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Original Article

The Impact of Berberine Loaded Selenium Nanoparticles on *K. pneumoniae* and *Candida albicans* Antibiotics Resistance Isolates

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Abstract

This study aimed to investigate the antibacterial and antifungal activities of selenium nanoparticles (SeNPs) and berberine (BBR) despite antibiotic resistance against *Klebsiella pneumoniae* and *Candida albicans*. Cells of *K. pneumoniae* and *C. albicans* were treated with solutions of different concentrations of each bare SeNPs, BBR, and BBR-loaded SeNPs (BLS) using the disk diffusion method. The results indicated that the activities of SeNPs, BBR, and BLS were statistically significant (P<0.05) when the concentration of all agents increased. Moreover, it was found that BLS had a statistically significant effect against *K. pneumoniae* and *C. albicans*, compared to SeNPs and BBR alone (P<0.05). The largest zones of inhibition of SeNPs were 14 and 16 mm toward *K. pneumoniae* and *C. albicans*, respectively, at the concentration of 20 Mml, compared to the concentrations of 10 and 15 Mml. Furthermore, BBR showed a maximum zone of inhibition at the concentration of 1,200 mg (15 mm for *K. pneumoniae* and 18 mm for *C. albicans*) and it was statistically significant in comparison with other concentrations of 400 and 800 mg. In addition, the BLS underwent a statistically significant increase (P<0.05) when the concentration increased and it registered a large zone of inhibition of 22 and 25 mm against *K. pneumoniae* and *C. albicans*, respectively, at 20 Mml of SeNPs: 1,200 mg BBR, compared to 10 Mml of SeNPs: 400 mg BBR and 15 Mml of SeNPs: 800 mg BBR. Based on the results of the current study, there was a statistically synergistic effect of BBR-loaded SeNPs, compared to that of BBR and Se nanoparticles, only in the case of both *K. pneumoniae* and *C. albicans*. This study is promising as a blueprint for the enhancement of weak antimicrobial agents and their return to their previous role as antibiotics.

Keywords: Berberine, *Candida albicans*, *K. pneumoniae*, Selenium nanoparticles

1. Introduction

Currently, selenium is recognized as a crucial micronutrient of human and animal vigor. Usage of selenium nanoparticles (SeNPs) has considerably increased in the scientific population typically via the study of their antitumor action; however, an excessive potential of this nanomaterial has been recently documented concerning its antibacterial and antifungal activities (1). Berberine (BBR) is a quaternary benzylisoquinoline alkaloid with many pharmacological impacts on the treatment of high blood pressure, cancers, microbial diseases, inflammations, human immunodeficiency virus, and cardiac illnesses. In medical trials, BBR is harmless at extreme dosages, however, it establishes poor bioavailability. Main deficiencies, such as inadequate absorption, fast breakdown, and quick systemic removal are responsible for lower BBR plasma and tissue levels.

Numerous investigators have assumed numerous approaches to overwhelm this matter and improve the bioavailability of BBR. These approaches include new preparation strategies; original medicine transfer systems, such as liposomes, nanosized dose formulae, phospholipid developments, mucoadhesive microparticles, and micro-emulsions; employment of adjuvants; and the strategy of the structural equivalent of BBR (2). *Klebsiella pneumoniae* is recognized to be ubiquitous in wildlife, one of the greatest pertinent opportunistic bacteria, which is a source of numerous human infections, like bloodstream infection, urinary tract infection (UTI), surgical-site infection, and pneumonia (3). Infections owing to multidrug resistance (MDR) and biofilm producers of *K. pneumoniae* are related to higher morbidity and mortality rates, compared to infections in sensitive isolates (4).

Fungi have appeared as the main source of human illnesses, and *Candida* persists as the greatest public cause of aggressive fungal infections in hospitalized patients due to numerous medical indexes, extending from mucocutaneous infections to aggressive illnesses. There are more than 150 recognized species of *Candida*, but *Candida albicans* are accountable for most of the fungal infections, spreading in diverse environmental areas with the possibility of growing antifungal resistance (5).

Recently, transmittable infectious diseases caused by pathogens have become a main source of morbidity and mortality worldwide owing to their resistance to several antibiotics. This has prompted creativities to grow original, alternative antimicrobial resources in order to resolve the problem of infection with multidrug-resistant pathogens. The nanomaterial-based formulation is concerned with its promising physicochemical characteristics, antimicrobial features, and brilliant biocompatibility (6).

The present study aimed to evaluate the activity of BBR loaded with SeNPs as potential antibacterial and antifungal agents through the chemical reduction synthesis of sodium selenite with ascorbic acid in the presence of vitamin E TPGS as a surface active agent. Afterward, the synthesized nanoparticles suspension was characterized in terms of spectrometry and morphology. Moreover, they were applied with some microorganisms, such as *K. pneumoniae* and *C. albicans* to investigate the synergistic antimicrobial activity of BBR-loaded SeNPs (BLS), compared to BBR and Se nanoparticles alone.

2. Materials and Methods

2.1. Synthesis of Selenium Nanoparticles

Ascorbic acid (AA) was used as a reducing agent for the synthesis of SeNPs. The solution of 0.3 M AA was freshly prepared by dissolving 0.529 g AA powder in 10 mL of deionized water. Afterward, an aliquot of vitamin E TPGS was mixed with 5 mL of 5 Mm of sodium selenite under magnetic stirring, and 5 mL of 0.3 M ascorbic acid was added drop by drop into the mixture to initiate the reduction. Subsequently, 10 ml of deionized water was added, and the solutions were gradually converted from colorless into intense orange-red by stirring for 24 h, followed by dialysis against 10-time-diluted phosphate buffer at pH 7.4 for 1 h (7).

The BLS was also prepared by the addition of equal amounts of SeNPs to equal amounts of BBR (1:1 volume ratio) in a conical flask, which was stirred for 30 min and then sonicated for 3 min at 30% amplitude of maximum power. The combination was centrifuged three times for 1 h at 14,000 rpm to eliminate unconjugated BBR. The final precipitate was suspended with distilled water for up to 20 ml to keep the fixed concentration of SeNPs in suspension.

2.2. Characterization of Selenium Nanoparticles

The SeNPs were characterized by numerous tests, including UV visible analysis, X-ray diffraction, and scanning electron microscope (SEM) (8).

2.3. Preparation of Concentrations

Different SeNPs and BBR concentrations were prepared using the serial dilution method according to the equation $V1 \times C1 = V2 \times C2$. Concentrations of SeNPs were 10 mM, 15 mM, and 20 mM, while the concentrations of BBR were 400 mg, 800 mg, and

1,200 mg. The concentrations in BLS were 10 Mml SeNPs: 400 mg BBR, 15 Mml SeNPS: 800 mg BBR, and 20 Mml Se NPs: 1,200 mg BBR.

2.4. Bacterial and Fungal Isolates

In total, 100 UTI infections were collected to identify *K. pneumoniae*, while 20 specimens of vaginal infections were obtained to identify *C. albicans*. It should be mentioned that the Vitek2 system made the identification. The *K. pneumoniae* and *C. albicans* isolates were tested against several antibiotics by disk diffusion method (Clinical and Laboratory Standards Institute, 2021) to determine their antibiotic resistance patterns (MDR, extensively drug-resistant, and pandrug-resistant). Afterward, the most resistant isolates were used to evaluate the activity of BBR, SeNPs, and BLS.

2.5. Determination of Zone of Inhibition

All the prepared concentrations of SeNPs, BBR, and BLS were used against MDR *K. pneumoniae* and *C. albicans* isolates. The disk diffusion method depended on this study, and the zone of inhibition was determined using a ruler. All tests for all concentrations were performed in triplicate (9).

2.6. Statistical Analysis

The statistical analysis was performed in SPSS software (version 28). Moreover, one-way analysis of variance analysis was used to determine the least significant difference values (P<0.05). It should be mentioned that the mean values were used to compare the results.

3. Results and Discussion

3.1. Synthesis and Characterization of Selenium Nanoparticles and Berberine-Loaded Selenium Nanoparticles

The SeNPs were chemically synthesized using the reduction method. Ascorbic acid was used as a reducing agent for the synthesis of SeNPs as it is safe and biocompatible in case of utilization with pharmaceutical formulations and capable of reducing selenite ions to form elementary selenium. However, the reduction could cause aggregation of selenium particles unless a surface active agent, such as vitamin E TPGS, is used to avoid the aggregation and agglomeration of selenium particles and form SeNPs instead. Such surfactants could help to make particles with a nanoscale range. The formed SeNPs were confirmed through the gradual color change from colorless into intense orange-red with stirring for 24 h.

Furthermore, dialysis was used to purify the prepared SeNPs and eliminate reactants, such as ascorbic acid and surfactant. These results are fully compatible with those of a study performed by Al-Shreefy, Al-Awady (7) in terms of particle size distribution, morphology, and optical properties. Therefore, BLS was achieved through the conjugation of SeNPs with BBR since electrostatic attraction forces between negatively charged SeNPs and cationic BBR were utilized.

Chemical synthesis of SeNPs from sodium selenite was tested by UV-Vis spectra, and the result showed the color changing from colorless sodium selenite to ruby red color in absorption maximum (kmax) at 200-300 nm. This is illustrated in figure 1 (blue line) as well as the conjugation of BBR into SeNPs (red line). The UV-visible spectroscopy performed on all the samples demonstrated that different aliquots were mainly absorbed in the visible violet-blue-green region of the spectrum. Hence, the samples exhibited a complementary color located in the orange-red region of the spectrum. This result was in agreement with those of a study conducted by Khiralla and El-Deeb (10).

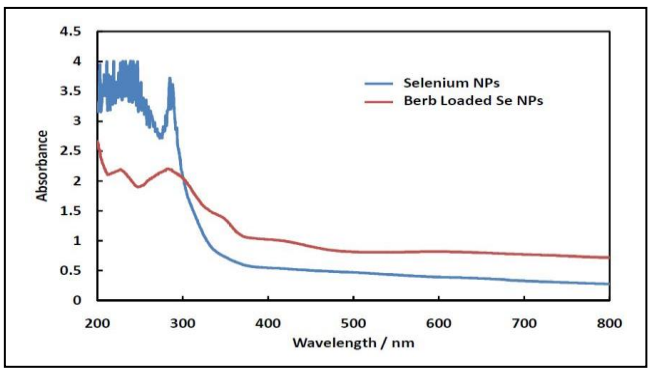

Figure 1. The UV- visible spectrum of chemical synthesis of Se nanoparticles upon reduction with ascorbic acid as a reducing agent and vitamin E TPGS as a stabilizing agent

The X-ray powder diffraction (XRD) pattern of SeNPs has shown one diffraction peak at around 23 degrees. The XRD pattern depicted that the dried sample of SeNPs was possibly nano-crystalline, which is in line with that of the standard selenium powder approving the formation of selenium. The particle size, D, was calculated based on the XRD data by using Scherrer's formula. Moreover, the crystallite size was calculated at 20 nm (11) as illustrated in figure 2. The XRD data confirmed that the formation of crystalline SeNPs showed broad and intense diffraction peaks with less intensity, which reflected the unprocessed crystallographic phase of SeNPs.

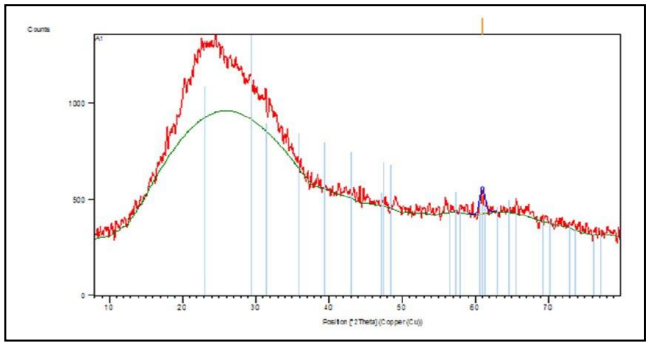

Figure 2. X-ray diffraction patterns (XRD) of chemical synthesized selenium nanoparticles (Se NPS) using ascorbic acid as a reducing agent in the presence of vitamin E TPGS as a surfactant

The SEM images of SeNPs were achieved using a SEM device, and the results are provided in figure 3. The photomicrograph of SeNPs confirmed the data of an almost monodispersed suspension of selenium nanoparticles with spherical shapes.

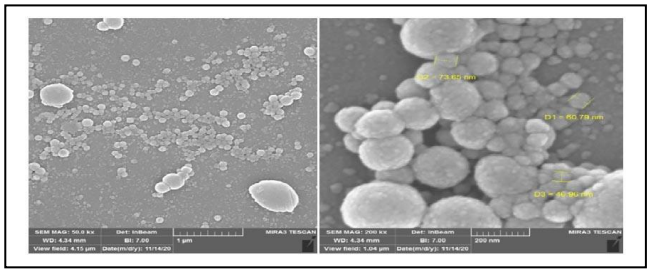

Figure 3. Scanning Electron Microscope images of selenium nanoparticles (Se NPs) synthesized by chemical reduction of sodium selenite with ascorbic acid using vitamin E TPGS as a surfactant

The SeNPs have shown round shape nanoparticles with a size of 60±15 nm, which is in line with the findings of a study performed by Verma and Maheshwari (12). Therefore, SEM revealed agglomerated spherical images with the spherical shape of SeNPs (13).

3.2. Identification of Bacterial and Fungal Isolates

The *K. pneumoniae* and *C. albicans* isolates were identified using the Vitek2 system. The results revealed that 34 (34%) isolates out of 100 specimens of UTI infection belonged to *K. pneumoniae*, 54 (54%) isolates other than *K. pneumoniae*, and 12 (12%) specimens showed nonbacterial growth. Moreover, 18 (45%) isolates out of 40 specimens of oral infections belonged to *C. albicans*, 14 (35%) isolates belonged to pathogens other than *C. albicans*, and 8 (20%) of specimens showed non-fungal growth as summarized in table 1.

Table 1. The prevalence of *K. pneumoniae* and *C. albicans* isolated from UTI and oral infections, respectively

UTI specimens (100)		
K. pneumoniae 34 (34%)	Other bacterial species 54 (54%)	Nonbacterial growth 12 (12%)
Oral infection specimens (40)		
C. albicans 18 (45%)	Other fungal species 14 (35%)	Non-fungal growth 8 (20%)

3.3. Antibiotics Resistance Pattern of *Klebsiella pneumoniae* and *Candida albicans*

Results of antibiotics resistance showed that all isolates of *K. pneumoniae* were MDR 34 (100%) while 7 (38.33%) of *C. albicans* isolates were MDR. The highest percentage of resistance was against Amoxicillin (100%) and Miconazole (83.33%) for *K. pneumoniae* and *C. albicans*, respectively. Meanwhile, the lowest percentage of resistance was 5.88% toward Imipenem for *K. pneumoniae* and 16.66% toward Amphotericin for *C. albicans*. Other percentages were within the range of 83.23-85.29% for *K. pneumoniae* and 72.22-22.22% for *C. albicans*, as presented in figures 4A and 4B.

The results of the *K. pneumoniae* were consistent with the findings of several previous studies (14-16). Furthermore, the resistance outcomes of *C. albicans*

were in agreement with those of some researchers (17-19). The variety of population revelation to antimicrobials with their germ-free culture and the nature of clinical specimens that were studied were considered the main motives for the different occurrence rates of microbial resistance in various studies (20).

Several factors affect the growth of antibiotic resistance, such as the use of antibiotics in the community, hospital, environment, agriculture, and animal production. Furthermore, since there is a possibility to buy antimicrobial agents without a prescription, they can be used excessively. Prolonged and intensive use of antibiotics in healthcare settings is considered a significant factor affecting the widespread of severe and dangerous nosocomial infections (21).

3.4. Evaluation of the Activity of Selenium Nanoparticles, Berberine, and Berberine-Loaded Selenium Nanoparticles

3.4.1. Against Multidrug-Resistant *Klebsiella pneumoniae*

The activity of SeNPs, BBR, and BLS was evaluated against MDR isolates of *K. pneumoniae*. The results revealed statistically significant differences (P<0.05) among the different concentrations of each agent, as shown in figures 5A, 5B, and 5C. The BBR showed the highest zone of inhibition (15 mm) at 1,200 mg, compared to the other two concentrations of 400 mg (6 mm) and 800 mg (12 mm) (Figure 4A). The sizes of the zones of inhibition of SeNPs were 0.0, 10, and 14 mm for 10, 15, and 20 mM, respectively (Figure 4B).

The used BBR was loaded with SeNPs (BLS), and its zone of inhibition statistically increased when the concentration increased and reached its highest value (22 mm) at 20 Mml: 1,200 mg, compared to the concentration of 10 Mml: 400 mg and 15 Mml: 800 mg where the zones of inhibition were 8 and 17 mm, respectively (Figure 4C). In addition, the 1,200 mg of BBR and 20 Mml of SeNPs showed statistically non-significant differences, while the BLS concentration of 20 Mml: 1,200 mg was statistically significant at P < 0.05, which indicated the synergistic impact of SeNPs when used in combination with BBR.

Sizes of inhibition zones were 15 mm, 14 mm, and 22 mm for 1,200 mg BBR, 20 Mml SeNPs, and BLS 20 Mml: 1,200 mg, respectively (Figure 4D). The outcomes illustrated in Figures 6A, 6B, and 6C confirmed the effect of the combination of SeNPs with BBR (BLS) at different concentrations when compared with SeNPs and BBR alone. Moreover, the zone of inhibition of BLS statistically improved at P < 0.05 as the concentration increased.

Filipović, Ušjak (3) documented that SeNPs had an antimicrobial impact on several gram-positive and gram-negative bacteria, including *K. pneumoniae*, and their results agreed with those of the current study. Findings of a study conducted by Rangrazi, Bagheri (22) showed that *K. pneumoniae* isolates were the most sensitive to SeNPS among gram-negative bacteria. Another study presented the antimicrobial effect of SeNPs on *K. pneumoniae* isolates which was compatible with those of the present study (9).

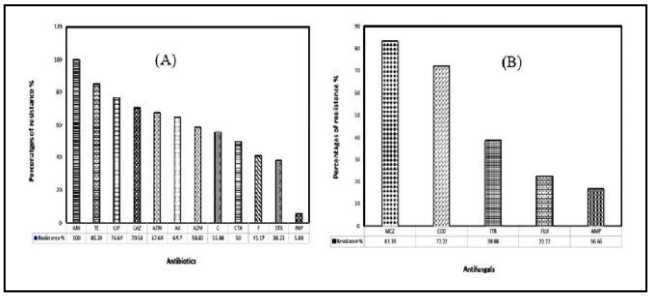

Figure 4. The percentages of antibiotics resistance tests: (A) for *Klebsiella pneumoniae* [(AM) Amoxicillin, (TE) Tetracycline, (CIP) Ciprofloxacin, (CAZ) Ceftazidime, (ATM) Aztreonam, (AK) Amikacin, (AZM) Azithromycin, (C) Chloramphenicol, (CTX) Cefotaxime, (F) Nitrofurantoin, (STX) sulfathiazole- trimethoprim, (IMP) imipenem]; (B) for *Candida albicans* [(MCZ) Miconazole, (ECO) Econazole, (ITR)Itraconazole, (FLU) Fluconazole and (AMP) Amphotericin]

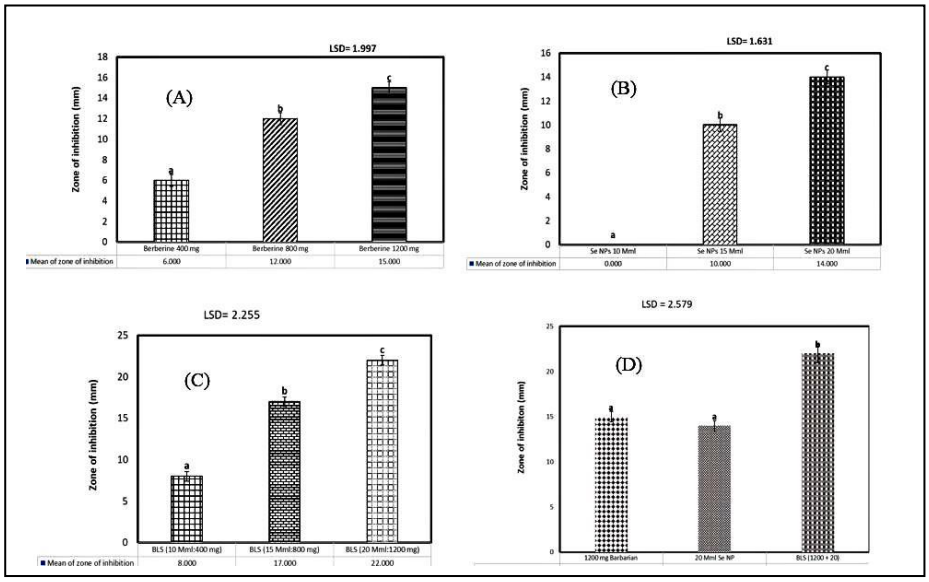

Figure 5. Mean of the zone of inhibition (mm) of XDR *Klebsiella pneumoniae*: **(A)** against berberine (400 mg, 800 mg and 1200 mg); **(B)** against selenium nanoparticles (Se NPs) (10 mM, 15 mM and 20 mM); **(C)** against Berberine loaded with selenium nanoparticles (BLS) (10 mM:400 mg, 15 mM:800 mg) and 20 mM:1200 mg). Different letters indicated statistically significant at P< 0.05, and similar letters indicated no statistically significant at *P*< 0.05

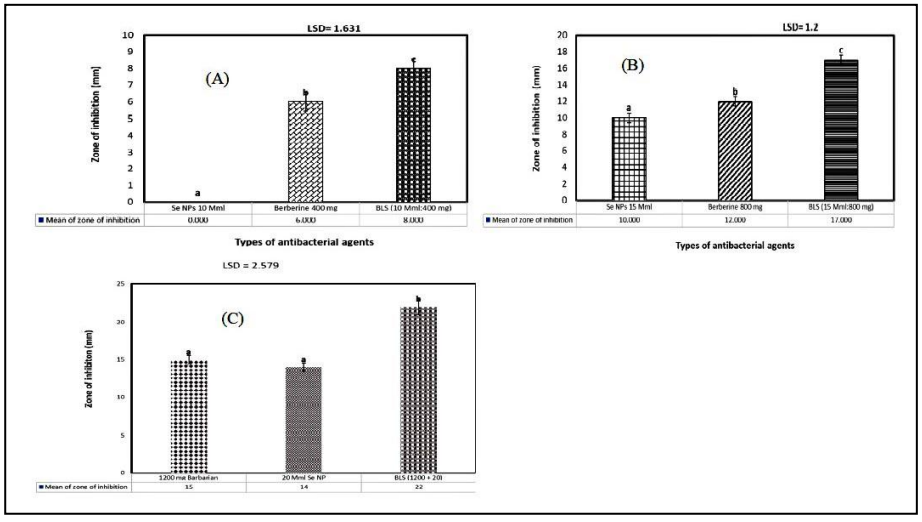

Figure 6. Comparison of Mean of the zone of inhibition of XDR *Klebsiella pneumoniae*: **(A)** against (Se NPs 10 Mml, berberine 400 mg, and BLS 10 Mml:400 mg); **(B)** against (Se NPs 15 Mml, berberine 800 mg and BLS 15 Mml:800 mg); **(C)** against (Se NPs 20 Mml, berberine 1200 mg and BLS 20 Mml:1200 mg). Different letters indicated statistically significant at P< 0.05, and similar letters indicated no statistically significant at P< 0.05

Employment of nanomaterials as antibacterial compounds is an excellent policy, particularly when transacting with chronic and hospital infections. The extensive employment of marketable antibacterials has developed MDR bacterial pathogens. Normally, numerous mechanisms of nanomaterial antibacterial action are documented, such as reactive oxygen species (ROS) generation, contact with cell walls by the alternation of permeability, cessation or decrease of the creation of proteins and DNA, and expression of genes responsible for breakdown processes (23).

When antibacterial action is accredited to ROS producers, these ROS classes can extra prevent DNA replication and amino acid creation and promote disruption in the microbial cell membrane (24). The principal benefit of nanomaterials as antibacterial compounds might be their capability to perform concurrently with these numerous mechanisms. Therefore, pathogens cannot develop resistance to these expressed mechanisms of action, contrary to commercial antibiotics (3).

In the current study, BBR displayed an antibacterial effect on MDR *K. pneumoniae* at different concentrations, and these outcomes corresponded with those of a study carried out by Tseng, Sun (25) who approved that the BBR had an antibacterial impact on carbapenems resistance *to K. pneumoniae*. In addition, the BBR in 25 and 50 μg/mL revealed decreasing cell growth of *K. pneumoniae*, and the *KmrA* efflux pump gene was hypersensitive to BBR stress (26).

According to a previous study, BBR in combination with ciprofloxacin exhibited a definite antibacterial effect on MDR *K. pneumoniae*, which might assist in the reduction of the dosage of ciprofloxacin, reduction of the development of ciprofloxacin-resistant *K. pneumoniae*, and decrease of the occurrence of medicine resistance (27). The BBR and protoberberine byproducts could be considered medicinally potent plant biomolecules with potent antimicrobial features (28). In this regard, numerous investigations have established the antibacterial action of protoberberine alkaloids (29).

The outcomes of the present study established the synergistic effect of BBR loaded with SeNPs (BLS); unfortunately, no studies have investigated the cooperative impact of BBR with SeNPs, but numerous studies have established the synergistic effect of SeNPs with other compounds. In a study that examined the activity of SeNPs with bovine serum albumin plus ascorbic acid, chitosan plus ascorbic acid, and glucose, the results showed the synergistic impacts of these formulations against gram-positive and gram-negative bacteria, including *K. pneumoniae* and demonstrated that SeNPs with chitosan were more effective on pathogens, compared to the particle size (3).

The gained selenium sulphide nanoparticles steadied by the Curcuma extract by pepper displayed higher bactericidal action, and these mixtures in pepper have been done synergistically with the potent materials in turmeric, enhancing the bactericidal action of the compounds (30). Findings of a previous study revealed that in the treatment of human dendritic cells and fibroblasts with SeNPs, the results did not indicate damage to cell viability, the improved release of ROS, or an essential rise in the production of pro-inflammatory and immunostimulatory cytokines. Hence, SeNPs are dependable applicants for harmless therapeutic applications alone or in combination with classical antimicrobials to prevent the growth of medical pathogens (31).

3.4.2. Against Multidrug-Resistant *Candida albicans*

Effects of SeNPs, BBR, and BLS on MDR *C. albicans* are illustrated in figures 7A, 7B, 7C, and 7D as well as figures 8A, 8B, and 8C. The BBR displayed a significant increase ($P<0.05$) in the inhibition zones when the concentration increased, and the zones of inhibition were 8, 13, and 18 mm for 400 mg, 800 mg, and 1,200 mg of BBR concentrations, respectively, as presented in the figure 7A. Moreover, the presented results exhibited the significant impact ($P<0.05$) of

SeNPs in 20 Mml concentration, compared to SeNPs in 10 and 15 mM. Moreover, the zones of inhibition significantly increased with an increase in the concentration of SeNPs, and the largest zone of inhibition was 16 mm for 20 Mml concentration of SeNPs. Meanwhile, the zones of inhibition were 0.0 and 11 mm for the concentrations 10 and 15 Mml, respectively, as shown in figure 7B.

The outcomes regarding the effects of BLS showed its significant effect (P<0.05) on MDR *C. albicans* with the largest zone of inhibition (25 mm) for the concentration of 20 Mml: 1,200 mg in comparison with the concentrations 10 Mml: 400 mg and 15 Mml: 800 mg with zones of inhibition of 12 and 19 mm, respectively. Furthermore, the cooperative impact of BLS was significant (P<0.05), compared to the effect of SeNPs and BBR alone (Figures 8A and 8B). Moreover, it was very clear in the highest concentration (20 Mml:1200 mg) in figures 7D and 8C where the results showed a non-significant difference between 1,200 mg BBR and 20 Mml SeNPS.

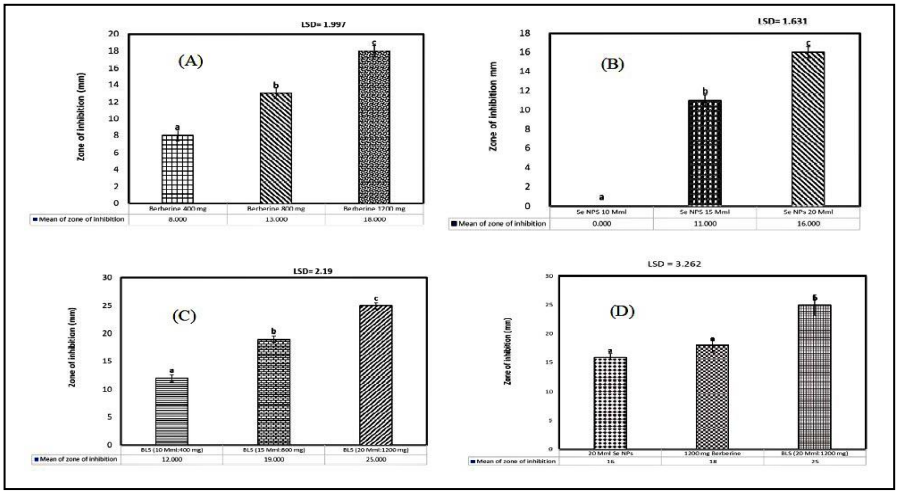

Figure 7. Mean of the zone of inhibition (mm) of MDR *Candida albicans*: **(A)** against berberine (400 mg, 800 mg and 1200 mg); **(B)** against selenium nanoparticles (Se NPs) (10 Mml, 15 mM and 20 Mml); **(C)** against Berberine loaded with selenium nanoparticles (BLS) (10 Mml:400 mg, 15 Mml:800 mg) and 20 Mml:1200 mg). Different letters indicated statistically significant at P< 0.05, and similar letters indicated no statistically significant at P< 0.05

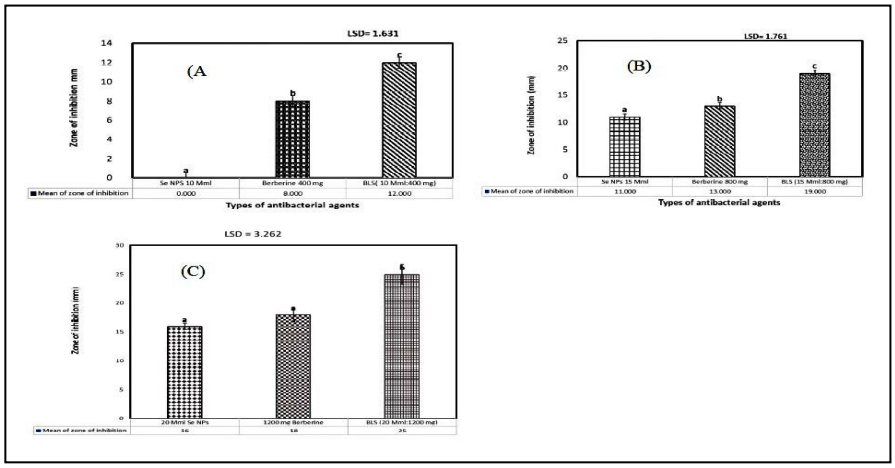

Figure 8. Comparison of Mean of the zone of inhibition of MDR *Candida albicans*: (A) against (Se NPs 10 Mml, berberine 400 mg and BLS 10 Mml:400 mg); (B) against (Se NPs 15 Mml, berberine 800 mg and BLS 15 Mml:800 mg); (C) against (Se NPs 20 Mml, berberine 1200 mg and BLS 20 Mml:1200 mg). Different letters indicated statistically significant at P< 0.05, and similar letters indicated no statistically significant at P< 0.05

Results of numerous studies have been parallel with those of the current study. According to one of the previous studies, the SeNPs showed the greatest potent effect on *C. albicans* (3). Abdel-Moneim, El-Saadony (9) approved that when the concentration of SeNPs increased, the zone of inhibition of *C. albicans* significantly increased as well. Outcomes of another study showed that biogenic SeNPs were beneficial when used as antifungal compounds toward *C. albicans*. A study revealed that SeNPs in the NDES extract of Curcuma positively repressed the growth of pathogenic fungi, including *C. albicans* (30). Hosseini Bafghi, Safdari (1) established that SeNPs were more active than silver NPs against *C. albicans*. Results of a recent study showed that SeNPs could inhibit 70% of *C. albicans* isolated from oral infections (8).

The BBR had a higher impact on fluconazole-resistant isolates of *C. albicans* which induced modifications to the safety of the plasma and mitochondrial membranes and might work at precise locations close to cell DNA, leading to death through apoptosis. Moreover, BBR might decrease the cell viability of *C. albicans* (32). The BBR displayed important antifungal action against *Candida* spp. and perhaps possessed original treatment activity as an antifungal compound or the main potent agent of antifungal medicines. Zhao, Yan (33) found that BBR at a small concentration (5.0 μg mL^{-1}) started to prevent the growth of *C. albicans* and at a higher concentration (75.0 μg mL^{-1}) completely repressed the growth of *C. albicans*. The repressive impact of BBR was strongly associated with its concentration, and the repressive impact of 256 μg ml^{-1} BH was better than that of 4 μg ml^{-1} fluconazole (34).

Unfortunately, no previous study has established the synergistic effect of BBR loaded with SeNPs. A study performed by Ha (35) revealed that nano BBR had a remarkable antifungal activity against clinical vaginal isolates of *C. albicans*, and its nano-formulation could be employed as a potent compound enhancement for the control of vulvovaginitis candidiasis. The decreasing oral bioavailability of BBR is one of the significant problems for market approval of BBR. The absorption, biodistribution, metabolism, and elimination studies of BBR have unfortunately shown poor absorption, rapid breakdown, and exclusion of BBR as the leading causes for its poor bioavailability.

One of the methods to solve the decreasing gastrointestinal absorption owing to the extremely intestinal first-pass effect follow-on in decreasing plasma levels of BBR comprises operative modulation of the P-gp mediated efflux of BBR. Studies have focused on the effective use of medicinal excipients that have P-gp repressive impacts. Numerous original dose formulae, like nanosized dose formulae, liposomes, micelles, and phospholipid multiplexes, are hopeful preparations that solve the difficulties of BBR by imparting extended circulation, improved penetrability, and resistance to metabolic operation.

Numerous strategies have enhanced the bioavailability of BBR, such as BBR-loaded solid lipid nanoparticles (36), polymer-lipid hybrid nanoparticles loaded with BBR phospholipid complex (PEG-lipid-PLGA NPs/BBR-SPC) (37), selenium-coated nanostructured lipid carriers (38), BBR-loaded solid pro-liposomes, and BBR-loaded chylomicron (39).

Efforts to improve the *in vitro* and *in vivo* effectiveness of BBR by structural alterations of the molecule and/or new preparations have been recently made. Therefore, the present study established that BBR loaded with SeNPs could be used as a potent antibacterial and antifungal agent and that BLS is a more suitable and inexpensive solution for the rise of opportunistic pathogens and MDR microorganisms towards the applied target medicines. In addition, the current study approved that SeNPs enhanced the activity and bioavailability of BBR toward bacteria and fungi. Furthermore, BLS is considered promising as a blueprint to enhance the weak antimicrobial agents and return to their previous role as antibiotics.

Authors' Contribution

Study concept and design: M. F. H.
Acquisition of data: M. J. K.
Analysis and interpretation of data: M. J. A.
Drafting of the manuscript: M. F. H.
Critical revision of the manuscript for important intellectual content: M. J. K.
Statistical analysis: M. J. K.
Administrative, technical, and material support: M. J. A.

Conflict of Interest

The authors declare that they have no conflict of interest.

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