Heliyon 10 (2024) e27018

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Biogenic synthesis of levofloxacin-loaded copper oxide nanoparticles using Cymbopogon citratus: A green approach for effective antibacterial applications

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ARTICLE INFO

Keywords:
Cymbopogon citratus
CuO NPs
Levofloxacin
Drug loading
Pathogens
Antibacterial

ABSTRACT

Despite the success of antibiotics in medicine, the treatment of bacterial infection is still challenging due to emerging resistance and suitable drug delivery system, therefore, innovative approaches focused on nanoparticles based antimicrobial drug delivery systems are highly desired. This research aimed to synthesize *Cymbopogon citratus* (*C. citratus*) aqueous extract-mediated copper oxide (CuO-Nps) conjugated with levofloxacin (LFX). The synthesized CuO NPs-LFX nano conjugate was confirmed by analysis using scanning electron microscopy (SEM), thermal gravimetric analysis (TGA), and infrared and ultraviolet/visible spectroscopy. Antibacterial activities were assessed in vitro through the agar well diffusion method against six bacterial strains of clinical relevance. CuO NPs confirmed by UV–Vis analysis absorption peak observed at 380 nm. TGA analysis showed 8.98% weight loss between the 400–800 °C temperature range. The functional group's presence was confirmed by FTIR analysis. Spherical shape nanoparticles with an average particle size of 55 nm were recorded by FESEM. Results from agar well diffusion assay showed that CuO NPs-LFX prohibited the development of both gram-positive and gram-negative bacteria at all established concentrations, and the antibacterial propensity was more pronounced as compared to bare CuO NPs, Levofloxacin and *C. citratus* aqueous extract alone. The results showed that gram-negative bacteria are more susceptible to CuO NPs-LFX nano conjugate and at 10 μgmL^{-1} concentration, form a 10.1 mm zone of inhibition (ZOI), whereas gram-positive bacteria on the same concentration form 9.5 mm ZOI. LFX-loaded CuO NPs antibacterial activity was observed higher than plant extract, bare CuO NPs, and standard drug (Levofloxacin). This study provides a novel approach for the fabrication of biogenic CuO NPs with antibacterial drug

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https://doi.org/10.1016/j.heliyon.2024.e27018
Received 6 October 2023; Received in revised form 20 February 2024; Accepted 22 February 2024
Available online 28 February 2024

levofloxacin and their usage as nano antibiotic carriers against pathogenic bacteria, especially antibiotic-resistant microbes.

1. Introduction

Considering worldwide medical advancements, infectious diseases brought on by invading pathogens still represent significant concerns about mortality and morbidity for world health. Antibiotic overuse and abuse have produced multidrug-resistant bacterial strains, which have contributed to the catastrophe [1]. According to their mechanisms, antibacterial nano-therapeutic techniques have been emphasized in the literature. In a recent review, Wang et al. discussed nanocarriers for antibacterial substances such as polymeric and MOF (metal-organic framework structures) nanoparticles that have associated with antibacterial activity and may increase activity as well as reduce the prevalence of antibiotic resistance [2]. The structure and functioning of NPs, especially metal NPs, is one of the interesting areas of nanotechnology study [3,4]. Because of its simplicity and capacity to display a variety of potentially important physical qualities, which are highly dependent on their form, sizes, and structure. CuO NPs have recently gained popularity over other NPs. An increasing number of pesticide formulations use copper and copper-based chemicals due to their strong biocidal properties, and several health-related uses are being investigated for and/or used [5]. These nanoparticles must possess special properties like extremely small dimensions and high dispersibility to produce enough copper-based compounds [6]. Due to the utilization of inexpensive resources, as well as its user and environmentally friendly attributes, alternatives to both physical and chemical procedures include biological synthesis. Species such as *Tamarindus indica*, *Phyllanthus amarus*, *Moringa oleifera*, *Azadirachta indica*, *Murraya koenigii*, *Centella asitica*, *Hibiscus rosasinensis* and were aided in the biosynthesis of CuO NPs previously [7–9]

The Lamiaceae family includes the aromatic medicinal herb *Cymbopogon citratus* [10]. Researchers have shown that *Cymbopogon* extracts can be used successfully to create metallic nanoparticles (NPs) such as silver, gold, copper, and cerium dioxide, which have shown advantages in terms of antibiosis and dye degradation [11,12]. Phytochemical analysis of *C. citratus* revealed that it possess several types of phytometabolites such as phenolic acid, flavonoids, stilbenes, tannins, alkaloids, proteins, lipids and sugars which can act as reducing, capping or stabilizing agents for biosynthesis of NPs [13]. The in vitro mechanism of nanoparticle synthesis mediated by plant extract involves nucleation, coarsening and bioreduction [14].

Levofloxacin (LFX) is a synthetic fluoro quinolone of the third generation with broad-spectrum antibacterial action that is used both intravenously and orally[15]. It works by preventing DNA gyrase and topoisomerase IV from functioning, making it effective alongside equally gram negative & gram positive bacteria [16]. Over the last few years, there have been numerous attempts to create innovative LFX loaded formulations using various polymers. several delivery mechanisms, such as nanoparticles [16,17]. In-situ gel with nanoparticles [18] nanohydrogels [19] have been studied to increase LFX bioavailability. It should be highlighted as well that evaluating the potential negative health effects linked to human exposure is crucial for encouraging the safe development of a new class of materials.

In the present study we extended our previous work of CuO NPs synthesis using *C. citratus* [20] to use it as drug delivery agent fabricated with levofloxacin (LFX) for potential antibacterial applications against pathogenic bacteria, which was not reported previously. We also characterized the effects of plant extract, CuO NPs, and LFX-loaded CuO nanoparticles on human pathogenic bacteria. The objectives of this study were to combine a therapeutic formulation with nanoparticles in vitro to increase drug availability, increase the effectiveness of drugs against gram-positive and gram-negative bacteria, and decrease the probability that resistance would develop.

2. Methods

2.1. Chemicals used

Chemicals of highest purity, such as sodium hydroxide (99%), nutritional agar, Levofloxacin, and clindamycin phosphate, were acquired through Sigma-Aldrich (Saint. Louis, MO, USA) and used directly without additional purification.

2.2. Preparation of extract

C. citratus plant leaves were collected from Chattar Kallas District Muzaffarabad and washed three times with DI water to remove dust particles and any other impurities attached. After washing, the plant leaves were dried on a cloth to remove extra water. For leaf extract preparation, the 40g of leaves were added to 500 mL DI water, kept at 45 °C until the color changed to lemon yellow. Whatman filter paper No.1 was used to filter the resulting crude extract, which was then cooled at room temperature. The clear extract was stored in the refrigerator in an airtight container.

2.3. CuO NPs synthesis

To synthesize CuO NPs, 1 g of copper chloride ($CuCl_2$) salt was dissolved in 100 mL of DI water, stirring for 20 min, and then adding 20 mL of *C. citratus* plant leaves extract by constant heating and stirring. The pH of the reaction mixture was maintained at 10 with the help of 0.2 M NaOH solution. After 30 min, the color of the solution changed from light green to greenish brown. The precipitates were

formed which were further kept at room temperature for 12 h. Following incubation, the water was removed from the surface using a pipette, and the wet precipitates were centrifuged three times using deionized water and then dried in an incubator at 37 °C. For future use, the dried NPs were kept in sealed containers. Using FTIR, TGA, SEM and UV–visible spectrophotometer analysis green synthesized nanoparticles were characterized.

2.4. Drug (levofloxacin) loading on synthesized CuO NPs

To prepare CuO NPs and levofloxacin conjugate, 1g of CuO NPs were dispersed in 20 mL of DI water to create a distinct suspension of nanoparticles. Levofloxacin 0.25g was dispersed in DI water to make a suspension. At room temperature, at 45 °C and 200 rpm, the particles were agitated for 40 min in a shaking incubator the drops of the levofloxacin solution were added under shaking. Reaction was continued until the formation of precipitates. The formation of precipitates indicates the formation of nanoparticles. Then these precipitates were collected and washed 3 times by using centrifuge (SBC0060–230V) at 2000 rpm. Collected NPs were dried at 37 °C in an incubator. For better dispersion in solvent, dried levofloxacin loaded CuO NPs further sonicated for 15 min using an ultrasonicator (Model W-225) at 1500 rpm. The filtrate dried in an incubator at room temperature, then placed in a polyethylene bottle for use. Drug-loaded nanoparticles were characterized using SEM and FTIR studies.

2.5. Characterization

Ultraviolet–visible spectrophotometer (Model Thermo Fisher Scientific Waltham, Massachusetts, USA, Shimadzu UV-800) which was used to study the light absorption between 200 and 800 nm. The chemical composition of the KBr pellet containing CuO NPs was investigated using an FTIR model (Nicolet 6700 Waltham, USA) in the 4000 to 400 cm-1 range. The surface morphology was investigated using a scanning electron microscope (SEM) model 5910 (Tokyo, Japan). A known quantity of CuO NPs heated to 900 °C with a 10 °C rise per minute using a PerkinElmer Model 6300 TGA analyzer (Hillsborough, NJ, USA) for thermogravimetric analysis (TGA).

2.6. Antibacterial assay

Utilizing agar well diffusion technique, the antibacterial activity of extract, pure and LFX-loaded CuO NPs was assessed according to the protocol [21]. From the Combined Military Hospital (CMH) Muzaffarabad, both gram-positive as well as gram-negative bacteria were isolated clinically. Six different bacterial species were selected for antibacterial screening in the current study: *Escherichia coli* (UTI), *Klebsiella pneumoniae* (Wound), *Serratia mercescens* (UTI) as gram-negative bacteria while *Staphylococcus aureus* (UTI), *Staphylococcus epidermidis* (UTI), and *Streptococcus pyogenes* (throat swab) as gram-positive bacteria. Various biochemical tests are used to identify all bacterial strains [22]. Pure cultures of all bacterial strains are kept in agar slants in a freeze-dried state and kept at 4 °C until use. All bacterial strains are subcultured overnight at 37 °C from their pure cultures on Mueller-Hinton Broth. The turbidity of the bacterial culture was adjusted to a freshly manufactured 0.5 McFarland turbidity standard [23] equivalent to (1.5×10^8 CFU ml^{-1}) bacteria. Under aseptic circumstances, sterile cotton swabs are used that swab each bacterial strain on agar plates made of Muller-Hinton, separately. A sterile (4 mm) polystyrene tip is used to create wells. Each well was loaded with different concentrations per volume of prepared solutions, i.e., 2, 5, 10 μg/mL separately, to investigate the effects of the solvent, plant extract, pure CuO NPs, and LFX loaded CuO NPs on the antibacterial activity. The impact of various doses was assessed for bare CuO NPs and LFX loaded CuO NPs. From all prepared concentrations 20 μL loaded separately in wells. The plates incubated at 37 °C overnight, and diameter of

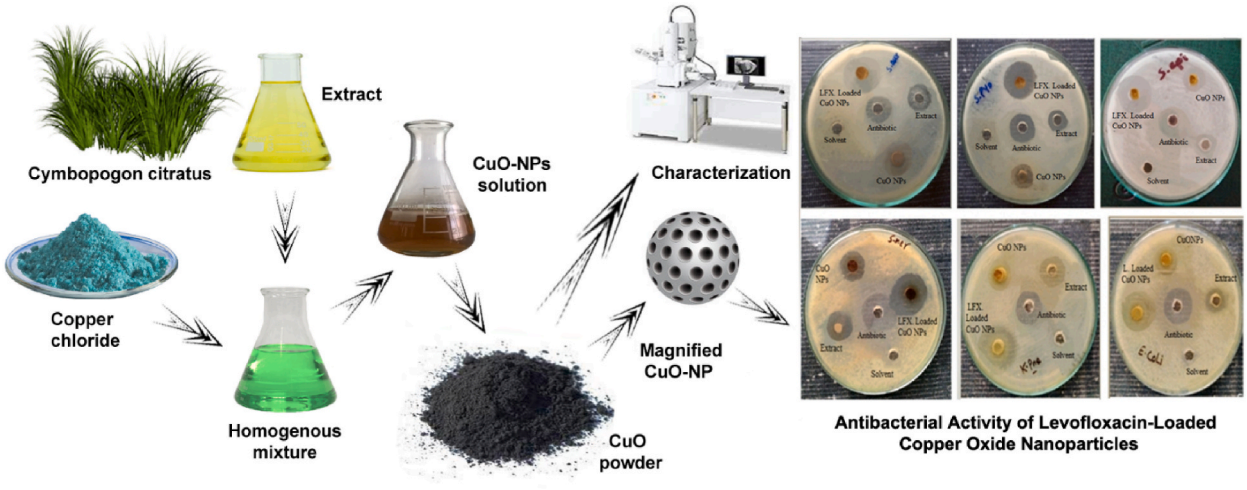

Fig. 1. Complete procedure from synthesis to applications-based study.

inhibition zone (ZOI) was measured with a calliper in millimetres. As a standard reference antibiotic, 20 μg/ml of clindamycin phosphate ($CliPO_4$) is used. Each experiment is performed three times (N = 3). The complete procedure followed from synthesis to analyzing antibacterial efficiency is demonstrated in Fig. 1.

2.7. Statistical analysis

Statistical analyses were conducted using GraphPad Prism version 9.2.0 (Graph[). To assess the efficacy of copper oxide nanoparticles (CuONPs) versus their LFX-modified variant (CuONPS-LFX) on Gram-positive and Gram-negative bacteria, a paired *t*-test was employed. The mean differences between the two treatments were evaluated, and a two-tailed p-value was calculated to determine statistical significance.

3. Results and discussion

Active phytochemicals in *C. citratus* extract are alcohols, long-chain hydrocarbons, ketone, esters, & aromatic compounds. Luteolin and its 6C and 7O apigenin, kaempferol, quercetin, isoorientin 2′ O-rhamnoside, glycosides, and isoorientin are significant flavonoids. It was discovered that hydroquinone, elimicin, catecol, chlorogenic acid, and caffeic acid are the main phenolic components in *C. citratus* [10]. Studies have shown that *Cymbopogon* extracts can be used successfully to create metallic nanoparticles (NPs) such as Cerium dioxide, silver, gold, copper, and other metals have shown to be beneficial for antibiosis and dye degradation [11]. The numerous uses of CuO NPs and the bioactives of *C. citratus* have prompted us to investigate the crude aqueous extract of *C. citratus* for the biosynthesis of CuO NPs, acts as a reducing, capping, and stabilizing agent. Levofloxacin loading could be a superior option for improving the surface modification of green synthesized CuO NPs' bactericidal activity.

3.1. UV. Vis spectrophotometry

Spectroscopy analysis performed using ultraviolet–visible light via electrostatic contact between a copper chloride solution and an aqueous extract, the investigation of the absorption spectra to identify structural changes and aggregate resulted in CuO nanoparticles formation. The absorption spectra (UV visible) of synthesized CuO-NPs recorded at wavelength ranging from 200 to 800 nm. CuO nanoparticles showed narrow and broad peaks at 265 nm and 380 nm, due to the resonance effects of surface plasmon. Leaf extract showed a broad absorption peak about 275 nm. Copper chloride is converted to CuO NPs by reduction in the leaf extract. Leaf extract phytochemicals have the potential to serve as the capping agent for the creation of stable CuO NPs. The absorption spectra of *C. citratus* extract and CuO NPs are displayed in Fig. 2. The outcomes match to those of the study of (D. K. [24]).

3.2. Thermogravimetric analysis (TGA)

CuO NPs' TG thermogram, which is depicted in Fig. 3, reveals an 8.98 percent loss of weight at two stages. Highly volatile chemical components and physically adsorbed water were lost during the initial, progressive weight loss, which took place in the 50–300 °C temperature range. The subsequent weight loss, which was observed at temperatures between 400 and 800 °C, is additionally related to the destruction of correlated bioorganic components that are found in the sample as a result of the usage of plant extract in the

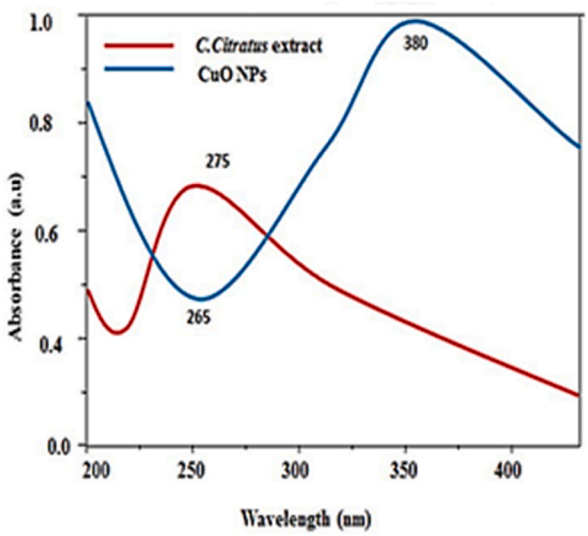

Fig. 2. Ultraviolet visible spectrophotometer images of *C.Citratus* plant extract and CuO NPs.

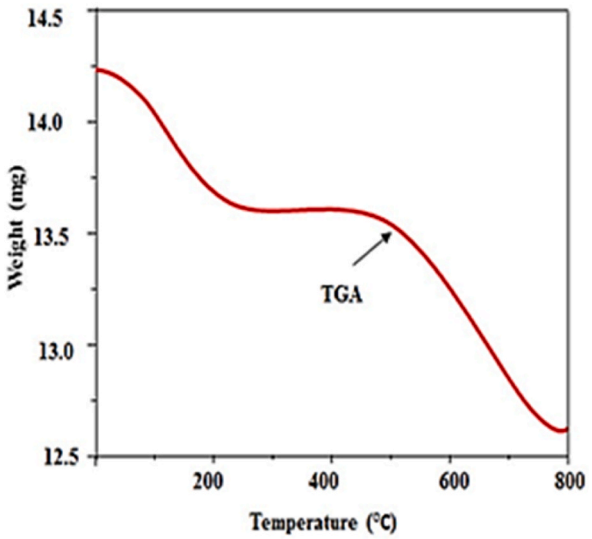

Fig. 3. Thermogravimetric analysis of CuO NPs.

creation of CuO NPs [25]. Our findings relate to ZnO nanoparticles that increased the PVA-CMC/ZnO nanocomposite EM loaded mat's thermal stability [26]. Higher thermal stability for CuO NPs is caused by strong contacts between the hydroxyl functional groups on extract and the CuO NPs.

3.3. Field emission scanning electron microscopy (FESEM)

Morphology and shape of bare and LFX loaded CuO nanoparticles are observed via FESEM analysis at two different magnifications (10 μm and 5 μm). The micrograph reveals that the particles had little agglomeration and were unevenly distributed due to drug loading. The majority of the particles have defined borders and come in a range of clear morphological sizes and forms. The defined boundaries were declining during drug loading, resulting in the particles covered in levofloxacin (LFX). CuO NPs' surface morphology resulting micrographs is shown in Fig. 4 (a, b) bare copper oxide nanoparticles Fig. 4 (c, d) LFX loaded copper oxide nanoparticles).

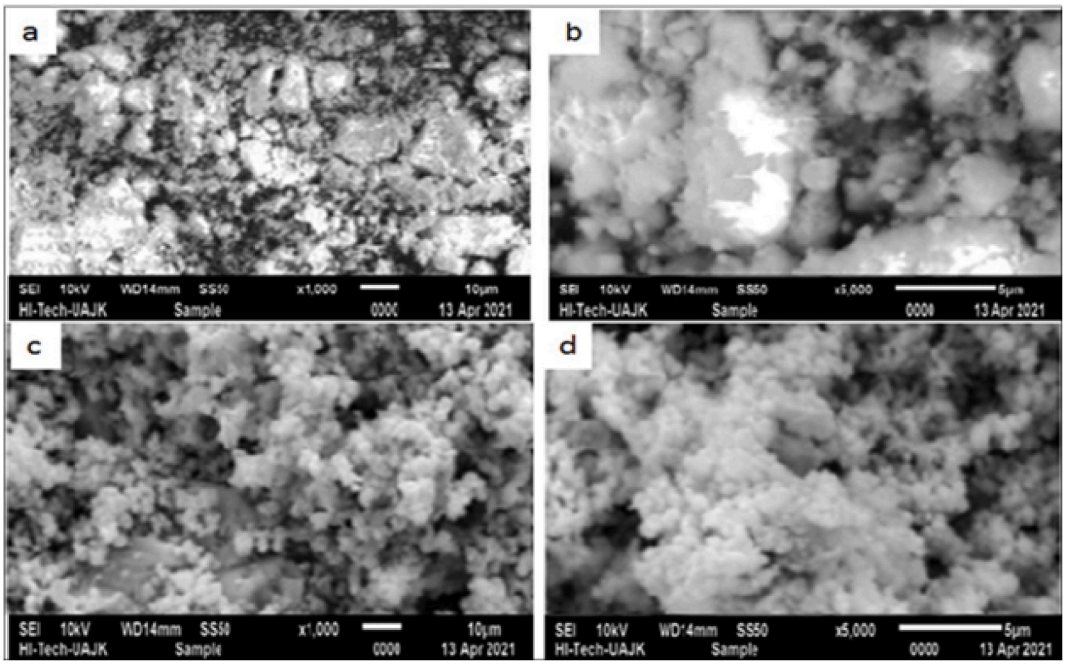

Fig. 4. SEM (a, b) CuO NPs at 10 μm and 5 μm magnification (b). LFX. Loaded CuO NPs at magnification 10 μm and 5 μm.

Due to the particles' uneven distribution, certain cavities may also be observed in the images. Higher magnification demonstrates a high rate of virtually spherical precipitation of the synthesized particles at increasing pH. The particles vary in size, which range from 42 to 65 nm, with a 55 nm average particle size.

3.4. Fourier-transform infrared spectroscopy (FTIR) analysis

Fig. 5 displayed FTIR spectra of green synthesized CuO NPs, Levofloxacin, and LFX loaded CuO NPs between 400 and 4000 cm^{-1} wavelengths, the spectra were captured. CuO NPs FTIR spectra showed peaks at 3510 due to the stretch of the aromatic primary amine N–H, 2175 due to the stretch of the thiocynate (-SCN), and 1190 because of bending O–H vibrations [27]. The stretching vibration of the Cu–*O*–Cu bond is the one that causes the band at 820 [28] While alkenes are represented by the peak at 2360, transmittance band C═C, C–F stretch is responsible for the peak at 1050. The N–H and O–H of phenolic compounds are represented by the bands that occurred in the spectrum of the drug-loaded sample at 3300 and 3600, respectively, while a narrow band is represented by the minor peak at 3670 [29].

3.5. Antibacterial activity

The antibacterial effect extract, CuO NPs and levofloxacin loaded CuO NPs evaluation of six different bacterial strains, including gram-positive (*S. pyogenes, S. aureus, S. epidermis*) while (*E. coli, K. pneumonia, S. mercescens*) as gram-negative bacteria. Various doses were used to treat the bacterial cultures (2,5,10 μg/mL) of leaves extract, pure CuO NPs, LFX loaded CuO NPs dispersed in Deionized (DI) water. Fig. 6(A-F) displays the zones of inhibition for all products against all bacterial species of both gram-negative and gram positive origin, while Table 1 provides a description of inhibition which shows the distinct zone of inhibition for the activity of the extract, CuO NPs, LFX loaded CuONPs and levofloxacin measured in millimetres (mm). The chosen bacterial species were examined to determine the dose-dependent activity and the experiment reveals that the activity increases with increasing concentrations of prepared NPs in the wells. Levofloxacin was used as standard drug for both types bacterial species (gram-positive and gram-negative). The antibacterial activity of *C. citratus* extract against both gram-positive and gram-negative bacterial strains is shown in Figs. 7 and 8. It was discovered that CuO NPs, activity was less than control substance, levofloxacin (9.3 ± 0.1 mm) as shown in Table 1. The maximum response was observed at 10 μg/mL concentration of CuO NPs. We observed that Gram positive bacteria at concentration 10 μg/mL *S. epidermis* displayed (8.2 ± 0.2 mm), while the least response at dose (2 μg/mL) is observed by *S. aureus* which displayed (4.6 ± 0.2 mm) while gram negative bacteria at highest concentration 10 μg/mL. *E. coli* displayed (9.3 ± 0.2 mm), while the least response at dose 2 μg/mL observed in *K. pneumonia* which showed (5.3 ± 0.05 mm), while LFX loaded CuO NPs have been reported to have more activity than regular drug and pure CuO Nps and plant extract. Gram positive bacteria *S. aureus* at maximum dose 10 μg/mL displayed (9.5 ± 0.2 mm) while the least response was observed by *S. pyogenes* at 2 μg/mL which showed (5.8 ± 0.05 mm) whereas in case of gram-negative bacteria the highest dose response was observed by *S. mercescens* at 10 μg/mL was (10.1 ± 0.14 mm) while least response at 2 μg/mL was observed by *K. pneumonia* which showed (7.4 ± 0.09 mm). Gram-negative bacteria are more easily destroyed by NPs than Gram-positive bacteria. Standard drug at highest (10 μg/mL) concentration show less activity (9.3 mm) than LFX loaded CuO NPs. The activity of plant extract, bare CuO NPs and drug loaded CuO NPs were combined studied for their effect under same conditions and concentrations.

The antibacterial effect of LFX loaded CuO NPs is more potent as compared to CuONPs alone and increases with increase in concentration.

Among gram positive negative bacteria, K. pneumonia form a ZOI of 7.4, 8.5, and 9.7(mm) diameter in response to 2, 5, and 10 μg/mL concentration of LFX loaded CuO NPs, respectively, whereas the same concentration of CuO NPs induce 5.3, 5.6 and 5.6 (mm) ZOI, respectively(Fig. 7A). Similarly *E.* form coli form ZOI of 7.6, 8.2, and 9.9 (mm) diameter in response to 2, 5, and 10 μg/mL

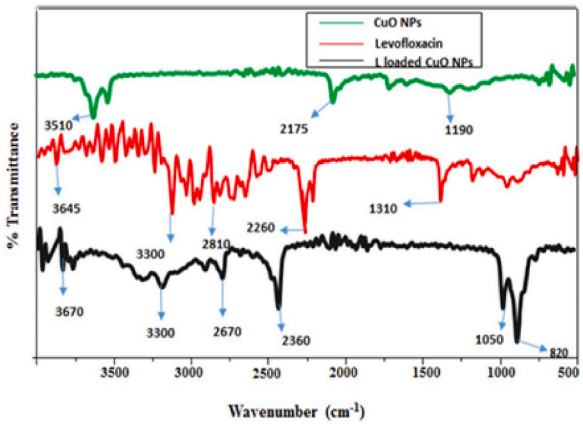

Fig. 5. FTIR spectra of CuO NPs, Levofloxacin antibiotic and LFX loaded CuO NPs.

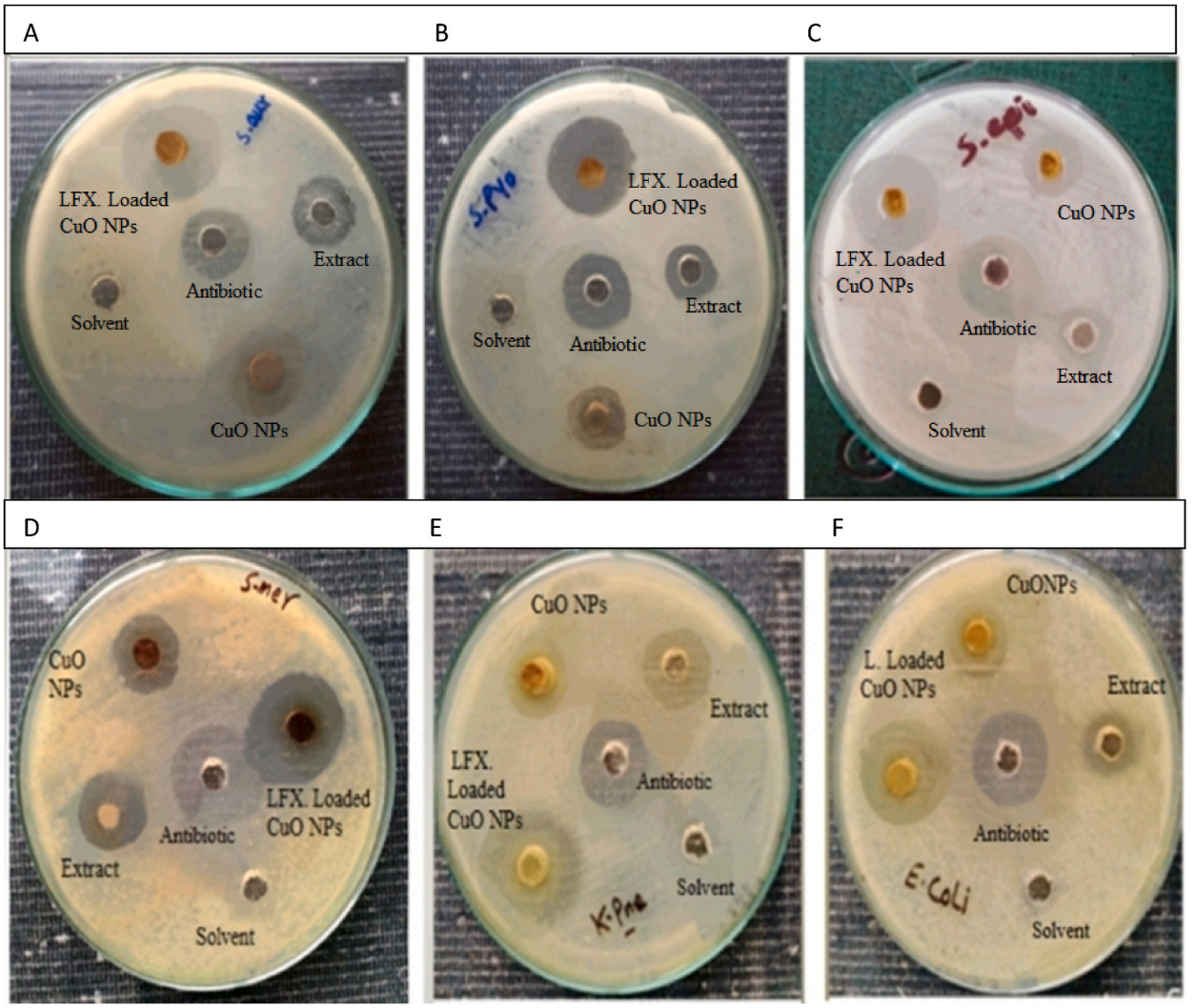

Fig. 6. Antibacterial activity of *C. citratus* extract, CuO NPs and LFX loaded CuO NPs A) *S. aureus* B) *S. pyogens* C) *E. epidermidis*, D) *S. mercescens* E) *K. pneumonia* F) *E. coli*, negative control (Solvent = DI water), positive control Levofloxacin, volume of solution = 20 μL.

Table 1
Average zone of inhibition (mm) formed by plant extract, CuO NPs and LFX loaded CuO NPs.

Samples	Concentrations (μgmL^{-1})	Gram positive bacteria			Gram negative bacteria		
		S. aureus	S. pyogens	*S. epidermis*	K. pneumonia	*E. coli*	S. mercescens
Plant extract	**2μgmL^{-1}**	4.6 ± 0.2	4.8 ± 0.0	4.4 ± 0.2	5.3 ± 0.7	5.2 ± 0.2	5.4 ± 0.2
	5μgmL^{-1}	4.6 ± 0.5	5.5 ± 0.4	5.6 ± 0.3	5.6 ± 0.6	5.5 ± 0.5	5.9 ± 0.4
	10μgmL^{-1}	5.2 ± 0.3	5.9 ± 0.4	5.3 ± 1.1	5.4 ± 0.4	5.6 ± 0.2	6.1 ± 0.3
CuO NPs	**2μgmL^{-1}**	4.52 ± 0.4	4.5 ± 0.4	4.3 ± 0.45	5.3 ± 0.05	5.3 ± 0.05	5.7 ± 0.3
	5μgmL^{-1}	5.3 ± 0.3	5.6 ± 0.2	5.7 ± 0.0	5.6 ± 0.1	5.6 ± 0.1	6.34 ± 0.2
	10μgmL^{-1}	7.4 ± 0.29	7.2 ± 0.2	8.2 ± 0.2	5.6 ± 0.0	5.6 ± 0.0	9.07 ± 0.1
LFX Loaded CuO NPs	**2μgmL^{-1}**	6.8 ± 0.08	5.8 ± 0.05	6.9 ± 0.6	7.4 ± 0.09	7.6 ± 0.12	7.7 ± 0.09
	5μgmL^{-1}	7.2 ± 0.26	7.6 ± 0.20	7.8 ± 0.1	8.5 ± 0.05	8.2 ± 0.29	8.4 ± 0.27
	10μgmL^{-1}	9.55 ± 0.2	9.15 ± 0.25	8.5 ± 0.5	9.7 ± 0.17	9.9 ± 0.28	10.1 ± 0.14
Levofloxacin	**10 μgmL^{-1}**	7.8 ± 0.19	8.5 ± 0.31	8.1 ± 0.33	8.4 ± 0.27	9.1 ± 0.3	9.3 ± 0.17

concentration of LFX loaded CuO NPs, respectively, whereas the same concentration of CuO NPs induce 5.5, 6.1 and 9.3 (mm) ZOI, respectively(Fig. 7B). Against *S. mercescens,* LFX loaded CuO NPs also form a greater ZOI than CuO NPs alone ((Fig. 7C).

Among gram positive bacteria, *S. aureus* form a ZOI of 6.8, 7.2, and 9.5(mm) diameter in response to 2, 5, and 10 μg/mL concentration of LFX loaded CuO NPs, respectively, whereas the same concentration of CuO NPs induce 4.52, 5.3 and 7.4 (mm) ZOI,

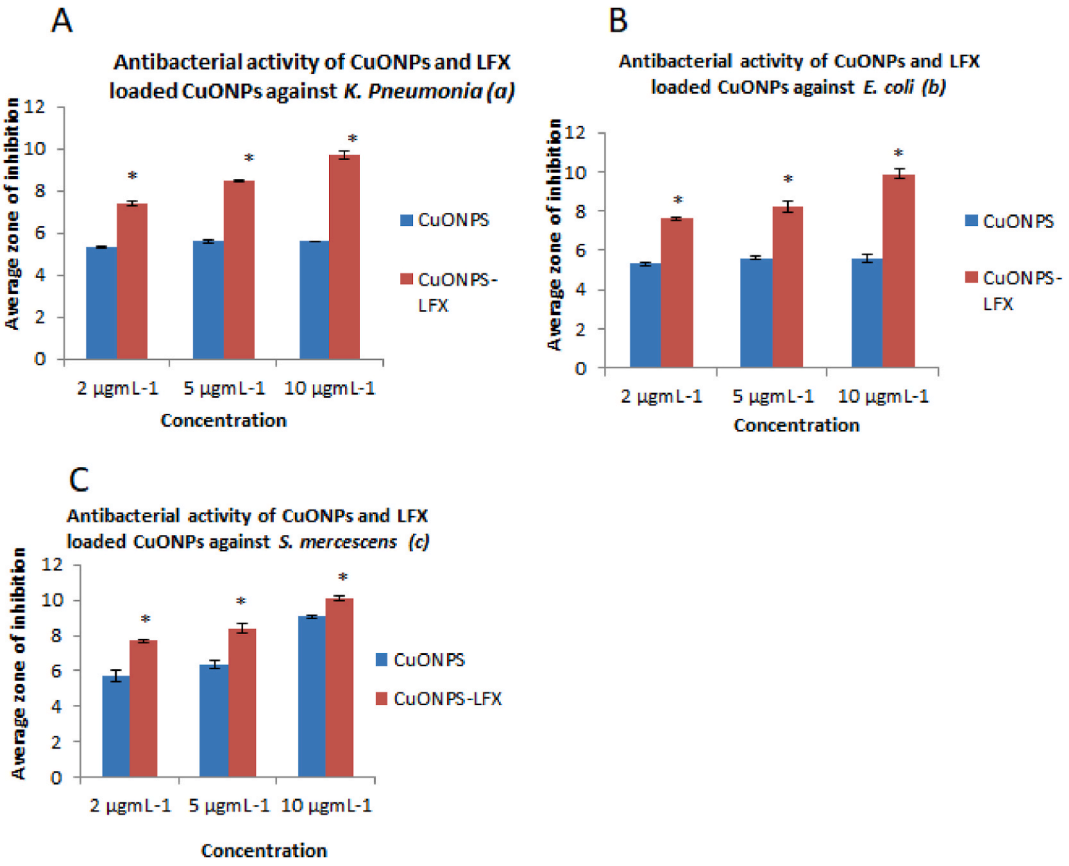

Fig. 7. CuO nanoparticles and LFX-loaded CuO NPs antibacterial efficacy against gram-negative bacterial species. (A) Comparative activity against *K. pneumonia*, (p-value 0.0348) (B) Comparative activity against *E. coli* (p-value 0.0388) (C) Comparative activity against *S. mercescens*, (p-value 0.0366).

respectively(Fig. 8A). Similarly *S. pyogenss* form a ZOI of 5.8, 7.6, and 9.15(mm) diameter in response to 2, 5, and 10 µg/mL concentration of LFX loaded CuO NPs, respectively, whereas the same concentration of CuO NPs induce 4.5, 5.6 and 7.2 (mm) ZOI, respectively (Fig. 8B). Against *S. epidermidis,* LFX loaded CuO NPs also form a greater ZOI than CuO NPs alone ((Fig. 8C).

The results showed an enhanced activity of LFX loaded NPs than the plant extract and CuO at different concentrations. The results also show the less activity of standard drug as compared to the LFX loaded nanoparticles.

The results of all test samples are consistent with past reports and effectively slowed down the growth of microorganisms [25,30]. The surface modification of green synthesized CuO with levofloxacin increased its efficiency to bacterial pathogens as shown in our results. Antibiotic (LFX) loaded CuO NPs demonstrated enhanced antibacterial activity as compared to a plant extract and bared nanoparticles when loaded in the same concentrations. Additionally, the CuO NPs loaded with LFX were found to be more effective than the reference medication (clindamycin phosphate) in our data. With 10 µg/mL concentration and a zone of inhibition of 9.21 mm, *S. mercescens*) was found to have the highest activity among Gram-negative bacteria. Differential cell wall and outer protective layer compositions of bacteria are associated with different antibacterial activity of NPs against certain bacterial species. Absence of an external protective layer may be the cause of CuO-NPs' enhanced action against Gram-negative bacteria. GPB's protective coating gives it more resistance to invading penetrating agents [31,32].

The following four-step mechanism underlies the antimicrobial effect of MNPs: (a) direct contact with the cell membrane; (b) change in membrane permeability and metal ions release, destabilization of the cell membrane (c) production of reactive oxygen species (ROS), (d) modulation of signal transduction pathways. Reactive oxygen species produced by the presence of copper (CuO) enter into the bacterial cell, to make it easier for copper (CuO NPs) to interact with the cell membrane. Disruptions brought on by CuO NPs in the bacterial cell membrane result in some faults in the bacterial cell, which inhibits bacterial species growth and may eventually end in their demise [33]. The bacterial cell membrane pore size in micrometer allows smaller sized CuO NPs in nanometers to easily penetrate into the cell membrane without any intervention [34]. CuO NPs may directly damage bacterial cell membranes by generating superoxide and hydroxyl radicals from metal oxides (CuO), as well as other radicals and reactive oxygen species [35]. The presence of carboxyl and amine groups on the surface of bacteria may attract Cu^{2+} ions to the cell. ([35,36]; J. [37]).Consequently, gene toxicity, damage to cells, or oxidative injury are all potential candidates for the antibacterial mechanism. Fig. 9 depicts the complete mechanism for potential toxicity of antibacterial agents.

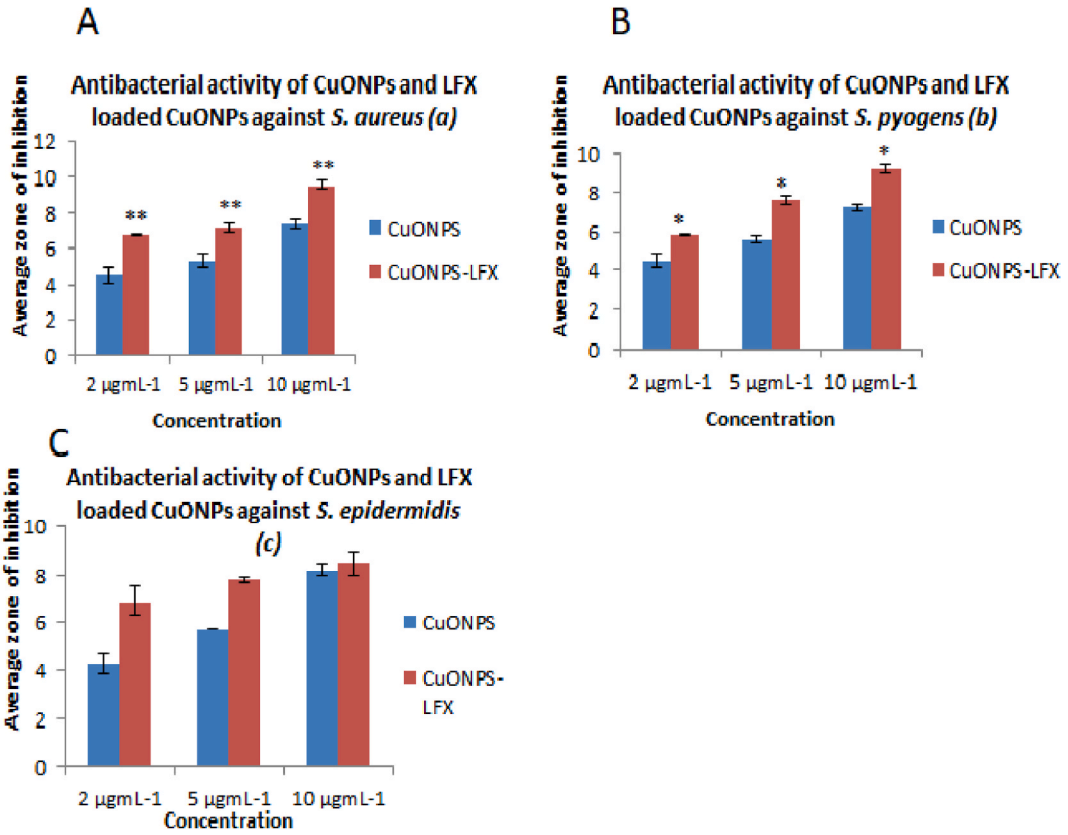

Fig. 8. CuO nanoparticles and LFX-loaded CuO NPs antibacterial efficacy against gram-positive bacterial species. (A) Comparative activity against *S. aureus*, (p-value 0.003) (B) Comparative activity against *S. pyogens* (p-value 0.0162) (C) Comparative activity against *S. epidermidis*, (p-value 0.1397).

Another possible mechanism affecting the antibacterial activity of green synthesized and antibiotic loaded nanoparticles are due to NPs that release Cu ions which interact with a crucial bacterial enzyme's thiol group, inactivating and ultimately killing microorganisms [31]. When light interacts with CuO NPs, it excites the electrons, which combine with absorbed oxygen to make oxygen ions, which then unite with an H_2O molecule to form H_2O_2. H_2O_2 enters the bacterial cell, impairs cytoplasmic functions, and kills the bacteria [38,39]. Positively, in our study, the concentrations employed for determining antimicrobial activity were at 2, 5, and 10. µg/ml, and the MIC values investigation were noted at 2 µg/ml. Since our CuO NPs-LFX nano-drug was created in the presence of Levofloxacin, it was effective at low concentrations and safe to use as an antibacterial agent. The combined effects of levofloxacin and copper ions were thought to be responsible for the increased activity. Gram negative bacteria were shown to be more bactericidal than Gram positive bacteria, and the order of activity observed in samples was LFX loaded CuO NPs > Bare CuO NPs > Plant extract.

3.6. Comparison with literature

The extracted data is summarized in Table 2 along with a comparison of the antibacterial activity of the nano drug produced CuO NPs reported in this work with those of other metal oxide NPs described in the literature. It is evident from the literature that only two to four bacterial species have been the subject of other investigations in the same field. The effectiveness of the *C. citratus* extract mediated CuO NPs and surface modified LFX loaded CuO NPs is highlighted by this data. Results clearly show that more zones of inhibition were seen in the current study than in the literature. The new aspect of our findings is the absence of any information about the formation of levofloxacin loaded CuO NPs in any published research.

4. Conclusion

To take advantage of the gram-positive and gram-negative bacteria targeting antibacterial activity, the compound LFX loaded CuO NPs nano drug was successfully produced in the current study using a modified procedure that proved successful in preserving the physicochemical characteristics of synthesized CuO nanoparticles. The advantages of nano sized CuO-NPs made of antibiotic and surface-coated with plant extract provide good nano carrier capabilities. This innovative synthesis of drug loaded CuO NPs has not been previously documented in the literature. The LFX loaded CuO had more effective antibacterial action against gram negative

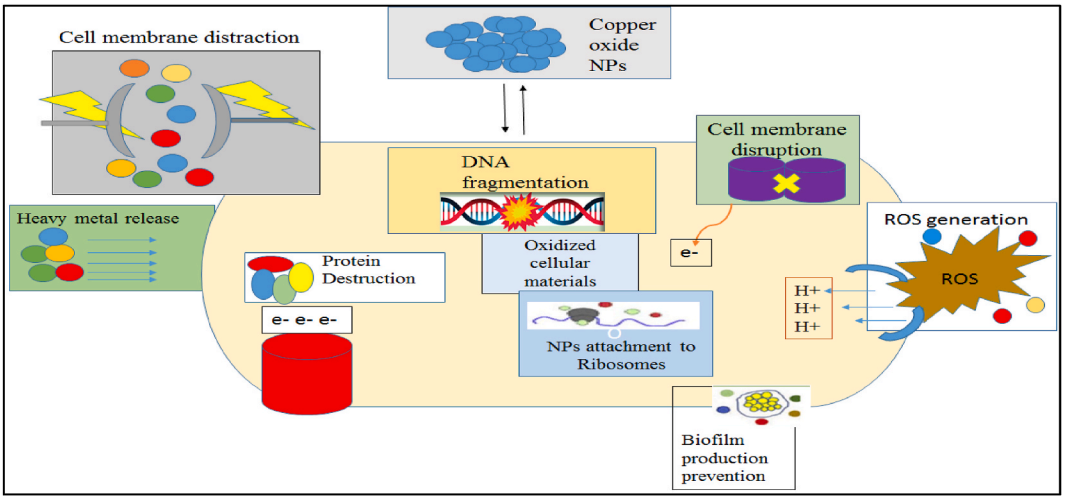

Fig. 9. Probable mechanism of toxicity induced by CuO NPs against bacteria.

Table 2
Comparison of the antibacterial efficacy of metallic NPs made with various medicines and using LFX-loaded CuO NPs synthesized in the current investigation to treat diverse infections.

NPs	Drugs	Antimicrobial activity	References
Copper oxide NPs	Levofloxacin	*S.aureus, S. epidermis, S. pyogenes, E.coli, S.mercescens* and *K. pneumonia*	Current study
Iron oxide NPs	Rifampicin and Tetracycline hydrochloride	*E. coli and S. aureus.*	[40]
Kefiran nanofibers	Doxycycline	*S. aureus and E. coli*	[41]
Citrate-capped AgNPs	Vancomycin	Gram-negative bacteria (*E. coli*), Gram-positive bacteria (*S. aureus*)	[42]
AgNPs	Neomycin, Typhimurium, Tetracycline, Enoxacin & Kanamycin	Microorganisms (*Salmonella*)	[43]
AgNPs	Tetracycline	*K. pneumoniae* and *S. aureus*	[44]
AgONPs	Moxifloxacin	*A. Niger, C. albicans, P. aeruginosa fungi. B. subtilis, S. aureus, E. coli bacteria*	[45]
AgO NPs	Ciprofloxacin	*S.aureus, E.coli*	[46]

bacteria in comparison to gram positive bacteria when compared to the conventional drug, bare CuO NPs, and plant extract. The combination action of the medication and nanoparticles increased the activity against the tested bacteria.

Data availability statement

All the relevant data supporting this article have been included in the manuscript.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Amina Jabeen: Writing – original draft, Software, Investigation, Formal analysis, Data curation. **Abdulhameed Khan:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Formal analysis. **Pervaiz Ahmad:** Writing – review & editing, Visualization, Validation, Resources, Methodology. **Awais Khalid:** Writing – review & editing, Writing – original draft, Validation, Software, Methodology. **Maha Saeed Ibrahim Wizrah:** Methodology, Software, Validation, Writing – original draft, Writing – review & editing. **Zeeshan Anjum:** Writing – review & editing, Writing – original draft, Validation, Resources, Formal analysis. **Satam Alotibi:** Writing – review & editing, Visualization, Validation, Methodology. **Bandar Hamad Aloufi:** Writing – review & editing, Visualization, Validation, Software, Methodology. **Abdulaziz M. Alanazi:** Writing – review & editing, Visualization, Validation, Software, Resources, Investigation. **Ohoud A. Jefri:** Writing – review & editing, Writing – original draft, Validation, Software, Methodology. **Mohamed A. Ismail:** Writing – review & editing, Visualization, Validation, Resources, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors extend their appreciation to the Deanship of Scientific Research at King Khalid University for funding this work through large group Research Project under grant number- RGP2/377/44.

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