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Protective Action of Gold Nanoparticles Against Ulcerative Colitis Induced by *Escherichia coli* in Rats

A Thesis

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

﴿ قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا ۖ
إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ ﴾

صدق الله العظيم

سورة البقرة : الآية(32)

Dedication

I dedicate these modest efforts to ....My beloved Family

To my greatest supporter …….My Father and Mother

To my wife and children who supported me all the time

To my supervisor who gave me all the support and paternal backing

To my dear sisters and brothers, who supported me

To my friends who encouraged and supported me all the time

To all my teachers who guided me

To everyone guide me to the knowledge

Omer Adel

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Declaration

I hereby declare that this thesis entitled **(Protective Action of Gold Nanoparticles Against Ulcerative Colitis Induced b *Escherichia coli* in Rats)** presented at the College of Veterinary Medicine-University of Diyala in 2025, is my original work, except for quotations and citations which have been duly acknowledged. I also declare that it has not been submitted previously or concurrently, for any other degree at the University of Diyala or other Universities.

Omar Adel Lafta

\ \ 2025

Abstract

Colitis is a worldwide distributed disease characterized by abnormal immune responses. Gold nanoparticles (GNPs) have recently been used in the medical field because their unique properties and low toxicity compared to other metals, (GNPs) have antibacterial and antioxidant properties, for these advantages GNPs have been chosen at this study for applying as antibacterial and immunomodulation agents. Additionally, GNPs have good stability and biocompatibility, enabling it to reach the targeted organ and exert their therapeutic effect after oral administration, especially in Inflammatory bowel disease (IBD).

This study aimed to investigate the gastrointestinal protective and treatment ability of orally administrated gold nanoparticles against *E. coli*-induced ulcerative colitis in rats.

After isolation of *E. coli* from cow's milk with clinical mastitis and used it to induce ulcer in rats**.** Protective ability of GNPs started by oral administration of Waster albino rats at two doses 5µg/Kg & 10µg/Kg as a prevention factor against *E.coli* induced ulcer compared with ulcer group and group administration omeprazole and evaluated the immunological effect of GNPs measured the level of Superoxide Dismutase (SOD), Malondialdehyde (MDA), Tumor Necrosis Factor-alpha (TNF –alpha), Transforming Growth Factor (TGF-β) and Prostaglandine E2 PGE2 by ELISA methods from the blood rats.

The treatment effect of GNPs was started by oral administration of *E coli* to Waster Albino rats for colitis induction, then the GNPs were administered daily for 15 days at two doses 5µg/Kg & 10µg/Kg, as a

treatment agent against *E. coli* induced ulcer compared with ulcer group, group administrated Omeprazole and sulfatrimethoprem, group administrated GNP dose of 10 μg/kg & omeprazole and group administrated of GNP dose 5 μg/kg & omeprazole. In a prevention experiment, rats were anesthetized in order to gather blood and tissues at the same day, and at the day 4^{th},8^{th},12^{th}, and 15^{th} after for treatment experiment to evaluate the immunological and pathological changes by measurements the level of Superoxide Dismutase, Malondialdehyde, Tumor Necrosis Factor-alpha, Transforming Growth Factor and Prostaglandine E2 by ELISA methods.

The experiments and the research work was performed with ethical guidelines established by Veterinary Medicine College at the University of Diyala started in 22\7\2024 and finished in 22\3\2025.

The result of protective ability shown that GNPs have aprotective effect against induced ulcer as dose dependent manner and have antioxidant properties through elevation of the SOD level and decrease the level of MDA compared with ulcer and omeprazole groups. Although the GNPs modulating the immune response by lowering the level of TNF –alpha , Transforming Growth Factor (TGF-β), and PGE2 significant compared with ulcer and omeprazole groups.

The result of the treatment effect of GNPs was shown that GNPs have antioxidant properties through the gradual significant elevation of the SOD dose dependently compare with ulcer and Omeprazole groups, and have the ability for scavenging the ROS by diminishing the level of MDA significantly compare Omeprazole and ulcer groups. In addition

the GNPs have anti-inflammatory effect through the decrease the TNF – alpha level dose dependently compare Omeprazole and ulcer groups. Also the GNPs modulates the immune response by significant lowering the level of TGF-β dose dependently compare with ulcer group and significant elevation the level of PGE2 dose dependently compare with ulcer and Omeprazole groups.

The results of the study were summarized that the gold nanoparticles have antibacterial and immunomodulation properties by enhancing the level of antioxidant enzymes and pro-inflammatory cytokines while reducing the level of inflammation by decreasing the inflammatory mediators.

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Table of abbreviations:-

Abbreviate	Meaning
(AgNPs)	Silver Nanoparticles
(APCs)	Antigen-Presenting Cells
(ATP)	Adenosine Triphosphate
(Au)	Gold
(AuNP)	Gold Nanoparticles
(CD)	Chron,s Disease
(DAMPs)	Damage-Associated molecular patterns
(DNA)	Deoxyribonucleic Acid
(ELISA)	Enzyme-linked Immunosorbent Assay
EP	E-Prostanoid
(EMB)	Eosin Methylene Blue
(GIT)	Gastrointestinal Tract
(GNPs)	Gold Nanoparticles
(GNPG1)	Gold Nanoparticles group one
(GNPG2)	Gold Nanoparticles group two
(IBD)	Inflammatory Bowel Disease
IL	Interleukin
(ILC)	Innate Lymphoid Cells
(IM)	Intramuscular
(LPS)	Lipopolysaccharide
(LSPR)	Longitudinalsurface Plasmon Resonance
(MDA)	Malondialdehyde
(N control)	Normal control
(NADPH)	Nicotinamide Adenine Dinucleotide Phosphate

(NK)	Natural killer cells
(NPs)	Nanoparticles
($O^{\cdot -}2$)	Superoxide Anion
(O2)	Oxygen
(OD)	Optical Density
(OMP)	Omeprazole Group
(OMPGNPG1)	groups treated with omeprazole 1mg/kg with trimethoprim 6mg/kg & sulfamethoxazol 30 mg/kg
(PAMPs)	pathogen-Associated molecular patterns
(PCR)	Polymerase Chain Reaction
(PGE2)	Prostaglandine E2
(PRRs)	Pattern Recognition Receptors
(PSB)	Phosphate Buffer Saline
(ROS)	Reactive Oxygen Species
(RNS)	Reactive Nitrogen Species
(rRNA)	Ribosomal Ribonucleic Acid
(SCC)	Somatic Cell Count
(SOD)	Superoxide Dismutase
(SPR)	Surface plasmon Resonance
(TGF-β)	Transforming Growth Factor
(Th)	T helper cells
(TLRs)	Toll-Like Receptors
(TNF-α)	Tumor Necrosis Factor-alpha
(Treg)	Regulatory T cells
(TSPR)	Transverse surface plasmon Resonance
(UV)	Ultra Violet
(U control)	Ulcer control
UC	Ulcerative Colitis
(WBCs)	White Blood Cells

Chapter One

Introduction

1.1Introduction

Antibiotic resistance is one of the most significant dangers to human survival, necessitating the development of novel therapies or more effective methods for managing infections. Antibiotic resistance is rapidly emerging as one of the most significant global health issues, with projections indicating over 10 million fatalities worldwide by 2050 (Fuller *et al*, 2020).

Nanotechnology has significant applications in the medical field as nanomedicine. Certain nanoparticles may be utilized in innovative diagnostic devices, imaging techniques, targeted therapeutics, pharmaceutical formulations, biomedical implants, and tissue engineering applications.Current high-toxicity medicines can be delivered with enhanced safety by nanotechnology, including chemotherapeutic agents for cancer (Haleem *et al*, 2023).

Nanotechnology possesses the capacity to provide the most efficient methods for the targeted delivery of compounds or the treatment of disorders directly, in a high regulated and secure method, facilitating the accumulation of nanoparticles and bioactive compounds in the intended tissue (Patra *et al*, 2018). Moreover, it aids in surmounting certain obstacles such as membranes and severe pH levels (Zhang *et al*, 2012). Inflammatory bowel disease (IBD) is inflammatory disease affect the gastrointestinal tract so it is called (inflammatory bowel disease), Over 4.9 million individuals worldwide suffer from it. Depend on the tissue involvement and pathological features that connect with the disease it is either ulcerative colitis or crohn's disease (Wang *et al*, 2023).

Inflammatory bowel disease includes many disorders like ulcerative colitis and Crohn's disease (CD) that affected the gastrointestinal (GI) tract. Ulcerative colitis distinguished by their clinical presentations, proposed pathogenic processes, and it is recurrent, non-transmural ,inflammatory and chronic feature that affected the colon manifested by diarrhea with blood through exacerbations. Crohn's disease is chronic, segmental, localized granulomatous disorders can include any region of the GIT that started with the mouth and end by the rectum. The clinical signs that conjugated with the disease depend on the region that affected and the severity characterized by fever, diarrhea with or without blood, sings of GI obstruction including pain and restlessness (Baumgart & Sandborn, 2007).

The causative agent of the IBD is complicated, however many studies indicated that there are many factors are related include immunity, food, microbes like (bacteria and viruses), many condition of the environment and genetic. Research that used laboratory animals for induce colitis suggest that the IBD is due to abnormal regulation of cellular immunity include either increase activity of effector lymphocyte cells (T lymphocyte) or decrease the activity of regulatory lymphocyte (T reg cells), result from these disorders elevation the production of proinflammatory mediators like tumor necrosis factor alpha and insufficient functions of the regulatory mediators (Mirsepasi-Lauridsen *et al*, 2019). During the inflammatory bowel disease (IBD) there is close relation between the oxidative stress and the inflammation, the reactive nitrogen species (RNS) and reactive oxygen species (ROS) are generated due to the activity of immune cells that responded to the defect. Oxidative stress is result from the inflammation through many ways like activation of nicotinamide adenine dinucleotide

phosphate (NADPH) oxidases inflamematory pathways and nitric oxide synthase.

In contrast the oxidative stress predisposed the inflammation through the induce the release of immune cells and inflammatory mediators in the intestinal barrier that exacerbate the disease (Bourgonje *et al*, 2020). Inflammatory cells like monocytes and neutrophils are accumulates in the intestinal wall and release of their cytokines that lead to development of oxidative stress through elevation of ROS and RNS (Chami *et al*, 2018). Therefore, restoring redox equilibrium to inhibit this cyclical reaction might be a viable approach for treating IBD.

Exogenous natural antioxidant enzymes have been used to neutralize ROS or suppress inflammation for IBD treatment which however are limited by low stability, high cost, and potential immunogenicity , the development of nanotechnology that produced a new nanomaterials that have enzymatic activities can protect and treated the IBD in accruable manner (Colgan & Taylor, 2010).

Gold nanoparticles (AuNPs) exhibit considerable potential therapeutics to gastrointestinal lining. Gold possesses a lengthy historical significance in therapy due to its exceptional biocompatibility and its antioxidant attributes. Advancements in nanotechnology have enabled the application of AuNPs in both imaging and therapy (Hassan *et al*, 2022). They have been extensively investigated for the treatment of diverse inflammatory diseases (Fujita *et al*, 2021). Include neuroinflammation (Di Bella *et al*, 2021). Autoimmune inflammation (Danscher & Rasmussen, 2023) and cutaneous inflammation (Dhandapani et al., 2023). Multiple trials

have demonstrated their effectiveness in treating inflammatory bowel illnesses (Dhandapani *et al,* 2023). An important benefit of GNPs is their remarkable stable, particularly at extreme decreased pH values. For these reasons is essential to be orally delivery, allowing for arriving the gut where they can apply their therapy on IBD pathophysiology. This attribute renders GNPs especially appropriate to tailored therapies in IBD therapy (Fontes *et al*, 2024).

1.2 Aim of the Study

This study aimed to identify the effect of Gold nanoparticles on the enhancement of immune response to prevent and treat the ulcers caused by *E.coli* in contaminated dietary

The aim was achieved by:

1- Investigating the gastrointestinal protective ability of Gold nanoparticles against *E.coli* induced ulcerative colitis.

2- Evaluating the ulcer healing ability of Gold nanoparticles against *E.coli* induced ulcerative colitis.

3- Estimating the potential of Gold nanoparticles on the enhancement of immune response to prevent and treat the ulcers caused by *E.coli* .

4. Agarose Gel Electrophoresis

Gel electrophoresis was used for the detection of amplified PCR products, which visualized with the aid of Ethidium bromide dye and UV transilluminator documentation system. Where one-gram agarose was added to 100 ml 1x TBE buffer to prepare agarose gel that loaded with PCR product, 3.5µl of extracted DNA was mixed with 1.5µl of loading dye (Bromo phenol blue) and loaded into the wells of the gel (Prifer, 1984), while for PCR product, each well was loaded with 5µl of the monoplex PCR products (Morovat *et al.,* 2009), DNA ladders were always run concurrently with each electrophoretic run to detect product size of PCR. DNA bands were visualized by UV transilluminator documentation system. TBE buffer was added to cover the gel in the electrophoresis boat and electrophoresis current runs for an hour at 7 volt/cm^2. After that, the agarose gel removed from the boat and visualized the DNA band by UV transilluminator documentation system and then photographed using digital camera (Mishra *et al.,* 2010).

الخلاصة:

التهاب القولون مرض منشر عالميا يتميز باستجابات مناعيه غير طبيعية و قد استخدمت جسيمات الذهب النانوية مؤخرًا في المجال الطبي نظرًا لخصائصها الفريدة وسميتها المنخفضة مقارنةً بالمعادن الأخرى، بالإضافة إلى خصائصها المضادة للبكتيريا ومضادات الأكسدة. ولهذه المزايا، اختيرت جسيمات الذهب النانوية في هذه الدراسة لاستخدامها كعوامل مضادة للبكتيريا وعامل مُعدّل للمناعة. بالإضافة إلى ذلك، تتميز جسيمات الذهب النانوية بثباتٍ وتوافقٍ حيويٍ جيدين، مما يُمكّنها من الوصول إلى العضو المستهدف وممارسة تأثيرها العلاجي بعد تناولها عن طريق الفم، وخاصةً في مرض التهاب الأمعاء. هدفت هذه الدراسة إلى دراسة القدرة الوقائية والعلاجية للجهاز الهضمي لجزيئات الذهب النانوية التي يتم تناولها عن طريق الفم ضد التهاب القولون التقرحي الناجم عن الإشريكية القولونية في الفئران

بعد عزل بكتيريا الإشريكية القولونية من حليب البقر المُصاب بالتهاب الضرع السريري، واستخدامها لإحداث قرحة لدى الجرذان. بدأت القدرة الوقائية لجسيمات الذهب النانوية بإعطاء جرذان بيضاء مُهقّة عن طريق الفم بجرعتين 5 ميكروغرام/كغ و10 ميكروغرام/كغ كعامل وقاية من القرحة المُسببة بالإشريكية القولونية، مقارنةً بمجموعة القرحة ومجموعة إعطاء أوميبرازول. وقاس مستوى إنزيم سوبر أكسيد ديموتاز، مالونديالدهيد ، عامل

نخـــر الـــورم ألفـــا ، عامـــل النمـــو المُحـــوِّل والبروســـتاجلاندين مـــن دم جـــرذان التجارب ، باستخدام طريقة الاليزة.

بـــدأ تـــأثير عـــلاج الجســـيمات النانويـــة الذهبيـــة عـــن طريـــق الإعطـــاء الفمـــوي لبكتيريـــا الإشـــريكية القولونيـــة للجـــرذان لإحـــداث التهـــاب القولـــون، ثـــم تـــم اعطـــاء الجســـيمات النانويـــــه الذهبيـــــة لمـــــدة 15 يومـــــا بجـــــرعتين 5 ميكروجـــــرام/كجم و10 ميكروجـــرام/كجم، كعامـــل عـــلاج ضـــد القرحـــة المُســـتحثة بالإشـــريكية القولونيـــة، مقارنـــــةً بمجموعـــــة القرحـــــة، المجموعـــــة التـــــي أُعطيـــــت أوميبـــــرازول وسلفاتريميثوبريم.

فـــي التجربـــة الوقائيـــة، خُـــدِّرت الجـــرذان لجمـــع عينـــات الـــدم والأنســـجة فـــي نفـــس اليـــوم، وفـــي اليـــوم الرابـــع والثـــامن والثـــاني عشـــر والخـــامس عشـــر بعـــد ذلـــك للتجربـــة العلاجيـــة لتقيـــيم التغيـــرات المناعيـــة والمرضـــية مـــن خـــلال قيـــاس مســـتوى إنـــزيم ســـوبر أكســـيد ديســـميوتاز المالونديالدهيـــد عامـــل نخـــر الـــورم ألفـــا ، وعامـــل النمـــو المُحوّل ، والبروستاجلاندين.

تـــم اجـــراء التجـــارب والاعمـــال البحثيـــة وفـــق الضـــوابط الاخلاقيـــة التـــي وضـــعتها كليـــة الطـــب البيطـــري فـــي جامعـــة ديـــالى وبـــدأت فـــي 2024/7/22 وانتهـــت فـــي 2025/3/22

أظهرت نتائج تأثير العلاج بجسيمات النانو الذهبية أن جسيمات النانو الذهبية لها خصائص مضادة للأكسدة من خلال الارتفاع التدريجي الكبير إنزيم سوبر أكسيد ديموتاز بشكل يعتمد على الجرعة المقارنة مع مجموعات القرحة وأوميبرازول، ولديها القدرة على إزالة الأكسجين التفاعلي المحدد عن طريق تقليل مستوى المالونديالدهيد بشكل كبير مقارنة بمجموعات أوميبرازول والقرحة. بالإضافة إلى ذلك، تمتلك جسيمات الذهب النانوية تأثيرًا مضادًا للالتهابات من خلال خفض مستوى عامل نخر الورم ألفا بشكل كبير وارتفاعًا ملحوظًا في مستوى البروستاكلاندين مقارنةً بمجموعة القرحة وأوميبرازول.

ولخصت نتائج الدراسة إلى أن جزيئات الذهب النانوية تمتلك خصائص مضادة للبكتيريا وتعديل المناعة من خلال تعزيز مستوى إنزيمات مضادات الأكسدة والسيتوكينات المؤيدة للالتهابات مع تقليل مستوى الالتهاب عن طريق تقليل الوسطاء الالتهابيين.

جمهوريـــــة العـــــراق
وزارة التعليم العالي و البحث العلمي
جامعة ديالـــــــــــى
كلية الطب البيطـــــري
فرع الاحياء المجهرية البيطرية

الفعل الوقائي لجزيئات الذهب النانوية ضد التهاب القولون التقرحي المستحدث بواسطة الإشريكية القولونية في الجرذان

رسالة مقدمه الى

مجلس كليه الطب البيطري\جامعه ديالى

وهي جزء من متطلبات نيل شهاده الماجستير في علوم الطب البيطري \ الاحياء المجهرية البيطرية

قدمها

عمر عادل لفته

بأشراف

أ.د ولاء نجم عبود

١٤٤٧هـ ٢٠٢٥م