The Antimicrobial Potential of Selenium Nanoparticles Singly and in Combination with Cinnamon Oil Against Fungal and Bacterial Causes of Diarrhea in Buffaloes

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Abstract | The antimicrobial potential of selenium nanoparticle (SeNPs) singly or in combination with cinnamon oil were evaluated against causes of diarrhea in buffaloes. The pathogens of *E. coli* and *C. albicans* were recovered from animal feeds, drinking water and feces of diarrheic animals at the top of all other isolates. The green synthesized selenium nanoparticles and cinnamon oil were had significant antimicrobial potential than traditional antibiotics against *E. coli* and *C. albicans*. The inhibitory concentrations of Se-NPs against *C. albicans* and *E. coli* were (0.4 and 0.3 mg/ml) and for Cinnamon oil were (0.5 %), respectively. The combination of SeNPs and Cinnamon oil completely inhibited the growth of *C. albicans* and *E. coli* sp. at (0.2 mg Se NPs / 0.2 % Cinnamon oil). It is concluded that the synergistic activity of metals NPs Se NPs / cinnamon oil were urgently required to overcome the microbial resistance against the traditional antibiotics and decrease the concentrations used of nanoparticles to avoid its toxicity for animals.

Keywords | Selenium nanoparticles, Synergy, Nano-emulsion, Cinnamon oil, Antimicrobial, Nanotechnology

INTRODUCTION

Nowadays, there is a huge worldwide interest in applications of nanotechnology in different fields of biomedicine related to human and animal health (Hassanen et al., 2019; Khalaf et al., 2019). Where, the animal health and their production represent the major role in food security for human consumption and of huge economic importance in developing countries (Patel et al., 2010; Hassan et al., 2020b). The microbial infections resulted from opportunistic bacteria and fungi have been common especially in human and animals which being affected by special conditions likes’ immune weakness. However, the fungal infections particularly by *C. albicans* and mycotoxigenic mold represent the widest spread causes of mycosis diseases of man and animals (Refai et al., 2014; Hassan et al., 2015, 2016, 2019a, 2020c). Moreover, several fungal and bacterial diseases adversely affect animal as mastitis, diarrhea and respiratory tract infections that resulted in decrease in their production and industry. The most important effects are related to economic losses due to decrease in milk yield (McDowell et al., 1995), diminished meat production due to diarrhea (Fagiolo et al., 2005) and respiratory disorders which are stress factors resulted in a bad production of animal (Quinn et al., 2002). The main recovered causes of animal diarrhea were *Staphylococcus* sp. *Streptococcus* sp., *E. coli*, *C. albicans*, *Aspergillus* sp. and *Penicillium* sp. (Yuan et al., 2012; Hassan et al., 2019a). However, in cases of calf diarrhea, enterotoxigenic *E. coli* (ETEC) are predominantly isolated which are producing toxin, *Salmonellae* sp. and *Y. enterocolitica* (Milnes et al., 2008). While, in water buffalo, *S. typhimurium* can induce a variety of clinical syndromes with different pathological lesions (Fagiolo et al., 2005). Some mold as members of *Aspergillus* sp., *Penicillium* sp.
Recently, nanotechnology enable the production of effective antimicrobial agents from Nano sized materials particularly metals (Tran and Webster, 2011; Hassan et al., 2020 a, b). In addition, several studies confirmed the antioxidant, antibacterial, and antifungal activities of several metal and metal oxide nanoparticles. Examples for these nanoparticles are zinc oxide nanoparticles (ZnO-NPs) (Hassan et al., 2020a), copper nanoparticles (CuO-NPs) (Hassan et al., 2017), silver nanoparticles (Ag-NPs) (Fouda et al., 2019), and gold nanoparticles (GNPs) (Hassan et al., 2020). Metals particularly selenium is the fundamental component or a micronutrient that used in disease treatment and could neutralize malignancy and decrease disease frequencies (Zonaro et al., 2015; Geoffrion et al. (2020). The selenium nanoparticles can be produced by seed of Mucuna pruriens gave NPs of nearly (100–120 nm) and had IC50 (60 μg/mL) for inhibition the cell viability at 48 h. (Menon and Shanmugam, 2019). They detected that the preparation of SeNPs by green methods are cost-effective and environmental friendly and can be utilized further for future biomedical applications. This micronutrient is the primary part of glutathione peroxidase (GSH-Px), a cancer-inhibiting catalyst, which is associated with the protection of cells from oxidative stress (Ramamurthy et al., 2013; Kumari et al., 2018). The SeNPs reported to be more efficient than bulk selenium in activating selenoenzymes and have reduced toxicity (Mary et al., 2016; Jay and Shaikat, 2018). There are other several uses of SeNPs as antioxidant, anticancer and antimicrobial agents and protection from heart diseases (Ramamurthy et al., 2013, Naziroğlu et al., 2017; Zhao et al., 2018). Recently, Meena et al. (2018) reported that the significant advantages of Nano-emulsions of oils are the simplicity, inexpensiveness, stability, versatility and the solubility of lipophilic substances that will protect them from degradation. Furthermore, the supplementation of oil with nanomaterials were successfully used in veterinary medicine as drug delivery agent and antimicrobial agents (Hassan et al., 2020a). Similarly, Abd-Elsalam and Khokhlov 2015, Hassan et al., 2020b) detected the antimicrobial potential of eugenol–Zinc Nano-emulsions against Fusarium sp. and E. coli. In addition, the contact of oil Nano-drops to the microbial membranes cause its adhesion, destruction and final death of pathogens (Meena et al., 2018). Therefore, the objectives of present article were to detect the prevalence of fungal and bacterial causes of diarrhea in buffaloes. The most prevalent microbial agents that recovered from the present study were used for evaluation the effects of SeNPs singly and in-combination with cinnamon oil in inhibition their activities. The minimum inhibitory concentrations were measured during all tests in comparison with traditional antibiotics. Moreover, the activities mechanisms of SeNPs and oils and their benefits were fully discussed.

**MATERIALS AND METHODS**

**Samples**

Ninety samples (30 of each of animal feeds, water, and feces) were obtained from a private animal farm suffering from diarrhea at Giza governorates aseptically in sterile McCartney bottles. The samples were transferred, as soon as possible, to the laboratory and kept in fridge until examination.

**Commercial antibacterial, antifungal and other chemicals**

A known commercial antibacterial, antifungal and reagents were purchased from Sigma Chemical Company (USA).

**Selenium nanoparticles and cinnamon oil**

The used SeNPs were synthesized by green method and were characterized by the laboratory of ALDRIK Sigma chemical company, USA. It was in amorphous powder form with 60 nm particle size. While cinnamon oil was purchased in crude form from Al Gomhorya chemical company, Egypt.

**Bacteriological and serological examination**

Samples were cultured onto MacConkey agar medium for 24 hrs. at 37°C, then a peptone water cultures were prepared from appeared colonies to inoculate biochemical tests (Quinn et al., 2002). While, serological identification for *E. coli* and *Salmonella* sp. was undertaken according to (Edwards and Ewing 1972; Neville and Bryant, 1986).

**Mycological examination**

The samples were prepared and examined for isolation of fungi as method as Refai et al. (2012). The samples were inoculated into Petri–dish plates contained Sabouraud’s dextrose agar (SDA) and incubated 3–5 days at 25–28°C and identification of appeared mold and yeast colonies were identified according to (Pitt and Hocking , 2009; Refai and Hassan, 2013).

Green synthesis and characterization of selenium nanoparticles (Inregole et al., 2010).
One 100 ml (10-1 M) sodium selenosulphate was treated with 10 ml 4% glucose solution and mixture was refluxed. The color of the solution changes from colorless to yellow after refluxing immediately and become orange after 30 minutes. The orange color solutions remained stable for months. The prepared nanoparticles were characterized via UV-visible spectra of each solution were measured in a SHIMADZU UV-1800 double beam digital spectrophotometer. XRD patterns were obtained on a Philips Xpert MPD X-ray diffractometer using Cu Kα (1.54059 Å) radiation with the X-ray generator operating at 45 kV and 40 mA. TEM images were obtained on JEOL 2010 microscopes. The TEM sample was prepared by dropping a sample suspension in ethanol on a Cu grid coated with a carbon film.

Measurement of MIC of prepared SeNPs and cinnamon oil against C. albicans and E. coli that isolated from diarrhea in buffaloes (CLSI 2008):

- Preparation of bacterial and fungal spore suspension of isolates (Koneman et al., 1992; Gupta and Kohli, 2003): Suspension of tested bacterial and fungal isolates were prepared from their cultures on MacConkey agar medium after incubation for 24h at 37°C for bacteria and on Sabouraud’s dextrose agar (SDA) for 1-3 days at 25°C for fungi, respectively. The bacterial colony and fungal mycelia/spore mat were washed off with a 6 ml of sterile distilled water and using sterile loop, the outer most layer of growth were scraped. This suspension was counted in haemocytometer slid and adjusted to 10⁷/ml colony forming unit considering the dilution factor.

- The minimum inhibitory concentration (MIC) of SeNPs and Cinnamon oil for the tested isolates were determined by a broth micro-dilution method based on the National Committee for Clinical Laboratory Standards (NCCLS) for bacteria (Balachandran et al., 2015) and for yeasts (NCCLS, M27-A2 2002). In sterile 12- x 75-mm plastic test tubes, 900 µl of RPMI 1640 broth medium or SD broth medium (for fungi) or nutrient broth (for bacteria) were added. Then, 100 µl of spore suspension added separately to adjust the inoculum of E. coli and C. albicans to (1 x 10⁸ cells/ml). 100 µl of SeNPs concentrations (0, 0.1, 0.2, 0.3, 0.4, 0.5 mg/L) or Cinnamon oil at levels of (0.0, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%), were added. Similar tests applied the traditional antibacterial and antifungal agents in the separate assays.

- Combination effects of Se-NPs and Cinnamon oil was performed as above-mentioned tests but 100ul was added from each. All the test tubes were incubated for 48 hrs.–5days at 28-30°C (for fungi) and for 24-48 h at 37°C (for bacteria) and the experiment was repeated twice. After end of incubation period, 5 µL of tested broth were inoculated on the sterile nutrient agar plates for bacteria and SDA plate for fungi and incubated at 37°C for 24 hr- 2 weeks. The turbidity of the growth in tubes was observed every 24 hrs. The growth was assayed by measurement of optical density and transmittance percentage of each tubes content at 405 nm using spectrophotometer. The MIC was the concentrations that remove the turbidity and decreased optical density (OD) and increased transmittance percentage (T%).

STATISTICAL ANALYSIS
The obtained data were computerized and analyzed for significance. Calculation of standard error and variance was according to SPSS 14, (2006).

RESULTS AND DISCUSSION
A total of ninety samples from animal feeds, drinking water and animal feces (30 each) were examined for isolation and identification of bacterial and fungal pathogens of diarrhea in buffalo. The tabulated results in (Table 1) illustrated that the incidence rates of bacterial species were (50%, 56.7 % and 83.3 %) for E. coli in animal feeds, drinking water and feces, respectively. While, it were (23.3 %, 16.7 % and 33.3 %) for S. typhi in samples, respectively. Meanwhile, E. coli and S. typhi were recovered from (63.3% and 24.4%) of total samples. However, isolates of E. coli, Salmonella sp. and Y. enterocolitica were recovered from cases of calf diarrhea (Milnes et al., 2008). While, in water buffalo, S. typhimurium can induce a variety of clinical syndromes with different pathological lesions (Fagiolo et al., 2005). Another study reported that S. typhi causing bacterial gastro enteritis and some Salmonella sp. have the multidrug-resistant (Yan et al., 2004, Scallan et al., 2011). On the other hand, the environmental pollution by fungi affect upon the growth rate and health of human and animals and cause several diseases as thrush, candidiasis, aspergillosis, dermatophytosis and mastitis, aneamia, carcinogenic, tremor-genic, hemorrhagic, pulmonary edema, immunosuppressive and hormonal effects (Hassan et al., 2016; 2019a; b, 2020b; Asfour et al., 2009). Currently, as observed in (Table 2), the incidence rates of molds and yeast species in animal feeds, drinking water and feces were (83.3 %, 56.7 % and 30 %), for A. flavus, (30 %, 83.3 % and 33.3 %) for A. oryzae and (80 %, 80 % and 86.7 %) for C. albicans, respectively. C. albicans were recovered from (68%, 40% and 16%) of diseased cases in buffaloes with mastitis, diarrhea and respiratory disorders, respectively. Hassan et al. (2014). Similar results were obtained by (Hassan et al., 2016, 2017, 2019a; 2020b) who recovered these fungi from cases of diarrhea and respiratory affections of buffaloes. In the present study, the most prevalent bacterial isolates in cases of diaper...
Table 1: Prevalence rates of bacterial species recovered from the examined samples

| Bacterial isolates | Types of samples (30 for each) | Animals feeds (30) | Drinking water (30) | Feces (30) | Total (90) |
|--------------------|---------------------------------|--------------------|---------------------|-----------|------------|
|                    | No | %   | No | %   | No | %   | No | %   | %   | %   |
| E. Coli            | 15 | 50  | 17 | 56.7| 25 | 83.3| 57 | 63.3|     |     |
| S. typhi           | 7  | 23.3| 5  | 16.7| 10 | 33.3| 22 | 24.4|     |     |

% calculated according to the number of samples examined (30)

Table 2: Prevalence rates of molds and yeast species isolated from the examined samples

| Molds and yeast species | Types of samples (30 for each) | Animals feeds (30) | Drinking water (30) | Feces (30) | Total (90) |
|-------------------------|---------------------------------|--------------------|---------------------|-----------|------------|
|                         | No | %   | No | %   | No | %   | No | %   | No | %   |
| A. flavus               | 25 | 83.3| 17 | 56.7| 9  | 30  | 51 | 56.6|     |     |
| A. ochraceus            | 9  | 30  | 25 | 83.3| 10 | 33.3| 44 | 48.8|     |     |
| C. albicans            | 24 | 80  | 27 | 80  |     |      | 77 | 85.5|     |     |

% calculated according to the number of samples examined (30)

rhea was E. coli that recovered from (63.3%) and fungi of C. albicans from (85.5%) of all examined samples.

Moreover, the mixed infection by E. coli with C. albicans in diarrheic buffaloes was investigated. The results revealed that out of 30 examined of feces 10 were showed mixed infection and 5 out of 30 feed samples had mixed infection. But, no mixed infection by E. coli with C. albicans was detected in water samples (Table 3). Okela, (2010) and Blanchard P.C. (2012) who detected mixed infection of E. coli and yeast in cases of bovine Diarrhea reported similar findings.

Table 3: Prevalence of mixed infection with E. coli & C. albicans recovered from diarrhoeic buffaloes

| Types of samples (30 for each) | Prevalence of E. coli & C. albicans |
|--------------------------------|-------------------------------------|
| Animals feeds (30)             | No = 5 | 16.7% |
| Drinking water (30)            | 0 | 0% |
| Feces (30)                     | 10 | 33.3% |

Table 4: Antibiotic sensitivity test of representative E.coli isolated from diarrhoeic buffaloes

| Antibacterial agents | Bacterial isolates | E. coli (20) |
|----------------------|--------------------|--------------|
|                      | R | S |
| Ofloxacin (10 μg)    | 0 | 20 | 100 |
| Amikacin (10 μg)     | 4 | 16 | 80 |
| Colestin (100 μg)    | 2 | 18 | 90 |
| Ampicillin (10 μg)   | 20| 0  | 0  |
| Kanamycin (10 μg)    | 16| 4  | 20 |
| Tetracyclin (30 μg)  | 19| 1  | 5  |
| Trimethoprim (25 μg) | 20| 0  | 0  |

No = Number % = Percent.  R = Resistant  S = Sensitive.

Table 5: Prevalence of mixed infection with E. coli & C. albicans recovered from diarrhoeic buffaloes

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Table 5: Antibiotic sensitivity test of representative *C. albicans* isolated from diarrhoeic buffaloes

| Fungal Isolates (Numbers) | Antifungal Agent       | Fluconazole (10 g) | Voriconazole (1 g) | Itraconazole (10 g) | Nystatin (100 g) | AmphotericinB (100 μg) |
|---------------------------|------------------------|--------------------|--------------------|--------------------|------------------|-----------------------|
| *C. albicans* (20)        | R S                    | R S                | R S                | R S                | R S              | R S                   |
| %                        | 20 0                  | 12 8              | 0 20               | 0 20               | 10 10            |
|                          | 100% 0                | 60% 40%           | 100% 100%         | 100% 50%          |

S = Sensitive  R = Resistant

Table 6: Optical density and Transmittance of treated *C. albicans* and *E. coli* by Se NPs

| Concentration of Se NPs (mg/l) | *C. albicans* | *E. coli* |
|-------------------------------|---------------|-----------|
| OD   | T%         | OD     | T%        |
| 0.0  | 2.06     | 0.87   | 2.06     | 0.87 |
| 0.1  | 0.15     | 10.1%  | 0.20     | 63.1 |
| 0.2  | 0.05     | 70.8%  | 0.03     | 93.3 |
| 0.3  | 0.02     | 95.5%  | 0.0      | 100  |
| 0.4  | 0.00     | 100%   | 0.0      | 100  |
| 0.5  | 0.00     | 100%   | 0.0      | 100  |

OD = Optical Density  T = transmittance

Figure 1: SEM pictures of Se–NPs showed the particle size and morphology (60 nm).

Figure 2: The UV-VIS absorbance spectra of Se-NPS (60nm) at 405 nm wave length

In the present study, SeNPs was evaluated for antimicrobial activities against the most predominantly isolated bacteria *E. coli* and fungi *C. albicans* from buffalo’s feed, drinking water and feces. The tabulated results in Table 6, Figure 3, 4, illustrated that the antimicrobial potential of Se-NPs against *C. albicans*, *E. coli* was concentration dependent. When the concentrations of SeNPs increased up to (0.5 mg/l), the OD of treated spore suspension were decreased until reach zero and T% increased to 100%. The inhibitory concentration of SeNPs against *C. albicans* was (0.4 mg/l) and it was (0.3 mg/l) for *E. coli* sp. Khiralla and El-Deeb (2015), detected the antimicrobial potential of Se-NPs against some bacterial food borne pathogens included *E. coli*, and *S. Typhimurium* and *S. Enteritidis* and MIC90 was (25 μg/ml for all). While, when the concentration of SeNPs reached (75 μg /ml), a complete inhibition of bacterial cells...
Table 7: Optical density and transmittance of treated C. albicans and E. coli by cinnamon oil.

| Concentration of cinnamon oil | C. albicans | E. coli |
|------------------------------|-------------|---------|
|                              | OD  | T%    | OD  | T%    |
| 0.0                          | 2.06| 0.87  | 2.10| 0.79  |
| 0.1%                         | 0.40| 3.98  | 0.37| 42.6  |
| 0.2%                         | 0.150| 10.8 | 0.22| 60.2  |
| 0.3%                         | 0.06| 87.1  | 0.04| 91.2  |
| 0.4%                         | 0.02| 95.5  | 0.02| 95.5  |
| 0.5%                         | 0.0 | 100   | 0.0 | 100   |

OD = Optical Density . T = Transmittance

Table 8: Optical density and transmittance of treated C. albicans and E. coli by Combination of Se NPs / cinnamon oil

| Concentration of SeNPs/CO | C. albicans | E. coli |
|---------------------------|-------------|---------|
|                           | OD  | T%    | OD  | T%    |
| 0.0                       | 2.06| 0.87  | 2.10| 0.79  |
| 0.1mg Se NPs/0.1% CO      | 0.40| 3.98  | 0.37| 42.6  |
| 0.1 mg Se NPs/ 0.2% CO    | 0.150| 10.8 | 0.22| 60.2  |
| 0.2 mg Se NPs/ 0.1% CO    | 0.06| 87.1  | 0.04| 91.2  |
| 0.2 mg Se NPs/0.2% CO     | 0.00| 100   | 0.00| 100   |

OD = Optical Density . T = Transmittance

Figure 3: O.D of treated C. albicans with gradual concentration of Se NPs

Figure 4: O.D of treated E. coli with gradual concentration of Se NPs.

growth occurred. Jay and Shafkat (2018) biosynthesized SeNPs and detected their anti-microbial activity against S. aureus and B. subtilis. They found that the MIC detected at (25 μl) and the highest zone of inhibition observed in S. aureus (32mm) and lower in B. subtilis (28mm) at concentration of 100μl SeNPs. While, Menon et al. (2020) detected antibacterial activity of SeNPs for wide range of bacterial strains. However, their antimicrobial activity have been detected against pathogenic bacteria, fungi and yeasts (Shahverdi et al., 2010, Harirhan et al., 2012, Beheshti et al., 2013; Hassan et al., 2019a b).

Regarding, the antimicrobial potentials of essential oil, they have antibacterial and antifungal activities (Vitoratos et al., 2013). They are able to control microorganisms related to skin and food spoilage, including Gram-negative and Gram-positive bacteria. Clove, cinnamon, mandarin, lime, and basil oils are the best examples that are commonly used as natural antibacterial and antifungal agents, attracting the growing interest of scientists for use as food preservatives (Ghosh et al., 2013).

Currently, the present results of the antimicrobial potential of Cinnamon oil against C. albicans and E. coli sp. (Table, 7 and Figure 5, 6), yielded that the Optical density and transmittance were also concentration dependent. When the concentration of Cinnamon oil increased up to (0.5 %), the OD of treated spore suspension was decreased till reach 100 % T. The inhibitory concentration of Cinnamon oil that inhibited the growth of C. albicans and E. coli sp. was 0.5 % . Similar findings were detected by Eugénia et al. (2009) as he detected the significant fungicidal effect of eugenol, against Candida, Aspergillus and dermatophytes including fluconazole-resistant strains. In other study, Wahba and Abd-khalilq, (2013) recorded that the clove oil in a pure state has a bactericidal and fungicidal effect where it inhibit growth of bacteria such as St. aureus, St. lentus , dermatophytes as Microsporum canis, Trichophyton mentagro-
phyte and T. verrucosum at concentrations of (25, 20, 15, 10, 5 μl), respectively.

Moreover, herein the antimicrobial potential of synergistic activity of SeNPs with Cinnamon oil was evaluated. The findings in (Table, 8 and Figure 7, 8) indicated that the required concentrations for growth inhibition of C. albicans and E. coli sp. in combination of Se NPs and Cinnamon oil were at rates of (0.2 mg Se NPs / 0.2 % Cinnamon oil) which was lower than if each used separately (Table, 6 -8 and Fig. 1-6). The significance for using low concentrations of Se NPs by conjugation with cinnamon oil was to ensure their nontoxicity for human and animal cells.

Figure 5: O.D of treated C. albicans with gradual concentration of Cinnamon oil

Figure 6: O.D of treated E. coli with gradual concentration of Cinnamon oil

Figure 7: O.D of treated C. albicans with combination of Se NPs and Cinnamon oil

Figure 8: O.D of treated E. coli with combination of Se NPs and Cinnamon oil

Hassan et al. (2017, 2019 b and 2020b), detected the more antimicrobial potentials of conjugation of ZnNPs with cinnamon oil or ozone than their single activities against bacteria and fungi. In addition, the essential oil additives loaded into mesoporous silica nanoparticles (MSNs) have the ability to suppress the growth of A. niger (Bernardos et al., 2015). The combination of Nano-emulsions and matrix of certain nanostructures such as lipids and polysaccharides were more effective in inactivation of E. coli than the traditional and classical emulsions and used lower doses (Salvia-Trujillo et al., 2017).

On the other hand, there are several mechanisms of antimicrobial activity of NPs included contact of NPs and penetration of the cell walls, destroying a microbial cell generating ROS release of metals ions and caused oxidative stress (Rudramurthy et al., 2016). The release of metallic ions resulted in depolarization of cell membranes, lipid peroxidation, protein oxidation, and DNA damage (Huang et al., 2020). Based on oxidative stress, Chang et al. (2012) found that NPs may enter the microbial cells via endocytosis process this related to induction ROS and the ions released by the nanostructures. While, Jay and Shafkat (2018); Hassan, et al. (2019b) reported that Se-NPs caused destruction of cell wall, leakage of cytoplasm contents and loss of treated fungal and bacterial cell functions as detected when they subjected to SEM. Zhao et al. (2018), found that a high stress due to accumulation of SeNPs on surface of cells stimulated the production of ROS which help in inhibition of bacterial cells. In general, the antimicrobial effect of nanoparticles occurs by two ways (Moraru et al., 2003). The first is the formation of H$_2$O$_2$ on the surface of NPs due to the possible formation of hydrogen bond between hydroxyl group of cellulose molecules of fungi and
bacteria. The synergistic and combination therapy of metals NPs as SeNPs with oils was urgently required to decrease the used concentration of nanoparticles, overcome the microbial resistant to traditional antibiotics, and resulted in more efficient antimicrobial activity of metal nanoparticles for the treatment of human and animal diseases.

Moreover, there will be several benefits of metallic nanomaterials to be used in improvement the biomedical applications. Although, data related to their harmful effects are not sufficient and special attention is required for known their toxicity risk before to biomedical applications. Hence, several toxicological studies are needed before nanotechnology applications in biomedicine and animal health.

CONCLUSION

The buffaloes’ diarrhea results in significant losses in animal health and causes important burdens to the country’s economy regarding to meat, milk, wool and leather industries. The frequent testing program of the animal feeds and other environmental factors for fungal and bacterial contamination is a critical demand. The metals nanomaterials are used as antimicrobial agents beside to other benefits strategies as diseases detection, diagnosis and therapy, additives to food, feeds and their products, and finally food safety. Our results detected that Se-NPs and cinnamon oil administration have significant antimicrobial potential against fungal and bacterial causes of diarrhea and their combination showed the requirement of lower concentrations from both to obtain the antimicrobial effects than their single form. Therefore, the synergistic therapies are needed to reduce the used doses of nanoparticles and hence overcome its toxicity and more efficient antimicrobial activities. Furthermore, the production of the production of SeNPs. Using glucose as a reducing agents and stabilizer avoid the aggregations of particles under ambient condition which can be used in large scale production and are safe method and environmentally friendly. The mechanisms of their activity are due to its penetration of cells membrane, damage of cytoplasmic contents, loss of function and cell kill. Hence, advanced and further investigations are required for direct treatment of farm animals by SeNPs in combination with other safe herbs and biological compound to avoid toxic effects of nanomaterials, which may result from misusing doses of nanoparticles. Moreover, the effects of green synthesized nanomaterial have long-term use on health need to concern and toxicity in surrounding environment and must be resolved in future. Therefore, the toxicity risk of nanomaterials must be determined before applications of green synthesized metallic nanomaterials in biomedicine for safe human and livestock health and their activities and production.

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