosan-turmeric group and all other treatment groups which indicates significant difference ($P < 0.05$) as shown in Figure 5.

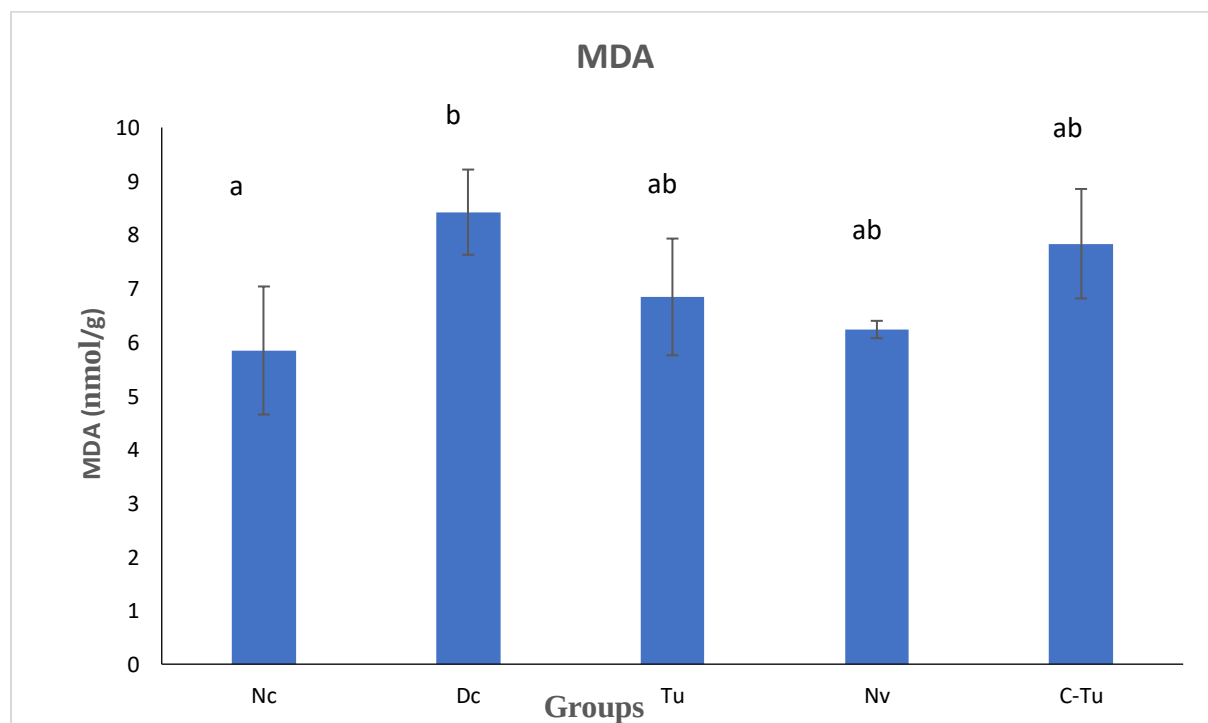


Figure 5: Malondialdehyde level of diabetic wounds treated with hydrogel formulation.

Bars with different letters are a significant difference ($p < 0.05$) using one-way ANOVA, followed by Tukey-Kramer post hoc tests.

4.1.2 Catalase activity

Figure 6 shows the result of the catalase activity of diabetic wound rats treated with chitosan hydrogel formulations. Treatment significantly increased the catalase activity when compared with diabetic control ($P < 0.05$). Furthermore, there was no significant difference between the treatment groups and the normal control.

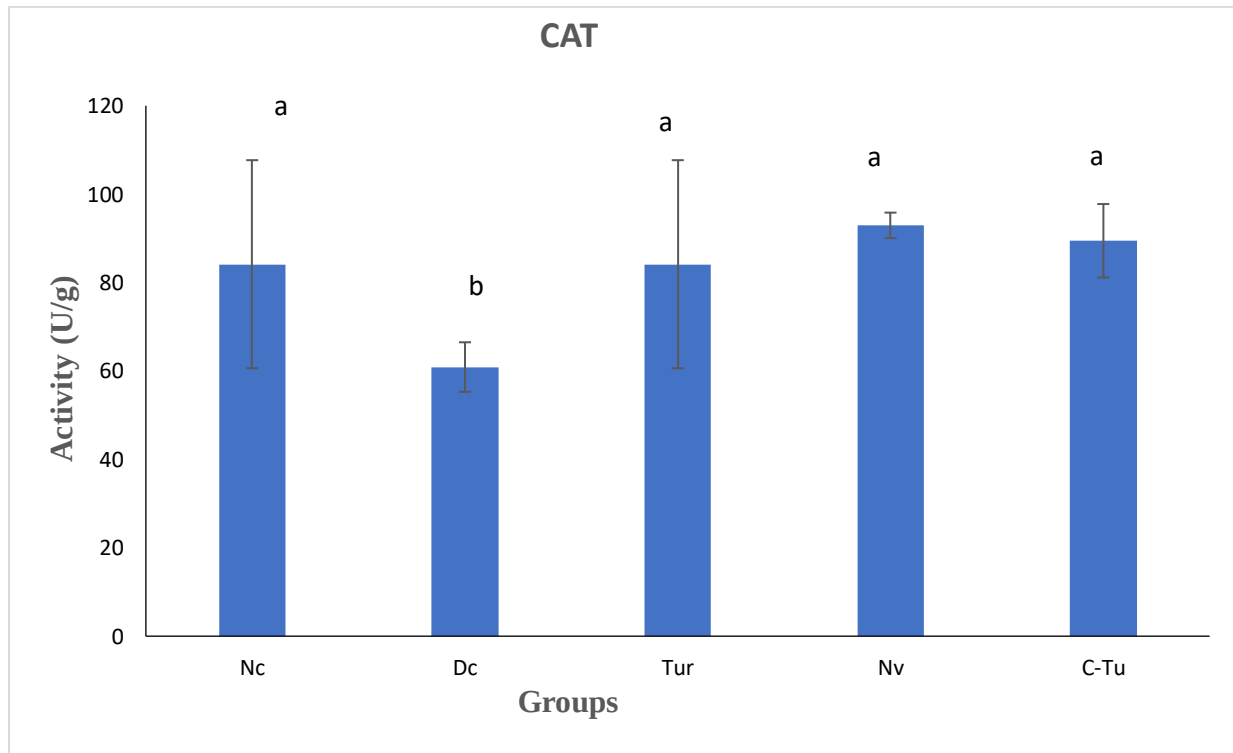


Figure 6: Catalase activity of diabetic wound rats

Bars with different letters are a significant difference ($p < 0.05$) using one-way ANOVA, followed by Tukey-Kramer post hoc tests.

4.1.3 SOD Activity

The result of the superoxide dismutase activity is depicted in Figure 7. It was observed that treatment with the hydrogel formulation significantly increased the activity of SOD in comparison with the diabetic untreated control ($P < 0.05$). The result further showed that the SOD activity of treated groups was not different compared to the normal control ($P > 0.05$), indicating normalized SOD activity.

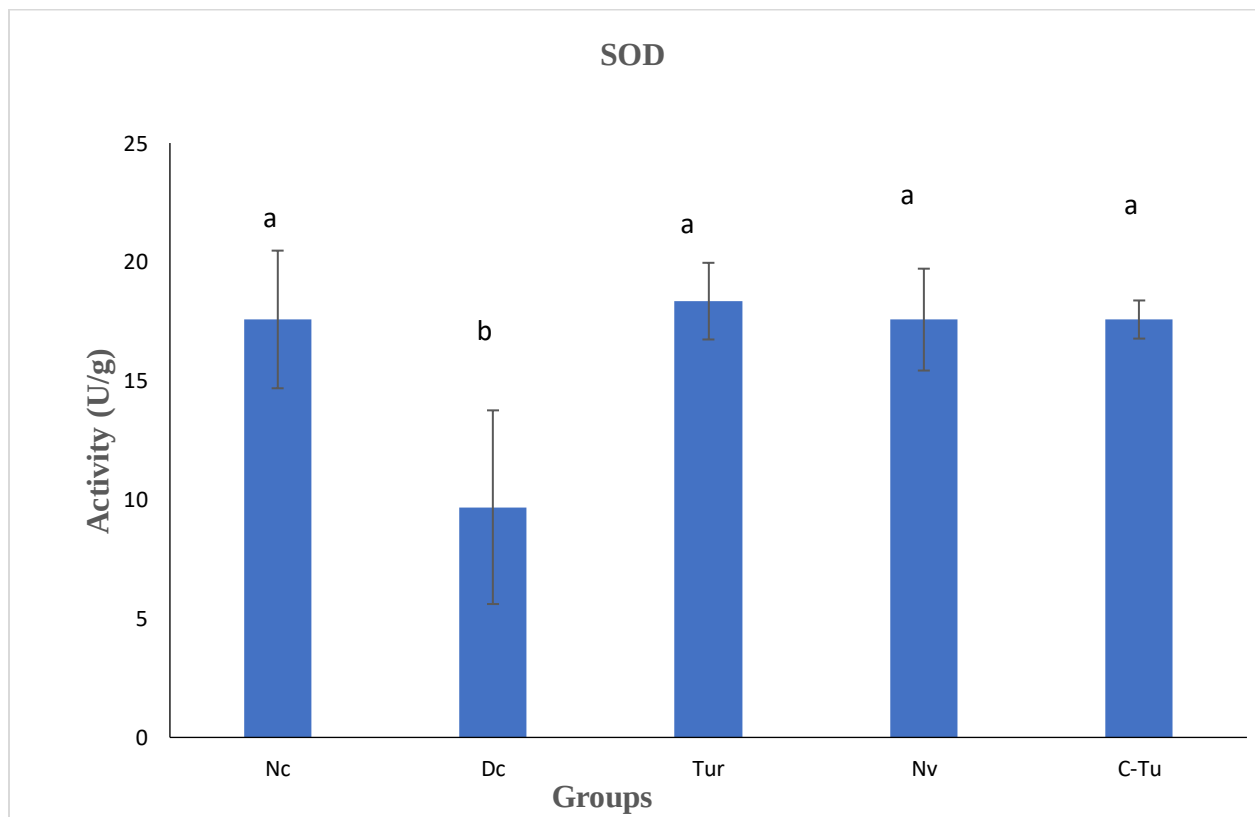


Figure 7: Superoxide dismutase activity of diabetic wound rats after treatment

Bars with different letters are a significant difference ($p < 0.05$) using one-way ANOVA, followed by Tukey-Kramer post hoc tests.

4.1.4 Glutathione levels

The result of the glutathione levels of the treatment group is compared to that of diabetic untreated is shown in figure 8 the level of the non-antioxidant enzyme was clearly higher than that of the chitosan-turmeric group and all other treatment groups, in which the results indicate significant difference ($p < 0.05$) as shown in Figure 8.

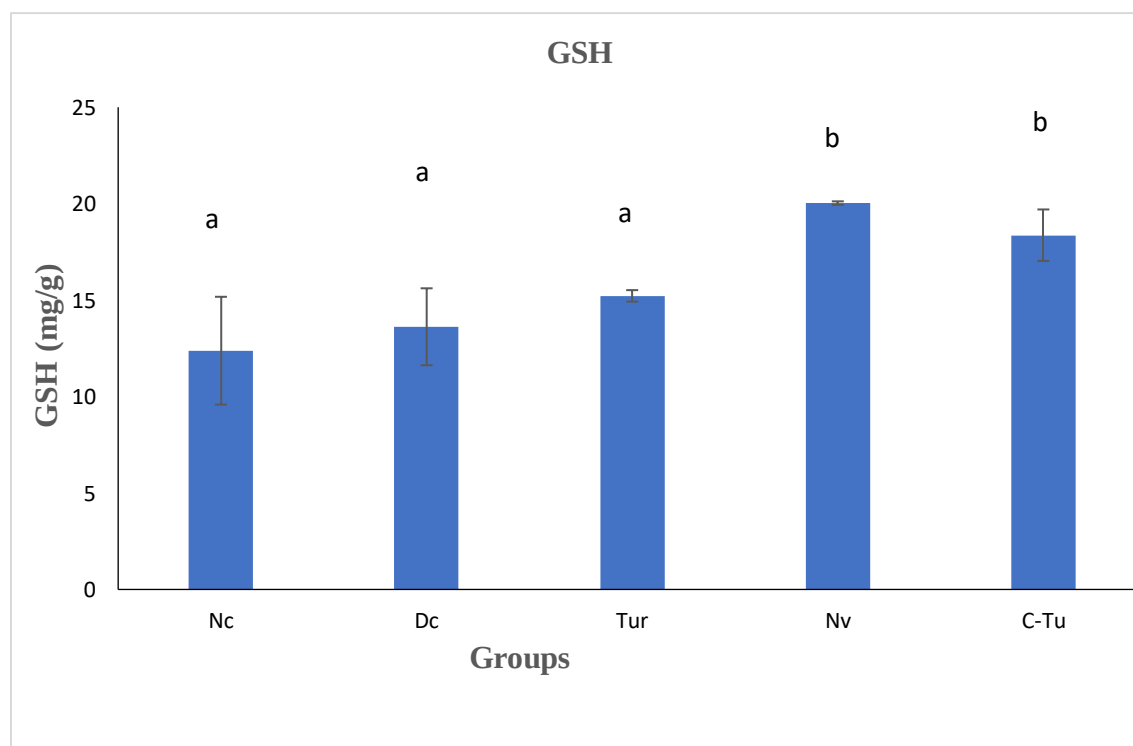


Figure 8: Glutathione level of diabetic rats with wounds treated with hydrogel formulation. Bars with different letters are a significant difference using one-way ANOVA, followed by Tukey-Kramer post hoc tests.

4.2 Discussion

This study demonstrates that chitosan hydrogel-loaded turmeric extracts exert a significant modulatory effect on oxidative stress biomarkers associated with diabetic wounds. The MDA level was reduced in the treatment group compared to the untreated diabetic group. This result could be attributed to the different types of bioactive compounds present in the plant extracts that were employed. This is consistent with existing literature showing that the active ingredients of turmeric, curcumin, effectively neutralize ROS, modulate redox-sensitive pathways, and mitigate lipid damage (Ashrafizadeh *et al.*, 2020). Earlier findings also show that chitosan possesses intrinsic antioxidant and anti-inflammatory properties (He *et al.*, 2025).

The significant increase in catalase (CAT) superoxide dismutase activity ($p < 0.05$) observed in the treated groups suggest that the formulations improve the expression of antioxidant enzymes involve in reducing oxidative stress compared with the untreated diabetic group, where the antioxidants enzyme activity is very low due to the impending rate of free radicals and this aligns with recent reports showing that antioxidant-loaded biopolymer hydrogels improve tissue remodeling, reduce oxidative injury, and create a more favorable microenvironment for angiogenesis and fibroblast proliferation thereby enhancing diabetic wound healing (Cui *et al.*, 2023; Maeso *et al.*, 2024).

The results also show higher glutathione (GSH) levels in the treatment group and a very significant decrease ($p < 0.05$) in the untreated diabetic group. Where these levels in the treated group were close to those of the healthy control group. Reduced GSH levels contribute to excessive ROS and delay the wound healing process (Ashrad *et al.*, 2025). It can be seen in the results that the chitosan-loaded turmeric extract increases the activity of these antioxidant enzymes far more than that of

the extract only, and this aligns with the report of Tarek *et al.*,(2025) work on grape seed extract loaded with chitosan hydrogel.

Interestingly, in some cases, there is no significant difference between the chitosan turmeric treatment group and the naïve treatment groups, which might have been due to two reasons. First, the chitosan itself possesses antioxidant properties, which are capable of scavenging free radicals and activating necessary pathways involve the reduction of oxidative stress, thereby competing for the same effectiveness as the chitosan loaded. Another thing is the rate of free radicals, antioxidants, and enzymes are expressed and activated in response to the number of free radicals in the body, so if there's excessive ROS in the wound tissues of these groups, then the body's antioxidant defense might be activated beyond measure. A similar case was reported by Ashrad *et al.*, (2025) work on aloe vera extract encapsulation in chitosan hydrogel. Overall, the topical application of chitosan-loaded turmeric hydrogel was the best choice for wound healing. It ameliorated the levels of antioxidant enzymes and decreased the levels of lipid damage in the wound tissues which is consistent to the report of Eze *et al.*, (2022) fabrication of chitosan nanoparticles with hunteria seed extracts. other works also reported that chitosan-loaded turmeric hydrogels enhance collagen deposition, angiogenesis, and epithelial regeneration while suppressing oxidative and inflammatory damage in diabetic wounds (Bai *et al.*, 2024).

4.3 Conclusion

Diabetes mellitus increases the generation of free radicals by glucose auto-oxidation and this depletes the activities of catalase and other antioxidants (Baynes & Thorpe, 1999). Indeed, there is still a research gap in how plant antioxidants can play an important role in their anti-oxidative effect against the hyperglycemia oxidative stress mediated diabetic complications (Yedjou *et al.*, 2023). In this study, chitosan hydrogel was loaded with turmeric extracts to treat diabetic wound in rats. Research has already shown that delayed wound healing in diabetes is as a result of oxidative stress, persistent inflammation, and others. In the present study, the test for the oxidative markers reveals that the chitosan hydrogel loaded turmeric extracts indeed has high level of efficacy in mitigating oxidative stress with the significant increase in the activity of CAT, GSH SOD and the lower levels of MDA.

4.4 Recommendations

Based on the finding of this study, the following recommendations are proposed

1. Further research should be carried out to ensure the efficacy of the hydrogel, and also assure its biocompatibility with the turmeric extracts.
2. Given that both turmeric and chitosan exhibit significant antioxidant properties, it is recommended that more research should be done to see if the loading of the extract in the hydrogel alters any properties of the extract.

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APPENDICES

RAW DATA

Table 1: MDA levels (nmol/g)

Sample	Concentration			
Control	5.89	4.80	6.73	5.96
Diabetes untreated	8.14	9.35	9.35	6.85
Turmeric extract	8.20	5.64	6.41	7.12
Naïve chitosan	6.09	6.41	6.21	
Chitosan turmeric extract	8.39	8.72	6.41	7.82

Table 2: GSH levels (mg/g)

Sample	Concentration			
Control	14.079	10.175	11.316	13.967
Diabetes untreated	13.510	10.088	13.455	17.412
Turmeric extract	15.263	14.912	15.088	15.614
Naïve chitosan	19.956	20.132	20.00	
Chitosan turmeric extract	19.035	19.825	16.798	17.807

Table 3: Catalase

Sample	Activity(U/g)			
Control	57.40	56.89	60.23	69.00
Diabetes untreated	89.9	63.9	67.8	115.0
Turmeric extract	89.9	63.9	67.8	115.0
Naïve chitosan	95.80	90.04	93.00	
Chitosan turmeric extract	80.4	92.8	99.3	85.3

Table 4: Superoxide dismutase activity (U/g)ss

Sample	Activity(U/g)			
Control	13.53	19.90	17.48	19.41
Diabetes untreated	8.93	6.04	14.08	
Turmeric extract	16.10	18.50	19.89	18.93
Naïve chitosan	18.88	18.82	15.10	
Chitosan turmeric extract	13.53	19.90	17.48	19.41