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Biosynthesis of a biovaccination based on copper nanocomposites and study of their effectiveness to protect against bacterial infections

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Dedication

To my parent

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Abstract

This work investigates the biosynthesis of a biovaccination based on copper nanocomposites (CuNPs) using *Salmonella enterica* and *Escherichia coli* bacteria and the study of their effectiveness to protect against bacterial infections.

Furthermore, UV-Vis and FT-IR spectroscopy, scanning electron microscopes (SEM) and EDX technique were used to verify the biosynthesis of copper nanoperticles. *In-vitro* antioxidant and anti-inflammatory activities of copper nanocomposites were evaluated by standard techniques.

In *in-vivo* study, twenty male albino Wistar rats were randomly diveded to five groups (four rats in each): (1) healthy rats (control), (2) rats infected with *S. enterica*, (3) rats infected with E. *coli*, (4) rats vaccinated with SE-CuNPs and subsequently exposed to *S. enterica*, and (5) rats vaccinated with EC-CuNPs and subsequently exposed to with *E. coli*. The biovaccination was done by intraperitoneal injection at a dose of 7 mg/kg. After a two-week period, all groups, except the control, were exposed to a bacterial dose. Some hematological, biochemical, enzymatic parameters, and oxidative stress markers were evaluated, along with conducting microscopic examinations of spleen tissue.

Results of *in-vitro* study revealed that EC-CuNPs and SE-CuNPs exhibited oval shapes with sizes ranging from 73 to 183 nm and from 135 to 202 nm, respectively. EC-CuONPs showed high antioxidant activity (IC_{50}= 61.86 µg/ml), while SC-CuNPs exhibited enhanced anti-hemolysis activity (IC_{50}= 40.1 µg/ml).

The biological findings indicate significant alterations induced by bacterial infection, including physiological changes and disturbances in biochemical parameters and antioxidant defense systems in various organs. Rats vaccinated with SE-CuNPs and EC-CuNPs showed significant improvements in biological parameters and histological analyses, suggesting the efficacy of vaccination in mitigating bacterial infection damage.

These findings suggest that both biovaccins SE-CuNPs and EC-CuNPs are effective in providing protection against bacterial infection with minimal side effects.

Keywords: *S. enterica*, *E. coli*, EC-CuNPs, SE-CuNPs, Nanovaccine, Bacterial infection

الملخص

يهدف هذا العمل إلى التصنيع البيولوجي للتطعيم الحيوي المعتمد على جزيئات النحاس النانوية (CuONPs) باستخدام بكتيريا *S. enterica* و*E. coli*، ودراسة تأثيرها الوقائي ومدى فعاليتها في الحماية من العدوى البكتيرية.

للتحقق من التخليق الحيوي لجسيمات النحاس النانوية، تم استخدام التحليل الطيفي للأشعة فوق البنفسجية (UV-Vis) والأشعة تحت الحمراء (IR)، والمجهر الإلكتروني الماسح (SEM)، وتقنية EDX. وتم تقييم الأنشطة المضادة للأكسدة والمضادة للالتهابات لهذه الجزيئات في المختبر.

في دراسة داخل الجسم الحي، تم تقسيم عشرين جرذاً ذكراً من فصيلة ألبينو ويستار عشوائيًا إلى خمس مجموعات (العدد = 4): مجموعة الشاهدة: الجرذان السليمة، مجموعة الجرذان المصابة بـ*S. enterica* ، مجموعة الجرذان المصابة بـ*E. coli* ، مجموعة الجرذان الملقحة بـ SE-CuONPs ومصابة ب *S. enterica*، مجموعة الجرذان الملقحة بـEC-CuONPs مصابة ب*E. coli* تم إجراء التطعيم الحيوي عن طريق الحقن تحت الصفاق بجرعة 7 ملغ/كغ. بعد أسبوعين، تمت إصابة المجموعات بجرعة بكتيرية باستثناء المجموعة الشاهدة. تم تقييم بعض المعايير الدموية، المعايير البيوكيميائية والإجهاد التأكسدي، إضافة إلى الملاحظة المجهرية لأنسجة الطحال.

أثبتت الدراسة المختبرية أن كلًا من ECuONPs وSCuONPs لها شكل بيضاوي وحجم يتراوح بين (73-183 نانومتر) و (135-202 نانومتر) على التوالي. علاوة على ذلك، أظهرت ECuONPs نشاطًا مضادًا للأكسدة أفضل بقيمة(IC_{50}=61.86 ميكروغرام/مل) بينما كان لـ SCuONPs نشاطًا مضادًا للانحلال أفضل (IC_{50}=40.1 ميكروغرام/مل).

تشير النتائج البيولوجية إلى تغيرات كبيرة ناجمة عن العدوى البكتيرية، بما في ذلك تغيرات فسيولوجية واضطرابات في المعايير البيوكيميائية وأنظمة الدفاع المضادة للأكسدة في عدة أعضاء، في حين أظهرت نتائج الفئران المحصنة بـ SE-CuONPs وEC-CuONPsتحسنا ملحوظا في المعايير البيولوجية والتحليلات النسيجية، مما يدل على فعالية الجسيمات النانوية .

تشير هذه النتائج إلى أن كلاً من اللقاحين الحيويين SE-CuONPs وEC-CuONPs فعالان في توفير الحماية ضد العدوى البكتيرية مع الحد الأدنى من الآثار الجانبية.

الكلمات المفتاحية: *S. enterica*، *E.coli*، EC-CuNPs، SE-CuNPs، اللقاح النانوي، عدوى بكتيرية

List of abbreviations

$^1O_2$: Singlet oxygen

ALAT: alanine aminotransferase.

ALP: alkaline phosphatase.

APCs: Antigen-Presenting Cells

APCs: Antigen-presenting cells

ASAT: aspartate aminotransferase.

BM-AuNPs: Bacterial Membrane-coated Gold Nanoparticles

Bregs: Regulatory B cells

CAT: Catalase

CBEU: Cytobacteriological exam of urine

CNPs: Chitosan Nanoparticles

Cu : Copper

CuONPs : Copper Oxide Nanoparticles

CuONPs : Copper oxide nanoparticles

CVD : Chemical vapor deposition

DCs: Dendritic Cells

DNA: Deoxyribonucleic acid

DPPH: 1,1-diphenyl-2-picryhydrazyl

ECuONPs: Copper oxide nanoparticles synthesised by E. coli

EPEC: Enteropathogenic Escherichia coli.

Fe_2O_3NPs: Iron Oxide Nanoparticles

FTIR: Fourier Transform Infrared spectroscopy

GI: Gastrointestinal

GPx: Glutathione peroxidase

GR: Glutathione reductase

GSH: Reduced Glutathion

GSSG: Glutathione disulfide

GYPA: Glycophorin-A

H_2O: Water

H_2O_2: Hydrogen peroxide

HOCl: Hypochlorous acid

HUS: Hemolytic uremic syndrome.

I.M: Intramuscular

IC_{50} : Inhibition concentration 50

ID : Intradermal

IFN-γ : Interferon-gamma

IL-10 : Interleukin 10

IL-6 : Interleukin 6

IL-8 : Interleukin 8

iNps : Inorganic nanoparticles

IP : Inhibition percentage

LNs: Lymph nodes

LPS: Lipopolysaccharide

MALT: Mucosal-associated lymphoid tissues

MCH: Mean Corpuscular Hemoglobin

MCHC: Mean Corpuscular Hemoglobin Concentration

MDA: Malondialdehyde

MDSCs: Myeloid-Derived Suppressor Cells

MHCII: Major histocompatibility complex class II

MNPs: Metal-based Nanoparticles

MØ: Macrophages

MPO : Myeloperoxidase

MZ : marginal zone.

NADPH: Nicotinamide adenine dinucleotide phosphate

NF-κB: Nuclear Factor Kappa B

NK: Natural Killer cells

NMs: Nanomaterials

NO: Nitric oxide

NPs: Nanoparticles

OH: Hydroxyl free radical

OMVs: Outer Membrane Vesicles

PAMPs: Pathogen-Associated Molecular Patterns

PCT: Procalcitonin

PLT: Platelets

PRR: Pattern Recognition Receptor

RBCs: Red Blood Cells

RNS: Reactive nitrogen species

ROS: Reactive oxygen species

ROS: Reactive Oxygen Species

RP: red pulp.

S.C: Subcutaneous

SCuONPs: Copper oxide nanoparticles synthesised by *S. enterica*

SOD: Superoxide dismutase

SSA: Specific surface area

STEC: Shiga toxin-producing Escherichia coli.

TGF-β: Transforming Growth Factor Beta

TLR4: Toll-like Receptor 4

TLR9: Toll-like Receptor 9

TNF-α: Tumor Necrosis Factor Alpha

Tregs : Regulatory T cells

UV-Vis : Ultraviolet–visible

WHO: World Health Organization

WP: white pulp

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Summary

Introduction

Introduction

Worldwide, millions of people die from infectious disease agents (Choudhary et *al.,* 2023) belonging to different classes of pathogens, including viruses, bacteria, fungi, and parasites (Maina et *al.,* 2020). Bacteria cause several deadly infectious diseases in humans, such as tuberculosis, cholera, tetanus, and pneumonia (Gupta et *al.,* 2022), so bacterial diseases are one of the leading causes of the rising fatality rate, mainly due to the multiple drug-tolerant pathogenic microorganisms brought by the overuse of traditional antimicrobial drugs, immunosuppressants, etc. (Qadri et *al.,* 2023). The development of vaccines is one of the greatest achievements of modern medicine, as these vaccines can prevent health-associated infections of all ages and in all countries, as the COVID-19 pandemic illustrates (Poolman, 2020; Gerberding & Haynes, 2021; Khan et *al.,* 2022), but several of these products demonstrate variable efficacy and safety in the field, require multiple doses, or are unstable under field conditions (Maina et *al.,* 2020).

Escherichia coli (*E. coli*) is known to cause diseases such as hemorrhagic enteritis and hemolytic uremic syndrome in animals, while *Salmonella* infections range from mild gastroenteritis to severe systemic illnesses like enteric fever. However, both bacteria are increasingly exhibiting resistance to antibiotics, posing a global challenge. Epidemiological studies on antibiotic resistance in E. coli vary widely in methodology, prompting a systematic review to assess its prevalence across different regions (Erb et *al.,* 2007; Guo et *al.,* 2024). The emergence of multidrug-resistant strains of Salmonella further complicates treatment efficacy and raises concerns about increased mortality rates (Eng et *al.,* 2015; Kiarely et *al.,* 2022).

Nanobiotechnology, a convergence of biology, medicine, and nanotechnology, is reshaping healthcare by advancing diagnosis, treatment, and disease prevention (Malik et *al.,* 2023). Nanomaterials possess remarkable physical, chemical, and biological characteristics, making them indispensable in modern medicine (Ahmed & Othman, 2024). Various manufacturing techniques, including chemical, physical, and green methods, have been developed for nanomaterial synthesis, with green methods utilizing biological materials for safer and more environmentally friendly production (Chopra et *al.,* 2022; El-Khawaga et *al.,* 2023). Bacteria are particularly notable for nanoparticle synthesis due to their advantages (Talebian et *al.,* 2023). Copper oxide (CuO) nanoparticles exhibit diverse properties such as

catalytic, anti-cancer, coating, and antibacterial effects, offering promising applications (Naz et *al.*, 2023).

In the first part of this study, it had an insight to the vaccine importance and new techniques for its development. Meanwhile, in the second part, it was based on the in-vitro evaluation of the biosynthesis of CuONP using *S. enterica* and *E. coli*, and used as a biovaccination against infection by the studied bacteria.

First part

Bibliographic synthesis

Chapter one

Vaccination

I.1. Definition

A vaccine is a substance used in healthy people to provide immunity against a disease (Seneff et *al.*, 2022) by stimulating the production of antibodies, developing cellular immunity, or both. It can be prepared from the pathogenic agent, its products, or a synthetic substitute treated to act as an antigen without inducing the disease (Opriessnig et *al.*, 2021).

I.2. Vaccine types

There are mainly four different types of vaccines that are licensed and widely used today that can be classified according to their nature (Dai et *al.*, 2019; Tong, 2019; Choudhary et *al.*, 2023), while there is a continuous development process in vaccine manufacturing as shown in figure 1.

I.2.1. Live attenuated vaccines

It is laboratory-weakened versions of the pathogenic agent (generally viruses rather than bacteria, as it is easier to control viral characteristics), resulting in long-term immunity and strong cellular and antibody responses with lower doses, e.g., the vaccines against measles, mumps, and chickenpox (Clem, 2011). Attenuated vaccines cannot be given to immunocompromised individuals because they produce an actual disease and have the possibility of reverting to the original virulent form of the pathogen (Vetter et *al.*, 2017).

I.2.2. Inactivated vaccines

The preparation of inactivated vaccines is obtained by exposing the pathogenic agent to a physical or chemical treatment resulting in a definitive loss of virulence without denaturing its immunogenicity, such as vaccines against rabies, so-called cellular or whole-cell pertussis, influenza, or hepatitis A (Speck, 2009). The inactivated vaccines are safer but tend to induce a moderate immune response, particularly in children and the elderly (Lamontagne et *al.*, 2022).

I.2.3. Subunit vaccines

These vaccines are based on single or multiple highly purified immunodominant antigens and specific components isolated from the pathogen to induce immunity (Menon et *al.*, 2021; De Pinho Favaro et *al.*, 2022), such as peptides, proteins, polysaccharides, and nucleic acids, which represent safer alternatives (Lemoine et *al.*, 2020). Polysaccharide antigens are the basis of vaccines that induce protective immune responses

against several bacterial infections, such as pneumonia and meningitis caused by *Streptococcus pneumoniae* (Pollard & Bijker, 2021). Also, subunit vaccines such as proteins, peptides, plasmid DNA, and mRNA have attracted much attention for immunization due to their high potency and specificity, like the anti-COVID-19 mRNA vaccine (Cao et *al.*, 2022).

I.2.4. Recombinant vaccines

These are created by using genetic engineering methods to create a vaccine that contains a specific, targeted piece of the pathogen's genetic material. These vaccines are more sustainable alternatives to antibiotics because they are highly specific and target only the pathogen without affecting other bacteria in the gut microbiome (Islam & Rahman, 2023), such as the hepatitis B virus recombinant proteins vaccine (Pollard & Bijker, 2021) and the human papilloma virus vaccine (Yadav et *al.*, 2018).

I.2.5. Toxoid vaccines

Harmful substances secreted by bacteria, called toxins, are inactivated with heat or chemical treatments (e.g., formaldehyde), ablating the biological effects of these toxins while preserving their physicochemical properties, overall structure, and immunogenicity (Blin, 2021). These vaccines induce immunity against the disease-causing toxin rather than the pathogen itself (Ghattas et *al.*, 2021), such as diphtheria and tetanus toxoids (Su et *al.*, 2019).

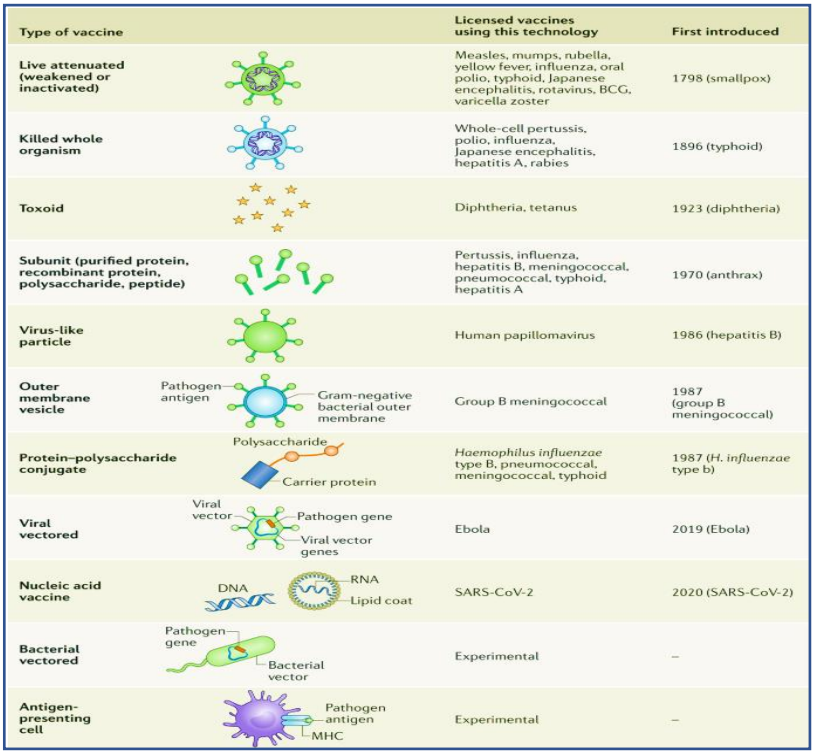


Figure 1: Different types of vaccine (Pollard & Bijker, 2021)

I. 3. Administration of vaccines

The vaccination must follow a suitable route that is optimal regarding safety, immunogenicity, and practicality (Herzog, 2014). Commonly, parenteral routes have been used to deliver vaccines, particularly through the intramuscular (IM) and subcutaneous (SC) routes (Cordeiro et *al.*, 2021) (figure 2).

I.3.1. Intramuscular (I.M.) administration

It is the most used and preferred route for the licensed vaccines (Kozak & Hu, 2023), as it is easy to perform and generally well tolerated, with a low risk for adverse reactions at the site of injection (Ols et *al.*, 2020).

I.3.2. Subcutaneous (S.C.) routes

It involves injecting under the skin into the adipose layer beneath the dermis (Lee et *al.*, 2023). The reduced dosages of various vaccines into the skin, compared with full dosages into muscle, have shown this tissue's dosage sparing ability, which is useful when vaccines are scarce or unaffordable in full dosages (Weniger & Papania, 2013), such as live-attenuated vaccines. The vaccine takes longer to reach the circulation after being deposited in fat that is less vascular than muscle. This decreased vascularity results in a slower and more sustained release, leading to a delay in processing by macrophages and eventual presentation to the T and B cells involved in the adaptive immune response (Kozak et *al.*, 2023).

I.3.3. Intradermal (ID) routes

It is a promising alternative to I.M. or S.C. vaccinations, including the Bacille Calmette-Guérin (BCG) vaccine for tuberculosis, reduced doses of rabies vaccines, and DNA vaccines (Kim et *al.*, 2012). The epidermis contains a high number of dendritic cells, and their stimulation results in the activation of both mucosal and systemic immunity (Peletta et *al.*, 2023). Novel needle-free technologies such as high-pressure stream injectors or electroporating are used in intradermal vaccination (Ahmed et *al.*, 2023).

I.3.4. Oral vaccination

Several human vaccines are already licensed and in use for delivery by oral ingestion, like vaccines for polio, cholera, rotavirus, typhoid, and adenovirus (Lemoine et *al.*, 2020). It is a preferred, painless, safe, and low-cost route for vaccination that does not require trained personnel for administration. The oral route commonly involves swallowing the vaccine,

which then passes through the gastrointestinal tract. Developing this type of vaccine must consider the harsh gastrointestinal (GI) conditions (stability in a highly enzymatic environment and resistance to site-specific pH) (Wallis et *al.*, 2019).

I.3.5. Intranasal vaccination

It delivers airborne particles via the nose or mouth for deposition onto the mucosal surfaces of the upper or lower airways. Since the beginning of immunization, the respiratory tract has been used for vaccine delivery, like the live-attenuated influenza vaccine. The major advantages of respiratory immunization are that it avoids needles and generally provides stronger mucosal immunity than parenteral immunization (Weniger & Papania, 2013).

Moreover, mucosal (oral, intranasal) vaccines are convenient for self-help vaccination, enhancing the comfort and compliance of individuals and reducing syringe usage and medical waste, due to which they are regarded as a resource-saving and environment-friendly strategy for a sustainable healthcare model (Nian et *al.*, 2022). In addition, the majority of human pathogens gain access across mucosal surfaces in the gastrointestinal, respiratory, or genitourinary tracts (Weniger & Papania, 2013), activating the mucosal-associated lymphoid tissues (MALT) (Peletta et *al.*, 2023).

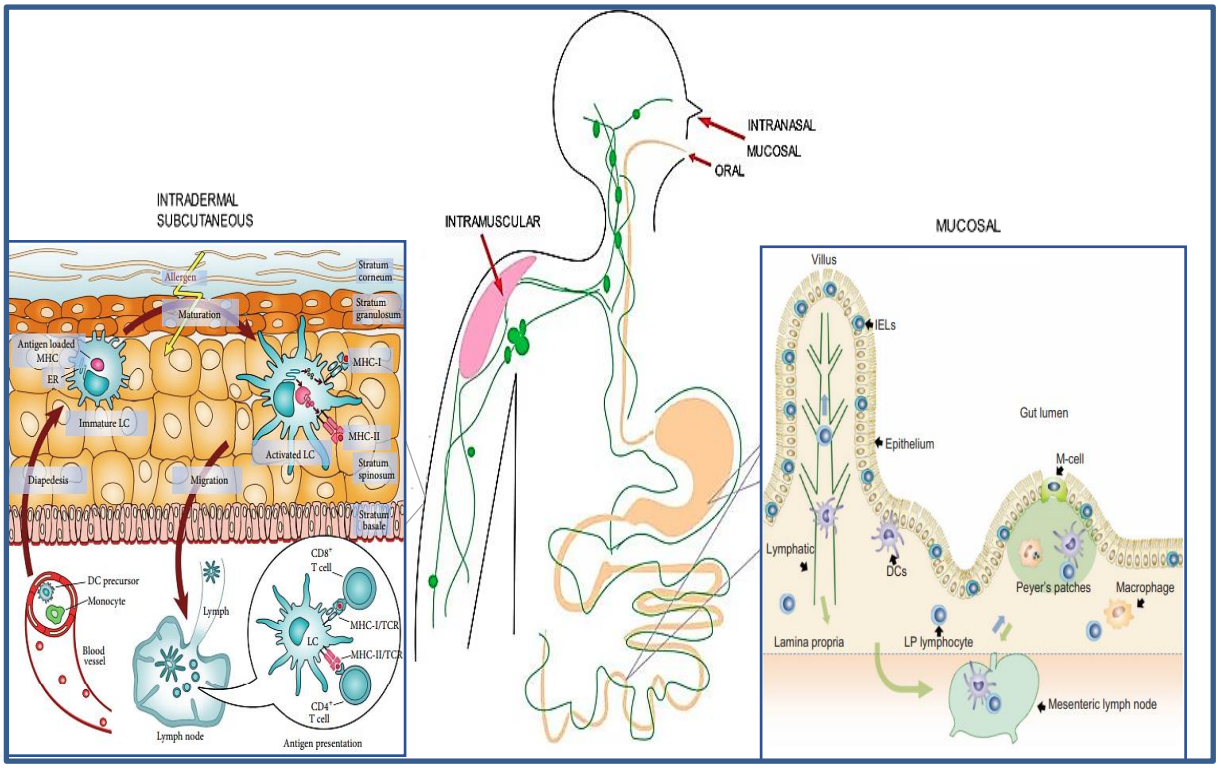


Figure 2: Vaccine Delivery Routes (Kozak & Hu, 2023)

I.4. Mechanism of action of a vaccine

Most vaccines are developed to mimic pathogens, thereby stimulating the individual's immunity to the disease (Yadav et *al.*, 2018) (figure 3).

Innate immunity is the first line of defense against pathogens that have entered the body. It is established within a few hours but is not specific to a particular pathogen and has no memory (Vetter et *al.*, 2017). In this type, bacterial vaccines stimulate the production of phagocytes such as macrophages (MØ) and neutrophils, which destroy the immunostimulants (Islam & Rahman, 2023). Specific immunity provides a second line of defense, generally at a later stage of infection, characterized by an extraordinarily diverse set of lymphocytes and antibodies able to eliminate virtually all recognized pathogens (Vetter et *al.*, 2017).

After vaccination, bacterial protein antigens reach draining lymph nodes and are taken up by dendritic cells that differentiate into active antigen-presenting cells (APCs), which contain preformed major histocompatibility complex class II (MHCII) molecules loaded with pathogen peptides (Osterloh, 2022). Activated CD4+ T helper cells release inflammatory mediators specific to the activated subpopulation (Th1, Th2, and Th17), facilitating the differentiation of B-cells into antibody-producing plasma cells or B-memory cells. In the systemic circulation, bacterial pathogens are exposed to circulating antibodies and complement factors that coat the bacterial cell surface, leading to their recognition and destruction by phagocytes (Poolman, 2020).

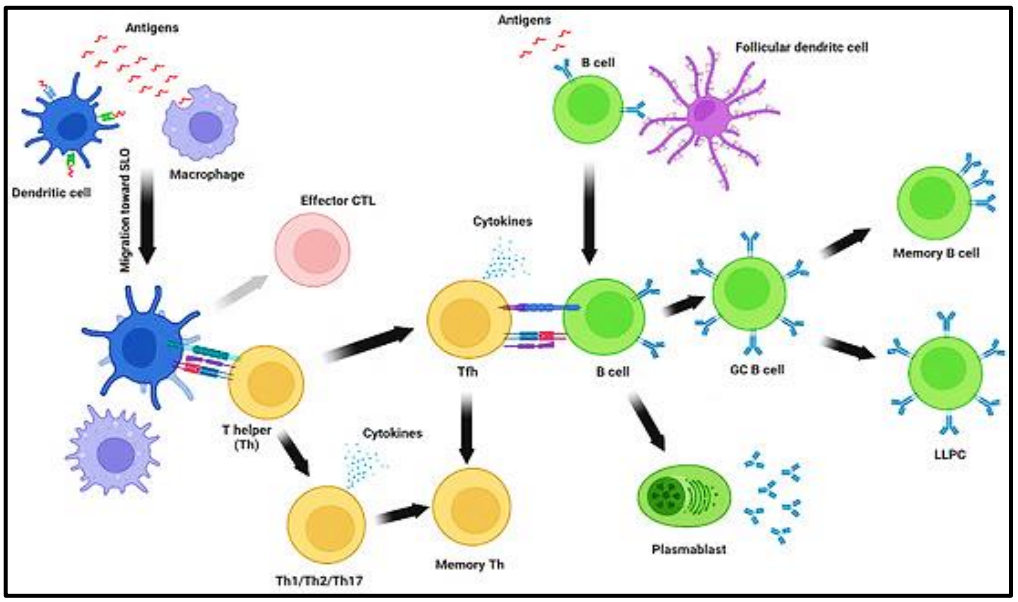


Figure 3: Mechanism of the Immune response to vaccines (Lamontagne et *al.*, 2022)

Chapter two

Escherichia coli and Salmonella enterica

II.1. *Escherichia coli*

II.1.1. Description

E. coli is a facultative anaerobic, rod-shaped bacterium of straight, rod-shaped, non-sporing, non-acid fast bacilli that exist in singles and pairs. Cells are typically rod-shaped, around 1 μm long, 0.35 μm wide, and 0.6–0.7 μm in volume. It belongs to the coliform family (Yu et *al.,* 2021; Basavaraju & Gunashree, 2023). Most strains are harmless and part of the normal intestinal flora, but some pathogenic serotypes can cause serious infections (Cobo-Simón et *al.,* 2023).

II.1.2. Classification of *Escherichia coli* (Faner et *al*.,2017)

Domain	Bacteria
Phylum	Pseudomonadota
Class	Gammaproteobacteria
Order	Enterobacterales
Family	Enterobacteriaceae
Genus	Escherichia
Species	*E. Coli*

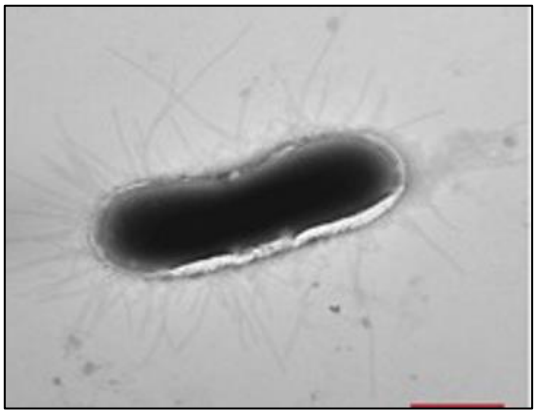


Figure 4 : *E. Coli* (Jauffred et *al.*, 2017)

II.1.3. Pathogenicity of *E. coli*

Pathogenic *E. coli* is divided into two groups: extraintestinal pathogenic *E. coli* (ExPEC) and intestinal pathogenic *E. coli* (InPEC) (Sora et *al.,* 2021). It can be categorized into various pathotypes (Table 1), depending on the presence of specific virulence factors, mechanisms of infection, tissue tropism, interactions with host cells, and clinical symptoms (Pokharel et *al.,* 2023).

Table 1: *E. coli* pathotypes and their associated symptoms (Geurtsen et *al.*, 2022)

Pathotypes of *E. coli*	**Clinical symptoms**
Enteropathogenic *E. coli* (EPEC)	A cause of acute and prolonged diarrhea in infants
Enterohemorrhagic *E. coli* (EHEC)	Can cause hemorrhagic colitis and hemolytic uremic syndrome (HUS)
Enterotoxigenic *E. coli* (ETEC)	A major cause of travelers' diarrhea
Enteroaggregative *E. coli* (EAEC)	A cause of acute and chronic diarrhea
Diffusely adherent *E. coli* (DAEC)	Associated with watery diarrhea in young children
Enteroinvasive *E. coli* (EIEC)	A cause of dysentery and watery diarrhea
Adherent-Invasive *E. coli* (AIEC)	It has been associated in the pathogenesis of inflammatory bowel disease (IBD)
Uropathogenic *E. coli* (UPEC)	A common cause of urinary tract infections (UTI)
Neonatal meningitis *E. coli* (NMEC)	A top cause of neonatal meningitis
Septicemia-associated *E. coli* (SEPEC)	bacteremia and sepsis
Avian pathogenic *E. coli* (APEC)	Can cause severe respiratory and systemic infections in poultry

II.2. *Salmonella enterica ssp*

II.2.1. Description

Salmonella enterica ssp. enterica is a subspecies of the bacterium *Salmonella enterica*, which is a species of the genus Salmonella. This subspecies includes many pathogenic serotypes, including serotypes Typhimurium, Enteritidis, and Paratyphi, which are responsible for serious diseases such as typhoid fever, paratyphoid fever, and salmonellosis. *Salmonella enterica ssp. enteric*a is a gram-negative bacterium, most often flagellated, which is part of the Enterobacteriaceae family (Porwollik et *al*., 2004).

II.2.2. Classification of *Salmonella enterica ssp*

Domain	Bacteria
Phylum	Pseudomonadota
Class	Gammaproteobacteria
Order	Enterobacterales
Family	Enterobacteriaceae
Genus	Salmonella
Species	*Salmonella enterica*
Subspecies	*Salmonella enterica subsp. Enterica* (Reimer et *al.,* 2021)

II.2.3. Pathogenicity of *Salmonella enterica ssp*

*Salmonella enterica subsp. enterica c*an cause several types of serious illnesses in humans. *Salmonella* species pose diverse pathogenic risks, primarily through gastroenteritis characterized by symptoms like diarrhea, abdominal pain, fever, and vomiting, which can escalate to severe outcomes in vulnerable populations. Additionally, Salmonella serotypes Typhi and Paratyphi cause severe enteric fevers with potentially life-threatening complications (Fàbrega et Vila, 2013). Salmonella bacteremia, particularly in immunocompromised individuals, can lead to serious conditions such as septic shock and endocarditis. Furthermore, *Salmonella* contributes to various localized infections. Some individuals may develop chronic carrier states, posing ongoing public health challenges. Understanding Salmonella's pathogenicity is crucial for effective prevention and management strategies (Eng et *al.,* 2015).

Chapter three

Nanoparticles

III.1. Nanotechnology

Nanotechnology has emerged as an important scientific achievement in the 21st century (Altammar, 2023), is a scientific discipline that employs diverse synthesis strategies to manipulate the structure and size of particles, crafting nanometric particles typically ranging from 1 to 100 nm in size (Jamkhande et *al*., 2019). It enables the precise manipulation of particle size, affording control over molecular bonds and chemical characteristics. Moreover, it facilitates intricate engagements with biological systems, exemplified by targeted drug delivery mechanisms (Malik et *al*., 2023). It exhibits a diverse array of applications spanning catalysis, energy storage, water treatment, agriculture, medicine and diagnostic methods.... etc (Sim & Wong, 2021; Joudeh & Linke, 2022).

Nanotechnology has revolutionized medicine, leading to significant progress in health care outcomes, including the treatment of cardiovascular disease, kidney disease, and cancer. In addition, gene therapy has greatly benefited from nanomedicines. Nanoparticles enable precise targeting of medications to the root cause of diseases, enhancing efficacy while minimizing side effects (Haleem et *al*., 2023).

III.2. Nanoparticles

Nanoparticles (NPs) are solid colloidal particles that can exist in varying shapes and sizes .their sizes range from about 1 nm to 100 nm (Hima et *al*., 2023).Depending on the overall shape these materials can be 0D, 1D, 2D or 3D (Khan et *al*., 2019).These nanomaterials also have unique chemical, physical and biological properties compared to their molecules on higher scales (Mekuye & Abera, 2023).They have a higher surface area to volume ratio, allowing greater concentration of atoms or molecules within a given volume, where the same effectiveness is achieved with the least amount of materials needed (Álvarez & Arenas, 2023).

III.3. Types of nanoparticles

III.3.1. Organic nanoparticles

Proteins, carbohydrates, lipids, polymers, or any other organic component fall under this class (Joudeh& Linke, 2022), some examples of these substances are: ferritin, liposomes, micelles, and dendrimer (Ealia & Saravanakumar, 2017). Additionally, three dimensional (3D) dendrimers have interior pores that can contain additional molecules, making the m potentially useful for medication delivery (Mekuye & Abera, 2023), they are also non-

toxic, biodegradable, sensitive to heat and light (Ijaz et *al*., 2020; Khan et *al*., 2022) (figure5).

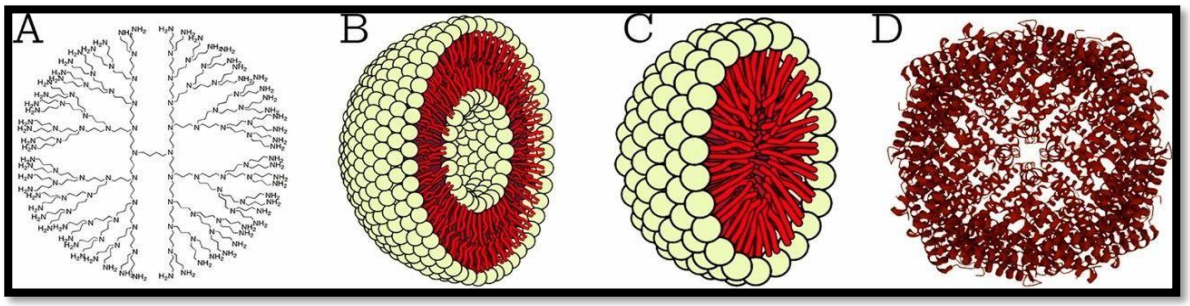


Figure 5: Types of organic nanoparticles (**Joudeh & Linke, 2022**)

A Dendrimers; B liposomes; C micelles; D ferritin

III.3.2. Carbon-based nanoparticles

NPs that are only composed of carbon atoms are known as fullerenes, carbon black NPs, and carbon quantum dots (figure 6), they are used in a wide range of application such as drug delivery, energy storage, bioimaging, photovoltaic devices, and environmental sensing applications to monitor microbial ecology or to detect microbial pathogens (Joudeh& Linke, 2022).

These materials are produced using a variety of methods, including chemical vapor deposition (CV D), arc discharge, and laser ablation (Mobeen et *al*., 2022).

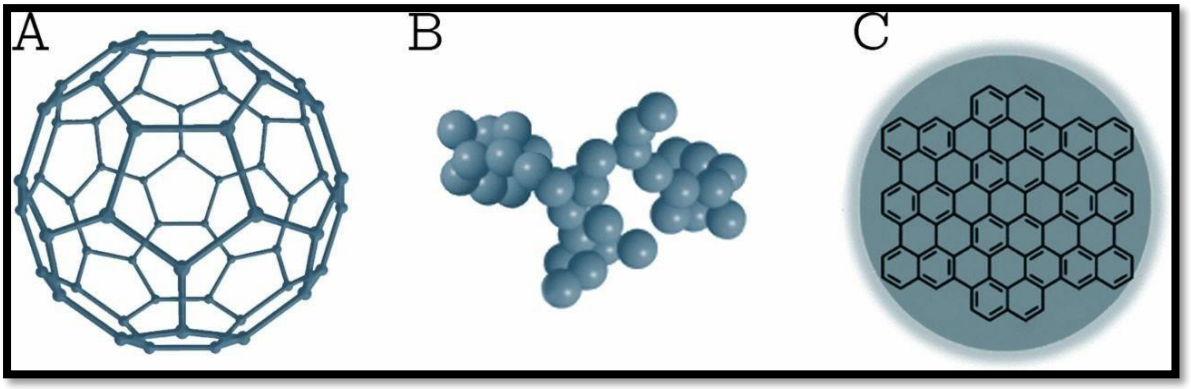


Figure 6: Types of carbon-based nanoparticles (**Joudeh & Linke, 2022**)

A C60 fullerene ; B carbon black NPs ; C carbon quantum

III.3.3. Inorganique nanoparticles

Non-carbon-containing nanoparticles are known as inorganic ones (Kumari & Sarkar, 2021). Among these NMs are metal, metal oxide nanoparticles, and semiconductors like silico n and ceramics (Jeevanandam et *al*., 2018), they exhibit remarkable physicochemical

qualities (magnetic, thermal, optical, and catalytic performance), as well as enormous surface areas and strong reactivity (Unnikrishnan et *al*., 2023; Yanar et *al*., 2023). Hydrophilic, nontoxic, and biocompatible with living systems are the benefits of inorganic nanoparticles, they are also more stable than organic nanoparticle (Alshammari et *al*., 2023).

INps are some of the most produced and utilized in commercially and their antibacterial and anti- cancer properties make them useful as medicinal agents (Bhatti et *al*., 2022).

III.4. Copper oxide nanoparticles (CuO NPs)

Copper (Cu) is an essential trace element for many physiological functions, such as energy production, hormone synthesis, melanin and hemoglobin formation, iron metabolism, and protection against oxidative damage. As a crucial cofactor for various enzymes and chaperones (Tulinska et *al*., 2022; Lu et *al*., 2024), addition it has a role in the development and differentiation of immune cells (Li et *al*., 2019).

Copper oxide nanoparticles (CuO NPs) are inorganic and exhibit greater stability compared to organic NPs (Naz et *al*., 2023). Their large surface-to-volume ratio contributes to their optical, catalytic, and magnetic properties (Kaningini et *al*., 2023). Additionally, CuO nanoscale materials display low toxicity toward living cells (Velsankar et *al*., 2020) and have an extended shelf life (Bukhari et *al*., 2021). These nanoparticles find various biological applications such as antimicrobial, antioxidant, antitumor, anticoagulant, and as drug delivery agents in biomedicine (Bulut Kocabas et *al*., 2021; Saha et *al*., 2023). Due to their small size, can readily, interact with the biomolecules present on the cell surface and intracellularly (Verma & Kumar, 2019).

III.5. Green synthesis nanoparticles

The process commonly known as "green" or "biological" nanoparticle synthesis harnesses biologically active agents such as plant materials, microbes, and various biowastes to fabricate metal nanoparticles (Altammar, 2023). Unlike chemical and physical methods, which rely on hazardous and costly chemicals, biosynthesis offers an environmentally benign alternative. This method is characterized by simplicity, and cost-effectiveness, resulting in the production of biocompatible nanoparticles with desired size and shape properties, non-toxicity, and scalability (Naz et *al*., 2023; Ivanova et *al*., 2024; Osman et *al*., 2024). Furthermore, this approach is notable for its one-step synthesis

capability. The process involves mixing a mineral salt solution with a natural biological extract, initiating a redox reaction that yields nanomaterials. Additionally, biosynthesis requires minimal energy input and can be conducted under ambient conditions (Abouzeid et *al*., 2023) (figure7).

Bacteria stand out as ideal candidates for synthesizing nanoparticles due to their numerous advantages, including high stability, rapid generation time, tolerance to various experimental conditions, ease of cultivation, resistance to toxic heavy metals, and capacity for large-scale sustainable nanoparticle production(Talebian et *al*., 2023).Where synthesis processes occur intracellular or extracellular (Lahiri et *al*., 2021) where, analysis 149 research articles published in the last five years (2018,2022) that used 72 different types of microorganism classes, including 44 bacteria, to produce MNPs, among them were *Streptomyces*, *Lactobacillus*, *Bacillus*, and *E.coli*.....etc (Carrapiço et *al*., 2023)

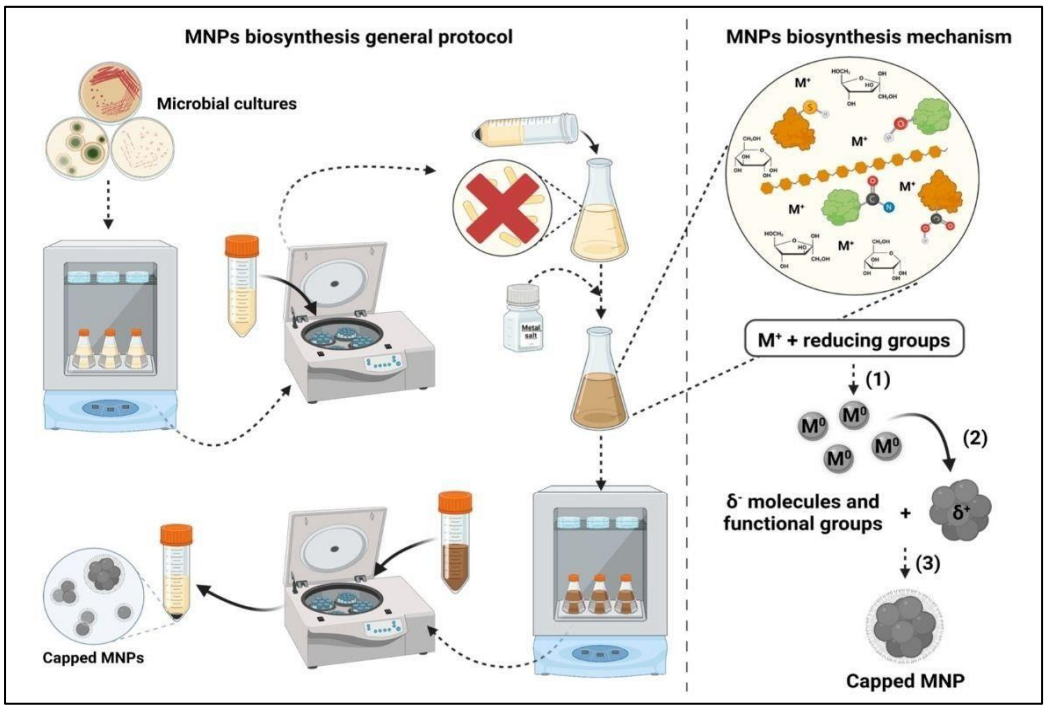


Figure 7: Green synthesis nanoparticles using microorganisms

(**Carrapiço et *al.*, 2023**)

III.6. Toxicity of nanoparticles

The synthesis of nanoparticles in chemical manufacturing necessitates the use of hazardous chemicals and solvents, posing environmental risks. Furthermore, resultant nanoparticles are tainted with chemical residues and yield perilous byproducts (Szczyglewska et *al*., 2023).

The physicochemical properties of Nps influence how they interact with cells and thus their potential toxicity, smaller nanoparticles have a higher specific surface area (SSA), they can interact with biological constituents like proteins, fatty acids, carbohydrates, and nucleic acids. The smaller size also likely allows for penetration into the cell, causing cellular damage. In some nanoparticles, toxicity has been shown to depend on both size and SSA (Huang et *al.,* 2017). In addition, charged NPs are more toxic to cells than their neutral counterparts, and in most cases, positively charged NPs are more toxic than negatively charged NPs (Awashra & Młynarz, 2023).

NPs enter the human body through the lung, intestinal system, or skin through ingestion or inhalation and then move within the body to various organs and tissues, thus triggering potential toxic effects. It is worth noting that the respiratory system appears as a pivotal target for the toxicity of NPs as it is the main gateway for inhaled particles, which causes Lung inflammation, heart and brain problems (Khan et *al*., 2019; Kumah et *al*., 2023**)**.

Second part

Experimental part

Chapter one

Materials and Methods

I.1. Materials

I.1.1. Microorganisms

Salmonella enterica and *Escherichia coli* isolated from patients were obtained from Al Nour Clinic, Department of Microbiological Analysis, and preserved in selective culture at 4 °C.

I.1.2. Animals

In this study, 8-week-old 20 male albino Wistar rats weighing between 156-235 g were sourced from the Institute Pasteur of Algiers. They were housed in cages within the animal room of the Department of Molecular and Cellular Biology at the Faculty of Nature and Life Sciences, Echahid Hamma Lakhdar-El-Oued University, Algeria. The animals underwent a 7- day adaptation period under standard conditions of room temperature with a 12-hour light/dark cycle. Throughout the study, the rats were given free access to food and water.

I.1.2.1. Study design

After a period of adaptation, the animals were divided into five groups (n = 4), as follows:

- Group I (control group): a non-vaccinated group injected intraperitoneally only with 0.5mL of physiological water.

- Group II (experimental *S. enterica* infection): were injected intraperitoneally with 0.5 mL of physiological water. After two weeks, we were exposed intraperitoneally to 200 µl suspension of the live bacteria *S. enterica*.

- Group III (experimental *E. coli* infection): were injected intraperitoneally with 0.5 mL of physiological water. After two weeks, we were exposed by injection to 200 µl of live *E. coli* suspension.

- Group IV (SE-CuNPs-vaccinated): were vaccinated by intraperitoneally injection (7 mg/kg) with SE-CuNPs synthesized by *S. enterica*. After two weeks, we were exposed by injection to 200 µl suspension of live *S. enterica*.

- Group V (EC-CuNPs-vaccinated): were vaccinated intraperitoneally with CuNPs synthesized by *E. coli* (7 mg/kg). After two weeks, we were exposed by injection to 200µl suspension of live *E. coli*.

The CuNPs solution was obtained by ultrasonic technique for 60 min at 60 °C, and their body weights were recorded twice a week during the experiment period.

I.1.2.2. Sacrifice, blood sampling and tissue collection

Four days after infection, the rats were sacrificed following a 16-hour fasting period. under slight anesthesia by chloroform (94%) by inhalation. During euthanasia, blood samples were collected from the animals and placed in EDTA tubes for hematological analysis and heparin tubes for biochemical analysis. The serum was obtained by centrifuging for 5 minutes at 2000 rpm, and blood sugar was measured by a glucometer. The weights of the organs were recorded, washed with saline (Nacl 0.9%), and frozen at -20 °C until use. The spleen tissues are fixed in 10% formaldehyde for histological analoysis.

I.1.3. Reagents and products

Hydrogen chloride (HCl), chloroform, sodium chloride (NaCl), ascorbic acid ($C_6H_8O_6$), Tris ($C_4H_{11}NO_3$), Riboflavin, Trichloroacetic Acid (TCA), Thiobarbituric Acid (TBA), Butylated Hydroxy Toluene (BHT), Nitroblue Tetrazolium (NBT), Dipotassium phosphate (K_2HPO_4), Potassium dihydrogen phosphate (KH_2PO_4), Ethanol, Ethyle nediaminetetraacetic acid (EDTA), physiological solution (Na Cl 0.9%), and distilled water.

I.2. Methods

I.2.1. Statistical study

This statistical study aims to conduct a comprehensive analysis of bacterial infections in El-Oued city for the period from January 1, 2022, to December 31, 2022. Data were obtained from the Microbiology Department of El Medjed Laboratory, located in the El-Oued region of Algeria. The study encompassed the results of a cytobacteriological examination of urine (CBEU) samples and coproculture samples, covering individuals of all age groups.

I.2.2. In vitro study

I.2.2.1. biosynthesis of copper nanocomposites

Fresh E. coli and S. enterica are cultivated at 37 °C in Bouillon LB-Luria liquid media for 48 h. After incubation, the cell biomass was separated from the culture broth by centrifugation (5000 rpm) for 10 min and washed twice with deionized water, which was used for the nanoparticle synthesis.

15 ml of aqueous copper sulfate (100 mM) was mixed with 5 ml of a suspension containing 300 µl of biomass (PH between 9 and 10). The reaction mixture was incubated with constant stirring at 30 °C. An observed brown-black color marked the formation of CuNPs at the end of 24 hours.

The resulting product was centrifuged (5000 rpm for 30 min), rinsed twice in sterile distilled water, and dried in an oven at 35 °C. Sterile deionized (DI) water was used to prepare solutions in all experiments, and sodium hydroxide (NaOH) and hydrogen chloride (HCl) were used in pH adjustment.

I.2.2.2. Characterization of Copper nanoparticles

The Cu nanoparticles synthesized using *E. coli* and S. enterica through a biological method were characterized using various techniques: the UV-Vis range of 200-600 nm, Fourier transform infrared spectroscopy (FTIR) to know the present functional groups associated with CuO in the range 400-4000 cm -1 and the morphological and elemental of NPs was determined using scanning electron microscopy (SEM) coupled with EDX.

I.2.2.3. Biological activity

I.2.2.3.1. DPPH antioxidant test

In vitro antioxidant activity was evaluated by measuring the scavenging power of the DPPH (1,1- diphenyl-2-picryhydrazyl) radical. A DPPH radical scavenging assay was employed to determine, by a spectroscopic method, the relative plant antioxidant ability.

The anti-radical activities of plant extracts were estimated according to the method of **Nwidu et *al.* (2017)**.

Stock solutions of the synthesized NPs (0.2 mg/ml) (1 mg/5 ml) were prepared and diluted to final concentrations of 200, 100, 50, 25, 12.5, and 6.25 µg/ml in distillated water. 1600µL of 0.1 mM DPPH in ethanol solution was added to 20µL of the sample or standard, and then mixed with 20µL of H2O. Ascorbic acid (as a control solution) over the concentration range of 1.56, 0.78, 0.39, 0.195, and 0.0975 mg/ml was assayed under similar conditions. The mixtures were incubated at 37 °C for 40 min in the dark. Sample absorbance was read at 517 nm.

The percentage inhibition {(A0-A1/A0) × 100}

I.2.2.3.2. Hemolysis assay

- Principle

The hemolysis assay was done as described by **(Vinjamuri et *al*., 2015)** to determine the protective effect of the antioxidant compound presented in the sample against membrane erythrocyte lysis, which was induced by 1X PBS. RBC lysis was detected by measuring the concentration of hemoglobin in blood plasma at 560 nm with a spectrophotometer.

- Procedure

1. 5 mL of blood was collected from healthy volunteers in tubes containing 5.4 mg of EDTA to prevent coagulation and centrifuged at 1000 rpm for 10 min at 4 °C.

2. Plasma is removed carefully, and the white buffy layer was completely removed by aspiration with the utmost care.

3. Then, the erythrocytes were washed three times with 1X PBS (pH = 7.4) for 5 min.

4. The washed erythrocytes were stored at 4 °C and used within 6 hours for the hemolysis assay.

5. Add 50 µL of 10 dilutions (100 µL erythrocytes suspension and 900 µL 1XPBS) of erythrocytes suspension mixed with 100 µL of test samples (0.81-200µg/mL) and with 100 µL of 1XPBS (as a control).

6. The reaction mixture is incubated in a 37°C water bath for 60 min.

7. 850 µL of 1XPBS was added to the reaction mixture, which was centrifuged at 300 rpm for 3 min.

8. The concentration of hemoglobin in the supernatant is measured at 560 nm by a spectrophotometer.

Expression of results

Hemolysis inhibition percentage (IP) was calculated as follows:

$$\textbf{IP (\%) = (100-(OD sample) / (OD control)) ×100}$$

I.2.3. In vivo study

I.2.3.1. Hematological parameter analysis

Hematological analysis by XN-330 (Sysmex). The metrics included the leucocytes, the erythrocytes, and the platelet line.

2.3.2. Biochemical and enzymatic parameters analysis

Urea, Creatinine, Cholesterol, Triglycerides, Alanine aminotransferase (ALAT), Aspartate aminotransferase (ASAT), Creatine phosphokinase (CPK), and alkaline phosphatase (PA).

I.2.3.3. Oxidative stress tests

I.2.3.3.1. Homogenate preparation

0.5 gram of tissues (liver, spleen, heart, and kidney) was grinded and homogenized in 5 ml of phosphate buffer solution (PBS, pH = 7.4). Homogenates were centrifuged at 5000 rpm for 15 min. The obtained supernatant was conserved at -20 °C.

I.2.3.3.2. Determination of Malondialdehyde (MDA) level

- Principle

The common method for the assessment of MDA level is the thiobarbituric acid (TBA) assay, where MDA forms a complex with two molecules of 2-thiobarbituric acid (TBA) in the presence of acidic medium and heat. A change in the color of the solution to a pink color is an indication of the presence of MDA (Yagi, 1976).

- Reagent

375 mg of TBA, 20 g of TCA, 0.01 g of BHT, 25 ml of 1N HCL, and 50 ml of distilled water were mixed in a beaker. The solution obtained was heated to 40 °C in a water bath until the TBA was completely dissolved, then transferred to a 100 mL flask, and the volume was filled up with distilled water.

- Procedure

Pipette into the glass vise test tubes 200 µl of sample and 800 µl of TBA reagent and close tightly. Heat the mixture in a water bath at 100 °C for 15 minutes. Then cool in a cold water bath for 30 minutes, leaving the tubes open to allow the gases formed during the

reaction to be removed. Centrifuge at 3000 rpm for 5 minutes and read the absorbance of the supernatant at 532 nm using a spectrophotometer.

- Expression of results

The concentration of TBARS was determined using the molecular extinction coefficient of MDA (å = 1.53 105 M-1 cm-1). The results were expressed in µmol/1.

MDA (µmol/mg of prot) = (OD sample /1.53 x 10^5) / mg of protein

I.2.3.3.3. Determination of reduced glutathione (GSH) level

-Principal

The level of reduced glutathione is determined according to **Weckbecker & Cory, (1988)**. By measuring the optical density results from the formation of 2-nitro-5-mercocapturic acid (TNB) from the reduction of dithio-bis-2-nitrobenzoic acid (DTNB), which is called Ellman reagent with SH groups exist in GSH briefly.

- Procedure

1. 800 µl of homogenate samples are added to 200 µl of salicylic acid (0.25%). Then centrifuged at 1000 rpm for 5 min.

2. Take 500 ml of supernatant and mix it with 1000 µl of tris buffer solution (tris 0.4 mol, NaCl 0.02 mol; ph = 8.9) and 25 µl of DTNB (0.01 mol/L).

3. Read the absorbance at λ = 412 nm after 5 minutes of incubation**.**

Expression of results

GSH concentration is expressed according to the following formula:

$$GSH\ (nM/mg\ \ of\ protein) = \frac{(OD \times 1 \times 1.525)}{13133 \times 0.8 \times 0.5 \times mg\ \ of\ protein} \times d$$

OD: Optical Density.

1.525: total volume of blend an ml.

13133: Absorption constant of SH groups at 412 nm.

0.5: volume of solution float an ml.

1: volume of protein mixture.

0.8: volume of homogeneous solution without protein exists in 1 ml.

GSH: concentration of glutathione.

d: dilution factor.

I.2.2.3.4 Determination of Super Oxide Dismutase (SOD) activity

- Principle

This test is based on the inhibition of NBT reduction by SOD. NBT is reduced by the superoxide anion O2-, and it is known that SOD neutralizes O2-, which inhibits the reduction of NBT (Beauchamp &Fridovich., 1971).

- Procedure

Collect in tubes	Blank	Sample
EDTA-Met (0.1mM, 13mM)	1000µL	1000µL
Phosphate buffer (50Mm)	1892,2 µL	1842,2 µL
Sample	-	50
NBT (75µM)	85,2µL	85,2µL
Riboflavin (2µM)	22,6µL	22,6µL

Expression of results

Inhibition percentage of NBT reduction by SOD as follows:

IP (%) = (OD blank – OD sample) / (OD blank) ×100

IP50%= 1UI of SOD

2.2.4. Histological study

Histological sections were done following the method of **Ishfaq et *al.* (2019)**, with a slight modification. 10% buffered formalin was used for fixing spleen samples, followed by dehydration with graded ethanol. The samples were processed for paraffin wax and cut into sections (5 µm thickness). After that, the sections were mounted on slides, stained with hematoxylin and eosin dye, and observed under a light microscope (10X and 40X magnifications).

I. 2.2.5. Statistical analysis

The results obtained are expressed as the mean ± standard error of the mean (Mean ± SEM). The analysis of the data was carried out by application of the Student's T test. All data in this study were examined by Minitab 13.0 software. $p < 0.05$ indicates statistically significant difference.

Chapter two

Results

II.1. Statistical study

II.1.1. CBEU and coproculture analysis according to the type of bacteria

Among the 5380 CBEU samples, *E. coli* exhibited the highest prevalence, comprising 64.15% of the isolates, whereas *K. pneumoniae* constituted 14.54%. On the other hand, *E. coli* accounts for 87% of infection cases in 432 coproculture samples (figure8).

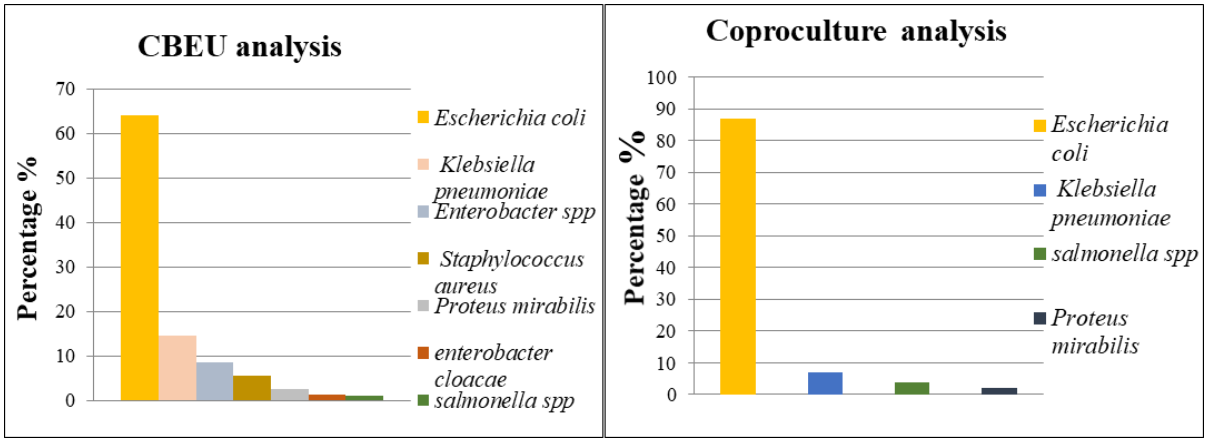


Figure 8: CBEU and coproculture analysis according to the type of bacteria

II.1.2. CBEU and coproculture analysis according to age

Figure 9 of the 5380 examined samples illustrates that the 65–75 age group showed the highest susceptibility to bacterial infection, accounting for 32% of cases. This was followed by the age groups 25–65 and 18–25, with infection rates of 23% and 12%, respectively. Among the 432 coproculture samples, the results showed that children aged 1 year had the highest infection rate (46% of all cases) and 30% of all cases at 3 months.

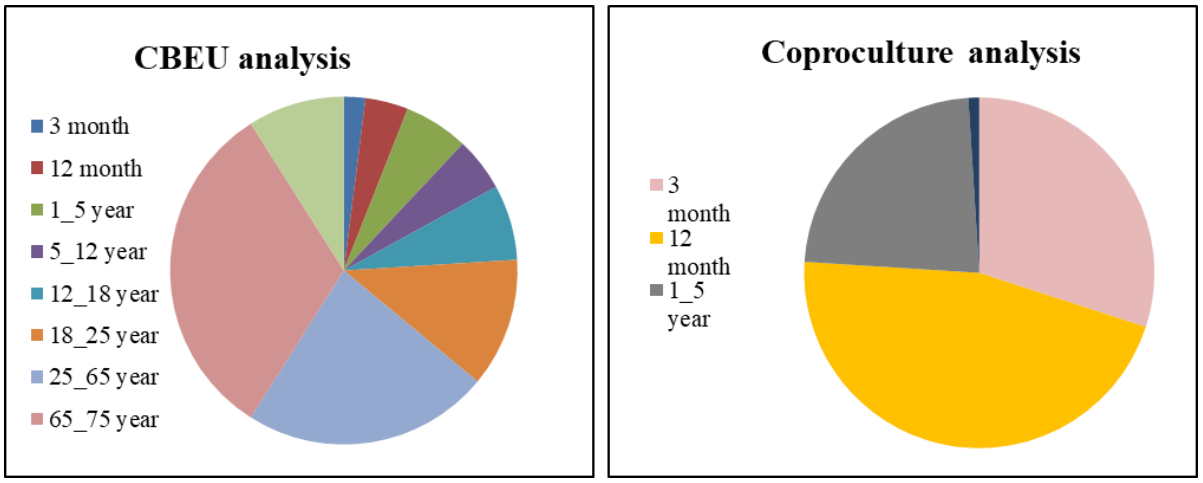


Figure 9: CBEU and coproculture analysis according to age

II.1. In vitro study

II.1.1. Characterization of copper nanoparticles

II.1.1. UV-VIS spectroscopy

The UV-Vis spectrum of CuONps synthesized from *E. coli* and *S. enterica* absorption spectra is shown in figure 10 and 11, respectively. The production of CuONPs was confirmed by a maximum absorption at 250 nm and 300 nm for ECuONPs and SCuONPs, respectively.

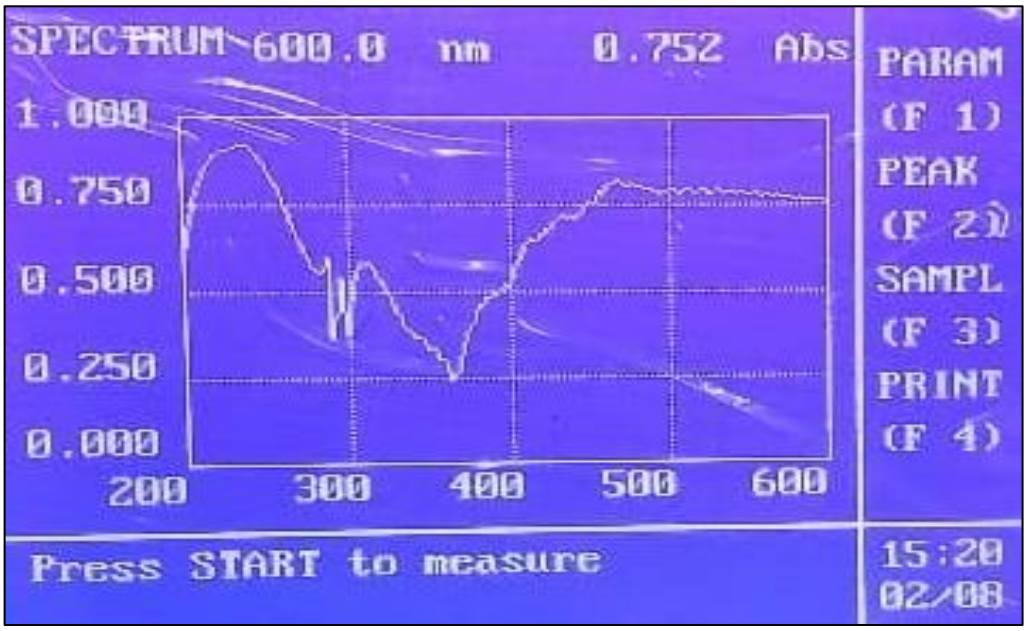


Figure 10: UV-Vis spectrum of EC-CuNP

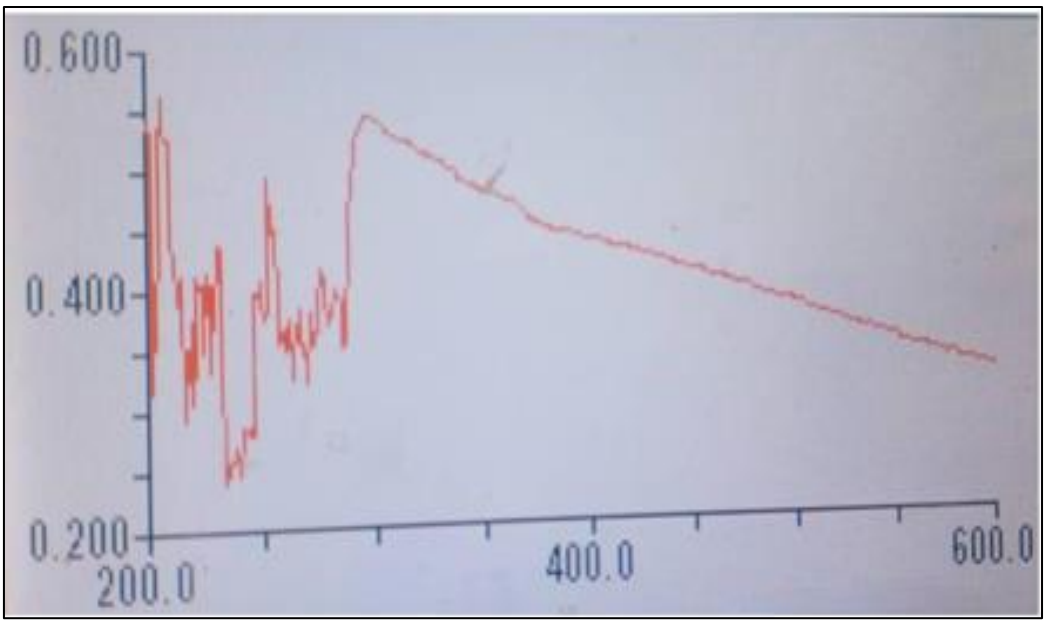


Figure 11: UV-Vis spectrum of SE-CuNP

II.1.1.2. FT- IR analysis

Figures 12 and 13 showed photographs of the FTIR spectroscopy recorded for SCuONps and ECuONPs, respectively. The presence of the band at 600 cm1 in both observed samples is indicative of the characteristic bands associated with the Cu-O bond.

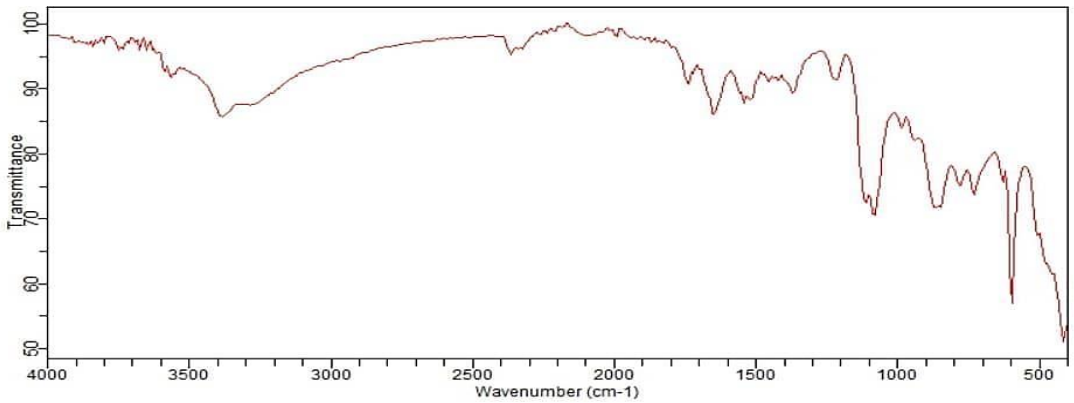


Figure 12: FTIR spectrum of SE-CuNPs

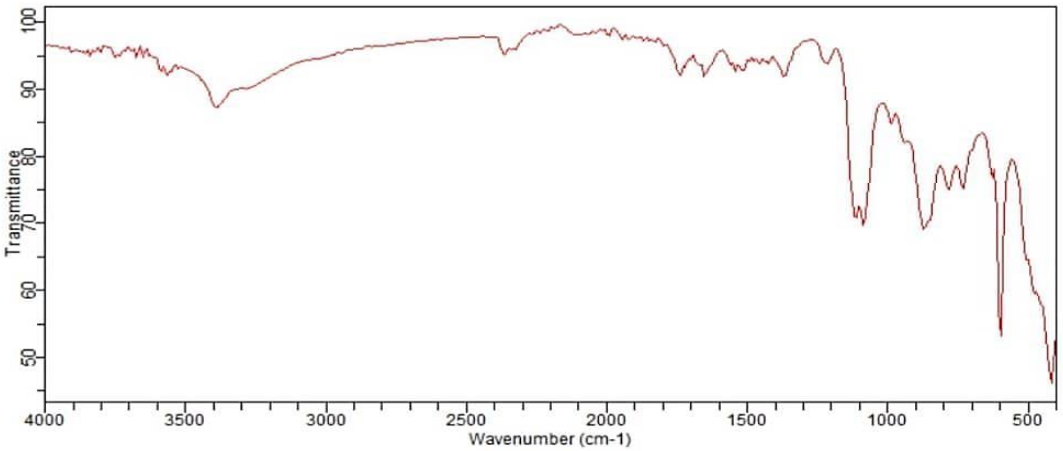


Figure 13: FT-IR spectrum of EC-CuNPs

II.1.1.3. Scanning electron microscopy and EDX

The SEM pictures of CuNPs biosynthesised by *E. coli* and *S. enterica* revealed that these nanoparticles had an oval structure and were between (73–183 nm) and (135–202 nm) in size, respectively. Additionally, the chemical compositions of CuO Nps were detected with EDX analysis, as shown in figure 14 and 15.

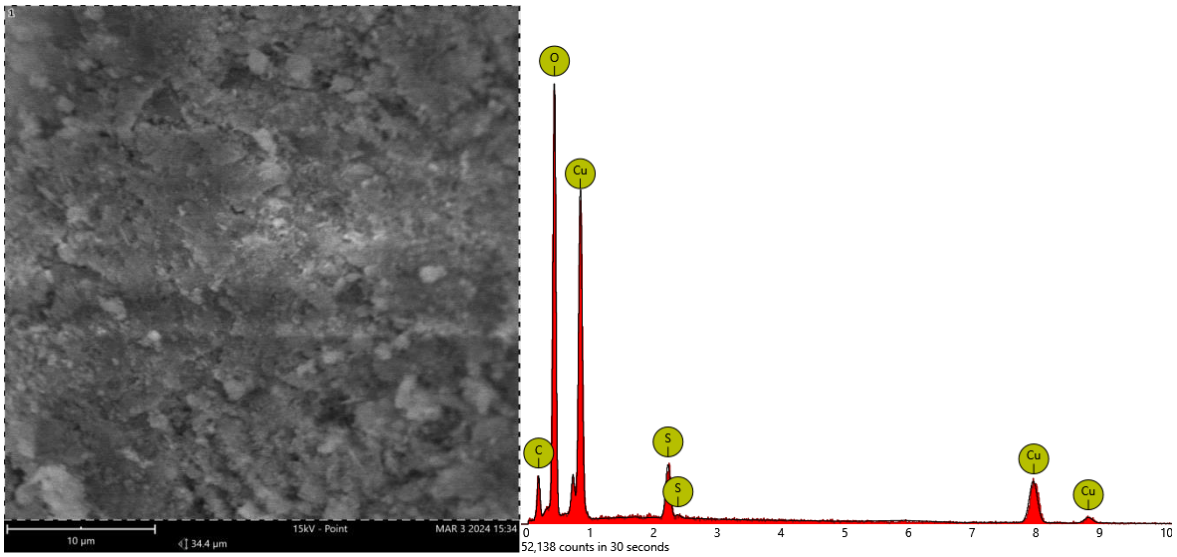


Figure 14: Scanning electron microscopy images of EC-CuNPs

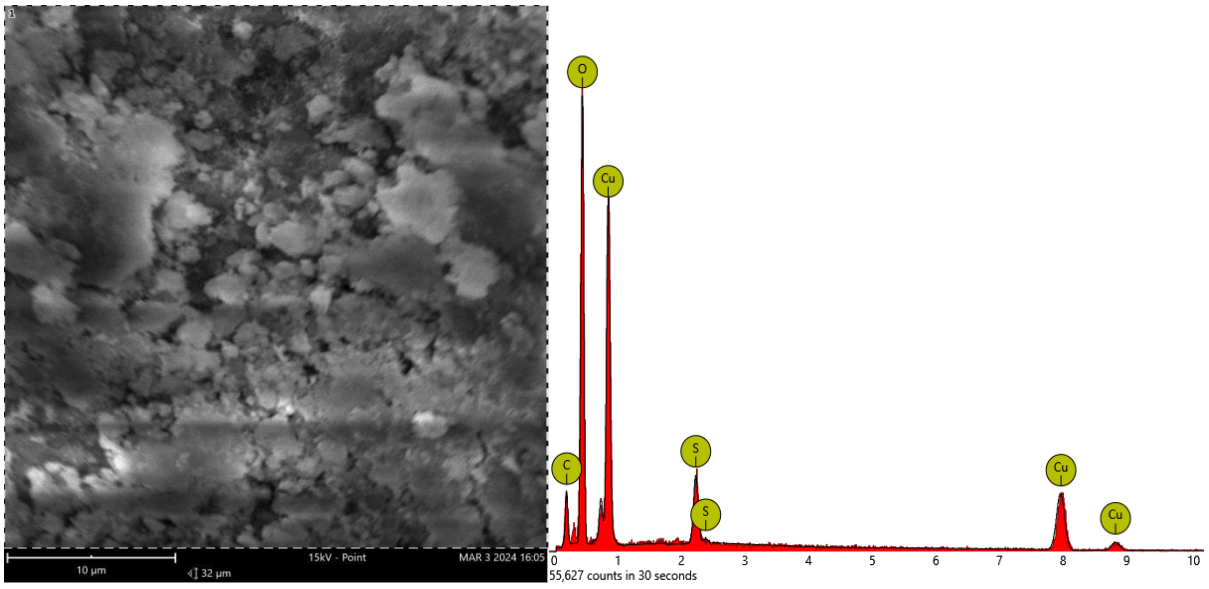


Figure 15: Scanning electron microscopy image of SE-CuNPs

II.2. Biological activities

II.2.1. Antioxidant activity

The DPPH tests revealed antioxidant activity in both SCuONPs and ECuONPs. The inhibition ratio (IP) curve of free radicals exhibited a proportional increase with the extract concentration (see Appendix). Moreover, the IC_{50} of S CuONPs (84.52 µg/ml) surpassed that of E CuONPs (61.86 µg/ml) (figure 16).

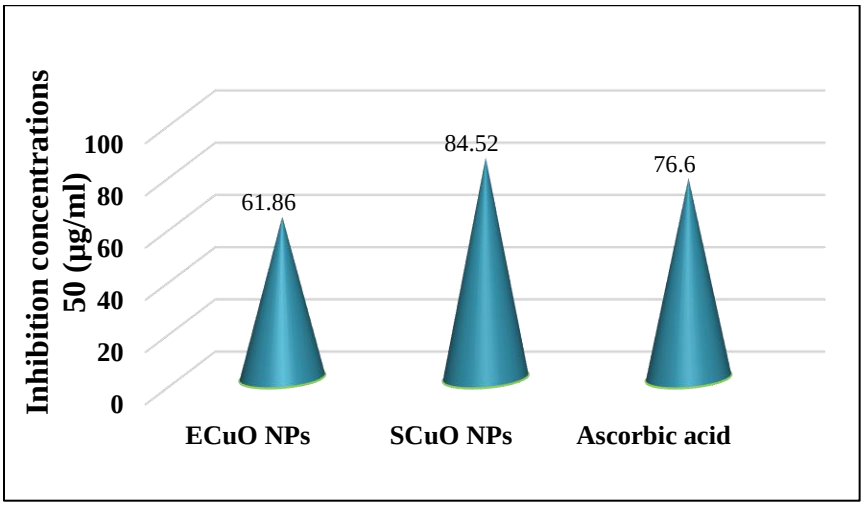


Figure 16: IC_{50} of the antioxidant activity of CuNPs

II.2.2. Hemolysis assay

From the results in figure 17, we observe that the synthesised CuONPs had different antihemolytic effects. The SE-CuNP showed the best anti-hemolytic activity, with the lowest IC_{50} (40.1 µg/ml) as compared to the EC-CuNP.

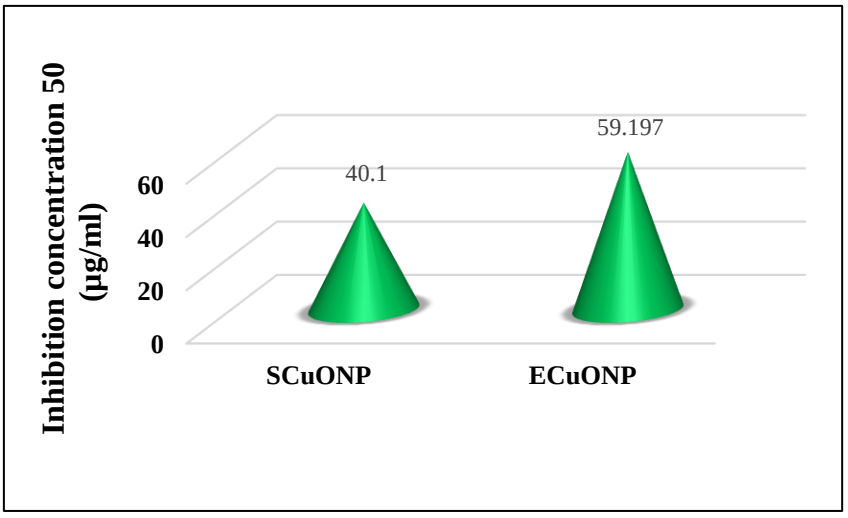


Figure 17: IC_{50} of the anti-hemolytic activity of CuNPs

II.2 In vivo study

II.2.1. Growth parameters

The analysis of body weight and relative organ weights is summarized in table **2** exposure to infection resulted in a significant reduction in weight gain among rats, whereas vaccinated groups exhibited no significant differences compared to controls. Relative organ weights indicated a significant increase in the weights of the liver, spleen, and kidneys($p<0.001$), with a slight increase in heart weight($p<0.01$) in the *S. enterica* group. In contrast, the *E. coli* group showed a highly significant increase in heart weight($p<0.001$), slight increases in liver and spleen weights ($p<0.01$, $p<0.05$), and a decrease in kidney weight($p<0.01$). In the SE-CuONPs-vaccinated group, significant improvements were observed in all organ weights ($p < 0.001$). Additionally, the EC-CuNPs-vaccinated group showed improvements in all organ weights except the spleen ($p < 0.01$, $p < 0.05$), comparde to infection group.

Table 2: Intial weight, Weight gain and Relative Organ Weights in control and experimental

	Control	***S. enterica***	***E. coli***	**SE-CuONPs**	**EC-CuONPs**
Initial body weight (g)	188±3.60	211.25±6.79	178.5±2.78	186±2.76	194±3.61
Body weight gain(g/day/rat)	1.024±0.103	0.5655±0.091 ***	0.619±0.077 ***	1.0952±0.1 **a3**	1±0.09 **b2**
Relative liver weight (g/rat)	2.2198±0.027	3.6636±0.041 ***	2.441±0.0562 **	2.4303±0.037 ***a3**	2.1767±0.065 **b2**
Relative spleen weight(g/rat)	0.26579±0.008	0.6804±0.382 ***	0.3348±0.022 *	0.3365±0.041 **a3**	0.3163±0.017 *
Relative heart weight (g/rat)	0.2896±0.006	0.30747±0.0045 **	0.31183±0.0004 ***	0.26707±0.008 ***a3**	0.33398±0.009 ****b1**
Relative kidney weight(g/rat)	0.6023±0.011	0.66235±0.001 ***	0.56339±0.009 **	0.53889±0.002 ***a3**	0.5986±0.009 **b2**

*p<0.05, **p<0.01, ***p<0.001: significantly different from control group. ^{a1}p<0.05, ^{a2}p<0.01, ^{a3} p<0.001: significantly different from *S. enterica* group. ^{b1} p<0.05, ^{b2} p<0.01, ^{b3} p<0.001: significantly different from *E. coli* group.

II.2.2. Hematological parameters

According to table 3, the results of the hematological analysis of the experimental groups showed a significant increase ($p<0.05$, $p<0.001$) in all leukocytes parameters compared with the control group. Compared with the *E. coli* group, there was a very high significant decrease ($p<0.001$) in WBC, lymphocytes, MID and granulocytes in the ECuONP group. On the other hand, there was no significant variation ($p>0.05$) in the lymphocyte and MID of the SCuONP group compared with the *S. enterica* group, while the WBC and granulocytes were highly reduced ($p<0.001$, $p<0.01$).

Table 3*:* Leukocyte line in blood of control and experimental groups

Parameters	Control	*S. enterica*	*E. coli*	SE-CuNP	EC-CuNP
WBC (10^9/l)	4.688± 0.238	6.7± 0.416**	7.125±0.462***	6.05±0.0567^{***a3}	5.95± 0.132^{***b3}
LYM (10^9/l)	3.875± 0.227	5.15±0.246***	5.537±0.463**	5.113±0.475^{*NS}	4.8± 0.113^{***b3}
GRAN (10^9/l)	0.125±0.0164	0.45±0.0463***	0.4± 0.0463***	0.3±0.0378^{**a2}	0.19875±0.00295^{***b3}
MID (10^9/l)	0.725± 0.126	1.087± 0.122*	1.325±0.161**	1.2±0.0756^{***NS}	0.95± 0.0189^{***b3}

NS: not significant. *$p<0.05$, **$p<0.01$, ***$p<0.001$: significantly different from control group. $^{a1}p<0.05$, $^{a2}p<0.01$, ^{a3} $p<0.001$: significantly different from *S. enterica* group. ^{b1} $p<0.05$, ^{b2} $p<0.01$, ^{b3} $p<0.001$: significantly different from *E. coli* group.

Table 4 illustrates that, compared to the control, there was a significant ($p<0.05$) to a very high significant decrease ($p<0.001$) of red blood cells (RBC), MCV, hemoglobin (HBG), and hematocrit (HCT), MCH with a high significant raise ($p<0.001$) in PLT and MCHC in the S. *enterica*, *E. coli* groups. In the ECuONP group, the MCV, HBG, MCH and HCT increased significantly high ($p<0.01$, $p<0.001$) simultaneously, with a significant decline in RBC, MCHC ($p<0.001$) and PLT ($p<0.01$) compared to the *E. coli* group. Also, the results of the SCuONP group showed no significant change ($p>0.05$) in HBG and PLT but a significantly high increase ($p<0.01$, $p<0.001$) in RBC, MCV, HCT, MCH while there was significant decline ($p<0.05$) of MCHC than the *S. enterica* group.

Table 4: Erythrocyte and Platelet line in blood of control and experimental groups

Parameters	Control	*S. enterica*	*E. coli*	SE-CuNPs	EC-CuNPs
RBC (10^{12}/l)	7.726± 0.12	7.197± 0.104***	7.5887±0.0348**	7.425±0.00945^{***a2}	7.27±0.0945^{***b3}
MCV (fl)	62.6± 0.543	57.663±0.189***	58.15± 0.22***	63.45±0.586$^{NS\ a3}$	66.8±1.21^{**b3}
HCT %	48.563±0.634	41.512±0.486***	44.5± 0.264***	44.038±0.558^{***a2}	48.55±0.246$^{NS\ b3}$
HGB (g/dl)	15.4± 0.164	13.687±0.0295***	14.787±0.103***	14.075±0.2^{***NS}	15.05±0.0567^{***b2}
MCH (pg)	19.9± 0.134	19.125± 0.224**	19.475± 0.168*	19.9± 0.176 $^{NS\ a2}$	20.7±0.189^{**b3}
MCHC(g/dl)	31.825±0.147	32.925± 0.298**	33.1± 0.139***	32.05± 0.362 $^{NS\ a1}$	31±0.265^{*b3}
PLT (10^{9}/l)	503.9± 19.4	624± 17.8***	629± 3.4***	496± 110 ^{NS}	607±4.54^{***b2}

NS: not significant. *p<0.05, **p<0.01, ***p<0.001: significantly different from control group. ^{a1}p<0.05, ^{a2}p<0.01, ^{a3} p<0.001: significantly different from *S. enterica* group. ^{b1} p<0.05, ^{b2} p<0.01, ^{b3} p<0.001: significantly different from *E. coli* group.

II.2.3. Biochemical parameters

II.2.3.1. Cholesterol, triglycerides, and blood sugar levels

Regarding biochemical parameters, tests for urea, creatine, cholesterol, triglycerides, and blood sugar were performed, in addition to the following enzyme tests: ASAT and ALAT. To support the results, in figure 18 we observed that the levels of blood sugar tests showed no significant difference(p>0.05) between the results of the two groups infected with *S. enterica* and *E. coli* bacteria compared to the control group. However, the groups vaccinated with ECuONP and SCuONP showed a significant (P < 0,001) increase (P < 0,05), respectively. As for the results of the groups infected with *S. enterica* and *E. coli* bacteria and the group immunized with ECuONP, they showed a significant increase (p<0.001) in cholesterol levels, with the exception of the group vaccinated with SCuONP showed that was no significant difference (p>0.05) compared with control group. For triglyceride results, all groups showed that a significant increase (p<0,001) for group infected with *S. enterica* and the other groups vaccinated with ECuONP and SCuONP and (p<0,01) for infected group with *E. coli* compared with control group.

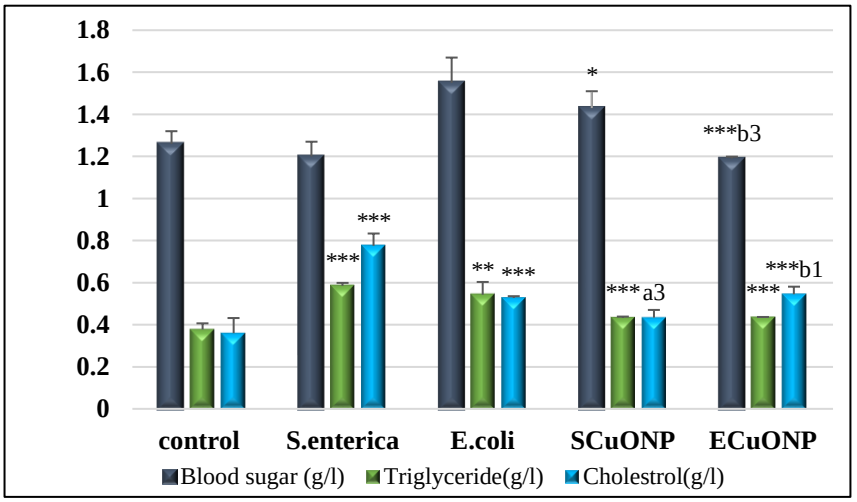


Figure 18: Cholesterol, triglycerides, and blood sugar of control and experimental groups

* p<0.05, **p<0.01, ***p<0.001: significantly different from control group. ^{a1}p<0.05, ^{a2}p<0.01, ^{a3} p<0.001: significantly different from *S. enterica* group. ^{b1} p<0.05, ^{b2} p<0.01, ^{b3} p<0.001: significantly different from *E. coli* group.

II.2.3.2. Urea and creatine levels

Figures 19 represent the levels of creatine and urea for the experimental groups as shown a significant decrease (p<0,001) in the levels of creatine for the group infected with *E. coli* and other groups vaccinated with ECuONP and SCuONP and a significant decrease (p<0,01) for group infected with *S. enterica* in the same analyse compared with control group.

Urea levels showed that a significant increase (p<0,001) for the group vaccinated with SCuONP and (p<0,01) fro infected group by *E. coli* and no significant difference for group infected with *S. enterica* and vaccinated group with ECuONP compared with control group.

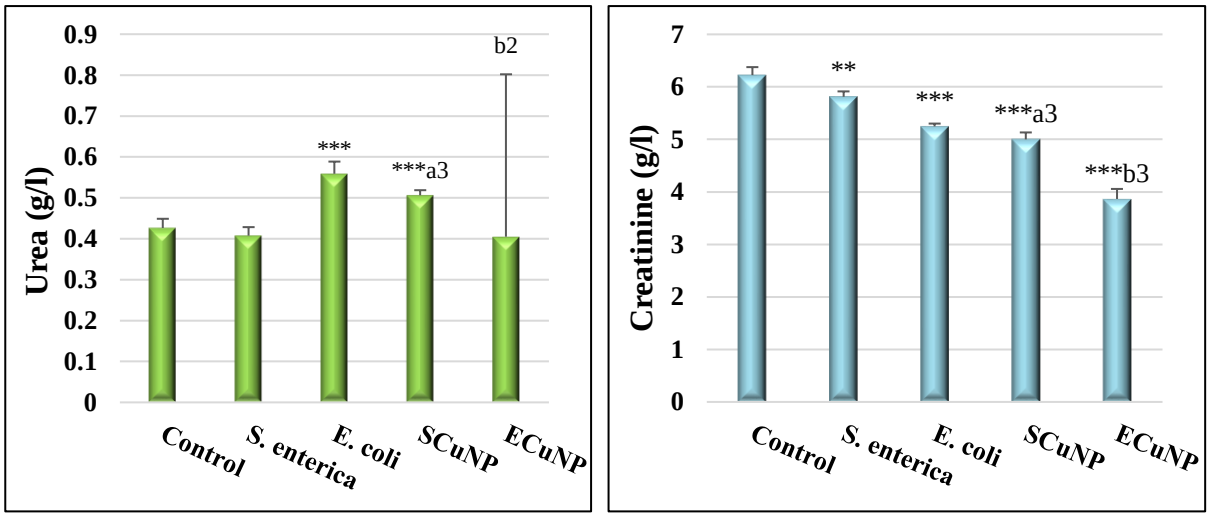


Figure 19: Urea levels and creatine activity in control and experimental groups

*p<0.05, **p<0.01, ***p<0.001: significantly different from control group. ^{a1}p<0.05, ^{a2}p<0.01, ^{a3} p<0.001 : significantly different from *S. enterica* group. ^{b1} p<0.05, ^{b2} p<0.01, ^{b3} p<0.001: significantly different from *E. coli* group.

II.2.4. Enzymatic parameters

The following figure (20) represents the results of ASAT and ALAT analysis, which showed in the levels of both analyzes a significant increase ($p<0.001$) for groups infected with *S. enterica* and *E. coli*, in contrast we observed no significant difference ($p>0,05$) for the groups vaccinated with ECuONP and SCuONP compared to control group.

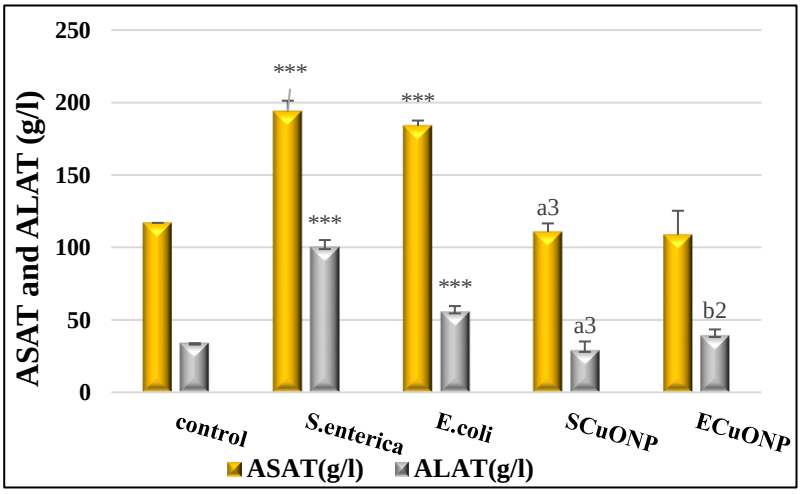


Figure 20: ASAT and ALAT activities in control and experimental groups

*p<0.05, **p<0.01, ***p<0.001: significantly different from control group. ^{a1}p<0.05, ^{a2}p<0.01, ^{a3} p<0.001: significantly different from *S. enterica* group. ^{b1} p<0.05, ^{b2} p<0.01, ^{b3} p<0.001: significantly different from *E. coli* group.

Figure 21 presented the results of CPK enzyme activity that showed no significant differences ($p > 0.05$) between the experimental groups and the control group, except for the SCuONP, which decreased significantly high CPK levels ($p<0.001$) compared to both the control and *S. enterica* groups.

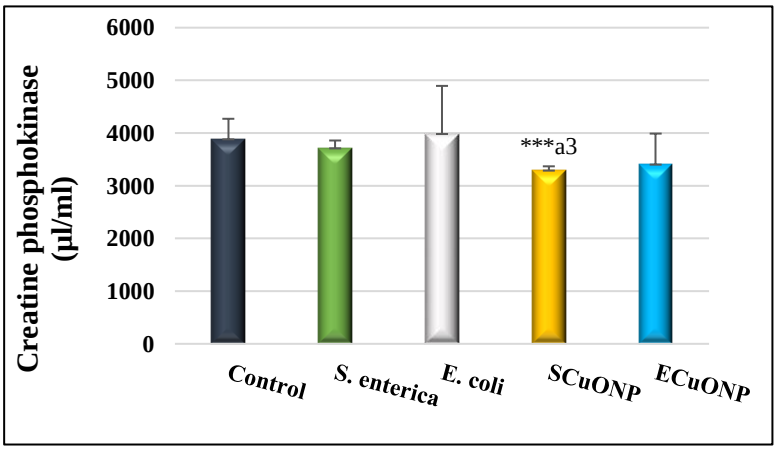


Figure 21: CPK enzyme activity in control and experimental groups

*p<0.05, **p<0.01, ***p<0.001: significantly different from control group. ^{a1}p<0.05, ^{a2}p<0.01, ^{a3} p<0.001: significantly different from *S. enterica* group. ^{b1} p<0.05, ^{b2} p<0.01, ^{b3} p<0.001: significantly different from *E. coli* group.

According to figure 22 of alkaline phosphatase (AP), only the *E. coli* group showed a high significant ($p<0.01$) reduction in PA levels compared to the control. Also, SE-CuONP induced a very high significant decline ($p<0.001$) in comparison to the *S. enterica* group. Meanwhile, the results demonstrated that there was a high significant increase ($p<0.001$) in AP in the EC_CuONP group compared to the *E. coli* group.

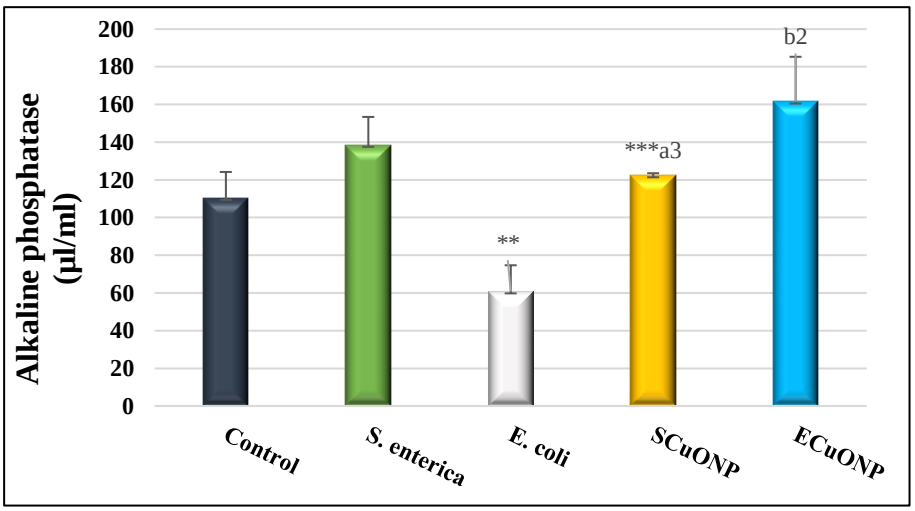


Figure 22: Alkaline phosphatase activity in control and experimental groups

*p<0.05, **p<0.01, ***p<0.001: significantly different from control group. $^{a1}p<0.05$, $^{a2}p<0.01$, ^{a3} p<0.001: significantly different from *S. enterica* group. ^{b1} p<0.05, ^{b2} p<0.01, ^{b3} p<0.001: significantly different from *E. coli* group.

II.2.5. Oxidative stress parameters

II.2.5.1. Malondialdehyde (MDA)

In the analyzed tissues (figure 23), MDA levels exhibited a significant increase in all organs for both infection groups compared to the control group ($p<0.001$-$p<0.05$), with the exception of the spleen in the *E. coli* group, where was not significant, and the *S. enterica* group, which showed a significant decrease ($p<0.001$). Conversely, compared to the infection group the SE-CuONP and EC-CuONP groups showed a significant decrease in MDA levels in the liver and heart ($p<0.001$, $p<0.05$) and in the spleen for the EC-CuONP group ($p<0.001$) but, this was not significant in the spleen for the SE-CuONP group, a slight increase in MDA levels was observed in the kidneys for both groups ($p<0.001$, $p<0.01$).

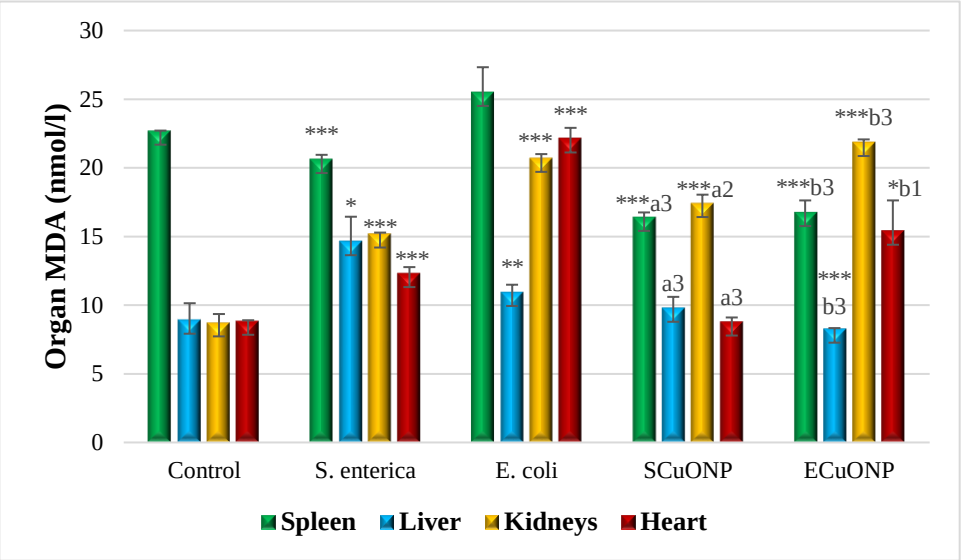


Figure 23: Tissues MDA concentration in control and experimental groups

*p<0.05, **p<0.01, ***p<0.001: significantly different from control group. ^{a1}p<0.05, ^{a2}p<0.01, ^{a3} p<0.001: significantly different from *S. enterica* group. ^{b1} p<0.05, ^{b2} p<0.01, ^{b3} p<0.001: significantly different from *E. coli* group.

II.2.5.2. Reduced glutathione (GSH)

The results of GSH levels in the different tissues represented in figure 24 show that, as compared to the control, there was a very high significant decrease in GSH levels (p<0.001) in all tissues of the *S. enterica* and *E. coli* groups but not in the liver of the *S. enterica* group. Furthermore, the GSH increased significantly high (p<0.001, p<0.01) in the different tissues of the SE-CuONP and EC-CuONP groups in comparison with the experimental infection groups, respectively.

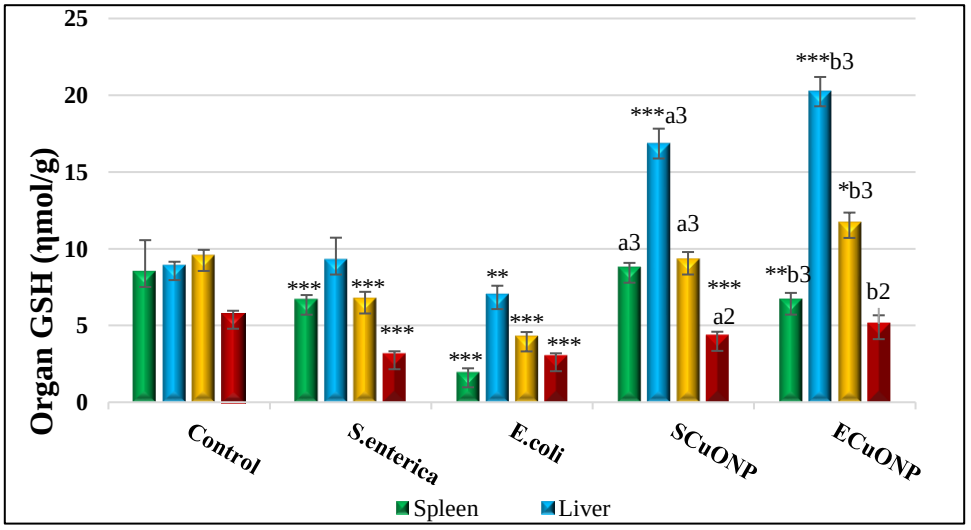


Figure 24: Tissues GSH concentration in control and experimental groups

*p<0.05, **p<0.01, ***p<0.001: significantly different from control group. ^{a1}p<0.05, ^{a2}p<0.01, ^{a3} p<0.001: significantly different from *S. enterica* group. ^{b1} p<0.05, ^{b2} p<0.01, ^{b3} p<0.001: significantly different from *E. coli* group.

II.2.5.3. Superoxide dismutase (SOD)

Compared to the control group, spleen and liver SOD activities showed a very high significant decrease ($p<0.001$) in both experimental infection groups, while only the E. *coli* group had a very high significant augmentation in heart and a decrease in kidney SOD activity (figure 25). Furthermore, SOD activity in the SE-CuONP group increased very high significant ($p<0.001$) in the spleen and high significant in the heart ($p<0.01$); in contrast, hepatic SOD activity showed a very high significant diminution ($p<0.001$) with no significant differences in the kidney in comparison to the S. *enterica* group. Also compared to the E. *coli* group, EC-CuONP induced no significant variation in liver and heart SOD activities, which were significantly raised ($p<0.05$) in the spleen. On the contrary, there was a high significant decline in SOD activity ($p<0.01$) in the kidney.

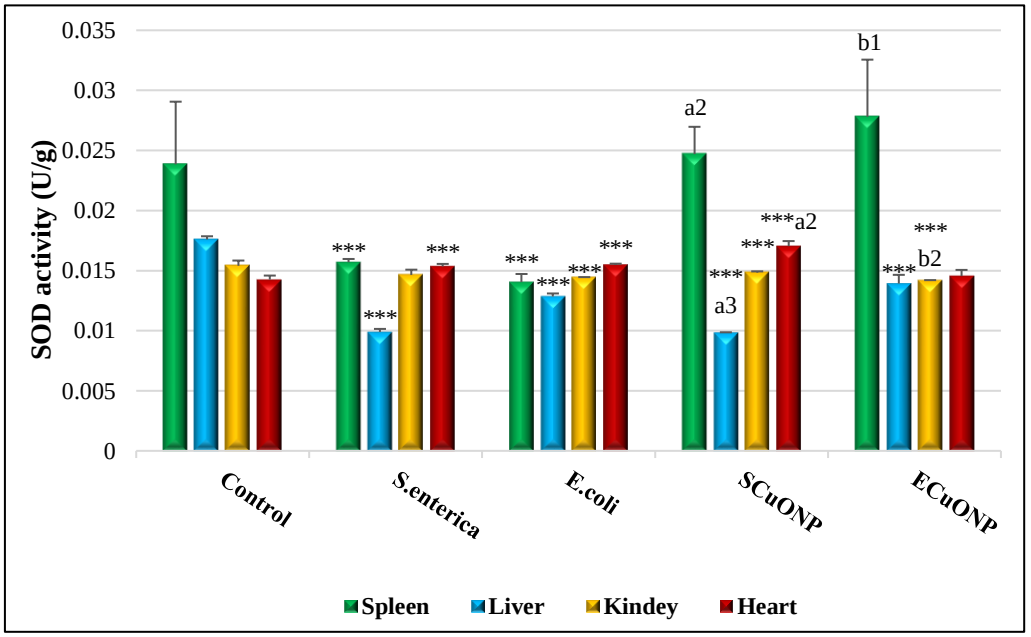


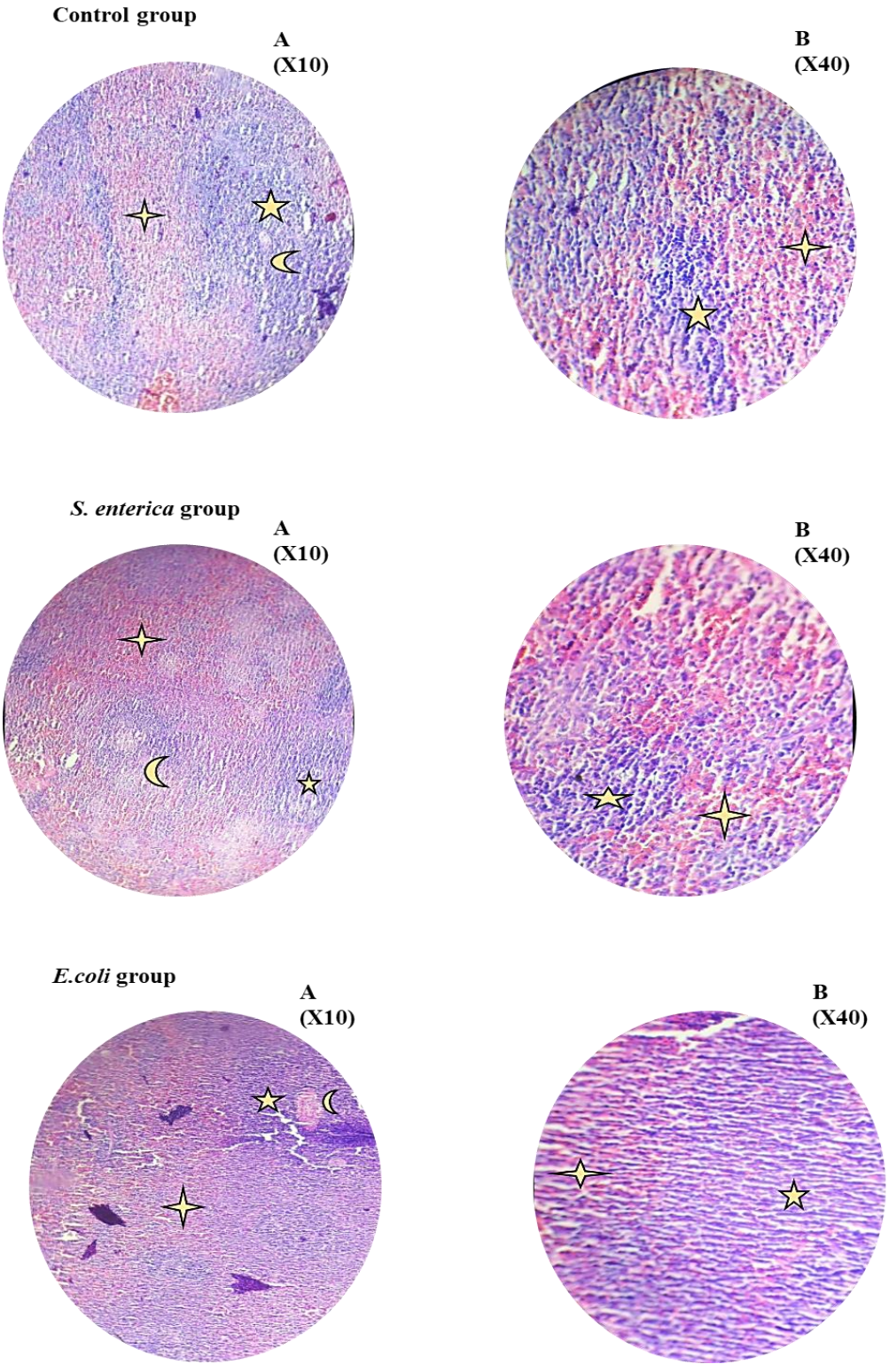
Figure 25: Tissues SOD activity in control and experimental groups

*p<0.05, **p<0.01, ***p<0.001: significantly different from control group. ^{a1}p<0.05, ^{a2}p<0.01, ^{a3} p<0.001: significantly different from *S. enterica* group. ^{b1} p<0.05, ^{b2} p<0.01, ^{b3} p<0.001: significantly different from *E. coli* group.

II.2.6. Histological analyzes

Histological analyzes of the spleen for the control group showed a very healthy structure and free of various abnormalities, which consisted of two pulps, a white pulp and a red pulp, which were normally interspersed, while the spleen sections of the two groups infected with *S.enterica* infection and the other with *E.coli* infection contained abnormalities in the pulps , as they were overlapping and an increase in the size of the white pulp, which caused the red

pulp to become confined and diminish in size compared to the spleen sections of the control group, and these changes disappeared completely in the rest of the groups vaccinated (figure 24).



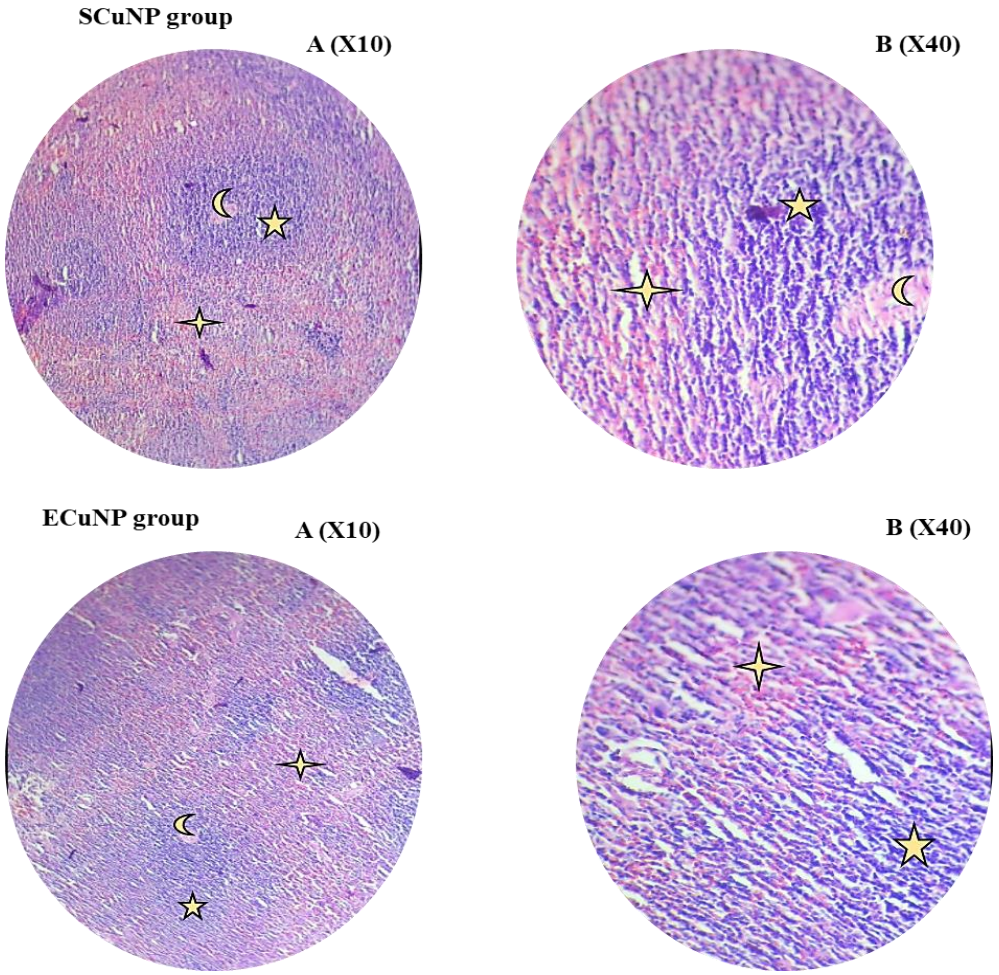


Figure 26: Photomicrographs of spleen section of all experimental groups stained with hematoxylin and eosin

(H&E), (A) : x**10** and (B) : x 40.

Central arteriole ★ White pulp ✦ Red pulp

Chapter three

Discussion

Discussion

Vaccination has been instrumental in reducing or eradicating many pathogens (Poria et *al.*, 2024). However, enthusiasm for *Escherichia coli* vaccines remains limited, despite their significance in scientific research (Cobo-Simón et *al.*, 2023; Pokharel et *al.*, 2023). While the recent COVID-19 pandemic has underscored the importance of vaccines, effective treatments for bacterial infections like *E. coli* remain elusive (Torres-Sangiao et *al.*, 2016). Nanotechnologies and molecular biology have revolutionized vaccine production processes, enhancing vaccine efficacy (Facciolà et *al.*, 2022). Although challenges persist in vaccine development and distribution (Poria et *al.*, 2024), integrating nanotechnology offers promising solutions to combat bacterial infections (Kim et *al.*, 2019).

This study aims to synthesize copper oxide nanoparticles (CuONPs) using a non-toxic, rapid, and eco-friendly approach and assess their potential as a vaccine against pathogenic *E. coli* and *Salmonella enterica*.

1. Statistical study

Infectious diseases have posed a threat to humanity since prehistoric times (de Nooijer et *al.*, 2023). Enteropathogenic *Escherichia coli* (EPEC) is a significant global cause of infections, primarily affecting infants and leading to acute and prolonged diarrhea (Gazi et *al.*, 2022). Enterohemorrhagic *Escherichia coli* (EHEC) is associated with severe conditions such as hemolytic uremic syndrome (HUS) and hemorrhagic colitis (Pokharel et *al.*, 2023). The World Health Organization reports that approximately 10% of patients with Shiga toxin-producing *Escherichia coli* (STEC) infections may develop HUS, which carries a mortality rate of 3 to 5%. HUS can also result in neurological complications, including seizures, stroke, and coma, in 25% of cases, and it is the leading cause of acute renal failure in young children (WHO,2018).

Salmonella remains one of the most prevalent causes of gastrointestinal infections globally and can manifest in two forms: gastroenteritis (Mestre et *al.*, 2021). The incidence of *Salmonella* infections varies across different regions, primarily due to differences in socio-economic status, hygiene practices, and water sanitation (Ngogo et *al.*, 2020). According to Zha et *al.* (2019), *Salmonella* is the second most common foodborne infection in the United States. In 2015, the number of *Salmonella* infections in Europe reached 94,625. Teklemariam et *al.* (2023) also confirmed that 99% of human salmonellosis cases are caused by *Salmonella enterica* ssp. enterica. Additionally, the WHO reports that *Salmonella* is responsible for more than 150,000 deaths annually worldwide (Bartsch et *al.*, 2023). Billah and Rahman (2024)

reported that the groups most at risk of infection with *Salmonella* are young children and the elderly with weakened immune systems. According to Milho et *al.* (2019), *Escherichia coli* and *Salmonella* are major foodborne pathogens frequently found in areas that are not cleaned regularly, such as the undersides of conveyor belts and pipelines, soil, produce wash water, and food products. Specifically, *Salmonella* is responsible for 23,000 hospitalizations and 450 deaths annually, while *E. coli* leads to 2,000 hospitalizations and 60 deaths each year in the United States.

2. Characterization of nanoparticles

2.1. UV-VIS spectroscopy

The initial detection of CuONPs production was indicated by a noticeable change in visual color, and UV-visible spectroscopy was employed for analysis. Previous results demonstrated absorption peaks for CuONPs synthesized by *E. coli* and *S. enterica* at approximately 250 nm and 300 nm, respectively. However, contrasting findings by Ghorbani et *al*. (2015) showed peaks at 365 nm and 565 nm using *E. coli* supernatant and *S. typhimurium*, respectively. These results were close to those of study by (Ghareib et *al*., 2018), which utilized *A. carneus biomass*, revealing peak at 315 nm. UV-Vis analysis is particularly sensitive to factors like size, shape, concentration, and refractive indices near the NP surface, owing to their unique optical properties (Fouda et *al*., 2022).

2.2 FT-IR analysis

The functional groups of EC-CuONPs and SE-CuONPs were analyzed via FTIR. An intense peak observed at 600 cm^{-1} corresponded to the Cu-O bond, confirming the successful biosynthesis of CuO nanoparticles. This finding is consistent with previous research by (Amin et *al*., 2021; Singh et *al*., 2023), who used aqueous leaf extract of *A. javanica* and a copper-tolerant bacterial isolate, respectively.

FTIR spectra of CuO NPs revealed absorption peaks at wavenumber positions of 3350 cm^{-1}, 2300 cm^{-1}, 1650 cm^{-1}, 1350 cm^{-1}, and 1100 cm^{-1}. The peaks in the 3500–3200 cm^{-1} region corresponded to the -NH groups of amine functional groups present in the protein contents of the bacterial plasma membrane and the -OH groups of hydroxyl functional groups (Solís-Sandí et *al*., 2023; Talebian et *al*., 2023). The C-O stretching vibrations of carboxylic acids, phenol, alcohols, ether, esters and typically shaw in the 1000-1320 cm^{-1} range (El-Ghwas, 2022). Additionally, the bands at 1543 cm^{-1} and 1647 corresponding to the bending vibrations of amide II the amide I bands of proteins, respectively (Abhang, 2020). Peaks at

2360.21 and 2341.64 cm^{-1} were attributed to the O=C=O (carbonyl bond group) (Huq & Akter, 2021).

The reduction of metal ions to metallic nanoparticles is primarily facilitated by various metabolites, including enzymes, proteins, phenolic compounds, organic acids, and polysaccharides (Rai et *al*., 2021). Similar to nanoparticles synthesized from aqueous plant extracts, the componeds of bacteria transform to CuONPs, which are responsible for reducing and stabilizing metal ions to form CuO NPs (Nzilu et *al*., 2023).

2.3. Scanning electron microscopy and EDX

To confirm the morphology and chemical composition of the surface of these molecules, we utilized SEM and EDX. EC-CuNPs and SE-CuNPs exhibit an oval shape, which is consistent with the study of (Suood et *al.*, 2021), where they used a type of fungus. However, these results contradict the predominant body of literature, which indicates that a spherical shape is typically achieved (Amin et *al.*, 2021; Ibrahim, 2023). Additionally, the sizes of EC-CuONPs and SE-CuONPs are 73–183 nm and 135–202 nm, respectively, which are similar with previously published data by (Ssekatawa et *al.*, 2022) using Camellia sinensis and Prunus Africana extracts, reporting size ranges of 3-192 nm and 4–576 nm, respectively. The concentration of reducing agents, the method and conditions of synthesis, and the presence of different chemical compositions all impact the size of the nanoparticles (Abhimanyu et *al.*, 2023). Element alanalysis via EDX showed that oxygen and copper are the primary constituents, with carbon and sulfur present in lesser amounts. These findings are similar to those of previous studies (Ali et *al.*, 2021; Eid et *al.*, 2023), with some differences. The green synthesis of CuO NPs is indicated by the high concentration of copper metal (Priya et *al.*, 2023).

3. Biological activities

The antioxidant potential of CuNPs was determined using the DPPH assay, with results compared to the standard ascorbic acid. The half-maximal inhibitory concentration (IC_{50}) values for SCuONPs and ECuONPs were found to be (84.52 µg/ml) and (61.86 µg/ml), respectively. These results do not align with those reported by (Alshami et *al*., 2023), who employed a new bacterial strain. However, the findings are consistent with studies conducted by (Ghareib et *al*., 2018; Ouidad et *al*., 2020).

CuNPs exhibit a significant ability to react with and reduce DPPH radicals. This is attributed to their high surface area-to-volume ratio, which enhances their capacity to accept

electrons from antioxidant molecules present in the CuNPs solution (AlSeidy & TajAldeen, 2021).

Regarding the anti-inflammatory test, the results revealed that bacterial-mediated CuNPs exhibited hemolysis inhibition activity, and SE-CuNP was better than EC-CuNP. In the present study, the synthesized nanocompounds showed better activity than the study of Angajala et *al.* (2014), in which they used *Aegle marmelos* Correa to biofabricate CuNPs. Also, the present study is consistent with the findings of Rehman et *al.* (2022), where CuNPs synthesized by *Cirsium arvense* showed no hemolytic activity.

4. Hematological parameters

Our results demonstrated a significant increase in the leukocyte line in both the *S. enterica* and *E. coli* infection groups compared with the control. The recognition and elimination of invading *Salmonella* and *Escherichia coli* are orchestrated by germ-line-encoded Toll-like Receptor 4 (TLR4), a type of pattern recognition receptor (PRR) present on the membrane of innate immune cells. TLR4 binds to conserved pathogen-associated molecular patterns (PAMPs), such as the lipopolysaccharide of gram-negative bacteria, initiating a cascade of complex inflammatory responses (Muthukumar et *al.,* 2014; Giordano et *al.,* 2020). This cascade leads to the release of various inflammatory cytokines, including TNF-α, IL-6, IL-8, and PCT, during bacterial infections. Neutrophils, as primary responders, play a pivotal role in the innate immune system's defense mechanisms by executing phagocytosis, degranulation of cytotoxic substances, and the generation of reactive oxygen species (ROS) (Li et *al.,* 2022). Toll-like receptors (TLRs), predominantly expressed on dendritic cells (DCs), recognize PAMPs, activating DCs and subsequently stimulating antigen-specific T and B cell responses (Pulendran et *al.,* 2021). Garfias-López et *al.* (2018) confirmed the importance of TLRs in adaptive immunity, highlighting the production of specific antibodies following parenteral immunization with bacteria such as *Staphylococcus aureus* and *E. coli*.

Furthermore, in *Salmonella* infections, neutrophils and Natural Killer (NK) cells produce IFN-γ, contributing to early resistance, while resident macrophages and dendritic cells recognize Salmonella components, inducing pro-inflammatory cytokine production and initiating the adaptive immune response (Kurtz et *al.,* 2017). Many studies have reported that *Salmonella* can be an intracellular parasite inside host cells and avoid destruction inside phagocyte cells like macrophages (resistance to oxidative burst used to kill and digest invading bacterial cells). Hence, cell-mediated immunity is induced, and specific activated

cytotoxic T lymphocytes will be produced (Al-Hamadany, 2021). Early innate immune responses to *Salmonella* involve inflammation, IFNγ secretion, and TLR-mediated activation of local tissue macrophages and dendritic cells (Wang et *al.*, 2020; Meijerink et *al.*, 2021).

E. coli infection induces significant increases in white blood cells, mediated by TLR4-mediated detection of *E. coli* lipopolysaccharide, resulting in NF-κB activation and cytokine production (Peltola et *al.*, 2006; Billips et *al.*, 2008). Studies by Hoerr et *al.* (2012) further support these findings, demonstrating elevated leukocyte counts and cytokine concentrations in *E. coli* infections.

Meanwhile, the groups immunized by SE-CuONPs and EC-CuNPs showed a significant decline in the leukocyte line parameters groups compared with the infection groups. It is likely that vaccines emulate infections, instigating a primary immune response that lays the groundwork for a subsequent secondary immune response upon exposure to the actual pathogen. Giesker and Hensel (2014) assert that the secondary immune response is characterized by its swiftness and potency, enabling more effective pathogen clearance compared to the primary response. A fundamental aspect of adaptive immunity, as underscored by Marshall et *al.* (2018), is the establishment of immunologic memory. This memory equips the host with the capacity to mount a rapid and robust immune response upon re-exposure to the antigen, thus bolstering the body's defense mechanisms. Subsequent encounters with the same antigen prompt a secondary response in both B- and T cells, activating pre-existing memory cells more swiftly, as elucidated by Ademokun and Dunn-Walters (2010).

Regulatory cells, including regulatory T cells (Tregs), regulatory B cells (Bregs), and myeloid-derived suppressor cells (MDSCs), are vital for maintaining immune tolerance and regulating immune responses during infections. They achieve this by producing inhibitory cytokines such as interleukin 10 (IL-10) and transforming growth factor beta (TGF-β), which suppress the production of pro-inflammatory cytokines and restrain the activation and proliferation of effector T cells (Goldmann et *al.*, 2024). This mechanism likely contributes to the observed decrease in leukocyte levels in the vaccinated groups following their secondary responses.

Nanovaccines play a pivotal role in enhancing targeted delivery, antigen presentation, and stimulation of the body's innate immunity, thus eliciting a robust T cell response while maintaining safety against infectious diseases (Das & Ali, 2021). Adjuvants, categorized into immunostimulants and delivery systems, activate antigen-presenting cells (APCs) via Toll-

like receptors (TLRs) and other pattern recognition receptors (PRRs), thereby augmenting adaptive immune responses (Zhao et *al.*, 2023). Metal-based nanoparticles (MNPs) serve as danger signals, enhancing immunotherapy efficacy by activating innate immune mechanisms (Naletova et *al.,* 2023). Pathogen-associated molecular patterns (PAMPs) mimic infectious disease symptoms, bolstering immune responses by interacting with PRRs on APCs (Seya et *al.,* 2022). Nanoparticle vaccines induce durable antigen-specific antibody responses, germinal center reactions, and T follicular helper (TFH) cell responses, ensuring long-lasting immunity (Pulendran et *al.,* 2021). Nanoparticles act as bystander vaccine delivery systems, stabilizing antigens, reducing vaccine doses, and facilitating gradual release for prolonged exposure to immune cells (Gheibi Hayat & Darroudi, 2019). Nanoparticles modulate leukocyte migration, promote antigen multivalency, and optimize particle size for enhanced bioavailability and prolonged absorption (Al Shoyaib et *al.,* 2019; Faisal et *al.,* 2022; Lamontagne et *al.,* 2022). Slow-release nanoparticle delivery systems enhance antibody responses by extending antigen exposure during germinal center induction (Tam et *al.,* 2016; Pulendran et *al.,* 2021). Immunization with Salmonella enteritidis-OMPs (outer membrane proteins) combined with an adjuvant enhances antibody responses against Salmonella infection, underscoring the adjuvant's role in improving vaccine efficacy (Khan et *al.,* 2003; Song et *al.,* 2024). Empty chitosan nanoparticles (CNPs) alone do not improve antibody responses without a Salmonella antigen (Acevedo-Villanueva et *al.,* 2021). BM-AuNPs (bacterial membrane-coated AuNPs) induce more robust E. coli-specific antibody responses and T cell activation than OMVs (bacterial outer membrane vesicles), highlighting their potential as effective vaccine carriers (Gao et *al.,* 2015). Nanoparticles offer targeted drug delivery, potent immune responses, and improved bioavailability, making them promising candidates for combating infectious diseases (Yadav et *al.,* 2018; Celis-Giraldo et *al.,* 2021; Lamontagne et *al.,* 2022).

Our data revealed that there were highly significant alterations in all the parameters related to erythrocytes and platelet lines in the *S. enterica* and *E. coli* infection groups when compared with the control. Agreeing with our study, *Salmonella enterica* serovar Typhimurium induces inflammatory features in the blood system, resulting in pathological deformations in RBCs and eryptotic RBCs, along with hyperactivation of platelets and alterations in hematological parameters (Silva et *al.,* 2019).

Inflammatory molecules interact with the RBC membrane, inducing morphological changes and eryptosis, which influences hemorheology and is associated with abnormal

coagulation, a hallmark of inflammation. This process is potentiated by oxidative stress, particularly from ROS generated during bacterial infections, which can induce anisocytosis and damage erythrocyte plasma membranes, potentially leading to hemolysis (Togan et *al.*, 2015; Stosik et *al.*, 2020; Johansson et *al.*, 2021). Also, the study of Serroukh et *al.* (2012) reported that ROS can alter the RBC membrane, affecting its deformability and contributing to inflammation. It was observed that bacterial and viral infections can induce hemolytic anemia in patients with glucose-6-phosphate dehydrogenase deficiency by promoting ROS production (Orrico et *al.*, 2023).

Glycophorin-A (GYPA) on erythrocyte surfaces may act as a decoy receptor, binding pathogens and sequestering them from important tissues, thereby reducing pathogen load (Morera & MacKenzie, 2011). Moreover, RBCs can express Toll-like receptor 9 (TLR9), bind pathogens, and accelerate erythrophagocytosis and innate immune activation (Chen et *al.*, 2023). According to Pretini et *al.* (2019), many pathogens, including *E. coli*, can bind glycophorins to avoid important tissues and facilitate clearance by the spleen.

Additionally, hemoglobin contains high-affinity binding sites for lipopolysaccharide (LPS), enhancing macrophage cytokine production and LPS binding (Anderson et *al.*, 2018). *E. coli* produce toxins that damage RBC membranes, especially through glycophorins acting as receptors, leading to RBC lysis (McCullough, 2014; Cané et *al.*, 2024).

In response to microbes, immune activation stimulates pro-inflammatory cytokine release, disrupting iron homeostasis (Lanser et *al.*, 2021).

Platelets, leukocytes, and lymphocytes are conventionally perceived as independently mediating hemostasis, inflammation, and immune regulation, respectively, yet they intricately interact in numerous physiological pathways (Yeaman, 2010). Platelets, primarily recognized for their role in hemostasis, actively engage in the innate immune response through a process termed immunothrombosis. Additionally, studies have shown that platelets contribute to the adaptive immune response by influencing T and B-cell recruitment, activation, and differentiation (Lombardi et *al.*, 2023). Recent findings further associate platelets with inflammation (Çelik et *al.*, 2023). Equipped with the machinery for both innate and adaptive immune responses, platelets demonstrate their versatility by responding to various pathogens through Toll-like receptors (TLRs), complement activation, and sensing immune complexes. Despite their anucleate nature and primary role in hemostasis, platelets play a central role not only in preventing bleeding but also in swiftly responding to microbial components, thereby alerting the immune system (Koupenova et *al.*, 2022).

Originating from bone marrow megakaryocyte cells, platelets, the smallest blood cells, are crucial in blood hemostasis and the coagulation process, with activated platelets participating in host defense mechanisms. During inflammation, platelet counts increase as part of the acute-phase reaction, often serving as markers for infectious and inflammatory diseases (Dwaya et *al.*, 2022). Platelets, measuring 2–5 µm and lacking a nucleus, derive their functionality from cytoplasmic RNAs, ribosomes, and mitochondria. They contain two types of granules: dense granules, which contain serotonin, nucleotides, and calcium, enhancing platelet activation, and α-granules, containing molecules promoting hemostasis and immunological functions such as von Willebrand factor and CD40 ligand (Scherlinger et *al.*, 2023). Upon activation during bacterial infection, platelets interact with vascular endothelial chemokine receptors, inducing leukocyte extravasation and promoting the formation of the NLRP3 inflammasome, resulting in the secretion of proinflammatory cytokines (Tokarz-Deptuła et *al.*, 2021). Furthermore, platelets play a vital role in the formation of neutrophil extracellular traps (NETs), facilitating bacterial clearance, and concentrating antibacterial factors (Gros et *al.*, 2015; Hamzeh-Cognasse et *al.*, 2015). This interaction between platelets and neutrophils leads to NET formation, contributing to both inflammation and thrombosis and ultimately aiding in the fight against bacterial infections.

The SE-CuNPS and EC-CuNPS groups demonstrated significant improvements in erythrocyte parameters and a decrease in platelets compared to the infection groups, suggesting a beneficial effect of biogenic nanoparticles. This improvement may be attributed to the hemolysis inhibitory activity of the nanoparticles. Interestingly, Raheem (2018) reported contrasting findings, indicating a decrease in erythrocyte parameters following immunization with AgNP. This underscores the importance of assessing nanomaterials' hemolytic effects, particularly given their potential for systemic entry (Pourahmad et *al.*, 2023).

Our study findings align with those of Tulinska et *al.* (2022), who found no significant variation in basic hematological parameters in the CuO NP-only exposed group compared to the control group. This confirms that hematological alterations were likely associated with the presence of the pathogenic antigen. Additionally, Zangiabadi et *al.* (2023) demonstrated the potent inhibitory effect of copper on the phosphorylation of key inflammatory signaling proteins, leading to a reduction in inflammatory cytokine production and excessive inflammation in macrophages. This mechanism may explain our synthesized nanocompounds'

effectiveness in reducing inflammatory anemia resulting from the host's response to pathogens.

Consistent with our study, Shah et *al.* (2021) illustrated a trend towards normalization of deviated PLT, MCH, and MCHC values following simultaneous inoculation with Fe2O3 NPs and bacterial pathogens. This underscores the potential in vivo therapeutic efficacy of Fe2O3 NPs against *S. aureus* and *E. coli*. Platelets play a crucial role in the host's immune system, trapping bacteria for destruction. However, excessive inflammation can lead to platelet activation and sequestration, resulting in a decreased platelet count (Assinger, 2014).

Iseki et *al.* (2000) highlighted the importance of copper in inhibiting NF-kB activation, a crucial pathway in inflammation regulation. Furthermore, copper nanoparticles synthesized from Catharanthus roseus leaf exhibited anti-inflammatory effects in vitro (Sultana et *al.*, 2023). Despite challenges in understanding nanoparticle modes of action (Bagheri-Josheghani et *al.*, 2022), studies have consistently emphasized copper's essential role in red blood cell production, iron metabolism, and body homeostasis (Milanino & Buchner, 2006; Azmat et *al.*, 2023; Pourahmad et *al.*, 2023).

5. Biochemical and enzymatic parameters

Salmonella and *E. coli* infections cause a significant increase in triglyceride and cholesterol levels (Fiser et *al.*, 1972; Dutta et *al.*,2009). These facts are consistent with the results of this study, as we observed a significant increase in triglyceride and cholesterol levels in the two groups infected with *S. enterica* and *E. coli* bacteria. A significant reduction in the levels of these parameters was also observed in the groups immunized with EC-CuNP and SE-CuNP, due to modulation of cholesterol homeostasis by the immune system as a means of amplifying the inflammatory response (Tall et *al.*, 2015).

Salmonella infection is considered a major global public health concern. The expenditures on disease prevention, treatment, and surveillance add to the economic burden of both developed and developing nations (Crump et *al.*, 2004). This is due to the following symptoms that patients may encounter: bradycardia, enlarged liver, enlarged spleen, and pink blotches on the chest and abdomen (Kuvandik et *al.*, 2009). In this study, we documented the levels of biochemical and enzymatic parameters (urea, creatine, ASAT and ALAT). We noted that there was a significant increase in the levels of urea, cholesterol, triglyceride, ASAT, ALAT, and ALP and a significant decrease in levels of creatine for the infected group by *S. enterica* compared with the control group, which indicated inflammation and damage to the liver and kidney dysfunction (Mazkour et *al.*, 2020*).* This is similar to what was found in the

study of Zeid et *al.* (2020), where it was identified as an increase in the levels of these biochemical parameters. Conversely, the results showed a significant decrease in the levels of these biochemical parameters for the vaccinated group by SE-CuONPs compared with the infected group, and this indicated that biochemical parameters were affected by vaccination (Kudair et *al.*, 2010).

Regarding the group infected with *Escherichia coli* bacteria, our study results were similar to the study by Sharma et *al*. (2015), which highlights the impact of *E. coli* infection on various biochemical parameters, indicating significant changes such as increased levels of urea, cholesterol, triglyceride, ASAT, ALAT, and ALP, along with decreased levels of creatine. These alterations suggest cell necrosis, including hepatocellular, myocardial, renal, pancreatic, and hemolytic necrosis.

Furthermore, Sugeçti (2021) corroborated these findings by identifying increased levels of these biochemical parameters in individuals infected with *Escherichia coli*. However, Aldali et *al.* (2023) explored the effects of vaccination using E CuONPs (copper oxide nanoparticles) on these biochemical parameters.

Their results indicated a decrease in the levels of these parameters compared to the infected groups, suggesting a potential protective effect of vaccination against the biochemical alterations induced by *E. coli* infection.

6. Oxidative stress parameters

Myeloid immune cells, such as monocytes, macrophages, and dendritic cells, are essential in the initial response to infections (Sahoo et *al*., 2024). The generation of ROS and RNS by phagocytic cells is a key component of the innate immune response (Abdulgafor et *al.*, 2018). During phagocytosis, neutrophils engulf pathogens within phagosomes and initiate a respiratory burst by activating both phagocytic membrane NOX complex and NADPH oxidase. This activation leads to the generation of superoxide anions (O_2^-), which are subsequently converted into hydroxyl radicals (OH·), singlet oxygen ($^1O_2$), and hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD). H_2O_2 serves as a substrate for myeloperoxidase (MPO), resulting in the production of hypochlorous acid (HOCl), a potent antimicrobial agent (Rajashekaraiah et *al*., 2021; Dagah et *al*., 2024).

ROS and RNS, are highly reactive and can damage DNA, proteins, and lipids (Deramaudt et *al*., 2013). Cells possess robust antioxidant defense mechanisms that include a variety of

enzymatic and non-enzymatic molecules, these molecules have significant capabilities to mitigate the harmful effects of ROS and other free radicals (Chakraborty et *al*., 2012).

In this study, we observed elevated MDA levels in all organs of rats infected with *S. enterica* and *E. coli*, except for the spleen. Malondialdehyde (MDA) is a biomarker indicative antioxidant status, lipid peroxidation and the intensity of oxidative stress (Wang et *al*., 2021; Merino et *al*., 2023). It also increases proinflammatory cytokines (Mueangson et *al*., 2023). This phenomenon can be attributed to the potential consumption of antioxidants to mitigate oxidative stress induced by infection. The enhanced production of ROS during infection threatens biomolecules by damaging nucleic acids, oxidizing proteins, and causing lipid peroxidation, consequently elevating MDA levels (Bahrami et *al*., 2016). These results are consistent with those reported by (Rishi et *al*., 2006; Tasdemir et *al*., 2013). Additionally, (Hendradewi et *al.*, 2020) confirmed that *E. coli* induces an increase in MDA levels.

Our results recorded a significant decrease in GSH levels in all organs of the infected groups, with the exception of the liver of the *S. enterica* group. Is in agreement with earlier findings (Shukla et *al*., 2012). Reduced levels of GSH can be attributed to two factors: either an increase in GSH consumption as a result of an infection with *S. enterica* and *E. coli*, or an increase in lipid oxidation products that may be linked to a decrease in NADPH availability, which is necessary for glutathione reductase (GR) to convert GSSG to GSH as a result of increased ROS production in the form of NO (Chakraborty et *al.*, 2012).

Superoxide dismutase (SOD) serves as a crucial defense mechanism against oxidative stress by scavenging potentially harmful free radical molecules (Abdulgafor et *al*., 2018). According to (Fodouop et *al*., 2015), superoxide radicals, either alone or in combination with H2O2, induce the oxidation of the catalase (CAT) and glutathione peroxidase (GSH-Px) enzymes, which subsequently leads to a reduction in SOD activity. This finding aligns with our observations, as we recorded a decrease in SOD activity in all groups infected with *S. enterica* and *E. coli*, with the exception of the kidneys in the Salmonella group. These results are consistent with the findings of (Rishi et *al*., 2006; Fodouop et *al*., 2015). Furthermore, decreased SOD activity is associated with increased inflammatory processes (Hwang et *al*., 2020).

Preventing pathogen specific infections and maximizing the pool of memory B and T cells to improve immunity against novel infections are the primary objectives of vaccination (Laupèze et *al.*, 2021). Our results demonstrate a significant decrease in MDA values in the liver and heart of both vaccinated groups and in the spleen of the ECuONPs-vaccinated group

compared to the infection group. These findings indicate a reduction in oxidative stress induced by infection, suggesting that the vaccine protects cells from oxidative damage.

Moreover, there was a significant improvement in GSH levels across all organs in the two vaccinated groups. GSH acts directly as an antimycobacterial peptide, because structurally similar to penicillin, functions as a NO carrier within macrophages (Morris et *al*., 2013). Also, it plays role such as catalase converting H_2O_2 into H_2O, and gives another pathway for detoxification by reducing organic peroxides to their equivalent alcohol (Rajashekaraiah et *al*., 2021). Additionally, our data recorded a significant increase in the levels of SOD in the spleen and heart of the ScuONPs group and in the spleen of the ECuONPs group. These findings indicate that reduce cellular redox imbalance and the bacterial load was significantly reduced compared to the infection group.

This reduction in bacterial load can be attributed to the rapid and effective immune response, facilitated by the prior recognition of the antigen by B and T lymphocytes. These antigenspecific cells underwent clonal expansion and differentiated into long-lived memory cells, enabling a swift and robust response upon re-exposure to the antigen (Sherwood, 2022). Immune memory B cells have two main components: (1) long-lived plasma cells that continuously secrete antibodies to defend against repeated challenges, and (2) memory B cells that are reactivated upon re-exposure to the antigen, rapidly producing antibodies to combat the pathogen (Guo et *al*., 2024). However, recent evidence suggests that innate immune cells can build long-term memory, termed "trained immunity," which produces hyper responsiveness upon restimulation, potentially serving as the underlying mechanism for vaccination-induced rapid protection (Gu et *al*., 2021). This process contributed to the reduction of oxidative stress.

Interestingly, both SE-CuNPs and EC-CuNPs are capable of inducing an immune response and exhibit antioxidant activity with IC_{50} values of 84.52 µg/ml and 61.86 µg/ml, respectively (in vitro part). This results in a positive synergistic effect on the immune system. Furthermore, copper plays a crucial role in the development and differentiation of immune cells, improves cardiovascular function, and participates in redox reactions and antioxidant processes (Collins, 2017; Li et *al*., 2019; Cheng et *al*., 2022).

7. Histological analyzes

The spleen is the largest peripheral lymphoid organ in the body. It can be roughly divided into the red pulp (RP, erythrocyte-rich area), white pulp (WP, lymphocyte-rich area), and marginal zone (MZ) (Fazhan et *al*.,2023).

This study showed that the spleen sections of the two groups infected with *S.enterica* infection and the other with *E.coli* infection contained abnormalities in the pulps, as they were overlapping and an increase in the size of the white pulp, which caused the red pulp to become confined and diminish in size which indicated that B and T lymphocytes may change their distribution and proportions in pulps (Rosche et *al*.,2015) and also shown in study of Guo et *al.* (2024) when the spleen sections of the 02 groupes vaccinated showed a healthy structure which indicated that Lymph nodes (LNs) are good targets for nanovaccines to stimulate immune cells, especially B and T cells, capable of eliminating pathogens in the body.

The secretion of specific antibodies against the nanovaccine depends on the ability of the nanovaccine to stimulate T helper 2 cells to produce cytokines and plasma B cells to produce antibodies against the vaccine. At this stage, the antibodies produced by the nanovaccine are caused by the nanoparticles themselves and the combined antigens. These nanovaccines are able to stimulate B cells that are distributed into plasma cells, causing them to produce specific antibodies against the antigens bound to the nanoparticles and NPs (Bagheri-Josheghani et *al.*, 2022).

Conclusion

Conclusion

The significance of vaccines cannot be overstated in preventing and controlling infectious diseases, especially amidst the challenges posed by drug resistance. With continuing scientific and technological advances and the use of nanotechnology in drug development and delivery, this approach is a promising area for vaccine manufacturing. Furthermore, the nanoscale size of these molecules allows targeted delivery to specific cells or tissues, which may enhance their efficiency and effectiveness, produce superior immune responses compared to conventional vaccines, and eliminate the need for multiple doses.

The successful biosynthesis of bacterial nanoparticles in our study was confirmed by UV-VIS, IR, and SEM analyses. These molecules also showed antioxidant and anti-hemolytic activity, protecting red blood cell membranes in vitro, enabling their positive synergistic effect with the immune system, and ensuring their safe use.

Both SE-CuONPs and EC-CuONPs showed significant improvements in biochemical, enzymatic, and hematological markers, as well as a reduction in oxidative stress after antigen re-exposure. These results indicate the effectiveness of these nanoparticles in enhancing the immune response by reducing the bacterial load, stimulating the immune system, and maintaining memory immunity. This preserved memory immunity enables the immune system to quickly recognize the antigen and produce antibodies efficiently, thus providing protection against pathogens. On the other hand, these molecules showed significant protection against tissue damage caused by oxidative stress and inflammation.

Finally, based on all of the above, the results indicate that both SE-CuNPs and EC-CuNPs showed immunization and protection against bacterial infection. Interestingly, they are made from natural materials, which reduces their side effects, makes them easy to store and use, and is inexpensive. Investing in vaccines not only enhances public health but also contributes to social and economic stability by reducing the health and economic burdens of diseases.

This study underscores the potential of nanotechnology in revolutionizing vaccine development and the importance of continued research in this field.

Prospectives:

Rigorous clinical testing and evaluation to assess the effectiveness, safety, and optimal administration of SE-CuNPs and EC-CuNPs.

Evaluation of storage periods and preservation conditions to ensure the stability and efficacy of nanoparticle-based vaccines.

Application of nanoparticle-based vaccine approaches to combat other bacteria and viruses, exploring the use of other metals such as gold and silver.

These prospective studies will further advance our understanding of nanoparticle-based vaccines and their potential for combating infectious diseases.

Bibliography

Abdulgafor, A. B., Owain, M. S., Hasan, M. S., Hussein, M. A., Aboud, Q. M., & Al-Zobaie, A. J. (2018). Liver, kidney function tests and oxidative damage during and after treatment of Salmonella typhimurium infection in experimental local rabbits. *International Journal of Pharmaceutical Quality Assurance, 9(5-6).*

Abhang, D. P. (2020). Antimicrobial and antioxidant properties of silver nano-particles synthesized from Escherichia coli. *Science, Technology and Development, 9.*

Abhimanyu, P., Arvind, M., & Kishor, N. (2023). Biosynthesis of CUO nanoparticles using plant extract as a precursor: characterization, antibacterial, and antioxidant activity. *Nano Biomedicine and Engineering*, *15*(4), 369–377. https://doi.org/10.26599/nbe.2023.9290027

Abouzeid, H. M., Julien, C. M., Zhu, L., & Hashem, A. M. (2023). Green synthesis of nanoparticles and their energy storage, environmental, and biomedical applications. *Crystals*, *13*(11), 1576. https://doi.org/10.3390/cryst13111576

Acevedo-Villanueva, K. Y., Renu, S., Shanmugasundaram, R., Akerele, G. O., Gourapura, R. J., & Selvaraj, R. K. (2021). Salmonella chitosan nanoparticle vaccine administration is protective against Salmonella Enteritidis in broiler birds. *PloS One*, *16*(11), e0259334. https://doi.org/10.1371/journal.pone.0259334

Ademokun, A. A., & Dunn-Walters, D. (2010). Immune responses: primary and secondary. Encyclopedia of Life Sciences. https://doi.org/10.1002/9780470015902.a0000947.pub2

Ahmed, N. A., & Othman, A. S. (2024). Green fabrication of ZnO nanoparticles via spirulina .platensis and its efficiency against biofilm forming pathogens. Microbial Cell Factories, 23(1), 92

Ahmed, T., Freitas, D., Huang, X., Qu, Q., Traverso, G., & Kirtane, A. R. (2023). Physical methods to overcome tissue barriers in vaccine delivery. *Vaccine Insights*, *02*(10), 363–380. https://doi.org/10.18609/vac/2023.050

Al Shoyaib, A., Archie, S. R., & Karamyan, V. T. (2019). *Intraperitoneal Route of Drug Administration: Should it Be Used in Experimental Animal Studies? Pharmaceutical Research, 37(1).* doi:10.1007/s11095-019-2745-x

Aldali, J. A., Alotaibi, B. A., Aldali, H. J., Alasiri, G. A., Alaseem, A., Almuqrin, A. M., Alshalani, A., & Alotaibi, F. T. (2023). Assessing the impact of COVID-19 vaccines on sickle cell anaemia patients: A comparative analysis of biochemical and haematological parameters. *Biomedicines*, *11*(8), 2203. https://doi.org/10.3390/biomedicines11082203

Al-Hamadany, W. S. (2021). Salmonella and the immune system. In IntechOpen eBooks. https://doi.org/10.5772/intechopen.95673

Ali, M., Ijaz, M., Ikram, M., Ul-Hamid, A., Avais, M., & Anjum, A. A. (2021). Biogenic Synthesis, Characterization and Antibacterial Potential Evaluation of Copper Oxide Nanoparticles Against Escherichia coli. Nanoscale Research Letters, 16(1). https://doi.org/10.1186/s11671-021-03605-z

ALSeidy, N. J., & TajAldeen, W. R. (2021). Activity of Biosynthetic Copper Oxide Nanoparticles by *Lactobacillus Plantarum* against Pathogenic Bacteria as Antibacterial Antibioflim and Antioxidant. Annals of the Romanian Society for Cell Biology, 25(6), 5224-5235.

ALSHAMI, H. G. A., AL-TAMIMI, W. H., & HATEET, R. R. (2023). The antioxidant potential of copper oxide nanoparticles synthesized from a new bacterial strain. Biodiversitas Journal of Biological Diversity, 24(5).

Alshammari, B. H., Lashin, M. M., Mahmood, M. A., Al-Mubaddel, F. S., Ilyas, N., Rahman, N., ... & Khan, R. (2023). Organic and inorganic nanomaterials: fabrication, properties and application. RSC advance ,13(20),13735-13785.

Altammar, K. A. (2023). A review on nanoparticles: characteristics, synthesis, applications, and challenges. *Frontiers in Microbiology*, *14*. https://doi.org/10.3389/fmicb.2023.1155622

Álvarez-Chimal, R., & Arenas-Alatorre, J. Á. (2023). Green Synthesis of Nanoparticles: a Biological approach. In *IntechOpen eBooks*. https://doi.org/10.5772/intechopen.1002203

Amin, F., Fozia, Khattak, B., Alotaibi, A., Qasim, M., Ahmad, I., Ullah, R., Bourhia, M., Gul, A., Zahoor, S., & Ahmad, R. (2021). Green Synthesis of Copper Oxide Nanoparticles Using Aerva javanica Leaf Extract and Their Characterization and Investigation of In Vitro Antimicrobial Potential and Cytotoxic Activities. Evidence-based complementary and alternative medicine : eCAM, 2021, 5589703. https://doi.org/10.1155/2021/5589703

Anderson, H. L., Brodsky, I. E., & Mangalmurti, N. S. (2018). The Evolving Erythrocyte: Red Blood Cells as Modulators of Innate Immunity. Journal of immunology (Baltimore, Md. : 1950), 201(5), 1343–1351. https://doi.org/10.4049/jimmunol.1800565

Angajala, G., Pavan, P., & Subashini, R. (2014). One-step biofabrication of copper nanoparticles from Aegle marmelos correa aqueous leaf extract and evaluation of its anti-inflammatory and mosquito larvicidal efficacy. RSC Advances, 4(93), 51459–51470. https://doi.org/10.1039/c4ra10003d

Assinger A. (2014). Platelets and infection - an emerging role of platelets in viral infection. *Frontiers in immunology*, *5*, 649. https://doi.org/10.3389/fimmu.2014.00649

Awashra, M., & Młynarz, P. (2023). The toxicity of nanoparticles and their interaction with cells: an in vitro metabolomic perspective. *Nanoscale Advances*, *5*(10), 2674–2723. https://doi.org/10.1039/d2na00534d

Azmat, F., Imran, A., Islam, F., Afzaal, M., Zahoor, T., Akram, R., Aggarwal, S., Rehman, M., Naaz, S., Ashraf, S., Hussain, G., Suleria, H. a. R., Ali, Q., Bibi, M., Batool, F., Gul, F., Amjad, N., & Shah, M. A. (2023). Valorization of the phytochemical profile, nutritional composition, and therapeutic potentials of garlic peel: a concurrent review. International Journal of Food Properties, 26(1), 2642–2655. https://doi.org/10.1080/10942912.2023.2251713

Bagheri-Josheghani, S., Bakhshi, B., & Najar-Peerayeh, S. (2022). The Influence of Nanoparticle on Vaccine Responses against Bacterial Infection. *Journal of Nanotechnology*, *2022*, 1–15. https://doi.org/10.1155/2022/6856982

Bahrami, S., Shahriari, A., Tavalla, M., Azadmanesh, S., & Hamidinejat, H. (2016). Blood levels of oxidant/antioxidant parameters in rats infected with Toxoplasma gondii. *Oxidative medicine and cellular longevity*, *2016.*

Bartsch, L. J., Borowiak, M., Deneke, C., Gruetzke, J., Hammerl, J. A., Malorny, B., Szabo, I., Alter, T., Nguyen, K. K., & Fischer, J. (2023). Genetic characterization of a multidrug-resistant *Salmonella enterica* serovar Agona isolated from a dietary supplement in Germany. *Frontiers in microbiology*, *14*, 1284929. https://doi.org/10.3389/fmicb.2023.1284929

Basavaraju, M., & Gunashree, B. (2023). Escherichia coli: An Overview of Main Characteristics. In IntechOpen eBooks. https://doi.org/10.5772/intechopen.105508

Beauchamp, C., & Fridovich, I. (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analytical biochemistry*, *44*(1), 276–287. https://doi.org/10.1016/0003-2697(71)90370-8

Bhatti, R., Shakeel, H., Malik, K., Qasim, M., Khan, M. A., Ahmed, N., & Jabeen, S. (2022). Inorganic nanoparticles: toxic effects, mechanisms of cytotoxicity and phytochemical interactions. Advanced Pharmaceutical Bulletin, 12(4), 757.

Billah, M. M., & Rahman, M. S. (2024). Salmonella in the environment: A review on ecology, antimicrobial resistance, seafood contaminations, and human health implications. *Journal of Hazardous Materials Advances*, *13*, 100407. https://doi.org/10.1016/j.hazadv.2024.100407

Billips, B. K., Schaeffer, A. J., & Klumpp, D. J. (2008). Molecular Basis of Uropathogenic Escherichia coli Evasion of the Innate Immune Response in the Bladder. Infection and Immunity, 76(9), 3891–3900. doi:10.1128/iai.00069-08

Blin, A. (2021). La fabrication d'un vaccin, un processus très encadré. *Actualités Pharmaceutiques*, *60*(606),45–48. https://doi.org/10.1016/j.actpha.2021.03.019

Bukhari, S. I., Hamed, M. M., Al-Agamy, M. H., Gazwi, H. S., Radwan, H. H., & Youssif, A. M. (2021). Biosynthesis of copper oxide nanoparticles using Streptomyces MHM38 and its biological 13:49applications. Journal of Nanomaterials, 2021, 1-16.

Bulut Kocabas, B., Attar, A., Peksel, A., & Altikatoglu Yapaoz, M. (2021). Phytosynthesis of CuONPs via Laurus nobilis: Determination of antioxidant content, antibacterial activity, and dye decolorization potential. Biotechnology and Applied Biochemistry, 68(4), 889-895.

Cané, L., Saffioti, N. A., Genetet, S., Millone, M. a. D., Ostuni, M. A., Schwarzbaum, P. J., Mouro-Chanteloup, I., & Herlax, V. (2024). Alpha hemolysin of E. coli induces hemolysis of human erythrocytes independently of toxin interaction with membrane proteins. *Biochimie*, *216*, 3–13. https://doi.org/10.1016/j.biochi.2023.10.008

Cao, P., Xu, Z. P., & Li, L. (2022). Tailoring functional nanoparticles for oral vaccine delivery: Recent advances and future perspectives. *Composites Part B: Engineering*, *236*, 109826. https://doi.org/10.1016/j.compositesb.2022.109826

Carrapiço, A., Martins, MR, Caldeira, AT, Mirão, J. et Dias, L. (2023). Biosynthèse de nanoparticules de métaux et d'oxydes métalliques à l'aide de cultures microbiennes : mécanismes, activité antimicrobienne et applications au patrimoine culturel. Microorganismes, 11 (2), 378.

Çelik, M., Ceylan, M. R., Altındağ, D., Dinçer, N. G., & Alkan, S. (2023). Diagnostic significance of hematological parameters in brucellosis. *Ķazaķstannyņ Klinikalyķ Medicinasy*, *20*(1), 50–55. https://doi.org/10.23950/jcmk/12929

Celis-Giraldo, C. T., López-Abán, J., Muro, A., Patarroyo, M. A., & Manzano-Román, R. (2021). Nanovaccines against Animal Pathogens: The Latest Findings. *Vaccines, 9*(9), 988. https://doi.org/10.3390/vaccines9090988

Chakraborty, S. P., Pramanik, P., & Roy, S. (2012). Staphylococcus aureus infection induced oxidative imbalance in neutrophils: possible protective role of nanoconjugated vancomycin. *International Scholarly Research Notices, 2012*

Chen, M., Leng, Y., He, C., Li, X., Zhao, L., Qu, Y., & Wu, Y. (2023). Red blood cells: a potential delivery system. Journal of Nanobiotechnology, 21(1). https://doi.org/10.1186/s12951-023-02060-5

Cheng, F., Peng, G., Lu, Y., Wang, K., Ju, Q., Ju, Y., & Ouyang, M. (2022). Relationship between copper and immunity: The potential role of copper in tumor immunity. *Frontiers in Oncology*, *12*. https://doi.org/10.3389/fonc.2022.1019153

Chopra, H., Bibi, S., Singh, I., Hasan, M. M., Khan, M. S., Yousafi, Q., ... & Cavalu, S. (2022). Green metallic nanoparticles: biosynthesis to applications. Frontiers in Bioengineering and. Biotechnology, 10, 874742.

Choudhary, P., Abusalah, M. a. H., Chopra, H., Sharma, A., Mustafa, S. A., Choudhary, O. P., Sharma, M., Dhawan, M., Khosla, R., Loshali, A., Sundriyal, A., & Saini, J. (2023). Nanovaccines: A game changing approach in the fight against infectious diseases. *Biomedicine & Pharmacotherapy*, *167*, 115597. https://doi.org/10.1016/j.biopha.2023.115597

Clem A. S. (2011). Fundamentals of vaccine immunology. *Journal of global infectious diseases*, *3*(1), 73–78. https://doi.org/10.4103/0974-777X.77299

Cobo-Simón, Marta., Hart, R., Ochman, H. (2023). *Escherichia Coli:* What Is and Which Are*? Molecular Biology and Evolution*, 40 (1), msac273. https://doi.org/10.1093/molbev/msac273

Collins, J. F. (2017). Copper: basic physiological and nutritional aspects. *In Molecular, Genetic, and Nutritional Aspects of Major and Trace Minerals (pp. 69-83)*. Academic Press.

Cordeiro, A. S., Patil-Sen, Y., Shivkumar, M., Patel, R., Khedr, A., & Elsawy, M. A. (2021). Nanovaccine Delivery Approaches and Advanced Delivery systems for the prevention of viral infections: From development to clinical application. *Pharmaceutics*, *13*(12), 2091. https://doi.org/10.3390/pharmaceutics13122091

Crump, J. A., Luby, S. P., & Mintz, E. D. (2004). The global burden of typhoid fever. *Bulletin of the World Health Organization*, *82*(5), 346–353.

Dagah, O. M., Silaa, B. B., Zhu, M., Pan, Q., Qi, L., Liu, X., ... & Lu, J. (2024). Exploring Immune Redox Modulation in Bacterial Infections: Insights into Thioredoxin-Mediated Interactions and Implications for Understanding Host–Pathogen Dynamics. *Antioxidants*, *13*(5), 545.

Dai, X., Xiong, Y., Li, N., & Jian, C. (2019). *Vaccine Types. Vaccines - the History and Future.* doi:10.5772/intechopen.84626

Das, A., & Ali, N. (2021). Nanovaccine: an emerging strategy. *Expert review of vaccines*, *20*(10), 1273–1290. https://doi.org/10.1080/14760584.2021.1984890

de Nooijer, A. H., Pickkers, P., Netea, M. G., & Kox, M. (2023). Inflammatory biomarkers to predict the prognosis of acute bacterial and viral infections. *Journal of critical care*, *78*, 154360. https://doi.org/10.1016/j.jcrc.2023.154360

De Pinho Favaro, M. T., Atienza-Garriga, J., Martínez-Torró, C., Parladé, E., Vázquez, E., Corchero, J. L., Ferrer-Miralles, N., & Villaverde, A. (2022). Recombinant vaccines in 2022: a perspective from the cell factory. *Microbial Cell Factories*, *21*(1). https://doi.org/10.1186/s12934-022-01929-8

Deramaudt, T. B., Dill, C., & Bonay, M. (2013). Regulation of oxidative stress by Nrf2 in the pathophysiology of infectious diseases. *Médecine et maladies infectieuses, 43(3)*, 100-107.

Dutta, K., Nandi, D., & Bishayi, B. (2009). Repeated systemic Escherichia coli infection enhances anti-oxidant response in hypercholesterolemic mice inducing cardiovascular inflammation. *Inflammation*, *32*(2), 89–98. https://doi.org/10.1007/s10753-009-9107-5

Dwaya, A., Urayet, A., Milad, M. B., & Alkout, A. M. (2022). *Platelet count as useful indicator for Gram-Type of bacteria causing urinary tract infection in women*. https://www.redalyc.org/journal/6920/692072545007/html/#redalyc_692072545007_ref24

Ealia, S. A. M., & Saravanakumar, M. P. (2017). A review on the classification, characterisation, synthesis of nanoparticles and their application. In IOP conference series: materials science and engineering (Vol. 263, No. 3, p. 032019). IOP Publishing.

Eid, A. M., Fouda, A., Hassan, S. E., Hamza, M. F., Alharbi, N. K., Elkelish, A., Alharthi, A., & Salem, W. M. (2023). Plant-Based copper oxide nanoparticles; biosynthesis, characterization, antibacterial activity, tanning wastewater treatment, and heavy metals sorption. *Catalysts*, *13*(2), 348. https://doi.org/10.3390/catal13020348

El-Ghwas, D. E. (2022). Short Communication: Characterization and biological synthesis of zinc oxide nanoparticles by new strain of Bacillus foraminis. *Biodiversitas*, *23*(1). https://doi.org/10.13057/biodiv/d230159

El-Khawaga, A. M., Elsayed, M. A., Gobara, M., Suliman, A. A., Hashem, A. H., Zaher, A. A., Mohsen, M., & Salem, S. S. (2023). Green synthesized ZnO nanoparticles by Saccharomyces cerevisiae and their antibacterial activity and photocatalytic degradation. *Biomass Conversion and Biorefinery*. https://doi.org/10.1007/s13399-023-04827-0

Eng, S., Pusparajah, P., Mutalib, N. A., Ser, H., Chan, K., & Lee, L. (2015b). Salmonella: A review on pathogenesis, epidemiology and antibiotic resistance. *Frontiers in Life Science*, *8*(3), 284–293. https://doi.org/10.1080/21553769.2015.1051243

Erb, A., Stürmer, T., Marre, R., & Brenner, H. (2007). Prevalence of antibiotic resistance in Escherichia coli: overview of geographical, temporal, and methodological

variations. *European Journal of Clinical Microbiology & Infectious Diseases*, *26*(2), 83–90. https://doi.org/10.1007/s10096-006-0248-2

Fàbrega, A., & Vila, J. (2013). Salmonella enterica serovar Typhimurium skills to succeed in the host: virulence and regulation. *Clinical microbiology reviews*, *26*(2), 308–341. https://doi.org/10.1128/CMR.00066-12

Facciolà, A., Visalli, G., Laganà, A., & Di Pietro, A. (2022). An Overview of Vaccine Adjuvants: Current Evidence and Future Perspectives. *Vaccines*, *10*(5), 819. https://doi.org/10.3390/vaccines10050819

Faisal, S., Jan, H., Abdullah, Alam, I., Rizwan, M., Hussain, Z., Sultana, K., Ali, Z., & Uddin, M. N. (2022). In Vivo Analgesic, Anti-Inflammatory, and Anti-Diabetic Screening of *Bacopa monnieri*-Synthesized Copper Oxide Nanoparticles. *ACS omega*, *7*(5), 4071–4082. https://doi.org/10.1021/acsomega.1c05410

Faner, R., Sibila, O., Agustí, A., Bernasconi, E., Chalmers, J. D., Huffnagle, G. B., Manichanh, C., Molyneaux, P. L., Paredes, R., Brocal, V. P., Ponomarenko, J., Sethi, S., Dorca, J., & Monsó, E. (2017). The microbiome in respiratory medicine: current challenges and future perspectives. *European Respiratory Journal/˜ the œEuropean Respiratory Journal*, *49*(4), 1602086. https://doi.org/10.1183/13993003.02086-2016

Fazhan Wang, Jia Lou, Xiaoke Gao, Lijing Zhang, Feifei Sun, Zhaoqing Wang, Tianjiao Ji, Zhihai Qin. (2023). Spleen-targeted nanosystems for immunomodulation, NanoToday, Volume 52, 101943, ISSN 1748-0132, https://doi.org10.1016j.nantod.2023.101943(https://www.sciencedirect.com/sciencearticle/pii/S1748013223001925)

Fiser, R. H., Denniston, J. C., & Beisel, W. R. (1972). Infection with Diplococcus pneumoniae and Salmonella typhimurium in Monkeys: Changes in Plasma Lipids and Lipoproteins. *The Journal of Infectious Diseases*, *125*(1), 54–60. https://doi.org/10.1093/infdis/125.1.54

Fodouop, S. P. C., Gatsing, D., Tangue, B. T., Tagne, R. S., Tala, S. D., Tchoumboué, J., & Kuiate, J. R. (2015). *Effect of Salmonella typhimurium infection on rat's cell oxidation and in*

vivo antioxidant activity of Vitellaria paradoxa and Ludwigia abyssinica aqueous extract. Asian Pacific Journal of Tropical Disease, 5(1), 38–46. doi:10.1016/s2222-1808(14)60624-1

Fouda, A., Hassan, S. E., Eid, A. M., Awad, M. A., Althumayri, K., Badr, N. F., & Hamza, M. F. (2022). Endophytic bacterial strain, Brevibacillus brevis-mediated green synthesis of copper oxide nanoparticles, characterization, antifungal, in vitrocytotoxicity, and larvicidal activity. *Green Processing and Synthesis*, *11*(1), 931–950. https://doi.org/10.1515/gps-2022-0080

Gao, W., Fang, R. H., Thamphiwatana, S., Luk, B. T., Li, J., Angsantikul, P., Zhang, Q., Hu, C. M., & Zhang, L. (2015). Modulating antibacterial immunity via bacterial membrane-coated nanoparticles. *Nano letters*, *15*(2), 1403–1409. https://doi.org/10.1021/nl504798g

Garfias-López, J. A., Castro-Escarpuli, G., Cárdenas, P. E., Moreno-Altamirano, M. M. B., Padierna-Olivos, J., & Sánchez-García, F. J. (2018). Immunization with intestinal microbiota-derived Staphylococcus aureus and Escherichia coli reduces bacteria-specific recolonization of the intestinal tract. *Immunology letters*, *196*, 149–154. https://doi.org/10.1016/j.imlet.2018.02.007

Gazi, M. A., Alam, M. A., Fahim, S. M., Wahid, B. Z., Khan, S. S., Islam, M. O., Hasan, M. M., Hasan, S. M. T., Das, S., Mahfuz, M., Haque, R., & Ahmed, T. (2022). Infection with Escherichia coli pathotypes is associated with biomarkers of gut enteropathy and nutritional status among malnourished children in Bangladesh. *Frontiers in Cellular and Infection Microbiology*, *12*. https://doi.org/10.3389/fcimb.2022.901324

Gerberding, J. L., & Haynes, B. F. (2021). Vaccine innovations — past and future. *The New England Journal of Medicine*, *384*(5), 393–396. https://doi.org/10.1056/nejmp2029466

Geurtsen, J., de Been, M., Weerdenburg, E., Zomer, A., McNally, A., & Poolman, J. (2022). Genomics and pathotypes of the many faces of Escherichia coli. *FEMS microbiology reviews*, *46*(6), fuac031. https://doi.org/10.1093/femsre/fuac031

Ghareib, M., Abu-Tahon, M., Abdallah, W. E., & Tallima, A. (2018). Green synthesis of copper oxide nanoparticles using some fungi isolated from the Egyptian soil. *ResearchGate*. https://www.researchgate.net/publication/328808805_Green_synthesis_of_copper_oxide_nanoparticles_using_some_fungi_isolated_from_the_Egyptian_soil

Ghattas, M., Dwivedi, G., Lavertu, M., & Alameh, M. (2021). Vaccine technologies and platforms for infectious diseases: current progress, challenges, and opportunities. *Vaccines (Basel)*, *9*(12), 1490. https://doi.org/10.3390/vaccines9121490

Gheibi Hayat, S. M., & Darroudi, M. (2019). Nanovaccine: A novel approach in immunization. *Journal of Cellular Physiology*. doi:10.1002/jcp.28120

Ghorbani, H. R., Mehr, F. P., & Poor, A. K. (2015). Extracellular synthesis of copper nanoparticles using culture supernatants of Salmonella typhimurium. *Orient. J. Chem, 31(1),* 527–529. https://doi.org/10.13005/ojc/310165

Giesker, K., & Hensel, M. (2014). Bacterial Vaccines. *Reference Module in Biomedical Sciences*. doi:10.1016/b978-0-12-801238-3.00141-0

Giordano, N. P., Cian, M. B., & Dalebroux, Z. D. (2020). Outermembrane lipid secretion and the innate immune response to Gram-negative bacteria. *Infection and Immunity*. doi:10.1128/iai.00920-19

Goldmann, O., Nwofor, O. V., Chen, Q., & Medina, E. (2024). Mechanisms underlying immunosuppression by regulatory cells. *Frontiers in Immunology, 15*. https://doi.org/10.3389/fimmu.2024.1328193

Gros, A., Ollivier, V., & Ho-Tin-Noé, B. (2015). Platelets in inflammation: regulation of leukocyte activities and vascular repair. *Frontiers in Immunology*, *5*. https://doi.org/10.3389/fimmu.2014.00678

Gu, H., Zeng, X., Peng, L., Xiang, C., Zhou, Y., Zhang, X., Zhang, J., Wang, N., Guo, G., Li, Y., Liu, K., Gu, J., Zeng, H., Zhuang, Y., Li, H., Zhang, J., Zhang, W., Zou, Q., & Shi, Y. (2021). Vaccination induces rapid protection against bacterial pneumonia via training alveolar macrophage in mice. *eLife*, *10*, e69951. https://doi.org/10.7554/eLife.69951

Guo, Y. N., Mou, R. W., Lu, M. H., Liang, S. S., He, Y. M., & Tang, L. P. (2024). Three different routes of EHEC O157:H7 infection were used to establish EHEC broiler model. *Poultry science*, *103*(4), 103561. https://doi.org/10.1016/j.psj.2024.103561

Gupta, S., Sharma, N., Naorem, L. D., Jain, S., & Raghava, G. P. S. (2022). Collection, compilation and analysis of bacterial vaccines. *Computers in Biology and Medicine*, *149*, 106030. https://doi.org/10.1016/j.compbiomed.2022.106030

Haleem, A., Javaid, M., Singh, R. P., Rab, S., & Suman, R. (2023). Applications of nanotechnology in medical field: a brief review. *Global Health Journal*, *7*(2), 70–77. https://doi.org/10.1016/j.glohj.2023.02.008

Hamzeh-Cognasse, H., Damien, P., Chabert, A., Pozzetto, B., Cognasse, F., & Garraud, O. (2015). Platelets and infections - complex interactions with bacteria. *Frontiers in immunology*, *6*, 82. https://doi.org/10.3389/fimmu.2015.00082

Hendradewi, S., Purwanto, B., Dirgahayu, P., Wasita, B., & Pamungkas, E. P. (2020). The effect of Escherichia Coli induction on superoxide dismutase (SOD) and Malondialdehyde (MDA) levels in acute rhinosinusitis white rats models. *Bali Medical Journal*, *9*(2), 542–545. https://doi.org/10.15562/bmj.v9i2.1772

Herzog, C. (2014). *Influence of parenteral administration routes and additional factors on vaccine safety and immunogenicity: a review of recent literature. Expert Review of Vaccines, 13(3), 399–415.* doi:10.1586/14760584.2014.883285

Hima Sree Buddhiraju, Dokkari Nagalaxmi Yadav, Sreenath Dey, Kalyani Eswar, Ananya Padmakumar et Aravind Kumar Rengan. (2023) Progrès dans l'administration de médicaments ciblés décorés de peptides : exploration du potentiel thérapeutique et des stratégies de nanoporteurs. Biomatériaux appliqués ACS.

Hoerr, V., Zbytnuik, L., Leger, C., Tam, P. P. C., Kubes, P., & Vogel, H. J. (2012). Gram-negative and Gram-Positive Bacterial Infections Give Rise to a Different Metabolic Response in a Mouse Model. *Journal of Proteome Research, 11(6), 3231–3245.* doi:10.1021/pr201274r

Huang, Y. W., Cambre, M., & Lee, H. J. (2017). The toxicity of nanoparticles depends on multiple molecular and physicochemical mechanisms. International journal of molecular sciences, 18(12), 2702.

Huq, M. A., & Akter, S. (2021). Bacterial mediated rapid and facile synthesis of silver nanoparticles and their antimicrobial efficacy against pathogenic microorganisms. *Materials*, *14*(10), 2615.

Hwang, J., Jin, J., Jeon, S., Moon, S. H., Park, M. Y., Yum, D. Y., ... & Oh, G. T. (2020). SOD1 suppresses pro-inflammatory immune responses by protecting against oxidative stress in colitis. *Redox Biology*, 37, 101760.

Ibrahim Arif, A. (2023). Biosynthesis of copper oxide nanoparticles using Aspergillus niger extract and their antibacterial and antioxidant activities. *Journal of Medicinal and Pharmaceutical Chemistry Research*, *5(7)*, 598-608.

Ijaz, I., Gilani, E., Nazir, A., & Bukhari, A. (2020). Detail review on chemical, physical and green synthesis, classification, characterizations and applications of nanoparticles. Green Chemistry Letters and Reviews, 13(3), 223-245.

Iseki, A., Kambe, F., Okumura, K., Niwata, S., Yamamoto, R., Hayakawa, T., & Seo, H. (2000). Pyrrolidine Dithiocarbamate Inhibits TNF-α-Dependent Activation of NF-κB by Increasing Intracellular Copper Level in Human Aortic Smooth Muscle Cells. Biochemical and Biophysical Research Communications, 276(1), 88–92. doi:10.1006/bbrc.2000.3452

Ishfaq, M., Chen, C., Bao, J., Zhang, W., Wu, Z., Wang, J., Liu, Y., Tian, E., Hamid, S., Li, R., Ding, L., & Li, J. (2019). Baicalin ameliorates oxidative stress and apoptosis by restoring mitochondrial dynamics in the spleen of chickens via the opposite modulation of NF-κB and Nrf2/HO-1 signaling pathway during Mycoplasma gallisepticum infection. *Poultry science*, *98*(12), 6296–6310. https://doi.org/10.3382/ps/pez406

Islam, M. S., & Rahman, M. T. (2023). A Comprehensive Review on Bacterial Vaccines Combating Antimicrobial Resistance in Poultry. *Vaccines*, *11*(3), 616. https://doi.org/10.3390/vaccines11030616

Ivanova, I. A., Daskalova, D. S., Yordanova, L. P., & Pavlova, E. L. (2024). Copper and Copper Nanoparticles Applications and Their Role against Infections: A Minireview. Processes, 12(2), 352.

Jamkhande, P. G., Ghule, N. W., Bamer, A. H., & Kalaskar, M. G. (2019). Metal nanoparticles synthesis: An overview on methods of preparation, advantages and disadvantages, and applications. Journal of drug delivery science and technology, 53, 101174.

Jauffred, L., Vejborg, R. M., Korolev, K. S., Brown, S., & Oddershede, L. B. (2017). Chirality in microbial biofilms is mediated by close interactions between the cell surface and the substratum. ˜the œISME Journal, 11(7), 1688–1701. https://doi.org/10.1038/ismej.2017.19

Jeevanandam, J., Barhoum, A., Chan, Y. S., Dufresne, A., & Danquah, M. K. (2018). Review on nanoparticles and nanostructured materials: history, sources, toxicity and regulations. Beilstein journal of nanotechnology, 9(1), 1050-1074.

Johansson, E., & B. Falk, A. (2021). Erythrocytes as Biomarkers of Virus and Bacteria in View of Metal Ion Homeostasis. IntechOpen. doi: 10.5772/intechopen.97850

Joudeh, N., & Linke, D. (2022). Nanoparticle classification, physicochemical properties, characterization, and applications: a comprehensive review for biologists. Journal of Nanobiotechnology, 20(1), 262.

Kaningini, A. G., Motlhalamme, T., More, G. K., Mohale, K. C., & Maaza, M. (2023). Antimicrobial, antioxidant, and cytotoxic properties of biosynthesized copper oxide nanoparticles (CuO-NPs) using Athrixia phylicoides DC. *Heliyon*, *9*(4), e15265. https://doi.org/10.1016/j.heliyon.2023.e15265

Khan, I., Saeed, K., & Khan, I. (2019). Nanoparticles: Properties, applications and toxicities. Arabian journal of chemistry, 12(7), 908-931.

Khan, M. I., Fadl, A. A., & Venkitanarayanan, K. S. (2003). *Reducing colonization of Salmonella Enteritidis in chicken by targeting outer membrane proteins. Journal of Applied Microbiology, 95(1), 142–145.* doi:10.1046/j.1365-2672.2003.01953.x

Khan, Y. H., Saifullah, A., & Mallhi, T. H. (2022). Bacterial vaccines. In *Elsevier eBooks* (pp. 530–544). https://doi.org/10.1016/b978-0-12-818731-9.00170-1

Khan, Y., Sadia, H., Ali Shah, S. Z., Khan, M. N., Shah, A. A., Ullah, N., ... & Khan, M. I. (2022). Classification, synthetic, and characterization approaches to nanoparticles, and their applications in various fields of nanotechnology: A review. Catalysts, 12(11), 1386.

Kiarely Souza, E., Pereira-Dutra, F. S., Rajão, M. A., Ferraro-Moreira, F., Goltara-Gomes, T. C., Cunha-Fernandes, T., Santos, J. D. C., Prestes, E. B., Andrade, W. A., Zamboni, D. S., Bozza, M. T., & Bozza, P. T. (2022). Lipid droplet accumulation occurs early following

Salmonella infection and contributes to intracellular bacterial survival and replication. *Molecular microbiology*, *117*(2), 293–306. https://doi.org/10.1111/mmi.14844

Kim, C. G., Kye, Y.-C., & Yun, C.-H. (2019). *The Role of Nanovaccine in Cross-Presentation of Antigen-Presenting Cells for the Activation of CD8+ T Cell Responses. Pharmaceutics, 11(11), 612.* doi:10.3390/pharmaceutics11110612

Kim, Y. C., Jarrahian, C., Zehrung, D., Mitragotri, S., & Prausnitz, M. R. (2012). Delivery systems for intradermal vaccination. *Current topics in microbiology and immunology*, *351*, 77–112. https://doi.org/10.1007/82_2011_123

Koupenova, M., Livada, A. C., & Morrell, C. N. (2022). Platelet and Megakaryocyte Roles in Innate and Adaptive Immunity. Circulation research, 130(2), 288–308. https://doi.org/10.1161/CIRCRESAHA.121.319821

Kozak, M., & Hu, J. (2023). The integrated consideration of vaccine platforms, adjuvants, and delivery routes for successful vaccine development. *Vaccines*, *11*(3), 695. https://doi.org/10.3390/vaccines11030695

Kudair, Mohammed & Al-Hussary, N.. (2010). Effect of vaccination on some biochemical parameters in broiler chickens. Iraqi Journal of Veterinary Sciences. 24. 10.33899/ijvs.2010.5604

Kumah, E. A., Fopa, R. D., Harati, S., Boadu, P., Zohoori, F. V., & Pak, T. (2023). Human and environmental impacts of nanoparticles: a scoping review of the current literature. BMC Public Health, 23(1), 1059.

Kumari, S., & Sarkar, L. (2021). A review on nanoparticles: Structure, classification, synthesis & applications. J Sci Res, 65(8), 42-46.

Kurtz, J. R., Goggins, J. A., & McLachlan, J. B.(2017). Salmonella infection: Interplay between the bacteria and host immune system. Immunology letters, 190, 42–50. https://doi.org/10.1016/j.imlet.2017.07.006.

Kuvandik, C., Karaoglan, I., Namiduru, M., & Baydar, I. (2009). Predictive value of clinical and laboratory findings in the diagnosis of the enteric fever. *The new microbiologica*, *32*(1), 25–30.

Lahiri, D., Nag, M., Sheikh, H. I., Sarkar, T., Edinur, H. A., Pati, S., & Ray, R. R. (2021). Microbiologically-synthesized nanoparticles and their role in silencing the biofilm signaling cascade. Frontiers in microbiology, 12, 636588.

Lamontagne, F., Khatri, V., St-Louis, P., Bourgault, S., & Archambault, D. (2022). Vaccination Strategies Based on Bacterial Self-Assembling Proteins as Antigen Delivery Nanoscaffolds. *Vaccines*, *10*(11), 1920. https://doi.org/10.3390/vaccines10111920

Lanser, L., Fuchs, D., Kurz, K., & Weiss, G. (2021). Physiology and Inflammation Driven Pathophysiology of Iron Homeostasis—Mechanistic Insights into Anemia of Inflammation and Its Treatment. Nutrients, 13(11), 3732. https://doi.org/10.3390/nu13113732

Laupèze, B., Del Giudice, G., Doherty, M. T., & Van der Most, R. (2021). Vaccination as a preventative measure contributing to immune fitness. *npj Vaccines*, *6*(1), 93.

Lee, Y., Jeong, M., Park, J., Jung, H., & Lee, H. (2023). Immunogenicity of lipid nanoparticles and its impact on the efficacy of mRNA vaccines and therapeutics. *Experimental & molecular medicine*, *55*(10), 2085–2096. https://doi.org/10.1038/s12276-023-01086-x

Lemoine, C., Thakur, A., Krajišnik, D., Guyon, R., Longet, S., Razim, A., Górska, S., Pantelić, I., Ilić, T., Nikolić, I., Lavelle, E. C., Gamian, A., Savić, S., & Milicic, A. (2020). Technological approaches for improving vaccination compliance and coverage. *Vaccines (Basel)*, *8*(2), 304. https://doi.org/10.3390/vaccines8020304

Li, C., Li, Y., & Ding, C. (2019). The role of copper homeostasis at the host-pathogen axis: from bacteria to fungi. *International journal of molecular sciences*, *20*(1), 175.

Li, Q., Chi, H., Shi, X., Gan, Q., Dalmo, R. A., Sun, Y., Tang, X., Xing, J., Sheng, X., & Zhan, W. (2022). Vaccine Adjuvants Induce Formation of Intraperitoneal Extracellular Traps in Flounder (Paralichthys olivaceus). *Frontiers in Cellular and Infection Microbiology*, *12*. https://doi.org/10.3389/fcimb.2022.875409

Lombardi, L., Maiorca, F., Marrapodi, R., Sabetta, A., Scafa, N., Pallucci, D., Miglionico, M., Romiti, G. F., Corica, B., Piconese, S., Polimeni, A., Pulcinelli, F., Cangemi, R., Visentini, M., Basili, S., & Stefanini, L. (2023). Distinct platelet crosstalk with adaptive and innate

immune cells after adenoviral and mRNA vaccination against SARS-CoV-2. *Journal of Thrombosis and Haemostasis*, *21*(6), 1636–1649. https://doi.org/10.1016/j.jtha.2023.03.003

Lu, J., Liu, X., Li, X., Li, H., Shi, L., Xia, X., ... & Yang, X. (2024). Copper regulates the host innate immune response against bacterial infection via activation of ALPK1 kinase. Proceedings of the National Academy of Sciences, 121(4), e2311630121.

Maina, T., Grego, E. A., Boggiatto, P. M., Sacco, R. E., Narasimhan, B., & McGill, J. L. (2020). Applications of nanovaccines for disease prevention in cattle. *Frontiers in Bioengineering and Biotechnology*, *8*. https://doi.org/10.3389/fbioe.2020.608050

Malik, S., Muhammad, K., & Waheed, Y. (2023). Nanotechnology: A revolution in modern industry. Molecules, 28(2), 661.

Marshall, J.S., Warrington, R., Watson, W. et al. An introduction to immunology and immunopathology. Allergy Asthma Clin Immunol 14 (Suppl 2), 49 (2018). https://doi.org/10.1186/s13223-018-0278-1

Mazkour, S., Shekarforoush, S. S., Basiri, S., Nazifi, S., Yektaseresht, A., & Honarmand, M. (2020). Effects of two probiotic spores of Bacillus species on hematological, biochemical, and inflammatory parameters in Salmonella Typhimurium infected rats. Scientific reports, 10(1), 8035. https://doi.org/10.1038/s41598-020-64559-3

McCullough, J. (2014). RBCs as targets of infection. Hematology, 2014(1), 404–409. doi:10.1182/asheducation-2014.1.404

Meijerink, N., Van Den Biggelaar, R. H. G. A., Van Haarlem, D. A., Stegeman, J. A., Rutten, V. P. M. G., & Jansen, C. A. (2021). A detailed analysis of innate and adaptive immune responsiveness upon infection with *Salmonella enterica* serotype Enteritidis in young broiler chickens. *Veterinary Research*, *52*(1). https://doi.org/10.1186/s13567-021-00978-y

Mekuye, B., & Abera, B. (2023). Nanomaterials: An overview of synthesis, classification, characterization, and applications. *Nano Select*, *4*(8), 486–501. https://doi.org/10.1002/nano.202300038

Menon, I., Bagwe, P., Gomes, K. B., Bajaj, L., Gala, R. P., Uddin, M. N., D'Souza, M. J., & Zughaier, S. M. (2021). Microneedles: a new generation vaccine delivery system. *Micromachines (Basel)*, *12*(4), 435. https://doi.org/10.3390/mi12040435

Merino de Paz, N., García-González, M., Gómez-Bernal, F., Quevedo-Abeledo, J. C., de Vera-González, A., López-Mejias, R., Abreu-González, P., Martín-González, C., González-Gay, M. Á., & Ferraz-Amaro, I. (2023). Relationship between Malondialdehyde Serum Levels and Disease Features in a Full Characterized Series of 284 Patients with Systemic Lupus Erythematosus. *Antioxidants (Basel, Switzerland)*, *12*(8), 1535. https://doi.org/10.3390/antiox12081535

Mestre, A., Cunha, G., Teixeira, N., Correia, C., & Abuowda, Y. (2021). Rhabdomyolysis Due to Salmonellosis: A Case Report of a Rare Presentation. *Cureus*, *13*(11), e19781. https://doi.org/10.7759/cureus.19781

Milanino, R., & Buchner, V. (2006). *Copper: Role of the "Endogenous" and "Exogenous" Metal on the Development and Control of Inflammatory Processes. Reviews on Environmental Health, 21(3), 153–215.* doi:10.1515/reveh.2006.21.3.153

Milho, C., Silva, M. D., Alves, D., Oliveira, H., Sousa, C., Pastrana, L. M., Azeredo, J., & Sillankorva, S. (2019). Escherichia coli and Salmonella Enteritidis dual-species biofilms: interspecies interactions and antibiofilm efficacy of phages. *Scientific reports*, *9*(1), 18183. https://doi.org/10.1038/s41598-019-54847-y

Mobeen, H., Safdar, M., Fatima, A., Afzal, S., Zaman, H., & Mehdi, Z. (2022). Emerging applications of nanotechnology in context to immunology: A comprehensive review. *Frontiers in bioengineering and biotechnology*, *10*, 1024871. https://doi.org/10.3389/fbioe.2022.1024871

Morera, D., & MacKenzie, S. A. (2011). Is there a direct role for erythrocytes in the immune response? Veterinary Research, 42(1), 89. https://doi.org/10.1186/1297-9716-42-89

Morris, D., Khurasany, M., Nguyen, T., Kim, J., Guilford, F., Mehta, R., Gray, D., Saviola, B., & Venketaraman, V. (2013). Glutathione and infection. *Biochimica Et Biophysica Acta. G, General Subjects/Biochimica Et Biophysica Acta. General Subjects (Online)*, *1830*(5), 3329–3349. https://doi.org/10.1016/j.bbagen.2012.10.012

Mueangson, O., Mahittikorn, A., Anabire, N. G., Mala, W., & Kotepui, M. (2023). Increased blood Concentrations of malondialdehyde in plasmodium Infection: A Systematic Review and Meta-Analysis. *Antioxidants*, *12*(8), 1502. https://doi.org/10.3390/antiox12081502

Muthukumar, R., Alexandar, V., Thangam, B., & Ahmed, S. S. S. J. (2014). A Systems Biological Approach Reveals Multiple Crosstalk Mechanism between Gram-Positive and Negative Bacterial Infections: An Insight into Core Mechanism and Unique Molecular Signatures. *PloS One*, *9*(2), e89993. https://doi.org/10.1371/journal.pone.0089993

Naletova, I., Tomasello, B., Attanasio, F., & Pleshkan, V. V. (2023). Prospects for the Use of Metal-Based Nanoparticles as Adjuvants for Local Cancer Immunotherapy. *Pharmaceutics*, *15*(5), 1346. https://doi.org/10.3390/pharmaceutics15051346

Naz, S., Gul, A., Zia, M., & Javed, R. (2023). Synthesis, biomedical applications, and toxicity of CuO nanoparticles. *Applied Microbiology and Biotechnology*, *107*(4), 1039–1061. https://doi.org/10.1007/s00253-023-12364-z

Ngogo, F. A., Joachim, A., Abade, A. M., Rumisha, S. F., Mizinduko, M. M., & Majigo, M. V. (2020). Factors associated with Salmonella infection in patients with gastrointestinal complaints seeking health care at Regional Hospital in Southern Highland of Tanzania. *BMC infectious diseases*, *20*(1), 135. https://doi.org/10.1186/s12879-020-4849-7

Nian, X., Zhang, J., Huang, S., Duan, K., Li, X., & Yang, X. (2022). Development of nasal vaccines and the associated challenges. *Pharmaceutics (Basel)*, *14*(10), 1983. https://doi.org/10.3390/pharmaceutics14101983

Nwidu, L. L., Elmorsy, E., Thornton, J., Wijamunige, B., Wijesekara, A., Tarbox, R., Warren, A., & Carter, W. G. (2017). Anti-acetylcholinesterase activity and antioxidant properties of extracts and fractions of Carpolobia lutea. Pharmaceutical Biology, 55(1), 1875–1883. https://doi.org/10.1080/13880209.2017.1339283

Nzilu, D. M., Madivoli, E. S., Makhanu, D. S., Wanakai, S. I., Kiprono, G. K., & Kareru, P. G. (2023). Green synthesis of copper oxide nanoparticles and its efficiency in degradation of rifampicin antibiotic. *Scientific reports*, *13*(1), 14030. https://doi.org/10.1038/s41598-023-41119-z

Ols, S., Yang, L., Thompson, E. A., Pushparaj, P., Tran, K., Liang, F., Lin, A., Eriksson, B., Karlsson Hedestam, G. B., Wyatt, R. T., & Loré, K. (2020). Route of Vaccine Administration Alters Antigen Trafficking but Not Innate or Adaptive Immunity. *Cell reports*, *30*(12), 3964–3971.e7. https://doi.org/10.1016/j.celrep.2020.02.111

Opriessnig, T., Mattei, A. A., Karuppannan, A. K., & Halbur, P. G. (2021). *Future perspectives on swine viral vaccines: where are we headed? Porcine Health Management, 7(1).* doi:10.1186/s40813-020-00179 -7

Orrico, F., Laurance, S., Lopez, A. C., Lefevre, S. D., Thomson, L., Möller, M. N., & Ostuni, M. A. (2023). Oxidative stress in healthy and pathological red blood cells. Biomolecules, 13(8), 1262. https://doi.org/10.3390/biom13081262

Osman, A. I., Zhang, Y., Farghali, M., Rashwan, A. K., Eltaweil, A. S., El-Monaem, E. M. A., Mohamed, I. M. A., Badr, M. M., Ihara, I., Rooney, D. W., & Yap, P. (2024). Synthesis of green nanoparticles for energy, biomedical, environmental, agricultural, and food applications: A review. *Environmental Chemistry Letters*, *22*(2), 841–887. https://doi.org/10.1007/s10311-023-01682-3

Osterloh A. (2022). Vaccination against Bacterial Infections: Challenges, Progress, and New Approaches with a Focus on Intracellular Bacteria. *Vaccines*, *10*(5), 751. https://doi.org/10.3390/vaccines10050751

Ouidad, A., Sara, C., & Samir, D. (2020). Biological properties and Acute Toxicity Study of Copper oxide nanoparticles prepared by aqueous leaves extract of *Portulaca oleracea (L).* Asian Journal of Pharmaceutical Research, 10(2), 89-94.

Peletta, A., Lemoine, C., Courant, T., Collin, N., & Borchard, G. (2023). Meeting vaccine formulation challenges in an emergency setting: Towards the development of accessible vaccines. *Pharmacological Research*, *189*, 106699. https://doi.org/10.1016/j.phrs.2023.106699

Peltola, V., Mertsola, J., & Ruuskanen, O. (2006). Comparison of total white blood cell count and serum C-reactive protein levels in confirmed bacterial and viral infections. *The Journal of Pediatrics, 149(5), 721–724.* doi:10.1016/j.jpeds.2006.08.051

Pokharel, P., Dhakal, S., Dozois, C.M. (2023). The Diversity of *Escherichia coli* Pathotypes and Vaccination Strategies against This Versatile Bacterial Pathogen. *Microorganisms*, *11*, 344. https://doi.org/10.3390/microorganisms11020344

Pollard, A. J., & Bijker, E. M. (2021). A guide to vaccinology: from basic principles to new developments. *Nature Reviews. Immunology*, *21*(2), 83–100. https://doi.org/10.1038/s41577-020-00479-7

Poolman, J. (2020). Expanding the role of bacterial vaccines into life-course vaccination strategies and prevention of antimicrobial-resistant infections. *Npj Vaccines*, *5*(1). https://doi.org/10.1038/s41541-020-00232-0

Poria, R., Kala, D., Nagraik, R., Dhir, Y. W., Dhir, S., Singh, B., Kaushik, N., Noorani, M. S., Kaushal, A., & Gupta, S. (2024). Vaccine development: Current trends and technologies. Life Sciences, 336, 122331. https://doi.org/10.1016/j.lfs.2023.122331

Porwollik, S., Boyd, E. F., Choy, C., Cheng, P., Florea, L., Proctor, E., & McClelland, M. (2004). Characterization of Salmonella enterica Subspecies I Genovars by Use of Microarrays. Journal of Bacteriology, 186(17), 5883–5898. https://doi.org/10.1128/jb.186.17.5883-5898.2004

Pourahmad, J., Salami, M. & Zarei, M.H.(2023). Comparative Toxic Effect of Bulk Copper Oxide (CuO) and CuO Nanoparticles on Human Red Blood Cells. Biol Trace Elem Res 201, 149–155 . https://doi.org/10.1007/s12011-022-03149-y

Pretini, V., Koenen, M. H., Kaestner, L., Fens, M. H. A. M., Schiffelers, R. M., Bartels, M., & Van Wijk, R. (2019). Red Blood Cells: Chasing Interactions. Frontiers in Physiology, 10. doi:10.3389/fphys.2019.00945

Priya, M., Venkatesan, R., Deepa, S., Sana, S. S., Arumugam, S., Karami, A. M., Vetcher, A. A., & Kim, S. C. (2023). Green synthesis, characterization, antibacterial, and antifungal activity of copper oxide nanoparticles derived from Morinda citrifolia leaf extract. Scientific reports, 13(1), 18838. https://doi.org/10.1038/s41598-023-46002-5

Pulendran, B., Arunachalam, P. S., & O'Hagan, D. T. (2021). Emerging concepts in the science of vaccine adjuvants. *Nature Reviews. Drug Discover/Nature Reviews. Drug Discovery*, *20*(6), 454–475. https://doi.org/10.1038/s41573-021-00163-y

Qadri, H., Shah, A. H., Alkhanani, M., Almilaibary, A., & Mir, M. A. (2023). Immunotherapies against human bacterial and fungal infectious diseases: A review. *Frontiers in Medicine (Lausanne)*, *10*. https://doi.org/10.3389/fmed.2023.1135541

Raheem, H. Q. (2018). STUDY EFFECT OF SILVER NANOPARTICLES ON SOME BLOOD PARAMETERS IN RABBITS. ResearchGate.https://www.researchgate.net/publication/324953850_STUDY_EFFEC_OF_SILVER_NANOPARTICLES_ON_SOME_BLOOD_PARAMETERS_IN_RABBITS,

Rai, M., Bonde, S., Golinska, P., Trzcińska-Wencel, J., Gade, A., Abd-Elsalam, K. A., Shende, S., Gaikwad, S., & Ingle, A. P. (2021). Fusarium as a Novel fungus for the synthesis of nanoparticles: mechanism and applications. *Journal of Fungi*, *7*(2), 139. https://doi.org/10.3390/jof7020139

Rajashekaraiah, V., Hsieh, C., & Pallavi, M. (2021). Modulations in Oxidative Stress of Erythrocytes during Bacterial and Viral Infections. In *IntechOpen eBooks*. https://doi.org/10.5772/intechopen.98236

Rehman, K. U., Khan, A. U., Tahir, K., Nazir, S., Albalawi, K., Hassan, H. M., Alabbad, E. A., Refat, M. S., Al-Shehri, H. S., & Aldawsari, A. M. (2022). Facile synthesis of copper oxide nanoparticles (CuONPs) using green method to promote photocatalytic and biocidal applications. *Journal of Molecular Liquids*, *360*, 119453. https://doi.org/10.1016/j.molliq.2022.119453

Reimer, L. C., Carbasse, J. S., Koblitz, J., Ebeling, C., Podstawka, A., & Overmann, J. (2021). BacDive in 2022: the knowledge base for standardized bacterial and archaeal data. Nucleic Acids Research, 50(D1), D741–D746. https://doi.org/10.1093/nar/gkab961

Rishi, P., Kaur, H., Tirkey, N., Chopra, K., Bharrhan, S., Chanana, V., & Koul, A. (2006). Are the increases in local tumour necrosis factor and lipid peroxidation observed in pre-starved mice infected with Salmonella typhimurium markers of increased liver damage? *Microbes and Infection*, *8*(7), 1695–1701. https://doi.org/10.1016/j.micinf.2006.01.025

Rosche, K. L., Aljasham, A. T., Kipfer, J. N., Piatkowski, B. T., & Konjufca, V. (2015). Infection with Salmonella enterica Serovar Typhimurium Leads to Increased Proportions of

F4/80+ Red Pulp Macrophages and Decreased Proportions of B and T Lymphocytes in the Spleen. PloS one, 10(6), e0130092. https://doi.org/10.1371/journal.pone.0130092

Saha, T., Mobarak, MB, Uddin, MN, Quddus, MS, Naim, MR et Pinky, NS (2023). Synthèse biogénique de NP d'oxyde de cuivre (CuO) exploitant l'extrait de feuille de carambole d'Averrrhoa et son activité antibactérienne potentielle. Chimie et Physique des Matériaux, 305, 127979.

Sahoo, D. K., Wong, D., Patani, A., Paital, B., Yadav, V. K., Patel, A., & Jergens, A. E. (2024). Exploring the role of antioxidants in sepsis-associated oxidative stress: a comprehensive review. *Frontiers in Cellular and Infection Microbiology*, *14*. https://doi.org/10.3389/fcimb.2024.1348713

Scherlinger, M., Richez, C., Tsokos, G. C., Boilard, E., & Blanco, P. (2023). The role of platelets in immune-mediated inflammatory diseases. *Nature Reviews. Immunology*, *23*(8), 495–510. https://doi.org/10.1038/s41577-023-00834-4

Seneff, S., Nigh, G., Kyriakopoulos, A. M., & McCullough, P. A. (2022). Innate immune suppression by SARS-CoV-2 mRNA vaccinations: The role of G-quadruplexes, exosomes, and MicroRNAs. *Food and Chemical Toxicology*, *164*, 113008. https://doi.org/10.1016/j.fct.2022.113008

Serroukh, Y., Djebara, S., Lelubre, C., Zouaoui Boudjeltia, K., Biston, P., & Piagnerelli, M. (2012). Alterations of the Erythrocyte Membrane during Sepsis. Critical Care Research and Practice, 2012, 1–7. doi:10.1155/2012/702956

Seya, T., Tatematsu, M., & Matsumoto, M. (2022). Toward Establishing an Ideal Adjuvant for Non-Inflammatory Immune Enhancement. *Cells*, *11*(24), 4006. https://doi.org/10.3390/cells11244006

Shah, A., Tauseef, I., Ali, M. B., Yameen, M. A., Mezni, A., Hedfi, A., Haleem, S. K., & Haq, S. (2021). In-Vitro and In-Vivo Tolerance and Therapeutic Investigations of Phyto-Fabricated Iron Oxide Nanoparticles against Selected Pathogens. Toxics, 9(5), 105. https://doi.org/10.3390/toxics9050105

Sharma, V., Jakhar, K. K., Nehra, V., & Kumar, S. (2015). Biochemical studies in experimentally Escherichia coli infected broiler chicken supplemented with neem

(Azadirachta indica) leaf extract. *Veterinary world*, *8*(11), 1340–1345. https://doi.org/10.14202/vetworld.2015.1340-1345

Sherwood, E. R., Burelbach, K. R., McBride, M. A., Stothers, C. L., Owen, A. M., Hernandez, A., Patil, N. K., Williams, D. L., & Bohannon, J. K. (2022). Innate Immune Memory and the Host Response to Infection. *Journal of immunology (Baltimore, Md. : 1950)*, *208*(4), 785–792. https://doi.org/10.4049/jimmunol.2101058

Shukla, G., Verma, I., & Sharma, L. (2012). Effect of *Salmonella enteric* Serovar Typhimurium in Pregnant Mice: A Biochemical and Histopathological Study. *Gastroenterology research*, *5*(3), 103–111. https://doi.org/10.4021/gr441w

Silva, A. F., Sousa, J. S., Cunha, P. L., Lima-Filho, J. V., Alencar, N. M., Freitas, C. D., Oliveira, C. L., & Ramos, M. V. (2019). Erythrocytes morphology and hemorheology in severe bacterial infection. *Memorias do Instituto Oswaldo Cruz*, *114*, e190326. https://doi.org/10.1590/0074-02760190326

Sim, S., & Wong, N. K. (2021). Nanotechnology and its use in imaging and drug delivery (Review). *Biomedical reports*, *14*(5), 42. https://doi.org/10.3892/br.2021.1418

Singh, D., Jain, D., Rajpurohit, D., Jat, G., Kushwaha, H. S., Singh, A., Mohanty, S. R., Al-Sadoon, M. K., Zaman, W., & Upadhyay, S. K. (2023). Bacteria assisted green synthesis of copper oxide nanoparticles and their potential applications as antimicrobial agents and plant growth stimulants. *Frontiers in Chemistry*, *11*. https://doi.org/10.3389/fchem.2023.1154128

Solís-Sandí, I., Cordero-Fuentes, S., Pereira-Reyes, R., Vega-Baudrit, J. R., Batista-Menezes, D., & Montes de Oca-Vásquez, G. (2023). Optimization of the biosynthesis of silver nanoparticles using bacterial extracts and their antimicrobial potential. *Biotechnology reports (Amsterdam, Netherlands)*, *40*, e00816. https://doi.org/10.1016/j.btre.2023.e00816

Song, C., Hu, J., Liu, Y., Tian, Y., Zhu, Y., Xi, J., Cui, M., Wang, X., Zhang, B., Fan, L., & Li, Q. (2024). Vaccination-Route-Dependent adjuvanticity of Antigen-Carrying nanoparticles for enhanced vaccine efficacy. *Vaccines*, *12*(2), 125. https://doi.org/10.3390/vaccines12020125

Sora, V. M., Meroni, G., Martino, P. A., Soggiu, A., Bonizzi, L., & Zecconi, A. (2021). Extraintestinal Pathogenic Escherichia coli: Virulence Factors and Antibiotic Resistance. Pathogens (Basel, Switzerland), 10(11), 1355. https://doi.org/10.3390/pathogens10111355

Speck, D. (2009). *Aspects spécifiques de la production dans le domaine des vaccins. Annales Pharmaceutiques Françaises, 67(3), 213–218.* doi:10.1016/j.pharma.2009.02.008

Ssekatawa, K., Byarugaba, D. K., Angwe, M. K., Wampande, E. M., Ejobi, F., Nxumalo, E., Maaza, M., Sackey, J., & Kirabira, J. B. (2022). Phyto-Mediated Copper Oxide Nanoparticles for Antibacterial, Antioxidant and Photocatalytic Performances. *Frontiers in bioengineering and biotechnology*, *10*, 820218. https://doi.org/10.3389/fbioe.2022.820218

Stosik, M., Tokarz-Deptuła, B., Deptuła, J., & Deptuła, W. (2020). Immune Functions of Erythrocytes in Osteichthyes. Frontiers in Immunology, 11. doi:10.3389/fimmu.2020.01914.

Su, J. R., Duffy, J., & Shimabukuro, T. T. (2019). *Vaccine Safety. Vaccinations, 1–24.* doi:10.1016/b978-0-323-55435-0.00001-x

Sugeçti, S. (2021), Biochemical and immune responses of model organism Galleria mellonella after infection with Escherichia coli. Entomol Exp Appl, 169: 911-917.https://doi.org/10.1111/eea.13092

Sultana, M., Nibir, A. I. S., & Ahmed, F. R. S. (2023). Biosensing and anti-inflammatory effects of silver, copper and iron nanoparticles from the leaf extract of Catharanthus roseus. *Beni-Seuf University Journal of Basic and Applied Sciences /Beni-Suef University Journal of Basic and Applied Sciences*, *12*(1). https://doi.org/10.1186/s43088-023-00358-9

Suood, A. M., Saleh, M. K., & Thalij, K. M. (2021). Synthesis of copper nanoparticles using Aspergillus niger and their efficacy against pathogenic Staphylococcus aureus. *IOP Conference Series. Earth and Environmental Science*, *910*(1), 012083. https://doi.org/10.1088/1755-1315/910/1/012083

Szczyglewska, P., Feliczak-Guzik, A., & Nowak, I. (2023). Nanotechnology–General aspects: A chemical reduction approach to the synthesis of nanoparticles. *Molecules/Molecules Online/Molecules Annual*, *28*(13), 4932. https://doi.org/10.3390/molecules28134932

Talebian, S., Shahnavaz, B., Nejabat, M., Abolhassani, Y., & Rassouli, F. B. (2023). Bacterial-mediated synthesis and characterization of copper oxide nanoparticles with antibacterial, antioxidant, and anticancer potentials. *Frontiers in Bioengineering and Biotechnology*, *11*. https://doi.org/10.3389/fbioe.2023.1140010

Tall, A. R., & Yvan-Charvet, L. (2015). Cholesterol, inflammation and innate immunity. *Nature reviews. Immunology*, *15*(2), 104–116. https://doi.org/10.1038/nri3793

Tam, H. H., Melo, M. B., Kang, M., Pelet, J. M., Ruda, V. M., Foley, M. H., Hu, J. K., Kumari, S., Crampton, J., Baldeon, A. D., Sanders, R. W., Moore, J. P., Crotty, S., Langer, R., Anderson, D. G., Chakraborty, A. K., & Irvine, D. J. (2016). Sustained antigen availability during germinal center initiation enhances antibody responses to vaccination. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(43), E6639–E6648. https://doi.org/10.1073/pnas.1606050113

Tasdemir, C., Tasdemir, S., Vardi, N., Ates, B., Onal, Y., Erdogan, S., Yucel, A., Aglamis, E., Yakupogullari, Y., Altıntas, R., & Karaman, A. (2013). Evaluation of the effects of ozone therapy on Escherichia coli-induced cytitis in rat. *Irish journal of medical science*, *182*(4), 557–563. https://doi.org/10.1007/s11845-013-0926-x

Teklemariam, A. D., Al-Hindi, R. R., Albiheyri, R. S., Alharbi, M. G., Alghamdi, M. A., Filimban, A. a. R., Mutiri, A. S. A., Al-Alyani, A. M., Alseghayer, M. S., Almaneea, A. M., Albar, A. H., Khormi, M. A., & Bhunia, A. K. (2023). Human salmonellosis: a continuous global threat in the Farm-to-Fork Food Safety continuum. *Foods*, *12*(9), 1756. https://doi.org/10.3390/foods12091756

Togan, T., Narci, H., Turan, H., Ciftci, O., Kursun, E., & Arslan, H. (2015). The impact of acute brucellosis on mean platelet volume and red blood cell distribution. *Jundishapur journal of microbiology*, *8*(2), e20039. https://doi.org/10.5812/jjm.20039

Tokarz-Deptuła, B., Palma, J., Baraniecki, Ł., Stosik, M., Kołacz, R., & Deptuła, W. (2021). What function do platelets play in inflammation and bacterial and viral infections? *Frontiers in Immunology*, *12*. https://doi.org/10.3389/fimmu.2021.770436

Tong, C. Y. W. (2019). Different types of vaccines. In *Oxford University Press eBooks*. https://doi.org/10.1093/oso/9780198801740.003.0061

Torres-Sangiao, E., Holban, A. M., & Gestal, M. C. (2016). Advanced Nanobiomaterials: Vaccines, Diagnosis and Treatment of Infectious Diseases. *Molecules (Basel, Switzerland)*, *21*(7), 867. https://doi.org/10.3390/molecules21070867

Tulinska, J., Mikusova, M. L., Liskova, A., Busova, M., Masanova, V., Uhnakova, I., Rollerova, E., Alacova, R., Krivosikova, Z., Wsolova, L., Dusinska, M., Horvathova, M., Szabova, M., Lukan, N., Stuchlikova, M., Kuba, D., Vecera, Z., Coufalik, P., Krumal, K., . . . Mikuska, P. (2022). Copper oxide nanoparticles stimulate the immune response and decrease antioxidant defense in mice after Six-Week inhalation. *Frontiers in Immunology*, *13*. https://doi.org/10.3389/fimmu.2022.874253

Unnikrishnan, G., Joy, A., Megha, M., Kolanthai, E., & Senthilkumar, M. (2023). Exploration of inorganic nanoparticles for revolutionary drug delivery applications: a critical review. *Discover Nano*, *18*(1). https://doi.org/10.1186/s11671-023-03943-0

Velsankar, K., Parvathy, G., Mohandoss, S., Kumar, R. M., & Sudhahar, S. (2022). Green synthesis and characterization of CuO nanoparticles using Panicum sumatrense grains extract for biological applications. *Applied Nanoscience*, *12*(6), 1993–2021. https://doi.org/10.1007/s13204-022-02441-6

Verma, N., & Kumar, N. (2019). Synthesis and biomedical applications of copper oxide nanoparticles: an expanding horizon. ACS biomaterials science & engineering, 5(3), 1170 11:381188.

Vetter, V., Denizer, G., Friedland, L. R., Krishnan, J., & Shapiro, M. (2017). Understanding modern-day vaccines: what you need to know. *Annals of Medicine (Helsinki)*, *50*(2), 110–120. https://doi.org/10.1080/07853890.2017.1407035

Vinjamuri S, Afshan S, Shekar S, Saraswathi VJ. (2015). Evaluation of hemolytic activity, atpase inhibitory activity and antitumor activity of tlc extract of lemon grass (cymbpogoncitratus). International journal of pharmacognosy and phytochemical research, 7(4), 785-788.

Wallis, J., Shenton, D. P., & Carlisle, R. C. (2019). *Novel approaches for the design, delivery and administration of vaccine technologies. Clinical & Experimental Immunology.* doi:10.1111/cei.13287

Wang, M., Qazi, I. H., Wang, L., Zhou, G., & Han, H. (2020). *Salmonella* Virulence and Immune Escape. *Microorganisms*, *8*(3), 407. https://doi.org/10.3390/microorganisms8030407

Wang, R., Li, S., Jia, H., Si, X., Lei, Y., Lyu, J., Dai, Z., & Wu, Z. (2021). Protective effects of cinnamaldehyde on the inflammatory response, oxidative stress, and apoptosis in liver of salmonella typhimurium-Challenged mice. *Molecules/Molecules Online/Molecules Annual*, *26*(8), 2309. https://doi.org/10.3390/molecules26082309

Weckbecker, G., & Cory, J. G. (1988). Ribonucleotide reductase activity and growth of glutathione-depleted mouse leukemia L1210 cells in vitro. Cancer Letters, 40(3), 257-264. Doi: 10.1016/0304-3835(88)90084-5.

Weniger, B. G., & Papania, M. J. (2013). Alternative vaccine delivery methods. In *Elsevier eBooks* (pp. 1200–1231). https://doi.org/10.1016/b978-1-4557-0090-5.00063-x

World Health Organization [WHO]. (2018, February 7).

Yadav, H. K. S., Dibi, M., Mohammad, A., & Srouji, A. E. (2018). *Nanovaccines formulation and applications-a review. Journal of Drug Delivery Science and Technology, 44, 380–387.* doi:10.1016/j.jddst.2018.01.015

Yagi K. (1976). A simple fluorometric assay for lipoperoxide in blood plasma. *Biochemical medicine,* 15(2), 212-216. Doi:10.1016/0006-2944(76)90049-1

Yanar, F., Carugo, D., & Zhang, X. (2023). Hybrid nanoplatforms comprising organic nanocompartments encapsulating inorganic nanoparticles for enhanced drug delivery and bioimaging applications. Molecules, 28(15), 5694.

Yeaman M. R. (2010). Platelets in defense against bacterial pathogens. *Cellular and molecular life sciences : CMLS*, *67*(4), 525–544. https://doi.org/10.1007/s00018-009-0210-4

Yu, D., Banting, G., & Neumann, N. F. (2021). A review of the taxonomy, genetics, and biology of the genus Escherichia and the type species Escherichia coli. *Canadian Journal of Microbiology*, *67*(8), 553–571. https://doi.org/10.1139/cjm-2020-0508

Zangiabadi, S., Chamoun, K. P., Nguyen, K., Tang, Y., Sweeney, G., & Abdul-Sater, A. A. (2023). Copper infused fabric attenuates inflammation in macrophages. PloS one, 18(9), e0287741. https://doi.org/10.1371/journal.pone.0287741

Zeid, M. a. A., Nasef, S. A., Gehan, I. E. A., & Hegazy, A. (2020). A Field Study on Biochemical Changes Associated with Salmonella Infection in Ducklings. *Journal of World's Poultry Research*, *10*(2s), 250–262. https://doi.org/10.36380/jwpr.2020.31

Zha, L., Garrett, S., & Sun, J. (2019). *Salmonella* Infection in Chronic Inflammation and Gastrointestinal Cancer. *Diseases (Basel, Switzerland)*, *7*(1), 28. https://doi.org/10.3390/diseases7010028

Zhao, T., Cai, Y., Jiang, Y., He, X., Wei, Y., Yu, Y., & Tian, X. (2023). Vaccine adjuvants: mechanisms and platforms. *Signal Transduction and Targeted Therapy*, *8*(1). https://doi.org/10.1038/s41392-023-01557-7

Annex

Annex 1 :

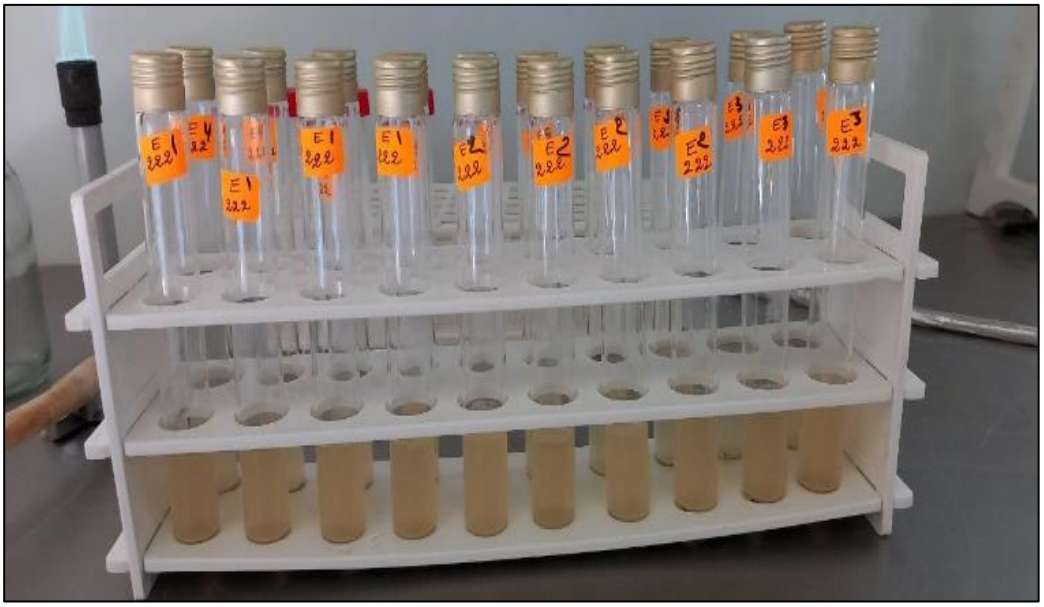


Figure 1: Bacterial cultivation in Luria broth liquid media (LB)

Figure 2: Ultrasonic bath

Figure 3: Centerfuge

Annex2

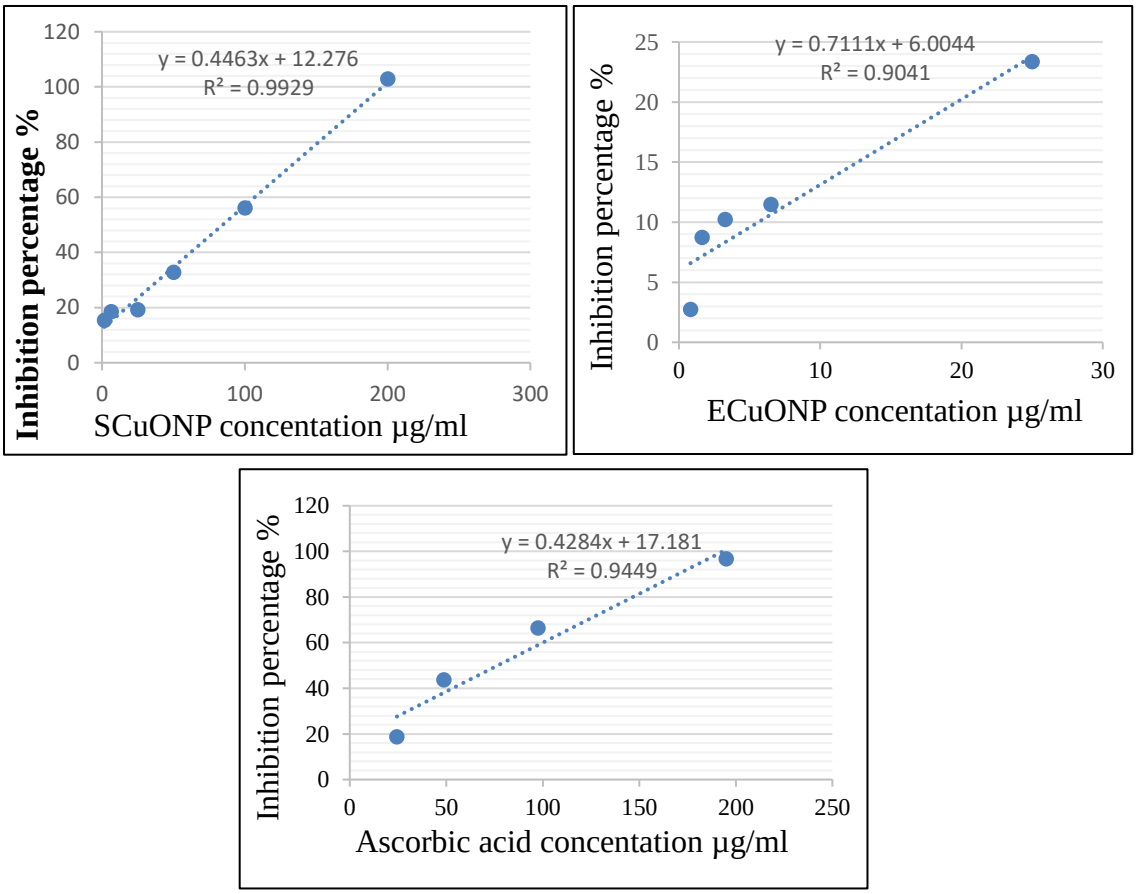


Figure 1: Percentage of DPPH radical inhibition as a function of concentrations of SCuNP, ECuNP and ascorbic acid to determine IC_{50}

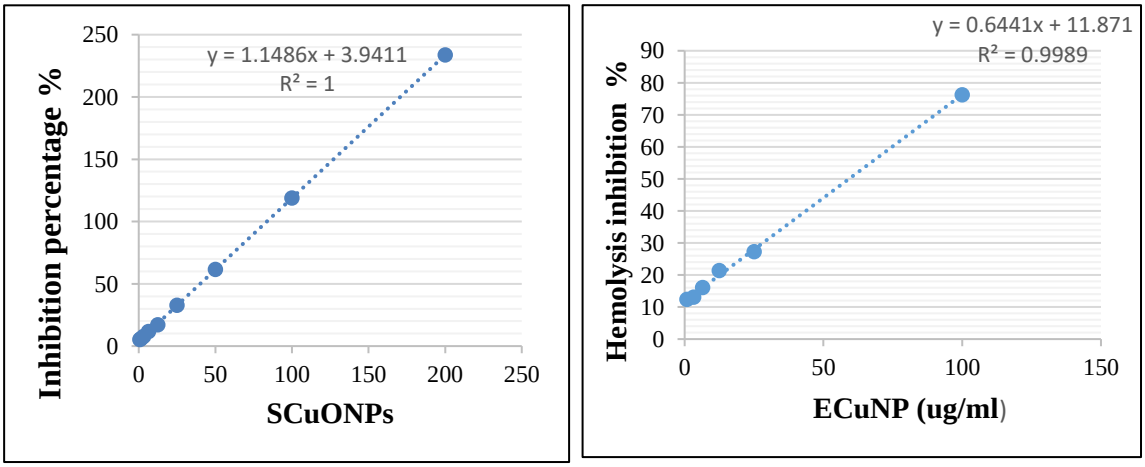


Figure 2: Inhibition of hemolysis as a function of concentrations of SCuNP, ECuNP and ascorbic acid to determine IC_{50}