



PREFACE

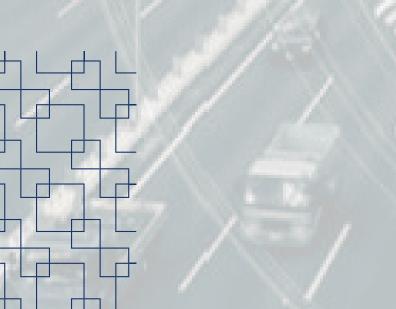


The V. International Rimar Congress of Pure and Applied Sciences "Rimar CONGRESS", was organized by Igdir University, in collaboration with Rimar Academy. The primary objective of this event was to compile and disseminate valuable scientific knowledge and make a meaningful contribution to the future.

Remarkably, a substantial number of researchers, both from local and international backgrounds, demonstrated their interest in this conference. The scientific committee meticulously reviewed the submissions and ultimately accepted a select group of individuals, totaling 18 applicants, 15 of them were accepted by the scientific committee.

The core of this conference was the presentation of 12 complete research papers, while the remaining articles and research findings are set to be featured in forthcoming issues of the MINAR Journal.

I would like to extend my sincere appreciation to all the contributors and scholars who played an essential role in making this conference a resounding success. Your dedication and valuable contributions are deeply respected and acknowledged



Editor in chief Prof. Dr. Ghuson Mohamed









INDEX

The Effect of Selenium/Zinc Oxide Nano Composites from Artemisia annua as Inhibitory Activity on Serratia marcescens Isolated from Catheter-Associated Bacteremia

Samira Gjir Jremich Riyam Wissam Hassan Dania Ali Azeiz

Study The Efficacy of COQ10 Enzyme with Vitamin E On Escherichia Coli Bacteria Isolated from Urinary Tract Infection

Taif H. Hassan Ali A. Ali

Evaluation of the Anti-Microbial Activity of Morinzhi Extract Against Some Human Pathogenic Bacteria

Ali A. MAHDI Mays M. HOOBI

Evaluation the vitamin **D3** and Renal function test and liver function Test Levels on Toxoplasma gondii (T. gondii) in Baghdad pregnant Women

Zainab Noaman Eyada Abbas Mosad Ajeed Sara Ali Mutashar Diyar Adel Lateef

Evaluating the Performance of IEEE 802.11ac and 802.11n in NS-3

Mustafa Mohammed Jassim Firas Mohammed Adress

1

11

25

33

40

88

INDEX

Association and Prevalence of Dyslipidemia in Type 2-Diabetes Mellitus patients in 51 **Baghdad Baraa Kasim Mohammed** Shaimaa Haidar Rabah Yusur Fadhil Shallal The Effect Albumin and Lipid Profile in The Type 2 Diabetes Mellitus with Hypertension in Iraqi Patients 62 Rawa M.M Taqi Wael dheaa kadhim Zeina Abdul-Ella **Nesreen Ahmed Nasser** 71 Assessment of Water Pollution in the Tigris River near Medical City Using Satellite images and Ground Measurements Rafah R. Ismail Maha A. Hameed Noor Z. Kouder 81 Efficiency of the Nano Particles of Aqueous Moringo Oleifera Extract in Antioxidants Levels in Male Albino **Rat Treated with Drug Cyclosporine** Haneen sameer fakher Zainab Shnewer Mahdi

Assessment of Vitamin B12 Levels in Pregnant Women with Toxoplasmosis

Anas H. Sadek



The Effect of Selenium/Zinc Oxide Nano Composites from *Artemisia annua* as Inhibitory Activity on *Serratia marcescens* Isolated from Catheter-Associated Bacteremia



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Abstract

The current study included testing the antibacterial effect of Artemisia annua (Sweet wormwood) and the two nanocomplexes (Artemisia annua /zinc oxide Nps) (Artemisia annua /selenium Nps) on Serratia marcescens isolated from catheter-associated bacteremia in hospitalized adults from many hospitals in Karbala city (Al-Hussein Teaching hospitals, ALTurky Hospital and Al-Kafeel Hospitals); Artemisia annua (sweet wormwood) and two nanocomposites, Artemisia annua mixed with zinc oxide nanoparticles (Artemisia annua/ZnO NP) and Artemisia annua mixed with selenium nanoparticles (Artemisia annua/Se NP), were investigated for their antibacterial effect. These compounds tested against Serratia marcescens, a bacterial isolate of blood-borne bacteria associated with catheters in hospitalized adults from patient admitted to hospitals. The bacterial isolates were identified phenotypically and biochemically. The two Nano-composites (Artemisia annua/ZnO NP and Artemisia annua/Se NP) prepared using green synthesis techniques. Synthesized compounds characterized by UV-Vis spectroscopy and Fourier transform infrared spectroscopy (FTIR). The study was conducted between December 2022 to the end of January 2023, the results found Liquid medium dilution was used to establish that the Artemisia annua had minimum inhibitory concentration (MIC) of 250 and 500 µg/mL, and the minimal inhibitory concentration (MIC) for Serratia marcescens against the Artemisia annua/zinc oxide nanoparticle (Art/ZnO NPs) composite was found to be 2000 μg/mL, as well as Artemisia annua/selenium nanoparticle (Art/Se NPs) composite showed MIC of 250 μg/mL against Serratia marcescens..

KEYWORDS

Serratia marcescens Artemisia annua Nanoparticles

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Introduction

Serratia marcescens is a Gram-negative, rod-shaped, aerobic bacterium belonging to the family Enterobacteriaceae, which had classically been considered a saprophytic bacterium; however, in the last 60 years it has become an agent of great clinical relevance, responsible for nosocomial outbreaks, causing a wide variety of infections such as: pneumonia, urinary tract infection, surgical wound infection, meningitis, endocarditis, and sepsis (1). It found in the intestinal microflora of animals and man, in the environmental factors and in reservoir like fomites, faucets, pipes, and water (2).

Outbreaks by *Serratia marcescens* have been reported, pointing to mechanical ventilation equipment, disinfectants, soaps and hands as potential sources of transmission, as well as fluids (catheter-associated bacteremia, contaminated serum, and multidose vials drugs injection) (3). In studies of *S. marcescens* outbreaks, breakage of aseptic technique and noncompliance with hand hygiene have been observed. The most important form of dissemination is person-to-person transmission (4). Studies have identified risk factors such as low birth weight, prolonged hospital stay, and invasive procedures (5).

Artemisia annua is a member of the Asteraceae family and grows best in temperate climates with short summers. The leaves of *A. annua* are between 3 and 5 centimeters in length and are divided into two or three small leaflets by deep cuts (6). The proposed action mechanism of *Artemisia annua* involves the cutting of endoperoxide bonds within it. (7).

With the presence of iron, this breakage occurs giving rise to various free radicals byproducts and one of them is hypervalent iron-oxo species, epoxides, aldehydes and dicarbonyl compounds in general the main importance of endoperoxide moieties present in *Artemisia annua* is highlighted by these events as well as emphasizing that iron must first act on this compound to produce reactive oxygen intermediates (ROIs) (8). Plants are an important source of many natural antimicrobials and the use of plants Medicines as antibiotics against various types of infections had preceded the use of medicines and drugs for a long time (9). Recent research has revealed that many plants previously thought to have medicinal properties can actually produce chemical compounds with antimicrobial or therapeutic properties as secondary metabolites such as *Artemisia annua*. Therefore, this study aimed to use different concentrations of Nano-composites of selenium and zinc oxide as an antimicrobial activity against *Serratia marcescens*, using a concentration of *Artemisia annua*

against pathogenic bacteria growing on the hospitals environment to test its inhibitory activity.

Materials and methods

Study design

Patients from 60 to 80 years of age Mean ± SD 76.4 ±8.56, with more than 24 hours of hospitalization during heart valve replacement catheter by specialized surgeon from many hospitals in Karbala city (ALHussein Teaching hospital, ALTurky Hospitals and ALKafeel Hospitals) during the period from 1/4/2023 to 1/4/2024.

Ethics approved

The presentation of the study was approved by the Scientific Committee and Ethics of the National Karbala Hospital. The personal data of the patients were kept strictly confidential.

Antibiotics susceptibility by agar diffusion methods:

In this study, all strain of *Serratia marcescens* obtained from clinical sample of patient who attended in the private heart centers analyzed. Following the recommendations of the National Committee for Clinical Laboratory Standards (10) and using agar diffusion procedures (11), the inhibition halos to the following antibiotics were measured: nanoparticle alone (Zno/or Sel nanoparticles), Nano-composite mixture (Zno/or Sel plus *Artemisia annua*). All samples obtained were sent to the Microbiology and Infectious Diseases Unit of the veterinary medicine college university of Karbala, and included in a computerized system called Whonet (WHOnet antibiogram software ver.22), belonging to the Surveillance Project of Bacterial Resistance to Antimicrobials in Iraq.

Preparation of Nano-composites

The procedure illustrated in (12) by 1 gram of *Artemisia annua* was digested in a vigorously stirred solution of zinc oxide in 50 ml distilled deionized water for 24 hours at room temperature. The ZnO solution dissolving 1 gram of the precursor was stirred for 18 hours at 40°C. The solution was put in a mixture at a temperature of 40 °C. The heating process was carried on in the plate of the magnetic stirrer the lover layer of the mixture changes its color from pale yellow to reddish, and then the permeability of the solution is measured in the

spectrophotometer. The mixture was incubated in shaker incubator, then the precipitation occurred the precipitate was separated by a centrifuge at a speed of 3000 cycle for 20 minute well. Finally, we washed the precipitation to get the fine powder.

Results and discussion

The results of Nano-composites applied and analyzed by the UV-visible spectroscopy. With the help of this method, it detected that the color of the solution, which was prepared, changed from yellow to reddish orange. The graphs ensure that the peak of the plasmon resonance is within the range of wavelength 300-450 nm. In addition, the fact that sets of spectra contain a sharp peak is due to the localization of the exciting LSP, which effectively scatters a local electric field at the specific wavelength of resonance occurrence. The graphical representation of the spectral pattern shown below as in Figure 1. It is obvious regarding the composition of the Nano-composites, including the main compound, which is a plant *Artemisia annua* and selenium, that there is visible deficiency. As a result, it can clearly see from the graph that there is an interaction between components. The result of the examination agrees with (13).

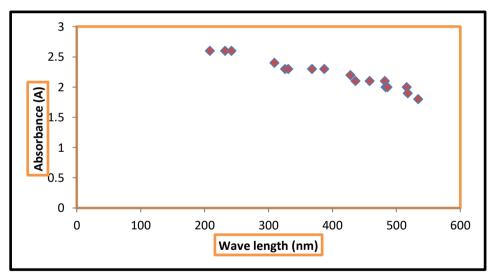


Figure (1) The spectral pattern of nano-composites (Artemisia annua / selenium).

The number of fluctuations of the secondary fluctuations is the main factor for the determination of the molecular weight of the nanoparticles and its determination the botanical extracts of phenidol, (Amine) and (Alcohol) and alcohol, Terpenoids, Alkaloids, and Carboxylic acid answerable for the stabilization and reduction of nanoparticle, figure (2) Many cities of the limitation are clear for 3400 , 2800 , 2900 , 1600 , 1200 cm-1 which mean the number is a multi-hydroxy chloride, carboxydial (COOH) and amine (NH 2) That is, we have

a compound, a hydrocarbon, an amine, or an amine generally, Carboxydialysis is deliberately modified by reduction and annealing, The specifics of the number of ways to get rid of the accumulated clutter are clear between 400-600 cm-1 figure 2, it was mean the crude in the among zinc oxide residues this result was agreement with (14).

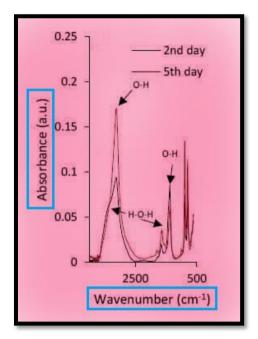


Figure (2) "Fourier-transform infrared (FT-IR) spectroscopy" for Nano-composites

After culturing in agar blood and incubating at 37°C for 24 hours, round and convex colony were obtained, it is shiny and red-color colonies due to appear its abilities to produce a reddish dark color pigments (15) figure 3. The culture and biochemical traits studied as well as the diagnostic Vitek were used, the colony is large and clear, the isolates were sub-cultured on CHROMagar™ *Serratia* medium, the colonies appeared Green-blue to metallic blue, indicating their inability to ferment MacConkey agar.

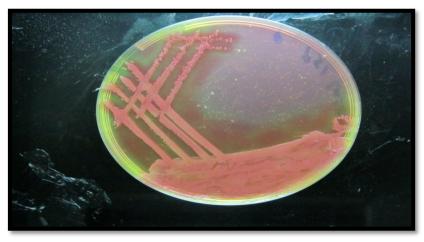


Figure (3) Reddish colonies of Serratia marcescens

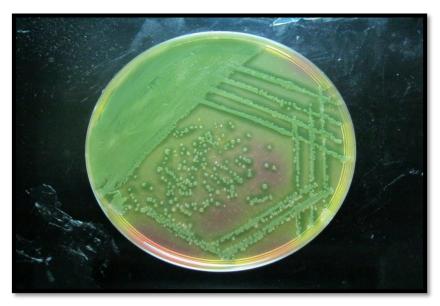


Figure (4) greenish colonies of Serratia marcescens

Serratia marcescens affected 17 patient have heart valve replacement catheter patients, age of the patients ranged from 60 months to 80 years, 5 of the cases were adults under 70 year of age and 12 of the cases were adults above 70 years; some of patients presented pneumonia associated with mechanical ventilation Ventilator-associated Pneumonia (VAP) 4/17, peritonitis 6/17, hypertension 6/17, and 1 patient with renal kidney failure (Table 1).

Table (1) Location of colonization and infection by *Serratia marcescens* among patient have heart valve replacement catheter.

Characteristic clinical	Number (No.)	Percentage (%)
VAP	4	23.5%
Peritonitis	6	35.3%
Hypertension	6	35.3%
Renal kidney failure	1	5.9%

Six concentrations of a series of nanoparticles (2000, 1000, 500, 250, 125, 62.5) μ g/mL were used by the dilution method, where the plant *Artemisia annua* concentration was 250 and 500 μ g/mL, on the other hand, *Art*/Zno NPs were given at 2000 μ g/mL, while the *Art*/Se NPs recorded for the MIC 250 μ g/mL, The results in Table (2) indicate that the effect of free *Artemisia annua* on *Serratia marcescens* a good reduction at 2 mg/ml concentration, which was (13.5 \pm 3.74) mm the effect of this plant was antimicrobial activity against bacterial infection (17), There are some studies using this plant extract as a treatment for fungal infections (18). At the same time, the concentration has an inhibition of 15.7 \pm 2.45 mm, and

the inhibition diameters can be seen in Table (2) when it used *Art*/Zno NPs, It is very important to known the Nano-composites preparation—was done by use 1gram of *Artemisia annua* to 1 gram of zinc oxide, as it was twice as concentrated in the Nano-composite, some of previous studies (19). Indicate that the degree of effectiveness of Nano-composites depend on the sizes, so the nanomaterial gave better result than the free substance. The results of Cur/Zno Nps inhibition were high, which indicates that there is a synergistic effect between ZnO and *Artemisia annua*, so thus, the formulated green synthesized Nano-composite, comprising of *Artemisia annua* and selenium Nano-composite, can implemented as an antibacterial agent as well as a medical drug.

The synergistic effect of this *Artemisia annua* and the released zinc ions with the Nano-composite formulation makes it a better candidate to be used in medical and antimicrobial uses than the mere use of the two on their own (20), Therefore, zinc ions and *Artemisia annua* may work synergistically to lead to an anti- and inhibitory effect against *Serratia marcescens* isolates. Selenium gave a good inhibition rate of mean (9.5± 2.61) mm against bacteria *Serratia marcescens*, Recently, some of indicated that selenium nanoparticles give a good effect against various types of bacteria like *Staphylococcus aureus* (16), *Enterococcus faecalis* (21), *Streptococcus spp.* (22).

Nano-selenium maked by green methods and reduced by Ascorbic acid give inhibition diameters calculated at 10.5± 1.92 mm in bacteria, according to what the study obtained (23) when he using Nano-selenium against gram negative bacteria. The reason for the difference in each of the two Nano-composites with the *Artemisia annua* is due to the belief that the number of different particles in the surface of the microbeads and the density of the particles in the microbeads and type of ions under the study (24).

Table (2) mean and standard deviation of diameters of inhibitory activity Nano-composite and nanoparticles against *Serratia marcescens* isolates.

Zinc Nps	Selenium Nps	Artemisia annua	Mean±SD
+	-	-	10.5± 1.92°
-	+	-	9.85±2.41 ^c
-	-	+	13.5 ± 3.74 ^c
+	-	+	15.7± 2.45 ^b
-	+	+	17.67±2.17 ^a

a,b Different letters mean significant differences (P< 0.05)

The concentration has an inhibition of (17.67±2.17) mm, and the inhibition diameters can be seen in Table (2) when it used *Sel*no NPs, It is very imortant to known the preparation of the nanocompsites were done by use 1 gram of *Artemisia annua* to 2 ml of selenium solution, we noticed that the mixture of the selenium element mixed with the *Artemisia annua* gave the best inhibition of bacteria *Serratia marcescens* compared with the rest of the single and mixed nanocomposites(25), due to their potent antibacterial and anticancer activities and relatively low toxicity, selenium nanomaterials like nanowires, nanorods, nanotubes, and nanoparticles has been put to use in a wide variety of settings (24).

In conclusion: The two nanoparticles (Art/Se Nps, Art/Zno Nps) proved to be effective in inhibiting bacteria *Serratia marcescens* studied and Art/Se Nps efficiency was more to the efficiency of Art/Zno Nps, so these two compounds can be used as an antimicrobial agents and in the medical drugs.

Acknowledgments: The author would like to acknowledge gratefully the anonymous referees for the constructive and useful comments that they have provided.

Ethical Considerations: The study was approved by the Research Ethical Committees of University of Karbala and National Karbala Hospital. Informed consent obtained from all participants and/or their legal guardians.

Funding: No external funds were received (private funding).

Conflict of Interest The author declare that they have no competing interests

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Study The Efficacy of COQ10 Enzyme with Vitamin E On Escherichia Coli Bacteria Isolated from Urinary Tract Infection



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Abstract

During a period between January and May of 2024, urine specimens were collected from several patients that suffering from urinary tract infections in different hospitals in Baghdad province (total number 150 specimen). A total of 63 specimen (42%) were positive cultures, while 87 (58%) showed negative cultures. It was found that the majority of infections were among female, where the percentage was female 62% and male 38%. Different bacteria were isolated from urinary tract infection, The most predominant bacteria were Escherichia coli followed by Klebsiella pneumonia, Staphylococcus aureus phenotypic characteristic on macConkey agar the results showed that the bacteria were lactose fermenter and gave pink, soft, shiny and sharp-edged colonies on the center of the differential macConkey agar while on nutrient agar showed large, circular, convex, grayish to white, moist, smooth and opaque colony, also it showed smooth form easily emulsifiable. On blood agar the most significant characteristic for differentiating of bacteria was cause no hemolysis on blood agar (gama hemolysis). The quantitative method showed inhibitory effect of COQ10 enzyme, the enzyme showed obvious effect in inhibiting bacterial isolates inhibition ratio was 30% against Klebsiella pneumoniae, 53% against E. coli and 50% against Staphylococcus aureus. The disk diffusion agar method on agar showed slightly effect and sample inhibition zone formed around the enzyme, even the highest concentration was used 400mg/ml.

KEYWORDS

COQ10 VIT E E.Coli UTI

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Introduction

Esherichia coli, a member of the bacterial family of Enterobacteriaceae, is the most prevalent commensal inhabitant of the gastrointestinal tracts of humans and warm-blooded animals, as well as one of the most important pathogens (1). E. coli is Gram-negative facultatively anaerobic rods (possessing both a fermentative and respiratory metabolism) and do not produce the enzyme oxidase (2).

Some virulent strains of *E. coli* produced lethal toxins that leads to cause serious disease and complications in their hosts (3). These strains can cause several diseases including cholecystitis, bacteremia, cholangitis, urinary tract infection (UTI) (4).

UTI are the most common extra intestinal *E. coli* infections and are caused by uropathogenic *E. coli* (*UPEC*), UTIs are classified into disease categories according to the site of infection: cystitis (the bladder), pyelonephritis (the kidney) and bacteriuria (the urine) (5).

Coenzyme Q (CoQ10) is an antioxidant that produces naturally by organism's body and used by cells for growth, maintenance, metabolism and producing energy. Coenzyme Q, also known as ubiquinone, (because it occurs in all animals and most bacteria). In humans, the most common form is coenzyme Q10 or ubiquinone-10. It is sold as a dietary supplement and also is an ingredient in some cosmetics and treatments some disease (6).

The oxidized structure of CoQ10 is 1, 4-Benzoquinone where Q refers to the quinone head and 10 refers to the number of isoprene repeats in the tail. The various kinds of CoQ10 may be distinguished by the number of isoprenoid subunits in their side-chains (7)

Figure (1) Chemical formula C59H90O4

Objective of this work

The aims of this work were to examine the effect of combined COQ10 enzyme and Vit E on the inhibition or reduce performance of Urinary tract bacteria especially E. coli. This bacteria lives in an acidic environment, and since the enzyme is an antioxidant, it is likely to have an effect on urinary tract infection, and this is what we search for it in this research, If the enzyme has an effect on UTI, it can be used in the future as a treatment for urinary tract infection.

MATERIALS AND METHODS

Materials

A total of sixty-three distinct pathogenic specimens obtained from patients in hospitals located in Baghdad, ranging in age and gender, between January and May of 2024. Suffered from urinary tract infection.

Isolation of bacteria: Bacteria are isolated from urine specimen by using cotton swab, that either inoculated in nutrient broth for increase rate of growth orstreaking on heart-brain infusion media which is considered enriched medium for support the growth of bacteria, it is also cultured on nutrient agar, macConky agar, blood agar and then incubated at 37C° for 24 hours-

Identification

Gram stain: Procedure to detect if the bacteria is gram positive or negative through using gram stain technique. bacterial smear is prepared on slide then crystal violet (1 minutes) are used, followed with iodine stain (1 minutes), ethanol appl for 15 seconds to remove the stain and finally safranin used (1 minutes), each process is followed with washing. Bacteria that appear pink in color, rod shaped bacilli may indicate gram negative (8).

Biochemical test

Catalase test: Place three drops of hydrogen peroxide (H2O2) on slide then single purified colony transported using a woody stick to the slide, bubbles formation on slide indicates positive result (9).

Oxidase test: Few drops of oxidase reagent are placed to filter paper then Single colony transported using a woody stick to the saturating filter paper with oxidase reagent. No color formation indicates negative result, while purple color formation indicates positive result (10)

Indole test: Peptone water poured in tube and inoculated with single colony and incubated at 37C° for 24hrs. After overnight incubation, 10 drops of Kovacs reagent were added, the formation of red ring at the top of the broth indicated positive result (11).

Motility test: To test for bacteria motility, use a sterile needle to pick a well-isolated colony and stab the medium to within 1 cm of the bottom of the tube. The needle must keep in the same line it entered as it is removed from the medium. Then incubate at 35°C for 18 hours or until growth is evident. A positive motility test is indicated by a turbid area extending away from the line of inoculation. A negative test is indicated by line growth along the inoculation line but no further turbidity (12).

Confirmed test using VITEK2 compact system

The Gram positive (GP) card is used for the automated identification of most significant non-spore-forming Gram-positive bacteria (primarily cocci). The GP identification card is based on established biochemical methods and newly developed substrates there are 64 biochemical tests measuring carbon source utilization, enzymatic activities and antibiotics resistance (13).

The Gram-negative card is based on established biochemical methods and newly developed substrates measuring carbon source utilization, enzymatic activities, and resistance of antibiotics. There are 47 biochemical tests and one negative control well (14).

Final identification results are available in approximately eight hours or less. Results were expressed as defined by the manufacturer as 96% to 100%, excellent identification; 93% to 95%, very good identification; 89% to 92%, good identification; 85% to 88%, acceptable identification; below 85%, no identification (15).

Procedure

Inoculum from a pure culture was prepared, in case of mixed cultures, a re-isolation step is required. It is recommended that a purity check plate was done to ensure that a pure culture was used for testing.

- Aseptically transferred 3.0 ml of sterile saline (aqueous 0.45% to 0.50% NaCl, pH 4.5 to 7.0) into a clear plastic (polystyrene) test tube (12 mm x 75 mm).
- A Sufficient number of morphologically similar colonies were transferred to the saline tube prepared in step 1 to Prepared the homogenous suspension of the organism with a density equivalent to a McFarland No. 0.5

Note: Age of suspension must not exceed 30 minutes before inoculating card.

Note: Suspension Turbidities Used for Card Inoculation (McFarland turbidity) must be range from 0.50-0.63.

- The suspension tubes and GP card were placed in the cassette.
- Followed local regulatory agency's guidelines for disposal of hazardous waste.

Qualitative screening of enzyme as antibacterial activity

The turbidity method was used which was based on the inhibition of a microbial growth in a fluid medium containing a uniform distribution of an antimicrobial compounds. The turbidity assay was performed to evaluate the sensitivity of the test pathogen in liquid culture according to (17). In this assay 100 μ l of inoculum of indicator bacteria (1.5×10⁸) CFU/ μ l was added to the test tubes containing 5 μ l of nutrient broth, then added 125 μ l of enzyme at different concentration and incubated at 37°C for 24hrs. After incubation the growth in terms of turbidity of the bacterial cultures was measured spectrophotometrically at 630 nm. The readings were measured by using spectrophotometer and compare with the control (tube containing nutrient broth and indicator bacteria) and inhibition of bacteria growth in tubes (containing the indicator bacteria and COQ10 enzyme).

Diffusion test on agar

Kirby Bauer disk diffusion method: Muller hinton agar are used to perform the test, then cultured with indicator bacteria (1.5×10⁸) CFU/ml by spreading method used cotton swab, after that several small circular piece of filter paper is cutting then sterilizing in oven. COQ10 enzyme are loaded on to sterile filter paper and placed on agar. (Figure 1) (18).

Millipore diffusion method: Muller hinton agar are cultured with indicator bacteria (1.5×10⁸) CFU/ml by spreading method used cotton swab, then five holes are forming in muller hinton

agar. Through using micropipette small amount of enzyme are transport into each hole. The culture media are incubation in incubator (at 37°C for 24 hrs) (Figure 2) (19).



Figure (2) COQ10 enzyme loaded on sterile filter paper and fixed on MHA agar



Figure (3) COQ10 enzyme loaded directly in hole formed in MHA for testing the enzyme activity against bacteria

RESULT

Isolation of Bacteria: During a period between January and May of 2024, urine specimens were collected from several patients that suffering from urinary tract infections in different hospitals in Baghdad province (total number 150 specimen). A total of 63 specimen (42%) were positive cultures, while 87 (58%) showed negative cultures. The result come in touch with the recent study which revealed that Out of 141 (31.6%) were yielded significant

bacteriuria (positive cultures) and 16 different species of bacteria that cause urinary tract infection were identified (20).

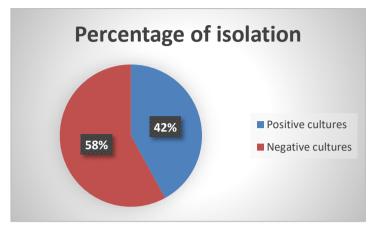


Figure (4) Percentage of isolation bacteria compared with negative cultures

It was found that the majority of infections were among female, where the percentage was female 62% and male38%, The results were in agreement with the studies (21) which revealed that the higher percentage of urinary tract infections (43%) was found among pregnant women.

Females are susceptible to get infection than men because of shorter urethra that allow pathogens to reach different site of urinary tract or may be to the external urethral meatus in women is mostly mucosa, moist tissue lining the inside of the vagina. This skin is thinner and more sensitive to get infection than most of the skin on the other body site.

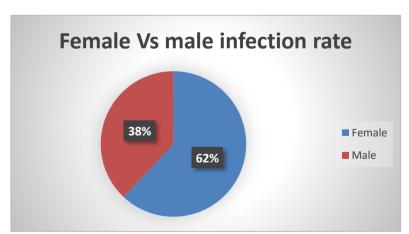


Figure (5) Distribution of infections according to gender

Different bacteria were isolated from urinary tract infection, The most predominant bacteria were *E. coli* followed by *Klebsiella pneumonia*, *Staphylococcus aureus* which all isolated from urine samples and cultured using cotton swab, as showed in Figure (5)

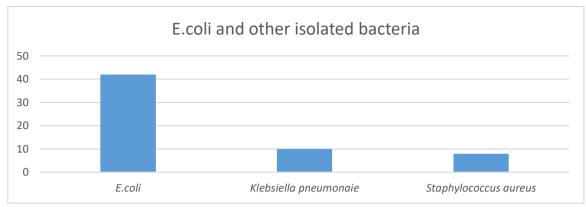


Figure (6) Types of bacteria isolated from UTI infections

Identification of Bacteria

Microscopic examination: The staining was carried out with gram stain, by preparing a smear from the colony Growing on agar medium then staining step performed followed by examination under microscope, the result showed gram negative, short, bacilli bacteria Figure (6) (22).

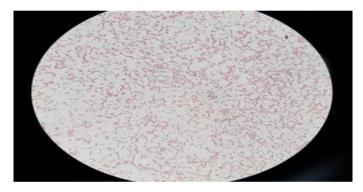


Figure (7) Gram strain of bacteria

Morphological identification: Isolation of *E. coli* were initially diagnosed based on phenotypic characteristic, after they were grown on each macConkey agar, nutrient agar and blood agar. On macConkey agar the results showed that the bacteria were lactose fermenter and gave pink, soft, shiny and sharp-edged colonies on the center of the differential macConkey agar, which contains bile salts and violet crystal, which allows the growth of Gram-negative bacteria, including the enteric family while inhibit the growth of gram-positive bacteria. On nutrient agar showed large, circular, convex, grayish to white, moist, smooth and opaque colony, also it showed smooth form easily emulsifiable. On blood agar the most significant characteristic for differentiating of bacteria was cause no hemolysis on blood agar (gama hemolysis) (figure7) (23).







E. coli cultured on nutrient agar

E. coli on blood agar (gamma hemolysis)

E. coli on macConkey agar

Figure (8) Showed morphology of E. coli on different media

Biochemical test: Test was performed and the result showed catalase positive which indicate the ability of bacteria to producing hydrogen peroxidase which breakdown reagent to produce water and oxygen. It showed positive result for indole test that indicates red color ring on the top of medium which result due to ability of bacteria to produce tryptophanase that degrading tryptophane into pyruvic acid, ammonia and indole (Table 3-1 and figure8) (24).

Table (1) Biochemical test of isolated bacteria

- (+) = positive
- (-) = negative

Type of bacteria	Catalase	Indole	Motility
Esherichia coli	+	+	+
Klebsiella pneumonia	+	-	-
Staphylococcus aureus	+	-	+

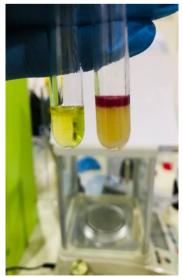






Figure (9) Showed biochemical test of E.coli (Indole test-Catalase test-Motility test)

Confirmed test using vitek system:

As showed in figure (9) all isolates of *E. coli* was checked using vitek system for confirmation of the diagnosis.

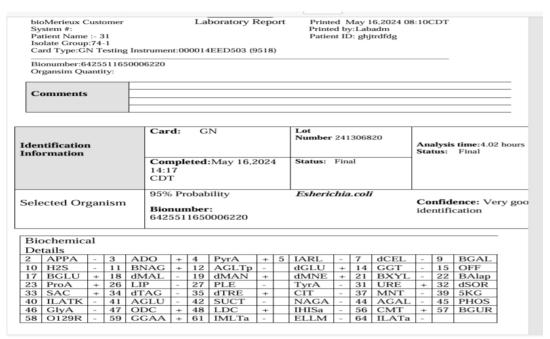


Figure (10) vitek system (gram negative) for confirmed test showed E. coli diagnosis and its biochemical test

Quantitive screening the effect of COQ10 enzyme on isolated bacteria

The inhibitory effect of COQ10 enzyme (Minimum inhibitory concentration), the enzyme showed obvious effect in inhibiting bacterial isolates. As shown in the table (3-2) inhibition ratio was 30% against *Klebsiella pneumoniae*, 53% against *E. coli* and 50% against *Staphylococcus aureus*.

Table (2) Minimum inhibitory concentration of COQ10 enzyme against some of pathogenic bacteria isolated from UTI cases

Bacterial species	Negative control	Treated (630nm)	bacteria	Inhibition %(MIC)
1. Staphylococcus aureus	1.332	0.66		50%
2. Escherichia coli	0,99	0.46		53%
3. Klebsiella pneumoniae	1.11	0.78		30%

Reduction in bacterial cells availability may referred to enzyme activity as antioxidant since no obvious recent or any previous studies examined its effect directly on inhibition of bacteria caused UTI infections and that is awesome study as ours know.

Recent study done in 2021 revealed the effect of enzyme as bacteriostatic on *Salmonella typhimurium* bacteria, The minimum inhibitory concentration of CoQ0 against *S. enterica serovars Typhimurium* was 0.1–0.2 mg/mL , also the study showed that CoQ0 effectively disrupted cell membrane integrity and induced morphological changes in the cell, resulting in hyperpolarization, decreased intracellular ATP concentrations, and cellular constituents leakage (25) , that revealed that the enzyme effective on pathogenic bacteria at very low concentration which come in line with our result and the difference in determine the concentration of optimum dose that cause reduction in number of bacteria may come back to experiment environment and source of specimens in which bacteria isolated from it.

Disk diffusion test on agar

The result of COQ10 enzyme inhibition of bacteria on agar showed slightly effect and simple inhibition zone formed around the enzyme as showed in (figure 10). Even though the highest concentration was used 400mg/ml.



Figure (11) Inhibition zone of COQ10 enzyme against pathogenic bacteria

The recent study revealed that inactivation of *S.typhimurium* bacteria and determine MIC and MBC was done by using LB broth, the result of slight inhibition on agar area may back to use Muller hinton agar which may prevent complete distribution of enzyme through agar since decrease percentage of agar may give best and good inhibition zone, since no previous study the inhibition zone on agar (25) also using DMSO to resolve enzyme may interfere with these result since using crude enzyme without any solvent prevent complete distribution.

Temperature, PH and other condition of the experiment in incubator may also interfere with the result, Guo and his group showed that the antibacterial activity of CoQ0 on Cronobacter sakazakii decreases as the temperature decreases from 55 to 25 C (26). There by,

using antibiotic as synergistic effect should be investigated to evaluation the effeictivness of enzyme on pathogenic bacteria caused urinary tract infection.

Conclusion

Although there are few clinical studies that study the effectiveness of CoQ10 supplementation in treatment of infectious diseases , our study showed the antibacterial efficacy of enzyme on *Escherichia coli, Klebsiella pneumoniae , Staphylococcus aureus* and Qunantitive method revealed reduction in bacteria number in tube method using spectrophotometer reading while disk diffusion experiment showed slightly effect on bacteria and need further investigation to optimize the conditions that interfere with enzyme affectivity . for this reason, its application in infectious causes treatment is feasible and it can present many advantages that contribute to the development of a potential new therapy for treatment or prevent bacterial proliferation in urinary tract infection or any other caused bacterial disease

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Evaluation of the Anti-Microbial Activity of Morinzhi Extract Against Some Human Pathogenic Bacteria

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Abstract

One of the biggest worldwide issues endangering public health is the rise in antibiotic resistance. As a result, more approaches to lessen infections are being investigated. The goal of this study is to investigate how Noni extract affects some pathogenic species. Noni extract, in its absolute concentration, was used, and sensitivity was checked for species of bacteria positive and negative to Gram stain, and for species of pathogenic fungi towards this extract, and then compared to the sensitivity of the same species used in the study towards types of antibiotics. The study showed that *Staphylococcus aureus* bacteria were sensitive to all types of antibiotics used whereas *Escherichia coli* bacteria were resistant to most of them; the sensitivity varied of other species used in this study. *Bacillus* and the used species of fungi in this study showed resistance to Noni extract, whereas *E. coli* and *S. aureus* showed obvious sensitivity to Noni extract. *Klebsiella* and *Pseudomonas aeruginosa* did not how clear sensitivity to the extract.

KEYWORDS

Morinda Citrifolia Noni Juice Gram Positive Sensitive Test

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Introduction

In Asian countries, Morinda citrifolia, a member of the coffee family (Rubiaceae), has long been used as a traditional medicinal plant. It is said to help with nearly every health issue (1,2,3), and its fruit is edible, with some people comparing its flavor to poor cheese (4).

Noni fruits have been identified as containing some active ingredients include iridoids, flavonoids, and phenolic compounds, particularly coumarins (5). Known for their anti-inflammatory properties, phenolic chemicals are secondary metabolites of plants. In vitro and in vivo, flavonoids have been found to have anti-inflammatory qualities (6, 7, 8). They are also recognized as natural antioxidants that show suppression of different enzymes linked to the generation of radical oxygen (9). Iridoids are metabolites of plants with a cyclopenta [c]paranoid skeleton based on a monoterpene structure. They are found in many therapeutic plants (10,11), and some of them have biological functions. Certain iridoids have been found to possess antioxidant properties (12). A wide range of human ailments, including high blood pressure, cancer, diabetes, gout, asthma, cough, sore throat, inflammation, arthritis, sore gums, fish poisoning, cuts, and deep wounds, have been treated using noni fruit juice, which has historically been fermented (13). Additionally, noni juice has different concentrations of minerals, vitamins, and other bioactive substances (14–16).

The extensive use of antibiotics in agriculture, medicine, and animal care is the main cause of the growth in antibiotic-resistant microorganisms. The issue is made worse by the abundance of novel antibiotics that target bacteria in different ways in order to get around the resistant genes. The search for novel, difficult-to-treat illnesses has become more difficult due to the viruses' declining effectiveness and resistance to antimicrobial medications. The two main culprits behind hospital-acquired infections are *E. coli* and Staphylococcus aureus. The human body is naturally home to these species. Nonetheless, certain types have the potential to cause illness and develop resistance to antibiotics (17–19).

A human pathogenic microbe has developed numerous resistances as a result of the careless use of antibiotic medications, which are frequently used to treat infectious disorders (20). Immune function is destroyed by smoking (21). Exposure to tobacco smoke increases the chance of contracting a number of bacterial and viral diseases (22, 23). Peripheral lymphocyte subsets of the blood may be significantly impacted negatively by smoking (24–25).

The potential of noni juice to shield cells from DNA damage may improve smokers' immune systems. However, the impact of noni juice on immune function extends beyond defending the cells that make up the adaptive immune system. For instance, during the first two weeks of life, whole blood phagocytic activity against Escherichia coli and Staphylococcus epidermis was markedly enhanced when newborn cattle (Holstein bull calves) were fed French Polynesian noni fruit puree (26).

Materials and Method

Morinzhi obtained from DXN company. Five different bacterial cultures of Gram positive (Staphylococcus aureus and Bacillus), Gram negative (Escherichia coli, Klebsiella and Pseudomonas aeruginosa) and two fungi (Candida albicans and Candida tropicalis) are used in this present study. The microbes were sub cultured into nutrient agar petri plates and incubated for 24 hours at 37°C for bacteria and 28°C for fungus (sabouraud dextrose agar). The agar plate bacterial colonies were transferred to test tubes containing 5 milliliters of sterile saline. The saline test tubes containing the bacterial cultures are compared to 0.5 MacFarland's standard for bacterial standardization. 9.5 grams of Muller Hinton agar were dissolved in 250 milliliters of distilled water to create 250 milliliters of Muller Hinton agar. The agar was autoclaved for 15 minutes at 121°C to sterilize it. The Muller-Hinton agar was aseptically placed onto the sterile petri dishes following sterilization. After the agar had solidified for a few minutes, each bacterial culture was aseptically swabbed onto the Muller-Hinton agar surface using sterile swabs, and they were left to dry. Making a Morinzhi disc with sterile Whatman filter paper to have antibacterial properties. The extract-containing discs were placed on the plate's surface using sterile forceps and carefully pushed to guarantee contact with the agar surface after being impregnated in morinzhi (sterile in a water bath for 15 minutes at 63 °C). Following a 24-hour incubation period at 37°C, the zone of inhibition was measured in millimeters.

The fungus is cultivated using the identical technique, with the exception that the incubation is carried out at 28°C for 24 hours rather than 37°C (27).

Sensitivity test for antibiotics

When using the Kirby Bauer method, test tubes containing five milliliters of sterile saline are filled with three to five bacterial colonies from the agar plates.. The saline test tubes

containing the bacterial cultures are compared to 0.5 MacFarland's standard for bacterial standardization. Three grams of Muller Hinton agar were dissolved in one hundred milliliters of distilled water to create one hundred milliliters of the agar. For fifteen minutes, the agar was autoclaved at 121°C to sanitize it. The Muller-Hinton agar was aseptically placed onto the sterile petri dishes following sterilization. After the agar had solidified for a few minutes, each bacterial culture was aseptically swabbed onto the Muller-Hinton agar surface using sterile swabs, and they were left to dry. To guarantee contact with the agar surface, the antibiotic disc (Trimethoprim, Azithromycin, Bacitracin, Vancomycin, Ciprofloxacin, and Gentamicin) was placed on the plate's surface using sterile forceps and gently pushed. For a whole day, the plate was incubated at 37°C. Millimeters were used to measure and observe the zone of inhibition (28).

Results and discussion

Table (1) shows that *S. aureus* was sensitive to all antibiotics used in the research with varying degrees ranged between (16mm) for Bacitracin to (31mm) for Gentamicin, whereas *Klebsiella* showed resistance to Vancomycin and Bacitracin, high sensitivity to Ciprofloxacin, and variable sensitivity to the rest of antibiotics used. *E. coli* bacteria showed high resistance to the majority of antibiotics used except Gentamycin which was effective against it. *Bacillus Spp* was resistant to Trimethoprim only and sensitive to the rest of the antibiotic types with sensitivity varied between (9mm) towards Bacitracin to (31mm) towards Azithromycin. *P. aeruginosa* was resistant to Trimethoprim and Vancomycin, highly sensitive to Ciprofloxacin and Azithromycin, and of medium sensitivity to Bacitracin and Gentamycin., Sensitivity test was performed for these antibiotics for comparison with Noni extract in this research.

Table (1) Sensitivity of bacteria species used in the research against different types of antibiotics.

Antibiotic	S. aureus	Klebsiella	E. coli	Bacillus	P. aeruginosa
Trimethoprim	27	15	R	R	R
Azithromycin	30	21	R	31	30
Bacitracin	16	R	R	9	10
Vancomycin	23	R	R	16	R
Ciprofloxacin	29	35	5	29	30
Gentamicin	31	21	23	26	13

Table (2) demonstrates the impact of noni extract on microbes used in the research; *S. aureus* exhibited high sensitivity (23mm) to it, more than that towards Bacitracin, whereas its sensitivity to Vancomycin was (23mm). Rivera et al, conducted a study in Europe on Noni juice

and proved the global success of this juice; Europeans reported its health benefits including reducing pain, increasing strength, minimizing injuries, enhancing sleep, improving digestion, and reducing symptoms of allergy and asthma (29), and this shows the high efficacy of Noni juice, hence, it is preferred over the use of antibiotics.

Klebsiella exhibited medium sensitivity (10mm) to the extract compared to the antibiotics; Bacitracin and Vancomycin, but less than that to Gentamycin, Azithromycin, Trimethoprim and Ciprofloxacin, and despite the medium sensitivity of this bacteria to Noni extract, yet the extract has different medical characteristic including being an antioxidant and anti-inflammatory (30).

E. coli exhibited high sensitivity to Noni extract that reached (20mm) compared to antibiotics used in this research to which it exhibited evident resistance; Azithromycin, Trimethoprim, Bacitracin and Vancomycin, and its sensitivity to Noni was higher than that to Ciprofloxacin but less than Gentamycin. This indicates the high efficacy of Noni extract compared to some types of antibiotics.

Bacillus bacteria exhibited resistance to Noni extract compared to the used antibiotics to which its sensitivity ranged between (9- 31 mm). Noni juice is one of the first complete nutrients approved by the new nutrients list of 1997 issued by the European Union (31); the government of China also authorized a single source of noni juice as a novel, secure food and resource that can boost immunity (32).

P. aeruginosa bacteria exhibited medium sensitivity to Noni extract compared to Trimethoprim and Vancomycin to which it showed resistance, and to the sensitivity to Bacitracin which was (10mm), and less than the effect of Gentamycin, Ciprofloxacin, and Azithromycin. Noni extract is preferred over the antibiotics that may have side effects such as fever, chill, ototoxicity, skin rash and megaloblastic anaemia (33). Obeng-Boateng et al and Oladeji et al confirmed the activity against microbes of Morinda citrifolia L extract, and this indicates that its compounds may exhibit strong bioactivity against pathogens such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Shigella*, and *Salmonella* (34,35).

Candida sp. Exhibited resistance to Noni extract compared to the antifungal Fluconazole used in the research of (Berkow and Lockhart, 2017) to which its sensitivity was (14-19mm) (36).

Morinda citrifolia fruit is widely used in traditional medicine not only as anti- germs but also against tumour and to protect from them, nowadays there are many pre-clinical experiments (animal models or in the laboratory); these studies opened new perspectives to understand this plant and its medical uses as this fruit may play a major role in treatments against cancer (37).

Table (2) The effect of Morinzhi on different kinds of microbes used

Morinzhi	S. aureus	Klebsiella	E. coli	Bacillus	P. aeruginosa		Candida tropicals
	23	10	20	R	11	R	R

Conclusion

The study proved that the absolute concentration of Noni extract has high effect in inhibiting the growth of *S. aureus* bacteria due to its ability to disrupt cell membrane and interfere with metabolic processes, and less effect towards *E. coli* due because its outer membrane may be vulnerable to noni extract which destabilize lipid bilayers, whereas Bacillus and the used species of fungi in this study showed resistance to this extract because *Bacillus* has thick peptidoglycan layer and spore-forming capability while for fungi may due to efflux pumps, and finally *P. aeruginosa* and *Klebsiella* showed medium sensitivity to Noni extract as a result of bioactive compound like iridoids may disrupt quorum sensing or membrane integrity.

Hence, we conclude that it is possible to use Noni extract to treat infections caused by *E. coli* and *S. aureus* due to its safe use, and instead of antibiotics that have side effects.

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Evaluation the vitamin D3 and Renal function test and liver function Test Levels on Toxoplasma gondii (T. gondii) in Baghdad pregnant Women



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Abstract

Toxoplasmosis during pregnancy poses notable risks, ranging from abortion to congenital defects. It's worth noting that insufficient vitamin D levels correlate with issues like bone fragility and osteoporosis, potentially raising the risk of maternal fractures while also affecting infant bone development. This study, therefore, aims to investigate any connection between toxoplasmosis seropositivity and irregularities observed in liver and kidney function tests, alongside vitamin D levels found in women's sera. To achieve accurate biomarker measurements, we plan to employ enzyme-linked immunosorbent assay (ELISA) techniques in conjunction with automated analysers. Methods: From the Central Public Health Laboratories in Baghdad Province, 60 women between the ages of 17 and 45 were selected to participate in this study. We took blood samples from this cohort of 60 women, which consisted of two groups from the Central Public Health Laboratories: 30 women who had contracted Toxoplasmosis and 30 women who did not. Toxoplasma gondii-related IgG and IgM antibody levels were assessed using the enzyme-linked immunosorbent assay (ELISA) method, a validated technique for identifying these specific antibodies. The ELISA microwell technique, renowned for its precise measurement of serum vitamin D, was also used to measure vitamin D concentrations. Monoband Inc., USA, provided this method. A spectrophotometer was also used to measure liver and kidney function, providing a comprehensive picture of the participant's overall health. This study show the result is statistically significant association (P = 0.001) between Toxoplasma gondii infection and reduced vitamin D₃ levels in pregnant women. Toxoplasmosis during pregnancy appears to be influenced by regional and socioeconomic factors. Importantly, diminished vitamin D levels may predispose expectant mothers to infection in toxoplasmosis viruse.

KEYWORDS

Toxoplasmosis

Vitamin D

Pregnant Women

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Introduction

Toxoplasmosis, caused by the protozoan parasite Toxoplasma gondii, is a widespread infection that continues to pose a public health concern. Global estimates suggest that nearly one in three individuals has been exposed to the parasite at some point, reflecting its extensive reach and the silent burden it imposes on human health. Although pre-pregnancy infection presents a specific risk, the first trimester is the most critical stage for congenital Toxoplasmosis. Toxoplasma gondii is often regarded as the most alarming infection to get during pregnancy. The ramifications of such an infection may be severe, potentially resulting in foetal termination or multiple congenital anomalies (6,7). Vitamin D, a fat-soluble prohormone, is gaining increasing focus due to its vital role in health. It mainly exists in two forms: vitamin D2 (ergocalciferol), which comes from plants, and vitamin D3 (cholecalciferol), which animals produce. It is significant because more than 80% of the vitamin D in the human body is made in the skin by exposure to ultraviolet B radiation. At the same time, the remaining is acquired via dietary sources (9,10). The significance of vitamin D for general health cannot be overstated. Its deficiency results in disorders such as rickets, osteocalcin deficiency, and osteoporosis (10). Vitamin D insufficiency is widely recognized as a significant public health concern due to its substantial health implications. Research indicates that vitamin D insufficiency is very prevalent, even in areas abundant in sunlight, especially among women and children (11).

Material and Method

This study was executed at Alkazmia Teaching Hospital from December 2023 to June 2024. This timeframe is critical when you consider how crucial it is to understand the potential impacts of infections like Toxoplasmosis, especially on maternal and fetal well-being, due to the heightened susceptibility of pregnant individuals. In terms of subjects and design, a controlled study was conducted to shed light on the effects of latent infections, specifically on the outcomes of pregnancy. One group comprised expectant mothers positively diagnosed with Toxoplasmosis; this underscores the importance of researching the relationship between infectious diseases and maternal health. The control group included other pregnant women whose ages ranged from 17 to 45 years. They served as a baseline against which the effects of the infection could be evaluated to both maternal and neonatal outcomes.

Result

The study examined 60 pregnant Iraqi women, divided equally into a toxoplasmosis-positive group and a healthy control group. As shown in Table (1), BMI was marginally lower in the patient group (22.22 \pm 3.27 kg/m²) compared to controls (27.65 \pm 6.14 kg/m²; p = 0.05). Vitamin D levels were significantly reduced in infected individuals (20.16 \pm 3.7 ng/ml vs. 34.38 \pm 6.28 ng/ml; p = 0.0001). Serum ALT, AST, and urea levels were significantly increased in the patient group. (31.32 \pm 5.01, 27.86 \pm 2.88, and 57.91 \pm 5.93 mg/dl, respectively) compared to controls (14.12 \pm 2.67, 18.91 \pm 1.98, and 17.65 \pm 2.33 mg/dl; p = 0.0001 for all). Toxoplasmosis-specific IgG and IgM levels were significantly higher in the patient group (6.065 \pm 1.55 and 2.41 \pm 1.28) than in controls (0.123 \pm 0.190 and 0.230 \pm 0.22). Table (2) also indicates negative correlations between BMI, age, IgM, and vitamin D, while IgG showed a positive association with vitamin D in the patient group. This analysis highlights key differences in age, BMI, vitamin D3, ALT, and AST between infected and healthy pregnant women.

Table (1) Comparison of clinical parameters between the patient and control groups.

Note: N.S. = Not Significant: Sig. = Significant: H.S. = Highly Significant.

Parameter	Mean + SD	Mean + SD	P VAL	
	CONTROL	PATIEINT		
Age	33.55+6.33	31.7+5.9	0.05	
ВМІ	22.22+3.27	27.65+6.14	0.05	
Vitamin d3	34.38+6.28	20.16+3.7	0.0001	
ALT	14.12+2.67	31.32+ 5.01	0.0001	
AST	18.91+1.98	27.86+2.88	0.0001	
Urea	17.65+2.33	57.91+5.93	0.05	
Toxo IGG	0.123+0.190	6.065+1.55	0.0001	
Toxo IGM	0.230+0.22	2.41+1.28	0.0001	

The difference was statistically significant at the 0.05 level. Specifically, the highly significant result was observed with p < 0.001 (H. Sig), while the nonsignificant result had p > 0.05 (N), and other important findings were noted with p < 0.05 (Sig).

Table (2) Correlation of Serum Toxoplasmosis IgG, IgM, and Vitamin D3 Levels Between Pregnant Women with Toxoplasmosis and Healthy Controls

VIT D3 ng/ml	Age	ВМІ	IGG	IGM
r	-0.201	-0.91	0.022	-0.311
р	0.23	0.001	0.05*	0.029*

Discussion

Toxoplasmosis remains a widespread parasitic infection affecting humans and other Endothermic animals globally. While many individuals may never experience symptoms due to the strength of their immune response, the infection's silent presence should not be underestimated. Our study reinforces the increasing body of research demonstrating that even subclinical Toxoplasmosis may subtly alter important biological markers—most notably, Serum concentrations of 25(OH)D(12,13).

A significant decline in vitamin D levels was observed in our analysis among infected individuals compared to healthy controls (p = 0.001). This finding aligns with patterns previously reported by Abdulwahab et al. and others. Interestingly, this reduction in vitamin D was not an isolated trend; it was strongly and inversely correlated with patient age, body mass index (BMI), and Toxoplasma-specific IgM levels. These patterns suggest a broader interplay between micronutrient status, immune responsiveness, and parasitic burden—especially relevant in women of reproductive age. Alessio et al. have shown that vitamin D is crucial for conception and healthy embryonic growth.(14,15)

Liver enzyme elevations (AST, ALT, ALP) were also evident in the toxoplasmosis group, suggesting a hepatic response to infection. While some researchers, such as Mikaeel and Mahmood, have observed similar patterns, others have reported more nuanced or even contrasting results. These discrepancies may reflect differences in the stage of infection, immune status, or underlying health conditions. Nonetheless, they serve as a reminder that Toxoplasmosis can have systemic effects beyond its typical associations with neurological or ocular disease.(16,17,18)

Perhaps more concerning is *Toxoplasma gondii*'s ability to infiltrate renal tissue, where it can trigger oxidative stress through the induction of nitric oxide and reactive oxygen species. This initiates an inflammatory cascade involving TNF- α , IL-1 β , and NF- κ B, leading to progressive tissue damage that may culminate in glomerulonephritis, tubular necrosis, or even chronic kidney disease. These effects are particularly relevant for individuals with undiagnosed infections and raise concerns regarding the renal health of at-risk populations,

such as patients undergoing dialysis—who may be more susceptible and thus warrant regular screening.(19,20)

In individuals with chronic liver disease (CLD), the situation may be even more precarious. An already compromised liver, burdened by persistent inflammation and metabolic dysfunction, may lack the resilience to counteract opportunistic infections like *T. gondii*. Our findings, consistent with those of El-Sayed et al., suggest a correlation between elevated hepatic enzymes and increased susceptibility to parasitic infection in CLD patients, underscoring the need for increased clinical vigilance.(19,21)

Collectively, these insights reinforce that even so-called latent or mild infections can leave behind tangible physiological consequences. Alterations in vitamin D status, liver enzyme activity, and markers of renal inflammation highlight the nuanced and often underestimated impact of *T. gondii*. Recognizing these interactions is vital for improving diagnostics, preventive care, and treatment—especially for immunocompromised individuals.(21)

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Evaluating the Performance of IEEE 802.11ac and 802.11n in NS-3



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Abstract

The IEEE 802.11 standards family has evolved to support the demand for higher-speed and robust wireless communications. Throughput was improved with IEEE 802.11n use of multiple-input multiple-output (MIMO) and channel bonding techniques, and by IEEE 802.11ac through wider channel bandwidths and multi-user MIMO (MU-MIMO). We present performance evaluation results of IEEE 802.11ac and IEEE 802.11n under a broad range of workloads, through networks of varying sizes, degrees of mobility and volume of interference using NS-3 simulations. We find that IEEE 802.11ac outperforms IEEE 802.11n in terms of throughput, latency, and efficiency in dense environments. . The IEEE 802.11ac (40 MHz, 80 MHz and 160 MHz) achieves highest throughput, reaching (105 Mbps, 245 Mbps and 600 Mbps) at 50 stations respectively. End-to-end delay is the total time taken by a data packet to reach from source to destination in the network, reaching (4.4,5.1,6.7,9.0,12.4,15.2) ms for (5,10,20,30,40,50) Number of stations respectively at IEEE 802.11ac (160 MHz).

KEYWORDS

IEEE 802.11ac
IEEE 802.11n
NS-3
Wireless Networks
MU-MIMO

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Introduction

A wireless network based on IEEE 802.11 standards provides high-speed communication. Next-generation Wi-Fi technologies have progressed over the last 20 years, with the goal of enhancing throughput, efficiency, and scalability in response to escalating data transmission requirements. IEEE 802.11n and IEEE 802.11ac have significantly improved on these three aspects, breaking new ground in terms of network performance, especially data rates, spectral efficiency and the dependability of networks have all been enhanced [1, 2, 26 and 28]. IEEE 802.11n (standardized in 2009) was a key milestone in this evolutionary process, as it brought multiple-input multiple-output (MIMO) technology, channel bonding (up to 40 MHz), and frame aggregation, allowing data rates of up to 600 Mbps [3, 20]. These enhancements offered better performing networks increased efficiency was especially significant for applications, which needed low latency and high throughput. Nevertheless, as connected devices proliferated, IEEE 802.11n encountered limitations in high-density environments, leading to congestion and interference that severely impacted network performance 4. IEEE 802.11ac, issued in 2013, subsequently extended Wi-Fi capabilities, responding to these challenges by introducing wider channels (80 MHz and 160 MHz), better modulation methods (256-QAM), and Multi-User MIMO (MU-MIMO) [6,21, 22, and 25]. These enhancements drove network aggregate throughput to grow beyond 1 Gbps, establishing IEEE 802.11ac applicability for high-bandwidth utilization like video streaming, cloud computing, and large-scale enterprise networks [7, 8]. Additionally, IEEE 802.11ac utilized beam-forming for improved signal transmission and reduced interference [9, 24 and 28].

One of the main reasons to simulate wireless networks is to study their behavior in the real world before deploying them in any environment of interest. Due to its accurate and flexible modeling environment when it comes to different network configurations and performance parameters NS-3 (Network Simulator-3) has emerged as the most adopted simulation tool for IEEE 802.11 protocol evaluation [10, 11]. Researchers can carry out simulations to model different network scenarios, mobility models, and congestion levels on NS-3 without having to invest in costly hardware infrastructure, as NS-3 works on simulation [12, 13 and 27]. This work compares IEEE 802.11n and IEEE 802.11ac performance in different scenarios, simulated using NS-3 to assess real-world effectiveness.

The performance analysis will focus on throughput, end-to-end delay, and packet loss under varying congestion and network density conditions. The analysis provides insights into the merits and drawbacks of IEEE 802.11ac compared to IEEE 802.11n which will help network engineers and researchers in their decision making of the deployment and enhancement of Wi-Fi network [14, 15, and 23]. IEEE 802.11 standards have revolutionised wireless networks, creating the high-speed connectivity that is so essential in modern communication. Wi-Fi technologies have come a long way from the early IEEE 802.11a/b/g standards to the recent IEEE 802.11n, and now IEEE 802.11ac, which promises more throughput and better efficiency in dense environments [1, 16, and 17]. MIMO technology and channel bonding was introduced with IEEE 802.11n, while 802.11ac enlarged these techniques with wider channels and MU-MIMO [2, 18]. NS-3 is commonly used for performance evaluation of simulated wireless networks allowing researchers to investigate various configurations and parameters without the need for physical infrastructure [3, 19]. The current study employs NS-3 to examine the practical performance comparison of both IEEE 802.11n and 802.11ac under different scenarios.

Literature Review

Several studies have analyzed IEEE 802.11 standards, focusing on their impact on throughput, latency, and power consumption. The main improvements of IEEE 802.11ac were emphasized by Park et al. [4], especially the addition of MU-MIMO, which enables simultaneous transmission to many clients. Gong et al. [5] showed that IEEE 802.11ac exhibits better spectral efficiency than that of its predecessors. Other work, e.g., Deng et al. [6, 27], that is used NS-3 to study the real-world applicability of 802.11ac in enterprise networks. Comparative studies such as (Wu et al. [7] have shown that video streaming performance over 802.11n and 802.11ac is different and 802.11ac helps reduce latency considerably. Shankaran et al. [8] explored MU-MIMO performance in dense network deployments and confirmed its benefits for overall throughput.

System Design

We use an NS-3 simulation model, as indicated in Table 1, in which a wireless access point (AP) connects to many stations (STAs) for assessment. Now let's set the key parameters of the simulation:

- Frequency Bands: 2.4 GHz (IEEE 802.11n) and 5 GHz (IEEE 802.11ac).
- Channel BWs: 20 MHz, 40 MHz (802.11n) and 160 MHz (802.11ac).
- MIMO Configurations: 802.11n 2x2 and 4x4; 802.11ac up to 8x8 MU-MIMO.
- Traffic Model: UDP flows with Constant Bit Rate (CBR).

Table (1) Simulation Parameters

Parameter	IEEE 802.11n	IEEE 802.11ac
Frequency	2.4 GHz, 5 GHz	5 GHz
Bandwidth	20, 40 MHz	20, 40, 80, 160 MHz
MIMO Config.	2x2, 4x4	Up to 8x8
Max Data Rate	~600 Mbps	~6.93 Gbps
Modulation	64-QAM	256-QAM

Network Architecture

We implemented the topology as depicted below in Figure 1 with following key components:

- Access Point (AP): The central node Wi-Fi coverage.
- Station Nodes (STAs): Multiple stations are connected to the AP.
- Traffic Type: Creates UDP or TCP traffic between STAs and AP.
- Simulation Area: Specified matrix-based placement (50m x 50m).
- Mobility Model: Random waypoint mobility for STAs.

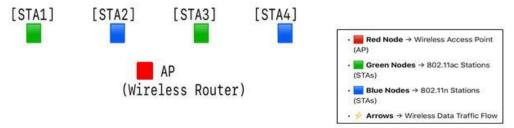


Figure (1) Illustrates the simulation topology used for our analysis.

Implementation in NS-3

The NS-3 network simulator is used and runs for 100 seconds, capturing key performance metrics also we implemented this topology using the WifiHelper and MobilityHelper modules:

- NodeContainer wifiApNode, wifiStaNodes;
- wifiApNode.Create(1);
- wifiStaNodes.Create(4);
- // Set up WiFi PHY and MAC
- WifiHelper wifi;
- YansWifiPhyHelper phy = YansWifiPhyHelper::Default();
- phy.SetChannel(channel.Create());
- // Set 802.11ac or 802.11n mode
- wifi.SetStandard(WIFI PHY STANDARD 80211ac);
- // Install on nodes
- NetDeviceContainer devices = wifi.Install(phy, wifiMac, wifiStaNodes);

Mathematical Model

Equation 1 provides an expression for the attainable data rate R in IEEE 802.11ac [9 and 10].

 $R = Ns \times Nb \times Nc \times B \times F$ coding(1)

Where:

- Ns = No. of spatial streams;
- Nb = Bits per symbol (modulation dependent),
- Nc = No. of subcarriers,
- B = Channel BW,
- Fcoding = Forward-error-correction-rate.

Simulation Results

Throughput Analysis

Throughput is one of the major metrics to assess the performance of wireless network. It indicates the successful data transmission per time (Mbps). For throughput analysis, we used NS-3 simulator making different your scenarios depending on (i) Channel Bandwidth: 20 MHz, 40 MHz (802.11n) vs. 20 MHz, 40 MHz, 80 MHz and 160 MHz (802.11ac) (ii) Modulation Scheme: 64-QAM (802.11n) vs 256-QAM (802.11ac) (iii) Number Of STAs: 5, 10, 20,30,40, 50

(iv) Traffic Type: UDP and TCP. Table 2 illustrates the average throughout obtained by both 802.11ac and 802.11n on various scenarios. The performance measurement results can be confirmed from Figure 2, as IEEE 802.11ac is performing much better than IEEE 802.11n in terms of throughput vs. number of stations for different bandwidth with number of clients through simulation time 100 seconds with traffic type UDP which proves that wider channels and enhanced MIMO are much better than narrow channels and limited MIMO support [11, 12, 18, 24].

Table (2) Throughput with different Scenarios

Scenario	IEEE 802.11	n IEEE 802.11ac (Max	% Improvement
	(Max Mbps)	Mbps)	
Single STA, 20 MHz	80 Mbps	95 Mbps	+18.7%
Single STA, 40 MHz	150 Mbps	220 Mbps	+46.8%
Multiple STAs (10), 40 MHz	120 Mbps	210 Mbps	+75.0%
Multiple STAs (50), 80 MHz	N/A	600 Mbps	-
Multiple STAs (50), 160 MHz	N/A	1.3 Gbps	-

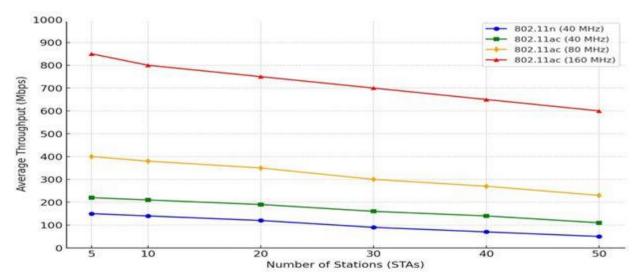


Figure (2) Shows throughput comparison of IEEE 802.11ac and IEEE 802.11n based on NS- simulations

Latency Performance

End-to-end delay is the total time taken by a data packet to reach from source to destination in the network. This metric is essential for real-time applications, such as video streaming, VoIP, and gaming. In Table 3, we present the average delay experienced by packets under different conditions in NS-3 simulation for this comparison. Figure 3 illustrates the lower end-to-end latency as compared to all IEEE 802.11ac. This is consistent with previous

research findings by Ni et al. [13] and Hsieh et al. [14], with a reported ~30% reduction in latency in IEEE 802.11ac networks.

Table (3)	End to End Delay	/ 802.11n vs.	802.11ac
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Number	IEEE 802.11n (40	IEEE 802.11ac (40	IEEE 802.11ac (80	IEEE 802.11ac (160
of STAs	MHz) Delay (ms)	MHz) Delay (ms)	MHz) Delay (ms)	MHz) Delay (ms)
5	15.1	10.7	7.0	4.4
10	20.4	13.1	8.2	5.1
20	35.0	18.3	10.4	6.7
30	50.6	25.2	14.1	9.0
40	72.3	32.7	19.2	12.4
50	90.5	41.6	25.7	15.2

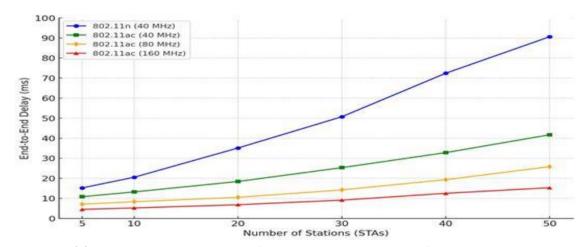


Figure (3) End-to-End Delay Comparison (IEEE 802.11ac vs. IEEE 802.11n) based on NS-3 simulations.

Packet Loss

Packet loss was measured under different congestion levels. IEEE 802.11ac's advanced channel access mechanisms led to a notable reduction in packet drops compared to IEEE 802.11n [15, 16]. Table 4 presents the percentage of packet loss under varying network loads. Figure 4 shows that with using 160MHz bandwidth in 802.11ac is optimal for high density environments.

Table (4) This table presents the percentage of packet loss under varying network loads.

Number of STAs		IEEE 802.11ac (40 MHz) Packet Loss (ms)		IEEE 802.11ac (160 MHz) Packet Loss (ms)
5	0.7	0.2	0.1	0.04
10	2.1	0.8	0.2	0.1
20	5.4	2.1	1.0	0.2
30	12.3	5.7	2.8	1.2
40	18.6	9.2	4.6	1.8
50	25.5	13.4	6.7	2.9

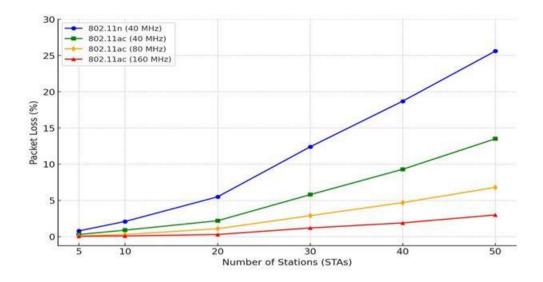


Figure (4) Packet Loss Comparison (IEEE 802.11ac vs. IEEE 802.11n) based on NS-3 simulations.

Conclusion

Our study shows that IEEE 802.11ac provides superior performance over IEEE 802.11n, especially in high-density scenarios. The higher throughput and lower latency make 802.11ac a more efficient choice for modern wireless applications. IEEE 802.11ac (160 MHz, 80 MHz and 40 MHz) reduces packet loss by (3.0%, 6.8% and 13.5%) at 50 stations respectively. Also, IEEE 802.11ac (80 MHz and 160 MHz) achieves the lowest delay as (25.8 ms and 15.3 ms) even at 50 stations respectively.

Future Work

- Investigation performance of IEEE 802.11ax and compare its against IEEE 802.11ac using NS-3
- 2. Integration of ML-based adaptive modulation techniques.
- 3. Impact of 802.11ac/ax coexistence with IoT applications.

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Association and Prevalence of Dyslipidemia in Type 2-Diabetes Mellitus patients in Baghdad



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Abstract

A prominent secondary cause of dyslipidemia, especially if glycemic control is compromised, is diabetes mellitus (dm), which is a significant risk factor for coronary artery disease and atherosclerosis. Fifty type 2 diabetic patients who visited a diabetes clinic in Baghdad participated in this cross-sectional research. Patients with secondary dyslipidemia from other recognized reasons were not included. Each patient's lipid profile and HbA1c data were taken from the clinic's records. Triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), and estimated low-density lipoprotein cholesterol (LDL-C) were all included in the lipid profile. Patients were deemed to have uncontrolled dyslipidemia if one or more of the aforementioned criteria fell outside of the ranges suggested by the recommendations for dyslipidemia. In this study, we found that 50 patients with dm type 2 studied, 30 (60%) were male and 20 (40%) were female. While cholesterol-lowering treatment (simvastatin at an average daily dose of 20 mg) was administered to all patients, 25 patients (50%) failed to meet all of their lipid profile goals. About 80% of patients had an LDL-C level of 1.8 mmol/l, which was the most common off-target lipid parameter. Combination dyslipidemia was the most prevalent kind of dyslipidemia (i.e., two abnormal lipid parameters) affecting a total of 25 out of 50 patients (50%) not reaching the recommended targets.

KEYWORDS

Typ2 Diabetes MellitusDiabetes Dyslipidemia Lipid profile

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Introduction

A metabolic disorder known as type 2 diabetes mellitus (T2DM) is typified by persistently high blood sugar levels brought on by anomalies in either insulin production or action, or both. The International Diabetes Federation claims that diabetes was a leading cause of mortality in 2015, accounting for approximately 5 million deaths due to diabetes and its related complications. Insulin is a key hormone responsible for regulating blood glucose levels^(1,2). A particular pattern of lipid abnormalities, such as increased triglycerides (TG), tiny, dense low-density lipoprotein (LDL) particles, and decreased levels of high-density lipoprotein (HDL) cholesterol, is also linked to type 2 diabetes^(3,4).

Plasma levels of LDL cholesterol in individuals with type 2diabetes are typically within the normal range. This is due to a shift in LDL particle distribution—A decrease in bigger LDL particles balances out an increase in tiny, dense LDL particles. Every element of diabetic dyslipidemia has been linked to an increased risk of developing cardiovascular disease (CVD), which continues to be the primary cause of death for those with type 2 diabetes^(5,6). Although most strategies aimed at reducing CVD-related morbidity and mortality have focused on lowering LDL cholesterol, other lipid abnormalities also play significant roles ⁽⁷⁾.

This study was conducted on 42 non–insulin-dependent diabetic patients (24 females and 18 males) without a control group for comparison. Data were collected from the Diabetes Center in Nasiriya City between November 2021 and February 2022. The research did not include patients with type 1 diabetes or those who were already on lipid-lowering drugs. Based on their glycated hemoglobin (HbA1c) levels, participants were divided into two groups: those with poor glycemic control (HbA1c > 7.0%) and those with good glycemic control (HbA1c < 7.0%) $^{(3)}$.

The data was analyzed using the SPSS software, and a significance level of p < 0.05 was set. Male study participants had a 43% prevalence of dyslipidemia, whereas female individuals had a 57% prevalence. The purpose of the study was to assess the connection between lipid profiles and glycemic management in individuals with type 2 diabetes. Gender-based analysis revealed no statistically significant differences in glycemic and lipid parameters between males and females, except for HDL cholesterol levels, which were significantly higher in females⁽⁸⁾. Moreover, recent studies have reported an increased secretion of apolipoprotein B (ApoB) in individuals with type 2 diabetes⁽⁹⁾.

A higher flow of free fatty acids (FFA) to the liver is assumed to be the cause of the increased production of lipoprotein particles containing apoB in people with type 2 diabetes. Furthermore, a decreased sensitivity to the inhibitory effects of insulin on apoB production is linked to insulin resistance. This resistance may be caused by a disturbance in the regulation of apoB breakdown or by a reduction in the activity of the microsomal triglyceride transfer protein (MTP), which is necessary for the generation of very low-density lipoprotein (VLDL) particles⁽¹⁰⁾.

Elevated plasma triglyceride (TG) levels can cause metabolic changes that result in decreased high-density lipoprotein (HDL) cholesterol and the creation of tiny, dense low-density lipoprotein (LDL) particles because of the increased endogenous synthesis of apoB-containing lipoproteins. The transfer of triglycerides from VLDL to HDL in exchange for cholesterol is facilitated by the cholesterol ester transfer protein (CETP) in a substrate-driven exchange. The resulting TG-enriched HDL particles are hydrolyzed by hepatic lipase (HL), which promotes their rapid catabolism and clearance from circulation ⁽⁹⁾.

Early identification and management of dyslipidemia in type 2 diabetes are essential for reducing cardiovascular complications. The goal of the current cross-sectional investigation was to evaluate the pattern and prevalence of hyperlipidemia in individuals with hyperglycemia. Patients who were referred from both public and private hospitals between July 2014 and June 2015 participated in the study, which was conducted in many labs around Pakistan. Lipid profiles were assessed for 200 individuals with a diagnosis of diabetes, 120 of whom were male (60%) and 80 of whom were female (40%). Male patients had a 97.18% prevalence of dyslipidemia, whereas female patients had an 87.15% prevalence. Of the guys who had dyslipidemia, 35% had isolated abnormalities, 47.5% had dyslipidemia including two lipid markers, and 17.5% had mixed dyslipidemia. These percentages were 16.25%, 51.25%, and 32.5% for females, respectively.

Overall, it was discovered that most people with hyperglycemia also had dyslipidemia. High triglycerides and low HDL cholesterol were the most prevalent dyslipidemic pattern in males, whereas raised LDL cholesterol and low HDL levels were more prevalent in females. Low HDL and high triglycerides were the most common lipid abnormalities in the entire sample. Because of the biological activity of its bioactive constituents, cinnamon has shown increasing scientific support in recent years for its potential advantages in controlling type 2

diabetes. In addition to summarizing the molecular processes by which cinnamon affects glucose and lipid metabolism, this study sought to investigate the impact of cinnamon on important clinical indicators associated with diabetes. Using internet databases including PubMed, Medline, and the Cochrane Library, a thorough literature search was carried out to find English-language research published between 2000 and 2022⁽¹⁰⁾. After screening titles and abstracts, relevant full-text articles were reviewed and included based on predefined inclusion criteria. The collective findings indicate that cinnamon supplementation improved both glycemic and lipid parameters. Clinical trials have also demonstrated anti-inflammatory properties of cinnamon, which may contribute additional benefits in diabetes management. Mechanistically, in vitro and in vivo studies suggest that cinnamon mimics insulin activity, enhances enzyme functions related to glucose metabolism, and reduces the intestinal absorption of cholesterol and fatty acids. However, comparison across studies remains challenging due to differences in cinnamon dosage, extract types, species, administration forms, and concurrent antidiuretic treatments ⁽¹¹⁾.

Methodology

The Design of the Study

An experimental study design was conducted on patients with type 2 diabetes in Baghdad. The study correlates with the effectiveness of prevalence and pattern of dyslipidemia in type 2 diabetes mellitus in Baghdad.

The sample of the study

A purposeful sample, which is non-probability, is chosen. Fifty patients were deemed to be part of the study sample. For the experimental trial, the patients were chosen at random. They are selected according to certain criteria that include age, gender, and years of disease Nevertheless, 10 normal samples were taken, 5 male samples and 5 female samples.

Sample collection

Samples were collected from different ages, men and women, and from different cases, and their ages ranged from 35 years to 50 years. After drawing blood samples, they were kept in special tubes. A period of two months, 50 samples were collected. Samples were divided

into 20 samples from women and 30 men disease Nevertheless, 10 normal samples were taken, 5 male samples and 5 female samples

Sample testing methods

Samples were collected and samples were added to the storage tube, the sample was gel tube, and it was transferred to Samples were examined to ensure that people had type 2 diabetes. Samples were examined on the spin xs device. One person with type 2 diabetes and 5 people with healthy results were examined in the glucose analysis device, and the results were confirmed.



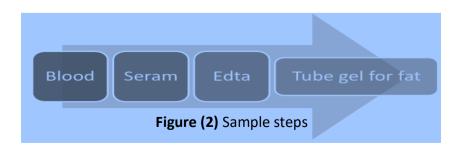
Figure (1) Automatic Biochemistry Analyzer

After confirming the results that people with diabetes, the lipid profile analysis was also examined for all people with diabetes.

The Pilot Study Takes the Following Consequences

Diabetes has long-term effects that develop gradually. The lower your blood sugar level and the longer you have diabetes, the more likely you are to experience complications. In the end, diabetic complications may be incapacitating or even fatal. Type 2 diabetes can really result from prediabetes.

Sample steps



Method

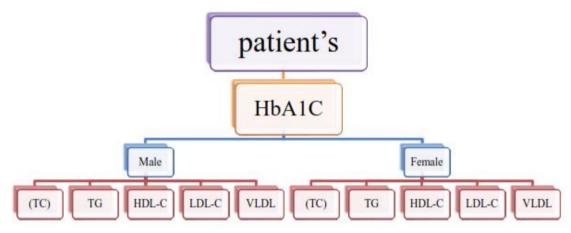


Figure (3) Method

Statistical analysis

The Statistical Package of Social Science (SPSS) will be used to examine the data that has been obtained. The data will be analyzed using the chi-square test.

Table (1) Statistics and results of patients who underwent tests and satisfactory analyzes.

,		, , ,
Age (50 – 35)	N	%
40 – 35	25	50.0
45 – 40	20	40.2
50 – 45	5	9.8
Gender	N	%
Male	30	60
Female	20	40
Total	50	100

A total of 50 male and female subjects, between the ages of 35 and 50, participated in the study. Tables below describe the data of people who have undergone age checks, and the largest age group was 35-50 years old (Issue 50). Where they represented (60%) men. women (40%.)

Table (2) The levels of HbA1C in patient and control

Haba1c	N	%
Number of patient	50	100
Patient age		
35-40	15	30
40-45	20	40
45-50	15	30
Normal	0	0
High	50	100
Total	50	100

 Table (3) Concentration of various lipids in men, women and the combined cohort

Cholesterol	Age	N	%
Number of patient		50	
Normal	35-40	20	40
Abnormal	40-50	30	60
Total		50	100
Control		10	
Triglycerides			
Number of patient		50	
Normal	35-44	25	50
Abnormal	45-50	25	50
Total		50	100
Control		10	
High Density Lipoprotein			
Number of patient		50	
Normal	35-46	32	64
Abnormal	46-50	18	36
Total		50	100

Table (4) the levels of LDL and VLDL in the patient and control

Low density lipoprotein	Age	N	%
Number of patient		50	
Normal	35-47	40	80
Abnormal	47-50	10	20
Total		50	100
Control		10	
Volume low density lipoprotein			
Number of patient		50	
Normal	35-44	38	76
Abnormal	44-50	12	24
Total		50	100
Control		10	

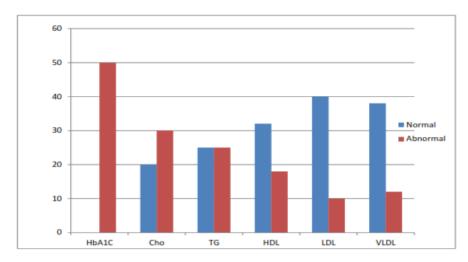


Figure (4) The levels of lipid profile (TC.,TG, HDL, LDL, VLDL) and HbA1C in patient and control

Results

In this chapter, the results of the study will be clarified. We have reached this study and the interpretation of each result

Discussion

Together with hyperglycemia and hypertension, dyslipidemia has also been linked to diabetes mellitus in the past. According to this study, 69.8% of individuals with type 2 diabetes had dyslipidemia. A greater incidence was recorded in Saudi Arabia (83.9%), whereas similar findings were obtained in other studies carried out in Karbala (73%) and Basra (70.5%). In contrast, a research conducted in China revealed that individuals with type 2 diabetes had a lower prevalence of dyslipidemia, at 59.3%.

Dietary practices, genetic predisposition, and lifestyle choices may all contribute to these differences in the incidence of dyslipidemia. In individuals with type 2 diabetes, the study discovered a high correlation between dyslipidemia and glycemic control, with dyslipidemia being more prevalent in those whose diabetes was not well managed. This finding aligns with previous research by Hyassat et al. ⁽¹²⁾.

Reduced high-density lipoprotein (HDL) and increased total cholesterol (TC), triglycerides (TG), and low-density lipoprotein (LDL) were linked to higher HbA1c levels. These findings were comparable to those from an earlier research by Alouffi. Regretfully, in spite of therapy and routine follow-up at the Diabetes Center every three months, the majority of

patients (70.4%) in this study had poor glycemic control. This high rate of uncontrolled diabetes was in line with previous research.

The patient's weight was another modifiable factor that positively correlated with HbA1c levels in a statistically meaningful way. discovered that when people with type 2 diabetes lost weight, their glycemic control improved, and when they gained weight, it deteriorated. ⁽¹³⁾. Every visit to the National Diabetes Center includes regular diabetes education. According to the participants in our study, they visited the National Diabetes Center frequently, so it's probable that additional visits will increase patients' knowledge of their condition and how to manage it. This might account for our study's lack of association ^(10, 14).

In this study, females had a higher mean HbA1c. Previous studies have shown a strong correlation between poor glycemic control and feminine gender. To assess the effect of type 2 diabetes on the lipid profile, more research is required ⁽¹⁵⁾.

Conclusion

Despite all patients receiving lipid-lowering treatment, the great majority of type 2 diabetes patients in the study had dyslipidemia and achieved suggested objectives. Patients with dyslipidemia require more extensive lipid-lowering treatment, including statin medication. In order to properly address dyslipidemia and lessen the burden, measures to decrease obesity and other lifestyle risk factors are also essential

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The Effect Albumin and Lipid Profile in The Type 2 Diabetes Mellitus with Hypertension in Iraqi Patients



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Abstract

Background Hypertension and diabetes are becoming increasingly common. Clinical trials have demonstrated the importance of tight blood pressure control among patients with diabetes. Methods A total of 90 samples 45 control groups and 45 patients groups with essential hypertension have diabetes. The plasma calcium and albumin, liver function test and renal function test levels were determined using spectrophotometer method., while plasma HBA1C level was determined using fine care method. Results the results showed that there is no statistical significance in BMI and HDL between the two groups. There is also a statistical significance in the age between the two groups. The results showed that the levels of albumin, calcium, showed a significant decrease in the patients group compared to the control group. The levels of uric acid, triglyceride, cholesterol, urea, creatinine and HBA1C were significant increase in patients group. Conclusion The study showed that decrease serum albumin, calcium ferritin level in patients groups than control groups and increased uric acid cholesterol, triglyceride, HDL, urea and HBA1C.

KEYWORDS

Serum Albumin Hba1c Diabetes Hypertension Calcium

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Introduction

An increased risk of atherosclerosis and its outcomes, including heart attacks and strokes, is connected with frequent co-morbidities such diabetes mellitus and hypertension [1]. Metabolic diseases such type 2 diabetes, hypertension, and dyslipidaemia are associated with higher rates of body fat [2]. Body mass index (BMI) criteria are currently at the heart of obesity treatment guidelines, with different treatment cut-off points depending on whether or not concomitant illnesses associated to obesity are present. In addition, a large percentage of people with metabolic illnesses are overweight or obese [3].

A multitude of blood lipid and lipoprotein abnormalities, such as low HDL cholesterol, an abundance of small dense LDL particles, and elevated triglycerides, are associated with type 2 diabetes and hypertension. A large number of people have these issues even when their LDL cholesterol levels are within the normal range. Many individuals develop type 2 diabetes because of these changes, which are a component of the insulin resistance syndrome, sometimes called the metabolic syndrome [4, 5].

Combining hypertension with type 2 diabetes is a recipe for cardiovascular disease and mortality. Patients with diabetes should take hypertension just as seriously as glycaemic control when coming up with therapy options because it is more common and increases the risk of complications [6]. Improperly controlled diabetes or hyperlipidaemia both increase the likelihood of macrovascular complications. Roughly a quarter of diabetes patients will have elevated lipid profiles [7]. Both diabetes mellitus and hypertension are associated with an increased risk of cardiovascular disease and stroke as people get older.(8) It is believed that the effects of insulin resistance on the kidneys and vasculature contribute to the frequently seen high blood pressure (BP) readings in T2D patients.eight (8)However, there is mounting evidence that hypertensive persons are more likely to have problems in glucose metabolism, suggesting that the pathogenic link between hypertension and diabetes mellitus is actually two-way. (9,10)

In addition to increasing healthcare expenditures and complicating treatment strategies, developing hypertension in diabetics greatly raises the risk for microvascular and macrovascular consequences.(11)

Although there is a marked decrease in microvascular and cardiovascular morbidity and mortality after blood pressure is lowered many diabetic patients still have their hypertension poorly managed. Possible causes of this finding include a lack of clarity about treatment goals and pathophysiological linkage, as well as clinical inertia, poor adherence to the recommended regimen, and a delay in hypertension diagnosis. (12,13),

Materials and Methods

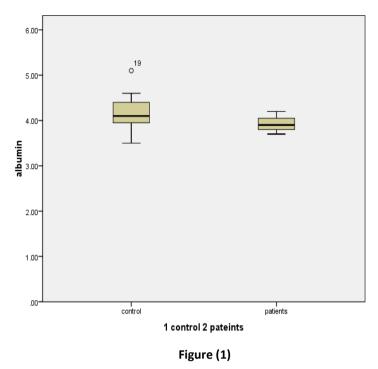
A case control study that included (90) samples divided to (45) healthy controls and (45) patients groups(type 2 diabetes mellitus with hypertension) were all included in this study. matched in terms of age, weight, and absence of illness. Patients who fulfilled the inclusion criteria and had type 2 diabetes and hypertension were sampled from the beginning of December 2023 to the end of May 2024. Clinical examinations and laboratory investigations confirmed the diagnoses made by the specialised physicians for all patients. Before the day of laboratory inquiry, each participant's blood was drawn, centrifuged to extract serum, and stored at -20 °C. Enzymatic methods on a completely automated biochemistry analyser (Spin120, Spinreact product) were used to quantify total cholesterol, triglycerides, uric acid urea, creatinin, albumin, and HBA1C. The VLDL cholesterol concentration was calculated using the Fridwald equation, which is triglyceride divided by 5.

Statistical analyses

A statistical analysis was conducted using SPSS, version 26, for the purpose of performing statistical analyses. Means and standard deviations (SD) were used to express the data. Statistical significance was determined when P < 0.05.

Results

A total of 90 participants were included in the study, the mean and standards error. The Results of the study showed that the albumin levels were significantly [p<0.05] lower in patients Group [3.91 \pm 0.033] when compared to control Group [4.19 \pm 0.07] (as illustrated in Figure 1).



ne calcium levels were significan

The Results of the study showed that the calcium levels were significantly [p<0.05] lower in patients Group $[8.86\pm0.535]$ when compared to control Group $[9.12\pm0.630]$ (as illustrated in Figure 2).

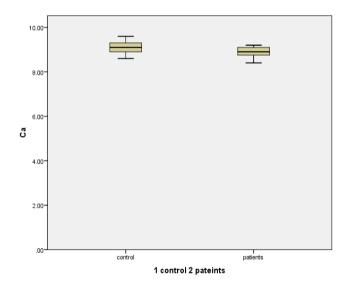


Figure (2)

The results demonstrated that the levels of HBA1C were noticeably higher in the patients' group $[9.00\pm0.407]$ compared to the control group $[4.89\pm0.065]$ (as shown in Figure 3), with a significance level of p<0.05.

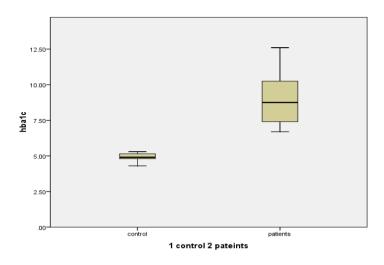


Figure (3)

The mean± SE age was 40.95±2.089 years, 32.5+1.92 years of the patients, controls were respectively. The mean± SE concentration of serum TRI, cholesterol was (212±6.67 mg/dL, 129+3.33 mg/dl), (10.1+199 mg/dl, 148+5.4 mg/dl) patients and controls respectively the showed highly significant p value was (0.0001) as shown Table 1 and the other hand. the mean± SE (urea, creatinine, uric acid) for patients and controls were respectively. was (44.3+0.751mg/dL, 38.7+0.44mg/dl), (1.02+0.39mg/dl, 0.72+0.0227mg/dl), (6.64+0.86 mg/dL, 4.88+0.15 mg/dl) the showed highly significant p value was (0.0001) as shown table 1.

Table (1) Comparison between control and patients groups with age, BMI, ca, uric acid, albumin, tri, cholesterol, HBA1C and urea (Mean± SE. value)

Variables	Control Mean + sE	Patients Mean+ SE	P value
Age	32.5+1.92	40.95±2.089	0.005
Bmi	31.4+1.07	33.56+1.09	0.176
Ca	9.12±0.630	8.86±0.535	0.003
Uric	4.88+0.15	0.86+6.64	0.0001
Albumin	4.19±0.07	3.91±0.033	0.002
Cho	148+5.4	10.1+199	0.0001
Tri	129+3.33	212±6.67	0.0001
Hdl	50.4+0.577	49.95+1.62	0.796
Urea	38.7+0.44	44.3+0.751	0.0001
Icrea	0.72+0.0227	1.02+0.39	0.0001
Hba1c	4.89±0.065	9.00±0.407	0.0001

Discussion

Identifying and controlling the risk factors for diabetes is an essential therapeutic aim because the disease and its complications place a heavy personal and public health burden. Lipid fractions and macrovascular consequences of diabetes, such as coronary artery disease, have been linked in multiple studies. (14) The world over, people understand that hypertension is one of the leading causes of cardiovascular disease, stroke, diabetes, and kidney disease. Obesity, glucose intolerance, and abnormalities in lipid metabolism are among the comorbidities that around 80% of hypertension patients experience. (15) Members of the control group and those with hypertension and type 2 diabetes were tested for lipid profiles, as well as fasting blood glucose, haemoglobin A1c, serum albumin, calcium, creatinine, and urea.

The calcium and albumin was significantly lower patients groups while uric acid, creatinine, urea, total cholesterol, LDL-cholesterol, and triglyceride are significantly higher among patients group when compared to the controls study group. Microvascular damage, brought on by hypertension, may play a role in the pathogenesis of diabetes (16, 17). Independent predictors of hyperglycemia were found to represent biomarkers of endothelial dysfunction; hypertension is highly connected with endothelial dysfunction, which is associated with insulin resistance. (18) Both the Lige song et al. (19) and the Anuradha K. et al. (20) research found that hypertension patients had considerably higher serum cholesterol and LDL levels and lower HDL levels. Similarly, Yamakado M et al. 21 found a statistically significant relationship between hypertension patients' lipid profiles and serum total proteins. The role of plasma albumin in obesity and T2D. Among healthy adult participants, reduced albumin, independent of %fat, was found to be associated with several expression markers of adipose tissue infammation, indicating that albumin may be refecting an immunological milieu which may be contributing to risk of T2D(21)

The current study, demonstrating that lower albumin is associated with higher glucose concentrations and elevates risk of incident T2D, indicates a potential mechanism that is both influencing albumin abundance and contributing to the pathogenesis of T2D. Several possibilities exist for such a mechanism. First, diabetes may result in decreased hepatic

albumin synthesis. Second, albumin could be reduced via mechanisms related to formation of glycated albumin [22]

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Assessment of Water Pollution in the Tigris River near Medical City Using Satellite images and Ground Measurements



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Abstract

This research examines water quality monitoring by satellite images in the Tigris River next to Medical City in Baghdad, emphasizing the geographical distribution of water quality data and examining the effects of hospital waste on the chemical characteristics of surface waters. Satellite images, is considered useful for detecting and monitoring rivers pollution. Sentinel-2 satellite image of Baghdad, Iraq, was used with ground resolution of 10 m in May 2021. ERDAS imaging 2014 software programming was utilized for Image processing to assess the water pollution in study area five regions were selected from a study area and then classified using the unsupervised K mean method by taking samples from five regions in study area physical, chemical parameters and Environmental variables for accurate analysis. Our findings demonstrate that hospital waste significantly affects acidity, conductivity, and dissolved solids. Field data and satellite images confirm that untreated wastewater from the Medical City complex is significantly degrading water quality in the Tigris River. Near the discharge point, shows sharp increases in turbidity, Chemical Oxygen Demand (COD), Total Suspended Solid (TSS), and nitrates, with a drop in Dissolved Oxygen (DO). Pollution spreads downstream along the same bank and even reaches the opposite bank, though with gradual dilution.

KEYWORDS

Pollution
Satellite Image
K_mean
Turbidity
Medical City

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Introduction

Tigris River is considered a vital resource for Baghdad's population, which exceeds 8 million residents. It supplies a significant portion of the city's drinking water, supporting domestic use, agriculture, and various industrial activities [1]. The river's water is essential for irrigating urban green spaces, parks, and agricultural lands surrounding the capital, contributing to food security and improving the urban environment. Rapid urbanization, industrial discharge, untreated sewage, and decreased water flow from upstream have led to increasing concerns about the river's water quality in Baghdad [2]. Protecting the Tigris is therefore critical for ensuring the city's water security, public health, and environmental sustainability [3].

Medical City in Baghdad, established in 1970, comprises several hospitals and is located in Bab Al-Muadham, Baghdad, Iraq. It is situated on the eastern bank of the Tigris River (Rusafa side), between the Sarafiya Bridge and the Bab Al-Muadham Bridge [4]. The release of untreated or inadequately treated medical wastewater, along with municipal sewage and solid debris, presents considerable threats to the river's water quality. This may result in the dissemination of pollutants, such as medications, infections, and chemical residues, so jeopardizing aquatic ecosystems and public health [5].

Many research studies have been carried out related to the wastewater from Medical City is directly impacting the Tigris River. A study in 2013 conducted by Baghdad University said that the wastewater from Medical City is directly impacting the Tigris River [6], while another study advocates for the immediate treatment of wastewater effluent by hospitals [7], and another studied examined the consequence of hospital wastewater discharge of Medical City, Baghdad on heavy metals concentration of the Tigris River during 2020. The results showed that the concentration of the heavy metals transcend the river maintenance system permissible of the Iraqi standard and the World Health Organization (WHO) [8].

Satellites offer robust instruments for the continuous monitoring and evaluation of water quality across extensive geographical and temporal dimensions. Satellite imaging enables the effective detection and analysis of critical indicators including as turbidity, algal blooms, and suspended sediments, even in inaccessible regions [9].

Classification is an information extraction strategy employed to categorize objects into predetermined groups. It is the most often employed fundamental decision-making task in human activity. A classification issue arises when an object must be allocated to a predetermined group or category based on multiple observed properties associated with that object. The classification plays a crucial role in satellite images analysis. Image categorization is a complex process that can be affected by several factors [10]. Unsupervised classification, known as clustering, and generates "spectral classes" that may not align with "information classes" identified by supervised classification [11]. K-Means unsupervised classification computes first class means uniformly dispersed across the data space and thereafter repeatedly clusters the pixels into the nearby class utilizing a minimum distance method [12]. Every iteration recalibrates class means and reclassifies pixels according to the updated means. All pixels are categorized into the nearest class if a standard deviation or distance threshold is established; in such instances, certain pixels may remain unclassified if they fail to satisfy the set requirements [13]. The operation persists until the pixel count in each class varies by less than the chosen pixel modification threshold or the maximum iteration limit is attained [14].

The primary goal of this study is to use a combination of field-based water quality measurements and satellite images to determine the extent to which the Tigris River in Baghdad's Medical City is polluted. The objectives of the project are to create pollutant distribution maps to aid in environmental monitoring and management, examine regional and temporal fluctuations in water pollution, and determine connections between indices produced from satellites and in-situ data.

Water Pollutions

Water pollutants are substances that diminish water quality, rendering it detrimental to humans, animals, and the environment. These contaminants are often categorized into various classifications: Physical pollutants encompass sediment, suspended solids, and thermal pollution, which influence the aesthetics and temperature of aquatic environments. Chemical pollutants encompass pesticides, heavy metals (including lead, mercury, and cadmium), industrial chemicals, and nutrients such as nitrates and phosphates [15]. These substances may be toxic and contribute to issues such as eutrophication. Biological pollutants encompass bacteria, viruses, and parasites derived from sewage or animal excrement, which

can lead to waterborne diseases. Radiological Pollutants: Radioactive materials capable of contaminating water as a result of nuclear operations or inadequate disposal of radioactive waste [16].

Study Area

The Medical City established in Bab al-Muadham (1970) On the Tigris River between the Sarafiya Bridge to the west and the Bab al-Muadham Bridge to the east at (33° 20' 53.7" - 33° 20' 50.3") N and (44° 22' 20.6" -44° 22' 56.6") E. It is including several hospitals located which dispose of their sewage directly into the Tigris River without treatment [17]. Five stations were selected in Tigris revere for sampling to make bacteriological and physicochemical studies on May 2021 as shown in (Figure 1).

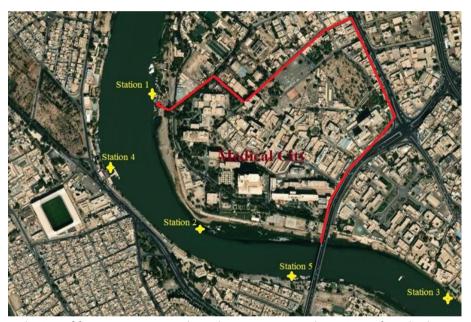


Figure (1) Distribution of five water stations along the Tigris River in the vicinity of Medical City, Baghdad.

Station 1 at (33° 22' 54"N, 44° 22' 20" E), about 150 m before the discharge point of Medical City, Station 2 at (33° 20' 37.8"N, 44° 22' 28" E) 500 m after discharge point, Station 3 at (33° 20' 29"N, 44° 23' 4.5" E),1500 m after discharge point, Station 4 at (33° 22' 45"N, 44° 22' 14" E), 250 m after discharge point in the opposite bank of the river), and Station 5 at (33° 20' 31"N, 44° 22' 42" E), 1000 m after discharge point in the opposite bank of the river.

Materials and Methods

In this work, using Earth Resource Data Analysis System (ERDAS) imagine. It's a mapping software company expert in Geographic Information Imaging. Software functions include introducing, observing, varying, analyzing raster and vector data sets and sophisticated tools

for digital analysis of satellite images [18]. ERDAS imagine 2014 is used in this work to apply (K-mean) unsupervised classification method to classify Sentinel 2 satellite images with 10 m spatial resolution, to mapping water quality parameter depending on the relationships between radiance data and water quality. Fieldwork was conducted on May 2021 to observe water pollution in five regions identified within the study area of the Tigris River. Five water samples were collected from each region; they were transferred to the laboratory within 2 hr. in sterilized 500 ml glass bottles for the bacteriological, physical, and chemical evaluated.

Results and Discussions

The satellite image of Baghdad city, specifically focusing on the Tigris River near the Medical City area, was acquired for May 2021. The image underwent preprocessing steps, including radiometric correction to eliminate noise and ensure the data's accuracy. The next step involved feature extraction, where specific spectral bands such as red, green, blue, and near-infrared (NIR) were selected for analysis. For image classification, an unsupervised classification method, specifically the K-Means clustering algorithm, was employed to categorize the pixels into distinct groups based on spectral similarity.

The resulting clusters were interpreted to represent different land covers, including clean water, polluted water, urban areas, and agricultural lands. The relationship between radiance data and water quality parameters was then analyzed, correlating spectral values with known water quality indicators such as turbidity, pH levels, and chemical concentrations. Finally, the classification results were validated by comparing them with available ground truth data. The final classification map, shown in Figure 2, highlights the spatial distribution of water quality variations along the Tigris River.

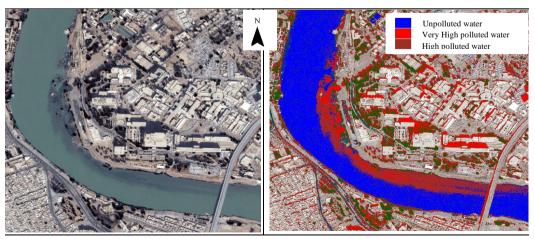


Figure (2) Classification image for the Tigris River beyond Medical City, Baghdad.

The classification image in Figure 2 shows the spatial distribution of water pollution levels in a segment of the Tigris River near Medical City in Baghdad. The water quality is classified into three categories based on pollution intensity, represented by different colors: Blue, these areas indicate relatively clean water with minimal or no detectable pollutants. They are farther from direct sources of contamination. Red refers to the reasons that show the highest level of pollution. This could be due to the direct discharge of untreated wastewater, hospital effluents, or industrial waste. The proximity to the Medical City suggests the possibility of medical or domestic discharge contributing to this pollution. Brown refers that these areas are also polluted but to a lesser extent than the red zones. They may represent areas where contaminants are diluted due to mixing or are slightly farther from direct pollution sources.

The spatial distribution refers to the right (inner) bank of the river appears to have more blue (unpolluted) zones, indicating cleaner water on that side. The left (outer) bank and especially sections close to Medical City show heavy contamination (red), suggesting pollution sources. The transition between colors suggests that pollution disperses downstream but may also settle in specific stagnant areas or bends of the river.

Water Pollution Tested

The investigation of water quality at the five sites indicates a distinct geographical distribution of pollution associated with the discharge from the hospital complex, as evidenced by field data in Table 1.

Table (1) Presents the results of water pollution tested of the five stations.

Station	Station 1	Station 2	Station 3	Station 4	Station 5
Indicator					
рН	7.5±0.16	6.8±0.25	7±0.38	7.3±0.21	6.6±0.16
Turbidity (FTU)	24±1.6	83±4.1	66±1.6	45±1.6	43±1.6
DO (mg/l)	7.1±0.16	3.6±0.16	4.9±0.16	4.3±0.16	4.9±0.16
BOD (mg/l)	3.6±0.16	5.7±0.16	5±0.16	4.2±0.16	4±0.16
COD (mg/l)	112±1.22	735±3.78	615±3.65	470±5.7	590±5.7
TDS (mg/l)	470±5.7	900±11.4	580±5.7	560±11.4	650±11.4
TSS (mg/l)	630±11.4	1350±11.4	1000±11.4	980±11.4	850±11.4
EC (μS/cm)	730±11.4	1360±14.8	1000±89.72	890±8.37	950±8.37
NO ₃ ⁻² (mg/l)	7±0.38	22±0.84	12±0.84	15±0.84	16±0.84

From the results in Table 1, it is observed that the analysis of samples from Station 1 indicates a high level of Dissolved Oxygen (DO), which reflects a healthy aquatic environment. Both Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) levels are relatively low, suggesting minimal organic or industrial pollution. Additionally, the values of Electrical Conductivity (EC), Total Suspended Solid (TSS), and Total Dissolved Solids (TDS) are also relatively low, indicating a reduced amount of dissolved salts in the water and the absence of agricultural pollutants, as shown by the low Nitrate (NO₃⁻²) concentration. The high turbidity level observed may be due to natural sediment movement, riverbed activity, or human activities within the river. Based on these findings, we can conclude that the water at station 1 is unpolluted and represents a healthy river environment, as corroborated by the satellite image (Figure 2), which shows this area free from apparent contamination.

Station 2, situated right next to the discharge outlet from the hospital, and shows a dramatic deterioration: turbidity, TSS, COD, and nitrate levels spike significantly, while dissolved oxygen drops sharply. This is a direct sign of heavy organic and chemical waste entering the river. In the classified image, this pollution plume is visible as dark red and brown tones along the left bank, indicating high concentrations of suspended material and urban runoff.

Station 3, also shows signs of pollution, although to a slightly lesser extent than Station 2. The turbidity here is lower than at Station 2. Dissolved oxygen (DO) levels remain low, and both BOD and COD values indicate ongoing non-organic pollution. Furthermore, the elevated levels of TDS, TSS, and EC suggest a high concentration of dissolved and suspended materials in the water. The increase in nitrate levels further supports the presence of pollutants spreading through the river.

Stations 4 and 5 both located on the right bank the water quality show partial recovery. While indicators such as turbidity, TSS, and EC remain elevated, there is a noticeable decline in COD and nitrate concentrations compared to Station 2. This gradual improvement, also visible in the satellite image as a fading of the reddish pollution signature, suggests dilution and dispersion of pollutants as the river flows onward.

Conclusions

The results of water sample analysis indicate a clear impact of the Medical City as a primary source of pollution in the Tigris River.

Station 2, located directly adjacent to the area likely receiving hospital wastewater discharge, recorded the highest levels of organic pollutants (BOD and COD) and suspended/dissolved solids (TSS and TDS), along with a significant drop in dissolved oxygen levels. This strongly suggests the presence of untreated or poorly treated medical effluents being discharged into the river.

Station 1 shows relatively healthy river conditions, with the highest dissolved oxygen levels and the lowest concentrations of contaminants. This supports the conclusion that pollution begins downstream of the Medical City area.

Satellite imagery used in the study reveals visible differences in water coloration near the Medical City, aligning with field measurements showing elevated levels of BOD, COD, TSS, and EC at stations located downstream from the hospital, and confirming the usefulness of satellite images in detecting and tracking river pollution.

The findings highlight the urgent need for a continuous environmental monitoring system around large medical institutions, particularly those located near water bodies, to ensure proper treatment of medical and liquid waste before it enters the aquatic environment.

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Efficiency Of the Nano Particles of Aqueous Moringo Oleifera Extract in Antioxidants Levels in Male Albino Rat Treated with Drug Cyclosporine



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Abstract

This study was conducted in an external laboratory of a veterinarian (Sigma Laboratories) for a period from 11/1/2023 to 5/1/2024 and included the use of Moringa leaves in the preparation of zinc nanoparticles and their characterization by conducting examinations, which are (UV visible absorption spectroscopy, FTIR spectroscopy, and scanning electron microscopy (SEM) techniques, X-ray diffraction analysis (XRD), and energy dispersive X-ray (EDX).

KEYWORDS

Antioxidants
Cyclosporine
Moringo Oleifera

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Introduction

Scientists have always used medicinal plants as an important treatment for many diseases, and because plants are directly related to humans, they have been used extensively to treat many diseases. Moringa oleifera was one of the most important trees that treated many diseases because it contains minerals, protein, beta-carotene, and antioxidant compounds (Horn et al., 2022)

Moringa oleifera is one of the most important plants that belongs to the Moriningaceae family and is characterized by its high medical and nutritional value. Its original home is India and its cultivation is widespread in tropical and subtropical regions. Later, its cultivation began all over the world (Radovich, 2010; Premi and Sharma, 2013). It is known as the miracle tree for its many therapeutic benefits and uses. It is used to reduce male infertility because of its role in oxidative stress, male hormone levels, steroid formation, and sperm formation (Mohlala et al., 2023). Nanotechnology holds great promise for providing a number of modern products with medical applications for early disease identification, treatment, and prevention. Nanoparticles have many characteristics in shape and size. Nanoparticles can be used due to the properties they possess in commercial products, which include food and drugs (Rashid et al., 2018). Nanoparticles have recently gained great importance due to their applications and that they are environmentally friendly, more effective, do not take a long time, and do not use toxic chemicals in the production of nanoparticles.

The green synthesis method includes (bacteria, yeast, fungi, algae, and plants). Parts of the plant, including the root and stem, were used. The leaf, seed and fruit (Rajesh Kumar et al., 2013) are used to manufacture nanoparticles because plant extracts are rich in phytochemical contents and act as antioxidants (Gnanjobitha et al., 2013; (Zong et al., 2014) and the reduction of zinc oxide nanoparticles (ZnO) from semiconductor metals and its importance has become famous in previous years due to its wide scope in various procedures such as the field of optics, medical devices and electronics. (Gunalan et al., 2012; Anbuvannan et al., 2015) Zinc oxide has an important role in that it can be easily prepared, inexpensive to prepare and safe to use. The US Food and Drug Administration has worked to recruit it as a metal oxide and there are many researchers who have used plant extracts in the production

of zinc oxide nanoparticles (ZnO PNS) such as (Citrus sinensis extract) (Luque et al., 2018) and tomentosa leaf extract (Sharmila et al., 2018).

Materials and working methods

The experiment used 48 adult male rats of the Ratuse ratus strain, aged 14 weeks, with weights ranging from (190 to 395) gm, and they were randomly distributed into 8 groups (6 rats per group). The first was the control group, the second was dosed with a nano extract (concentration 0.5 mg/kg), the third group was dosed with a nano extract (concentration 1 mg/kg), the fourth group was dosed with a nano extract (concentration 0.5), and after 8 hours it was dosed with cyclosporine, the fifth group was dosed with a nano extract (concentration 1), and after 8 hours it was dosed with cyclosporine, the sixth group was dosed with cyclosporine only, the seventh group was dosed with a regular extract at a concentration of 300, and the eighth group was dosed with a regular extract, and after 8 hours it was dosed with cyclosporine, respectively. Note that the dosing process continued for 60 days, once daily.

Groups of experimental animals

The animals were divided into 8 groups of 48 rats, 6 rats per group, with similar weights, as shown below:

- The first group: (control group) was given tap water and fodder for 60 days (6 males)
- The second group: was given orally with nano extract (Morinca leaves) at a concentration of (0.5 mg/kg) of body weight for 60 days (6 males)
- The third group: was given orally with nano extract (Morinca leaves) at a concentration of (1 mg/kg) for 60 days (6 males)
- The fourth group: was given nano extract (Morinca leaves) at a concentration of (0.5 mg/kg) and after 8 hours with cyclosporine
- The fifth group: was given nano extract (Morinca leaves) at a concentration of (1 mg/kg) of body weight and after 8 hours with cyclosporine
- Group 6: dosed with cyclosporine only
- Group 7: dosed with the normal extract at a concentration of 300 mg/kg of body weight
- Group 8: dosed with the normal extract and after 8 hours with cyclosporine

The dosing process for the animals was done using a plastic tube connected to the top of a push tube (stomach tube), after withdrawing the material (Morinca aloe vera leaf extract, nano zinc material and cyclosporine) into the push tube and given to the animals by inserting the plastic tube orally through the esophagus and then reaching the stomach to ensure that the material enters and is completely taken up.

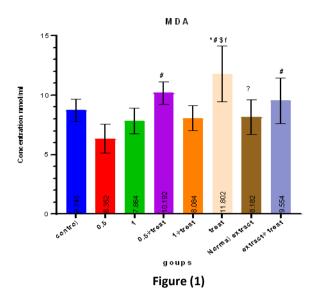
Sacrifice rat animals and collect blood samples Sacrifice rat animals

Upon completion of the experiment for 60 days, the rats were fasted for 24 hours, then the rats were anesthetized with chloroform and sacrificed after the animals were weighed on a regular scale and the abdominal cavity was opened using scissors and a surgical scalpel, then blood was drawn from the heart by what is known as a heart puncture. A sufficient amount of blood was obtained using a 5 ml plastic medical syringe and the blood was placed in Gel tube test tubes and left for half an hour until it clotted. It was then taken to a centrifuge at a speed of 3000 rpm for a quarter of an hour to separate the serum, which was stored in special tubes for freezing at -20 degrees for the purpose of conducting biochemical tests that include antioxidants (SOD, Gpx, MDA) and hormonal levels (LH, FSH, Testosterone)) and then The epididymis and testicles were removed and placed in a Petri dish containing a physiological saline solution to avoid drying them out. Then the fatty materials attached to them were removed and dried well using filter papers. The weights of the organs were taken using a sensitive electronic balance and they were preserved in a 10% formalin fixation solution for the purpose of conducting histological studies on them.

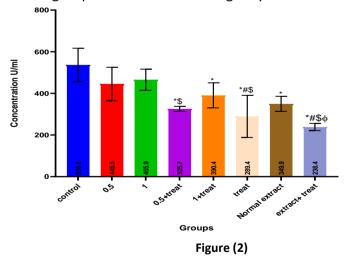
The results

The results of the current study showed a significant decrease (P<0.05) in the average concentration of malondialdehyde (MDA) for males treated with (nano extract concentration 0.5, nano extract concentration 1, normal extract) compared to the control group, while it showed a significant increase (P<0.05) for the group of males treated with the drug only compared to the control group.

It showed the presence of significant differences for the groups treated with (nano extract concentration 0.5 + cyclosporine, nano extract concentration 1 + cyclosporine, normal extract + cyclosporine) with the group of rats treated with the drug only, as shown in the following figure



The results of the current study showed a significant decrease (P<0.05) in the average glutathione peroxidase (GPX) enzyme for rats in the following groups (nano extract concentration 0.5, nano extract concentration 1, normal extract) compared with the control group, and also a significant decrease (P<0.05) for the group of male rats treated with the drug only compared with the control group, and showed a significant decrease for the groups treated with (nano extract concentration 0.5 + cyclosporin, and nano extract concentration 1 + cyclosporin) with the group treated with the drug only. As shown in the following figure



Discussion

A significant decrease (P<0.05) was observed for the groups treated with the nano-extract concentration (0.5 and 1) compared to the control group. This was supported by the researcher (Iqbal. et al 2024), who indicated a significant decrease in malondial dehyde (MDA)

for rats treated with the nano-extract compared to the control group. He indicated that nano-zinc oxide has received great attention due to its non-toxicity and stability, as it absorbs ultraviolet rays effectively. While there was a significant increase (P<0.05) for the groups treated with cyclosporine only compared to the control group, this is what the researcher (Robert.J et al., 2005) stated, where he indicated that giving cyclosporine leads to a significant increase in malondialdehyde by 15% in the plasma, and he showed that the concentration of MDA reached its peak two hours after dosing and then declined to the midline after six hours, which was interpreted as the possibility that the absorption of cyclosporine is faster, which explains the increase in the concentration of MDA after two hours).

Since the increase in the level of the enzyme malondial dehyde MDA leads to damage resulting from severe oxidation, which can lead to infertility and an increase in the tumor necrosis factor TNF-alpha, which gives an indication of the presence of inflammation.

These results showed a significant decrease (P<0.05) for the groups treated with the nano-extract concentration (0.5 and 1) compared to the control group. These results are consistent with (Wulandari et al., 2019). It can be concluded that there is a clear difference in GPX activity between the groups and there is a significant effect of Moringa aloe vera leaf extract on GPX activity, as increasing the dose of Moringa aloe vera leaf nano-extract leads to a significant increase in GPX activity. There was also a significant decrease in rats treated with cyclosporine only compared to the control group. This was shown by the researcher (Robert. J. et al., 2005), who indicated that taking cyclosporine for 7 days leads to a significant decrease (P<0.05) in the concentration of Gpx in red blood cells.

Conclusions

The strong efficacy of the nano-extract (0.5 and 1) concentration was proven as a preventive treatment for rats before cyclosporine doses. The nano extract of Moringa alba leaves had a strong effect on reducing the weight of rats: it caused changes in the biochemical parameters (antioxidants).

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Assessment of Vitamin B12 Levels in Pregnant Women with Toxoplasmosis

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Abstract

infection with Toxoplasma gondii causes toxoplasmosis. It can be transmitted congenitally, fecal matter or by the consumption of uncooked infected meat. Infection during pregnancy can cause severe damage like miscarriage and birth problems. In certain Arab nations, it has an impact on women. For instance, reports of a 34.7% toxoplasmosis infection rate among pregnant women in Baghdad, Iraq, have been made. Vitamin B12 is a water-soluble vitamin that your body needs to perform vital functions. This vitamin dissolves in water and leaves the body daily through urine. Your liver can store vitamin B12 for several years. This study aimed to measure the effect of toxoplasmosis on serum vitamin B12 and its correlation with IgG, IgM anti-Toxoplasma gondii antibody in pregnant women. Sixty pregnant women were enrolled in this case -control study; 30 women negative 30 positive toxoplasmosis. Their ages ranged from 15 - 40 year the blood samples were collected in private laboratory in Baghdad between September 2024 and December 2024. IgG, IgM anti-Toxoplasma gondii antibody and vitamin B12 levels were determined by using enzyme-linked immune sorbent assay (ELISA). The results showed highly significant increase in IgG and IgM anti-Toxoplasma gondii antibody (P value = 0.0001) between patients (4.065+1.55, 3.09+1.06) and control groups (0.347+0.190, 0.378+0.22), vitamin B12 concentrations appeared significant lowering (P value < 0.05) in patient's groups (314.1+113.7) when compared to control group (326.3+66.28). Also, vitamin B12 showed negative correlation with IgM anti-Toxoplasma gondii antibody but no correlation with age, IgG anti-Toxoplasma gondii antibody and BMI.

KEYWORDS

Toxoplasma gondii Toxoplasmosis Vitamin B12 Pregnancy

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Introduction

Toxoplasma gondii infect approximately one-third the global population and often undetected and remains clinically silent (asymptomatic in most immunocompetent hosts). But can develop acute disease manifest like fever, malaise, and posterior uveitis. (1,2).

T. gondii, the parasite which cause toxoplasmosis, reproduces through the asexual and the sexual cycles. The first cycle happens in true hosts, such as cats but the asexual cycle happens between humans and warm-blooded animals, such as birds (3).

Serological determination of IgM and IgG still the common test used to check pregnant women for toxoplasmosis. For acute infection IgM is used while indication immunity to the occurrence and severity of re-infection IgG levels is measured. The first antibodies which increase after 1 week of infection is IgM which used as a sign for acute infection while the Immunity against the incidence and severity of re-infection is indicated by IgG levels which seems after 2 weeks of infection (4).

During gestation vitamins, minerals, and trace elements are crucial for preserving mother health and fetal development (5).

Vitamin B12 is a water-soluble vitamin, it is known as Cobalamin because it contains the mineral cobalt (6,7). In plasma, vitamin is bound either to transcobalamin (around 20%) or bound to a circulating form of haptocorrin (the remaining major fraction). The transcobalamin-bound B12 fraction (holotranscobalamin) is internalized by CD320 antigen receptor or LRP2 receptor. Inside the cells, B12 is converted into the active form (8).

Vitamin B12 play an important role in hematopoiesis and maintenance nervous system health. Also, it keeps nerve and brain development and function. Maternal vitamin B12 is essential for fetal brain and spinal cord growth and function throughout pregnancy in order to prevent severe neural tube defect like: pina bifida (incorrect formation of baby's spine), Anencephaly (incorrect formation of brain and the spinal cord), Encephalocele (parts of the brain begins to drove out) (9,10). So, its deficiency can adversely affect both the mother and baby (11).

Insufficient studies have measured the direct correlation between B12 deficiency and toxoplasmosis in pregnant women, particularly in areas like Iraq where T. gondii is highly

prevalent. So, this research aims to study the impact of T. gondii infection on vitamin B12 levels in pregnant women and discovering the correlations with IgM and IgG antibodies.

Material and Method

This case-control study contains 60 pregnant women: 30 cases pregnant women with toxoplasmosis and 30 pregnant women without toxoplasmosis. All the participants aged 15 - 40 year. Women with previous abortion, hypertension, diabetes mellitus and any chronic disease were excluded from the study. Blood samples were collected after taking their permission in private laboratory. All samples were tested for IgG and IgM anti-Toxoplasma gondii antibody and vitamin B12 by enzyme-linked immune sorbent assay (ELISA). Age, weight and hight was recorded then body mass index BMI was calculated as follow:

$$BMI = \frac{weight(Kg)}{hight^2(m)}$$

Results

The age, body mass index (BMI), S. Vitamin B12, IgG and IgM anti-Toxoplasma gondii antibody of all groups are listed in table 1. The link between serum vitamin B12 and IgG, IgM anti-Toxoplasma gondii antibody in patients group are listed in table 2.

Table (1) The age, body mass index, S. Vitamin B12, IgG and IgM of all groups.

Parameter	Control	Patients	P-val
Age	36.55+14.33	33.7+10.9	0.431
ВМІ	29.5+4.27	31.88+5.14	0.233
Vitamin B12	326.3+66.28	314.1+113.7	0.002
IgG	0.347+0.190	4.065+1.55	0.0001
IgM	0.378+0.22	3.09+1.06	0.0001

All data are presented as the means \pm SD, P \leq 0.05 considered statistically significant. SD, standard diviation.

Table (2) The link between serum Vitamin B12 and toxoplasmosis IgG, IgM in patients group

		age	BMI	IgG	IgM	
Vit B12	r	-0.201	-0.122	0.031	-0.218	
	р	0.46	0.52	0.321	0.029	٦

The result showed no significant differences in age and BMI between healthy and patient's groups (p > 0.05). But the result revealed a high significant difference in S. Vitamin

B12 between control and patient's groups (p = 0.002). Also, there is very high significant differences in both IgG and IgM anti-Toxoplasma gondii antibody between control and patient's groups (p = 0.001). Also, the result showed a negative correlation between vit B12 and IgM anti-Toxoplasma gondii antibody.

Discussion

No enough studies were done to conduct the correlation of vitamin B12 and toxoplasmosis. This study found that serum vitamin B12 significantly decreased in pregnant women infected with toxoplasmosis when compared with control group. This result is agreed with Sayl and Alawadi study, 2024 which present a significant lowering of vitamin B12 in infected women (12).

The decreasing in serum vitamin B12 in pregnant women infected with Toxoplasma gondii can be affected by several factors which in relation with the infection's impact on nutrient metabolism and immune response:

- The infectious disease triggers oxidative stress and inflammatory reactions in pregnant women, which may elevate the body's requirement for antioxidants and minerals, potentially leading to a depletion of vitamin B12 levels. Studies have shown increased oxidative stress markers in T. gondii infected pregnant women, suggesting a higher consumption or lower availability of protective nutrients like vitamin B12 (13).
- T. gondii infection may affected by vitamin B12 metabolism or absorption and other related nutrients like folate, which are critical for cognitive function. This finding was supported by Berrett et al., 2017 (14).
- Although studied on vitamin D consider more common but infections like toxoplasmosis can interrupt multiple vitamins status such as vitamin B12 due to its effects on immune function, nutrient absorption, and metabolism (15,16).

Conclusion

This study is one of the infrequent studies that shows the changes of vitamin B12 levels in pregnant women infected with toxoplasmosis and measure its correlation IgM, IgG anti-Toxoplasma gondii antibody, Age and BMI.

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