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Research Article

Topically Applied Luteolin /Quercetin-Capped Silver Nanoparticle Ointment as Antileishmanial Composite: Acceleration Wound Healing in BALB/c Mice

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Cutaneous leishmaniasis is a major health problem in several parts of the world. However, existing drugs have various therapeutic effects on different *Leishmania* species and also cause many side effects. This study focuses on the use of the aqueous extract of quercetin in producing silver nanoparticles for the experimental treatment of cutaneous leishmaniasis in laboratory mice. Silver nanoparticles (AgNPs) were synthesized using quercetin and characterized by UV-Vis spectroscopy, DLS, FTIR, and FESEM. We investigated the antileishmanial activity of green synthesized AgNPs *in vitro* and *in vivo*. Synthesized AgNPs from quercetin macromolecules showed promising antileishmanial activity at an IC50 of 125 μg/ml against promastigotes *in vitro*. In the *in vivo* study, *L. major*-infected BALB/c mice were treated topically with luteolin/quercetin-capped silver nanoparticle (AgNPQct) ointment for 21 consecutive days. As a result, this ointment reduced the inflammatory response compared to the untreated group ($p < 0.05$). The size of lesions in the luteolin/AgNPQct group showed a slight increase compared to other groups ($p < 0.01$). Biogenic silver nanoparticles along with the luteolin macromolecule can be an effective and reliable candidate for the design of antileishmanial drugs in the future.

1. Introduction

Leishmaniasis is an important neglected zoonotic disease that is transmitted to humans by phlebotomine sandflies. The disease affects humans in three major clinical forms that have different complications and outcomes: cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), and visceral leishmaniasis (VL) [1].

At present, the treatment of leishmaniasis is based on pentavalent antimonials that are associated with severe side effects such as cardiac, liver, kidney, and blood disturbances if administered intramuscularly [2, 3]. These drugs are also expensive, and full recovery requires their continued use, which imposes a high cost of treatment. Also, drug resistance has been observed in leishmaniasis endemic regions, and this problem has challenged drug development [4].

Various drugs have been used as alternative treatments against the clinical forms of leishmaniasis. The main alternative drug in cases of antimony treatment failure of VL is liposomal amphotericin B (L-AmB), which, in recent years,

has been practically replaced by antimony compounds in the treatment of Kala-azar in India [5]. This drug has also been widely used in the treatment of MCL. However, there is no consensus on the alternative treatment of CL in this field, and several drugs, such as paromomycin, miltefosine, pentamidine, imiquimod, amphotericin B, and ketoconazole, have been used in different parts of the world [6]. These drugs also have side effects, and each has limited therapeutic effects against specific species of *Leishmania*. So there is still a need for more effective drugs with less toxicity and ease of use. In recent years, there has been growing interest in natural products such as plants and their derivatives for the treatment of leishmaniasis [7–9]. Antileishmanial activity of various plants has been attributed to the presence of compounds such as flavonoids, alkaloids, steroids, triterpenoids, naphthoquinones, quinones, terpenes, lignans, and saponins [10].

Flavonoids are natural plant secondary metabolites with a polyphenolic structure. They have a high therapeutic value against several diseases [2, 11–17]. Among flavonoids, quercetin and luteolin have received considerable attention due to their strong antioxidant and anti-inflammatory properties, ability to scavenge free radicals, stimulation/inhibition of enzyme activities, and signal transduction pathways [18–20]. These two compounds can also induce topoisomerase II-mediated kinetoplast DNA minicircle cleavage in trypanosomatids such as *Leishmania*, both in promastigotes and intracellular amastigotes [21].

Among the different aspects of nanomedicine, the synthesis of silver nanoparticles (AgNPs) provides more effective particles with various applications [22]. Chemical procedures for synthesizing AgNPs require toxic chemicals, expensive equipment, and capping agents for the stabilization of nanoparticles [23]. In comparison, the use of green materials such as bacteria, fungi, proteins, polypeptides, algal extracts, and plant extracts as reducing agents in the synthesis of AgNPs is cost effective and ecofriendly [24–26]. Green synthesis of AgNPs based on plant extracts containing phytochemicals such as terpenoids, flavonoids, phenolics, aldehydes, carboxylic acids, and enzymes can act as a reducing and stabilizing agent as well as a capping agent in the biosynthesis of AgNPs [26–29]. The antimicrobial and antiprotozoal effects of silver nanoparticles depend on their ability to produce reactive oxygen species (ROS), which play an important role in killing pathogenic microbes [30, 31].

So far, many studies have been conducted on the effectiveness of silver nanoparticles on *Leishmania* wounds, but no similar study has been performed with this combination (quercetin-capped nanosilver and luteolin). In this study, an attempt has been made to use quercetin for synthesis of AgNPs to explore the antileishmanial activity of these nanoparticles. Since quercetin has a low solubility in water, we decided to synthesize quercetin-capped nanosilver and investigate its effect. We further investigated the *in vivo* effectiveness of luteolin/quercetin-capped silver nanoparticle (AgNPQct) ointment against cutaneous leishmaniasis lesions in mice.

2. Materials and Methods

2.1. Materials. Silver nitrate (AgNO3) and quercetin were purchased from Sigma-Aldrich (USA). Luteolin was purchased from Golexir Company in Mashhad, Iran.

2.2. Quercetin-Mediated Synthesis of AgNPs. Silver nanoparticles were synthesized from quercetin. For this purpose, the effect of the two amounts of extracts (2 and 4 mM) and 1 mM of an aqueous solution of silver nitrate was investigated to obtain the optimum values. The volume of 1 ml of quercetin extracts (2 mM) and 9 ml of 1 mM of $AgNO_3$ was selected as the optimal. Briefly, to prepare these AgNPs, 10ml of quercetin (2 mM) was added dropwise to 90 ml of silver nitrate (1 mM) solution. This solution was placed on a shaker with constant rotation (120 rpm) at room temperature for 24 h in a dark place. The change of the reaction mixture color indicated that AgNPs were synthesized from quercetin. After synthesis, nanoparticle-containing solutions were centrifuged at 8,000 rpm for 10 min. The sediment was washed three times with distilled water at 8,000 rpm for 10 min to separate AgNPs from other components of the solution. The dried AgNPs were kept in a microtube at room temperature for the next steps [32, 33].

2.3. Characterization of Synthesized AgNPs

2.3.1. Ultraviolet-Visible (UV-Vis) Spectrum Analysis. UV-Vis spectrophotometry is the most important and simplest technique to confirm the formation of AgNPs. Green silver nanoparticles were scanned in the wavelength range of 300–700 nm by UV-visible spectroscopy (Epoch microplate spectrophotometer, BioTek, VT, USA) to monitor the reduction of pure Ag^+ ions.

2.3.2. Dynamic Light Scattering (DLS) and Zeta Potential. The hydrodynamic size of the phytofabricated AgNPs and the polydispersity index (PDI) were evaluated using nanoparticle-tracking analysis, as determined using Brookhaven ZetaPlus (Brookhaven 90Plus, USA) at a temperature of 25°C, by using dynamic light scattering (DLS) measurements. The net surface charge was measured by the zeta potential.

2.3.3. Fourier-Transform Infrared (FTIR) Spectroscopy. The FTIR analysis of synthesized AgNPs was performed using a spectrum RX 1 instrument (Perkin-Elmer, USA), and data were recorded between wave numbers ranging from 500–4000 cm^{-1}.

2.3.4. Field Emission Scanning Electron Microscopy (FESEM). Silver nanoparticles were analyzed by using a field emission scanning electron microscope (FESEM) (SIGMA VP, ZEISS, Germany) from Day Petronic Co (Tehran, Iran). Thin films of AgNPs were placed onto a carbon-coated copper grid, and then, the films on the FESEM grid were allowed to dry for analysis.

2.4. Assessing the Antileishmanial Activity of Synthesized AgNPs

2.4.1. Parasite Culture. *Leishmania major* promastigotes (MRHO/IR/75/ER) were cultured in the Roswell Park Memorial Institute-1640 (RPMI-1640) medium supplemented with 12–15% FBS, 100 U/ml penicillin, and 100 mg/ml streptomycin at 26°C ± 1. Cultures were passaged after 4 days of incubation. The growth of promastigotes was monitored daily using an inverted microscope.

2.4.2. Determination of Effects of Ag-NPs on the Proliferation of Promastigotes. One ml of culture media (RPMI-1640) containing 1×10^6 promastigotes was added to each of the 4 tubes. A hundred μL of silver nanoparticles (1 mg/ml), quercetin (5 mg/ml), and glucantime (50 mg/ml) as the positive control was added to each tube; one tube was considered untreated (negative control). After 24, 48, and 96 hr incubation at 26°C, antileishmanial efficacies were determined by counting the number of promastigotes with a hemocytometer.

2.4.3. Cytotoxicity Assay of L. major Promastigotes. The antileishmanial activity of synthesized AgNPQct, quercetin, luteolin, and glucantime was measured by the MTT test. Parasites were resuspended in RPMI-1640 medium supplemented with 10% FBS, 100 U/ml penicillin, and 100 mg/ml streptomycin and seeded (1×10^5 promastigotes/well) in 96-well flat bottom microplates. The parasites were exposed to different concentrations of AgNPQct (3.125–200 μg/ml), quercetin (0.1–5 mg/ml), luteolin (0.003–0.1 mg/ml), and glucantime (100 mg/ml) at 26°C for 48 h. Finally, the cells were subjected to the MTT assay. 10 μL of MTT (5 mg/ml) was added to each well, and the plate was incubated again at 37°C in dark for 4 h. Then, 100 μL of dimethyl sulfoxide (DMSO) was added to each well, and the absorbance values were determined at 570 nm by using an ELISA plate reader. The results were expressed as percentage cell viability.

$$\text{Cell viability \%} = \frac{\text{Mean OD of samples}}{\text{Mean OD of control}} \times 100. \quad (1)$$

2.5. In Vivo Assay

2.5.1. Preparation of AgNPQct-Based Ointment. A total of 1 gr of Vaseline as an ointment base was weighed and melted at 70°C in a water bath. 0.5% AgNPQct and 0.15% luteolin at 50°C were added and mixed with melted Vaseline. Four batches of the ointment were prepared: Vaseline, 0.5% AgNPQct, and 0.15% luteolin (batch A), Vaseline and 0.5% AgNPQct (batch B), Vaseline and 0.15% luteolin (batch C), and negative control with neither nanoparticle nor luteolin (batch D).

2.5.2. Ulcer Induction. A total of 30 female BALB/c mice (6–8 weeks old) were provided by the Pasteur Institute of Iran in Tehran, Iran. The mice were kept under controlled environmental conditions (25 ± 2°C temperature and 12 : 12-hour dark-light cycle). The mice had free access to water and a standard diet. The infection in mice was induced by the promastigote form of *L. major*. To do that, 0.1 ml of a suspension consisting of 2.5×10^6 promastigotes was injected subcutaneously into the top of the mouse's tail base using an insulin syringe. The lesions were observed after about 3 weeks of inoculation.

2.5.3. Treatments. The infected mice were randomly divided into five groups ($n = 6$) that received different treatments: Vaseline, 0.5% AgNPQct, and 0.15% luteolin (group A) every day; Vaseline and 0.5% AgNPQct ointment (group B) daily; ointment contained Vaseline and 0.15% luteolin/day (group C); Vaseline as a negative control (group D); and glucantime (300 mg/ml; 100 μl) (injection) as a positive control (group E) daily. Each formulation was topically applied every 24 hours and continued for 21 days from the day those cutaneous lesions appeared on the mouse tail. The size of the lesions was measured every 7 days.

2.5.4. Measurement of the Lesions' Size. From the beginning of treatment, the lesions were photographed every 7 days to determine their size. To calculate the wound area, the photographs were analyzed in using Digimizer 4.2.6.0. For each experimental condition, the mean and standard deviation were calculated [34, 35].

2.5.5. Histological Analysis. At the end of the treatment, the mice were euthanized with a high dose of ether. Thick skin specimens from the wound sites were excised, fixed in buffered formaldehyde, dehydrated with graded ethanol, and cleaned with xylol. They were then embedded in paraffin wax and stained with hematoxylin and eosin (H&E) and finally used for pathological assessments.

2.6. Data Analysis. Data were statistically analyzed using IBM SPSS v26 software and one-way analysis. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Nanoparticles. The reduction process of silver nitrate (1 mM) solution using quercetin was observed by the color change from yellow to dark brown. UV-visible spectroscopic analysis of synthesized AgNPs showed that the absorption peak is in the range of 430–450 nm, which confirms the formation of silver nanoparticles (Figure 1(a)). The hydrodynamic diameter and the zeta potential of synthesized AgNPs analyzed using DLS are shown in Figure 1(b). The average size and the zeta potential of AgNPQct were 310 nm and −10.23 mv, respectively (Figures 1(b) and 1(c)). The FTIR spectra of synthesized AgNPs in Figure 1(d) show the existence of multiple functional groups including bands at 3746, 3437, 2924, 2861, 2364, 1618, and 1101 cm^{-1}. The band at 3746 cm^{-1} in the spectra corresponds to N-H stretching of amines, the band at 3437 cm^{-1} represents OH stretch,

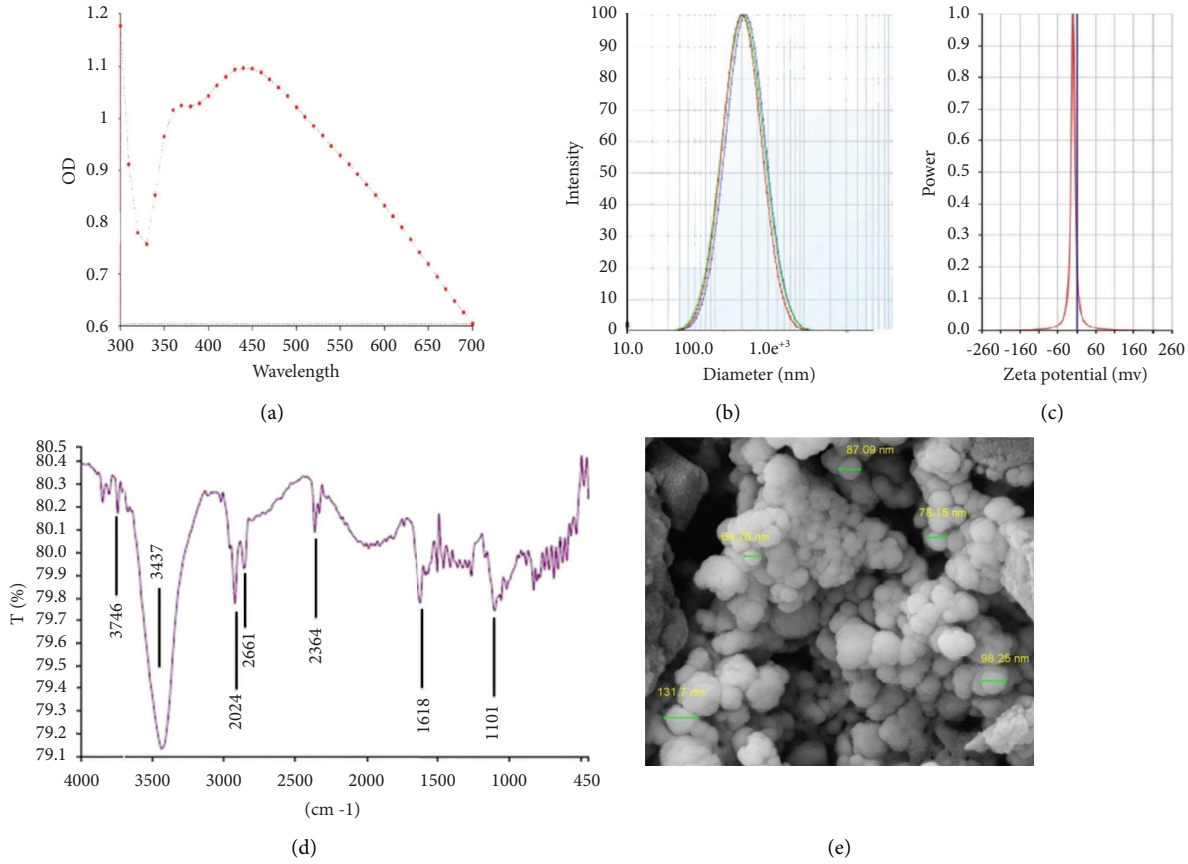

Figure 1: Properties of synthesized AgNPs of quercetin: UV-Vis absorption spectrum (a), particle size distribution (DLS) (b), zeta potential measurement (c), FTIR spectrum, (d) and FESEM image (e).

indicating alcohol and phenol, the bands at 2924 and 2861 cm^{-1} connote methylene C-H asymmetric stretch, the band at 1618 cm^{-1} in the spectra represents C-N and C-C stretching, indicating proteins, and finally, the band at 1101 cm^{-1} is the proof of skeletal C-C vibrations.

FESEM was employed to identify the shape and distribution of green synthesized silver nanoparticles. Scanning electron micrographs indicated that the synthesized silver nanoparticles were spherical nanoparticles. Individual nanoparticles can aggregate to form larger silver particles. This aggregation occurred due to the presence of cell components on the surface of nanoparticles and acts as a capping agent. The average size of AgNPQct was 77 nm (Figure 1(e)).

3.2. Antileishmanial Activity

3.2.1. In Vitro Results. The antileishmanial activity of quercetin, AgNPQct, and glucantime on the proliferation of *L. major* promastigotes was measured at various time intervals (24, 48, and 72 hr). The analysis result showed that the promastigote count in the first 24 hr of incubation with AgNPQct was significantly decreased in comparison with the untreated group ($p < 0.01$), and after 72 hr of incubation, cytotoxicity was 100%. Extracts of quercetin showed cytotoxicity with 67.5% after 48 hr and 82% after 72 hr (Figure 2).

MTT analysis showed that both the aqueous extract from quercetin and AgNPQct had significant activities against promastigotes. Synthesized AgNPQct exhibited 50% cell inhibition (IC50) at 125 μg/ml, whereas the aqueous extract from quercetin and luteolin exhibited 50% cell inhibition (IC50) at 150 and 13 μg/ml, respectively.

3.2.2. In Vivo Results. The mean area of lesions was calculated every 7 days at the beginning of treatment. As shown in Figures 3 and 4, the size of the lesions increased in all cases but showed a slight increase in the group treated with luteolin/AgNPQct ointment, while the increase in the size of the lesions in the untreated group was significant ($p < 0.01$).

(1) Histological Analysis. After 21 days of treatment of *L. major*-infected mice with luteolin/AgNPQct ointment, histological evaluations were performed. In the untreated group, macrophages containing *Leishmania* amastigotes and free parasites were present in much higher numbers. Furthermore, large numbers of inflammatory cells, including macrophages, lymphocytes, neutrophils, and eosinophils, were detected in the dermis of these mice (Figure 5(d)). In contrast, in the group of mice treated with luteolin/AgNPQct ointment, tissue granulation was established, and accordingly, the number of lymphocytes and neutrophils was significantly lower and the number of fibroblasts and

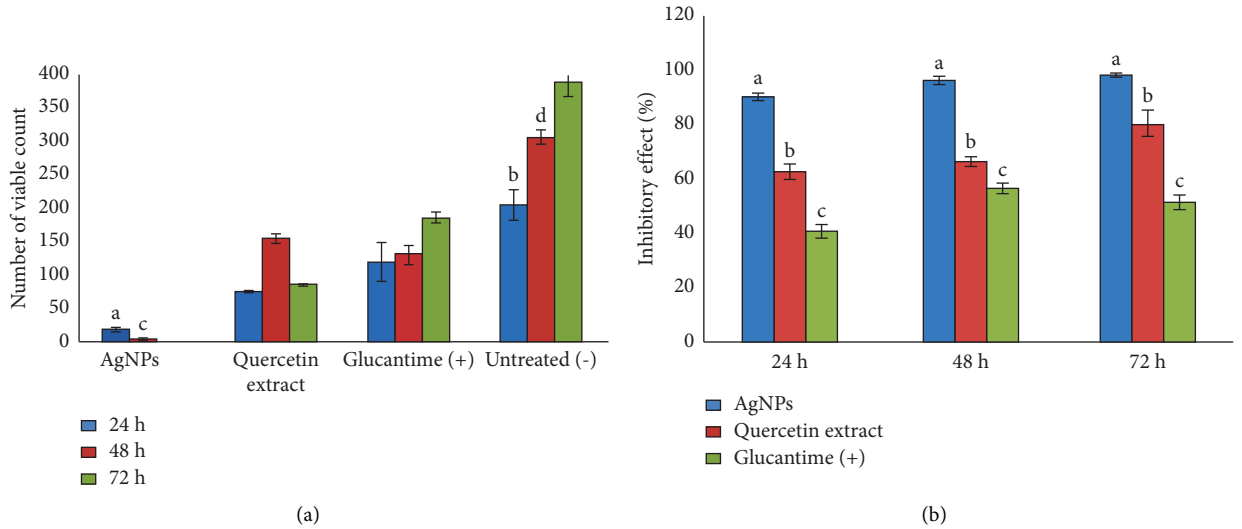

Figure 2: Time-dependent cytotoxicity of synthesized AgNPs and quercetin against *L. major* promastigotes. The number of viable cells in controls and treated samples at different time intervals (24, 48, and 72 hr) (a). The percent of growth inhibition after exposure to silver nanoparticles, quercetin, and glucantime at various time intervals (24, 48, and 72 hr) (b).

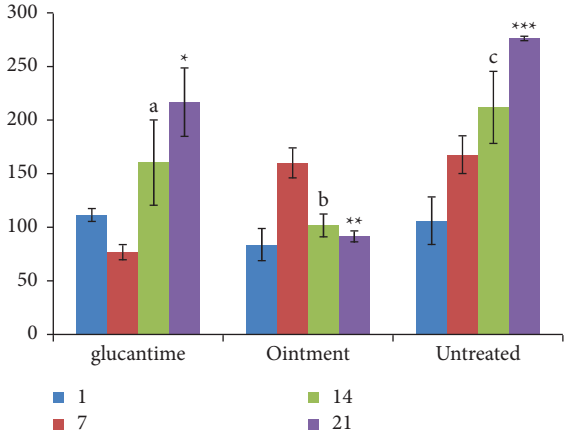

Figure 3: Changes in the surface area of the lesions (mean ± SE) of different experimental groups during treatment with luteolin/AgNPQct ointment.

fibrocytes was significantly higher than those in the other groups ($p < 0.05$) (Figure 5(a)). A high content of collagen deposition in the luteolin/AgNPQct group was demonstrated, but collagen deposition was sparse in the untreated group.

4. Discussion

Among various nanomaterials, metallic nanoparticles such as silver nanoparticles can be widely used as alternative therapeutic agents against infectious diseases [36]. There are several physical and chemical methods for synthesizing nanoparticles, but unfortunately, the use of hazardous substances in these processes is inevitable, so there is a need for finding eco-friendly and cost-effective methods with high efficiency. Biosynthesis of nanoparticles using plants is safer, more secure, and healthier than using bacteria, fungi, and yeasts. These biomolecules, as reducing, capping, and stabilizing agents, which are bound on the surface of AgNPs may change the surface chemistry of AgNPs and interfere with the AgNPs' behavior in response to their biological environment. The cytotoxicity of biogenic AgNPs was observed to have a wide range of IC50 values. It is important to note that a number of factors, including AgNP size distribution, shape, and surface chemistry, may affect the cytotoxicity of biogenic AgNPs in order to explain this observation [26, 37–42].

In this study, we synthesized Ag nanoparticles mediated by quercetin aqueous extracts. In our results, the size of Ag nanoparticles in FESEM analysis was smaller than that in DLS analysis. DLS measures the hydrodynamic diameter, while FESEM gives the actual size of nanoparticles. The zeta potential values of synthesized AgNPs indicate the stabilization of AgNPs. In this study, the negative zeta potential value (−10.23 mV) may be due to the adsorption of bioactive components of quercetin as capping agents because these components mainly consisted of negatively charged groups [43].

FTIR spectroscopy was performed to ascertain the possible functional groups involved in the capping and efficient stabilization of silver nanoparticles. Based on FTIR spectral results, it can be suggested that biological molecules such as flavonoids and other phenolic compounds and their functional groups are responsible for reducing and stabilizing AgNPs in an aqueous medium. These findings are consistent with the findings of Ullah et al. (2018), Patra et al. (2014), and Ahmad et al. (2016) [44–46].

In this study, a three-week treatment of cutaneous lesions of *L. major*-infected BALB/c mice with luteolin/AgNPQct ointment significantly reduced macrophages loaded with amastigotes and neutrophil infiltration compared with the untreated group. This treatment also significantly increased collagen deposition and the number of fibroblasts and fibrocytes. In the glucantime-treated group

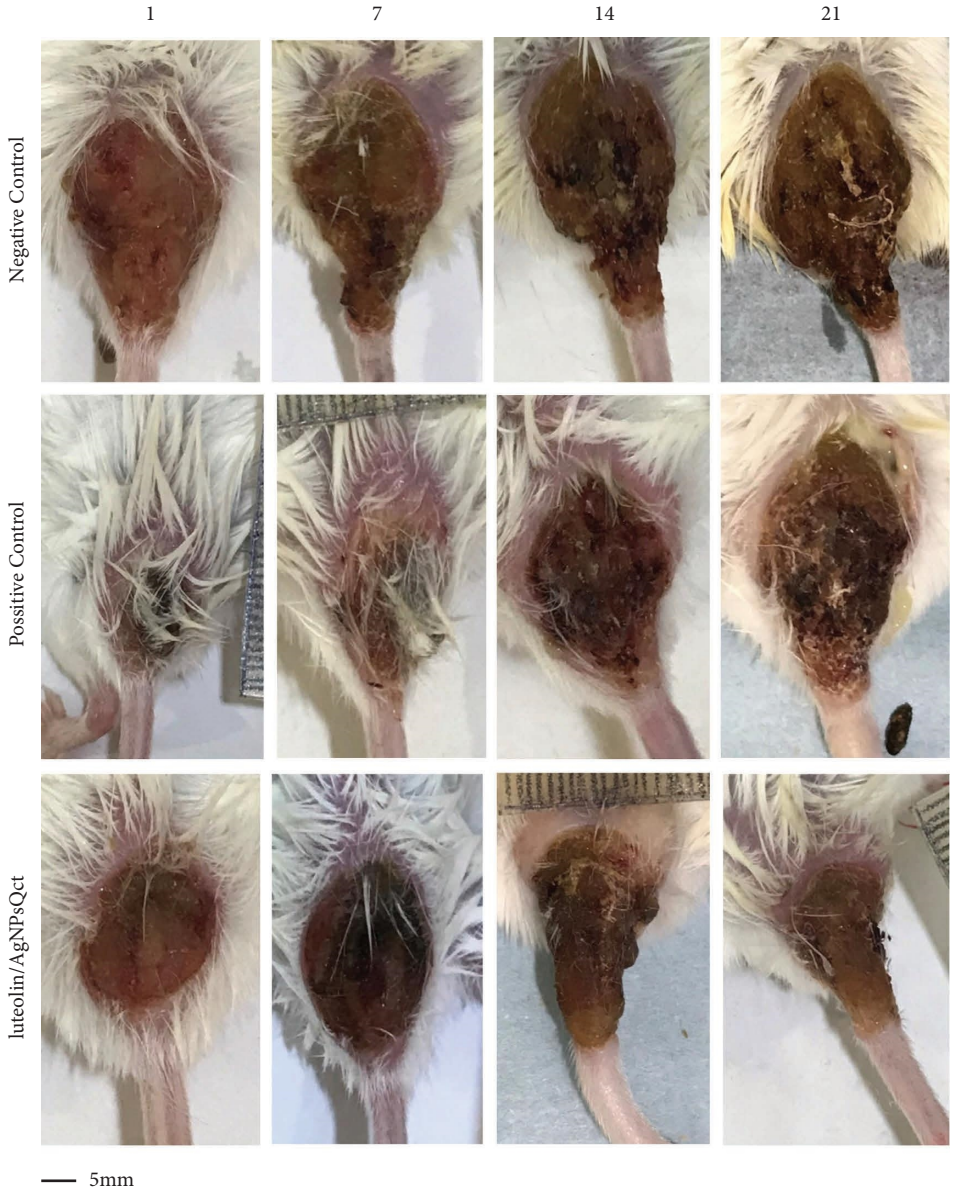

Figure 4: The image of tail-base wound in mice. Negative control (without treatment) vs. positive control (treatment with glucantime) and the ointment group (treatment with luteolin/AgNPQct ointment) on 1, 7, 14, and 21 days.

(Figure 4(e)), the number of fibroblasts and fibrocytes and the alignment of granulation tissue in animal lesions were lower than those in the luteolin/AgNPQct-treated group, so luteolin/AgNPQct ointment seems to be superior to glucantime in rapid wound healing. Several studies have been published on the antileishmanial activities of synthetic and biogenic silver, gold, and titanium oxide nanoparticles [44, 46–48]. There are some possible explanations for the high antileishmanial activity of biological nanoparticles. Nitric oxide (NO) is a free radical in biological systems. Nanoparticles can produce high levels of nitric oxide (NO) which reacts with the superoxides of living organisms. The produced nitrate (NO_3^-) during this process has toxic effects on the chemical composition or structure, surface morphology, protein synthesis, and lipid peroxidation of *Leishmania* cells [49, 50]. Another point is that increased local ROS production may be responsible for the loss of mitochondrial membrane integrity and cell death [30]. In addition, metal nanoparticles have an increased surface-to-volume ratio and can easily bind to sulfur- and phosphorus-containing groups and increase their antileishmanial activity [47, 51].

Quercetin and luteolin are two important members of flavonoids with the marked antileishmanial activity [21, 52]. These two macromolecules can exert their antileishmanial activity by inducing topoisomerase II-mediated kinetoplast DNA cleavage in *Leishmania* cells and leading to apoptotic-like death. Furthermore, quercetin and luteolin can induce the death of parasites by increasing the production of reactive oxygen species (ROS) [21, 52–54]. Among the studies on the effect of quercetin on *Leishmania* amastigotes, Vilanova et al. (2012) evaluated the growth inhibitory effect

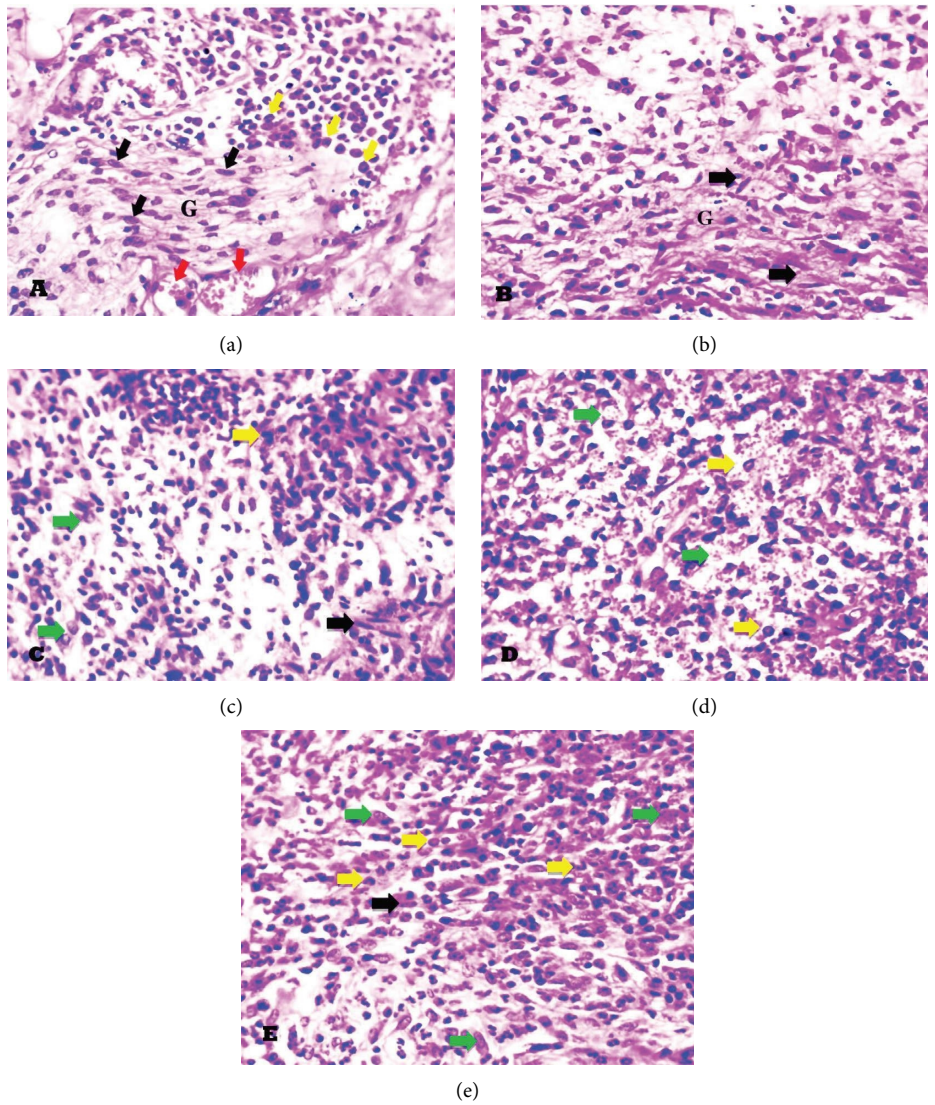

Figure 5: Histological sections from the skin of BALB/c mice infected with *L. major* and treated with luteolin/AgNPQct ointment (a), AgNPQct (b), and luteolin (c) for 21 days compared to the untreated (d) and glucantime (e) groups (H&E, ×400). Black arrow: fibroblast, red arrow: blood vessel, green arrow: amastigotes, and yellow arrow: neutrophils.

of quercetin on *L. infantum* amastigotes to be approximately equivalent to the effect of amphotericin B as a control drug in the macrophage culture medium. In another study, the use of various quercetin compounds in the oral treatment of *L. amazonensis*-infected mice had inhibitory effects on the growth of skin lesions and also significantly reduced the load of amastigotes [55].

Evaluating the effects of quercetin on *L. braziliensis*-infected macrophages, Cataneo et al. (2019) [53] stated that the use of this flavonoid reduced the number of parasites in infected macrophages, decreased TNF levels, and increased serum IL-10 levels without modulating NO production, while reducing iron is used to amplify parasitic amastigotes. However, in the study by Muzitano et al. (2009) [56], quercetin failed to inhibit the growth of skin lesions in laboratory mice, despite its inhibitory effects on the growth of promastigotes *in vitro*.

Based on many studies, it seems that quercetin nanoparticles are a good platform to increase their bioavailability in drug delivery systems [57]. According to the study by Awad et al. (2021) [58], using silver nanoparticles in the treatment of subcutaneous lesions caused by *L. major* in mice had more inhibitory effects on preventing the growth of these lesions than Pentostam and chemical nanoparticles. According to the researchers' hypothesis, the wider surface area of these particles, their very small size, and the ability to bind sulfur- and phosphorus-containing groups increase antileishmanial effects. Similarly, the significant antileishmanial activity of AgNPQct in the present study may be due to the synergistic effects of capping agents on the surface of silver nanoparticles.

The present study is a report on the synthesis of AgNPs using quercetin as a reducing and capping agent to produce ointment with luteolin and synthesized AgNPs. Our study

suggested that luteolin/quercetin-capped silver nanoparticle ointment can be effective against promastigotes and amastigotes of *L. major*.

5. Limitations

Although this ointment can be effective along with other treatments, there are several challenges that limit large-scale production and subsequent applications. Some of the major challenges are as follows:

(i) Detailed optimization studies on reactants and process parameters are required to control the size and shape of NPs.

(ii) The involvement of each metabolite of plant extracts and cellular components of microorganisms in the synthesis of NPs should be completely analyzed.

(iii) Scale-up of NP production for commercial purposes using green synthesis methods needs to be prioritized.

Also, one of the limitations of using silver nanoparticles in the treatment of patients is the potential toxicity of silver nanoparticles in multiple systems, including the skin, eyes, kidneys, respiratory system, hepatobiliary system, immune system, and reproductive system. To overcome this limitation, AgNPs' biocompatibility and possible cytotoxicity must be evaluated in greater detail in order to create safer and more biocompatible AgNP-based drugs.

6. Conclusions

In this study, the synthesis of silver nanoparticles is a cost-effective and eco-friendly green approach. The aqueous extract from quercetin was successfully used as a reducing and capping agent without the use of any aided supportive chemicals. The use of luteolin/AgNPQct ointment *in vivo* showed that the immunomodulatory and antileishmanial effects of the ointment could accelerate wound healing and reduce the parasitic load and inflammatory responses.

The findings of this study conclude that combination therapy with luteolin/AgNPQct ointment may be a more effective and economic strategy for the treatment of cutaneous leishmaniasis. In addition, combination therapy could reduce the amount and cost of expensive treatments, thus providing a safe, effective, and economic alternative to the current antileishmanial treatment.

Abbreviations

AgNPs: Silver nanoparticles
AgNPQct: Luteolin/quercetin-capped silver nanoparticles
CL: Cutaneous leishmaniasis
MCL: Mucocutaneous leishmaniasis
VL: Visceral leishmaniasis
L-AmB: Liposomal amphotericin B
ROS: Reactive oxygen species
UV-Vis: Ultraviolet-visible
DLS: Dynamic light scattering
PDI: Polydispersity index
FTIR: Fourier-transform infrared
FESEM: Field emission scanning electron microscopy
RPMI-1640: Roswell Park Memorial Institute-1640
DMSO: Dimethyl sulfoxide.

Data Availability

All the data generated or analyzed during this study are included in this published article.

Ethical Approval

This study was approved by the Ethics Committee of the Birjand University of Medical Sciences (IR.BUMS.REC.1398.349).

Consent

No consent was required for this study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Farshid Abedi, Mohammad Yahya Hanafi-Bojd, and Mehdi Karamian designed and supervised the study. Effat Alemzadeh and Mohammad Yahya Hanafi-Bojd performed the laboratory assays. Mohammd Amin Ghatee, Mehdi Karamian, Mina Hemmati, and Azadeh Ebrahimzadeh were involved in drafting the manuscript and analysis and/or interpretation of the data. Azadeh Ebrahimzadeh revised the manuscript critically for important intellectual content. All the authors have read and approved the final version of the manuscript.

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References

[1] B. Sakib, "Croft simon L, boelaert marleen. leishmaniasis," *The Lancet*, vol. 392, no. 10151, pp. 951–970, 2018.

[2] M. Shahinozzaman, N. Taira, T. Ishii, M. A. Halim, M. A. Hossain, and S. Tawata, "Anti-inflammatory, anti-diabetic, and anti-Alzheimer's effects of prenylated flavonoids from Okinawa propolis: an investigation by experimental and computational studies," *Molecules*, vol. 23, no. 10, p. 2479, 2018.

[3] S. Singh-Phulgenda, P. Dahal, R. Ngu, B. J. Maguire, A. Hawryszkiewycz, and S. Rashan, "Serious adverse events following treatment of visceral leishmaniasis: a systematic review and meta-analysis," *PLoS Neglected Tropical Diseases*, vol. 15, no. 3, Article ID e0009302, 2021.

[4] A. Ponte-Sucre, F. Gamarro, J.-C. Dujardin, M. P. Barrett, R. López-Vélez, and R. García-Hernández, "Drug resistance and treatment failure in leishmaniasis: a 21st century challenge," *PLoS Neglected Tropical Diseases*, vol. 11, no. 12, Article ID e0006052, 2017.

[5] J. Berman, "Liposomal amphotericin B treatment and the leishmaniases," *The American Journal of Tropical Medicine and Hygiene*, vol. 101, no. 4, p. 727, 2019.

[6] M. Azim, S. A. Khan, S. Ullah, S. Ullah, and S. I. Anjum, "Therapeutic advances in the topical treatment of cutaneous leishmaniasis: a review," *PLoS Neglected Tropical Diseases*, vol. 15, no. 3, Article ID e0009099, 2021.

[7] J. F. Peixoto, Y. J. Ramos, D. de Lima Moreira, C. R. Alves, and L. F. Gonçalves-Oliveira, "Potential of Piper spp. as a source of new compounds for the leishmaniases treatment," *Parasitology Research*, vol. 120, no. 8, pp. 2731–2747, 2021.

[8] S. Raj, S. Sasidharan, S. Balaji, V. K. Dubey, and P. Saudagar, "Review on natural products as an alternative to contemporary anti-leishmanial therapeutics," *Journal of Proteins and Proteomics*, vol. 11, no. 2, pp. 135–158, 2020.

[9] A. Salehabadi, M. Karamian, M. H. Farzad, and M. H. Namaei, "Effect of root bark extract of Berberis vulgaris L. on Leishmania major on BALB/c mice," *Parasitology Research*, vol. 113, no. 3, pp. 953–957, 2014.

[10] V. Gouri, S. C. Pandey, D. Joshi, V. Pande, S. Upreti, and M. Samant, *Natural Products as a Novel Source for Anti-leishmanial Drug Development. Pathogenesis, Treatment and Prevention of Leishmaniasis*, Elsevier, Amsterdam, Netherlands, 2021.

[11] J. Solnier and J.-P. Fladerer, "Flavonoids: a complementary approach to conventional therapy of COVID-19?" *Phytochemistry Reviews*, vol. 20, no. 4, pp. 773–795, 2021.

[12] H. M. Waheeb, G. M. Sulaiman, and M. S. Jabir, "Effect of hesperidin conjugated with golden nanoparticles on phagocytic activity: in vitro study," in *Proceedings of the AIP Conference Proceedings*, AIP Publishing LLC, Antalya, Turkey, May 2020.

[13] A. A. Al-Jubori, G. M. Sulaiman, A. T. Tawfeeq, H. A. Mohammed, R. A. Khan, and S. A. Mohammed, "Layer-by-layer nanoparticles of tamoxifen and resveratrol for dual drug delivery system and potential triple-negative breast cancer treatment," *Pharmaceutics*, vol. 13, no. 7, p. 1098, 2021.

[14] M. R. Farahpour, N. Mirzakhani, J. Doostmohammadi, and M. Ebrahimzadeh, "Hydroethanolic Pistacia atlantica hulls extract improved wound healing process; evidence for mast cells infiltration, angiogenesis and RNA stability," *International Journal of Surgery*, vol. 17, pp. 88–98, 2015.

[15] M. R. Farahpour, S. Sheikh, E. Kafshdooz, and A. Sonboli, "Accelerative effect of topical Zataria multiflora essential oil against infected wound model by modulating inflammation, angiogenesis, and collagen biosynthesis," *Pharmaceutical Biology*, vol. 59, no. 1, pp. 1–10, 2021.

[16] M. R. Farahpour, M. Vahid, and A. Oryan, "Effectiveness of topical application of ostrich oil on the healing of Staphylococcus aureus-and Pseudomonas aeruginosa-infected wounds," *Connective Tissue Research*, vol. 59, no. 3, pp. 212–222, 2018.

[17] M. R. Farahpour, S. Hesaraki, D. Faraji, R. Zeinalpour, and M. Aghaei, "Hydroethanolic Allium sativum extract accelerates excision wound healing: evidence for roles of mast-cell infiltration and intracytoplasmic carbohydrate ratio," *Brazilian Journal of Pharmaceutical Sciences*, vol. 53, 2017.

[18] H. A. Mohammed, G. M. Sulaiman, S. S. Anwar, A. T. Tawfeeq, R. A. Khan, and S. A. Mohammed, "Quercetin against MCF7 and CAL51 breast cancer cell lines: apoptosis, gene expression and cytotoxicity of nano-quercetin," *Nanomedicine*, vol. 16, no. 22, pp. 1937–1961, 2021.

[19] M. Sobhani, M. H. Farzaei, S. Kiani, and R. Khodarahmi, "Immunomodulatory; anti-inflammatory/antioxidant effects of polyphenols: a comparative review on the parental compounds and their metabolites," *Food Reviews International*, vol. 37, no. 8, pp. 759–811, 2021.

[20] G. M. Sulaiman, "In vitro study of molecular structure and cytotoxicity effect of luteolin in the human colon carcinoma cells," *European Food Research and Technology*, vol. 241, no. 1, pp. 83–90, 2015.

[21] B. Mittra, A. Saha, A. Roy Chowdhury, C. Pal, S. Mandal, and S. Mukhopadhyay, "Luteolin, an abundant dietary component is a potent anti-leishmanial agent that acts by inducing topoisomerase II-mediated kinetoplast DNA cleavage leading to apoptosis," *Molecular Medicine*, vol. 6, no. 6, pp. 527–541, 2000.

[22] A.-C. Burduşel, O. Gherasim, A. M. Grumezescu, L. Mogoantă, A. Ficai, and E. Andronescu, "Biomedical applications of silver nanoparticles: an up-to-date overview," *Nanomaterials*, vol. 8, no. 9, p. 681, 2018.

[23] S. Iravani, H. Korbekandi, S. V. Mirmohammadi, and B. Zolfaghari, "Synthesis of silver nanoparticles: chemical, physical and biological methods," *Research in pharmaceutical sciences*, vol. 9, no. 6, p. 385, 2014.

[24] S. G. Daghian, M. R. Farahpour, and S. Jafarirad, "Biological fabrication and electrostatic attractions of new layered silver/talc nanocomposite using Lawsonia inermis L. and its chitosan-capped inorganic/organic hybrid: investigation on acceleration of *Staphylococcus aureus* and *Pseudomonas aeruginosa* infected wound healing," *Materials Science and Engineering: C*, vol. 128, Article ID 112294, 2021.

[25] S. Mahmoudabadi, M. R. Farahpour, and S. Jafarirad, "Effectiveness of green synthesis of silver/kaolinite nanocomposite using Quercus infectoria galls aqueous extract and its chitosan-capped derivative on the healing of infected wound," *IEEE Transactions on NanoBioscience*, vol. 20, no. 4, pp. 530–542, 2021.

[26] A. Roy, O. Bulut, S. Some, A. K. Mandal, and M. D. Yilmaz, "Green synthesis of silver nanoparticles: biomolecule-nanoparticle organizations targeting antimicrobial activity," *RSC Advances*, vol. 9, no. 5, pp. 2673–2702, 2019.

[27] E. Alemzadeh, M. Karamian, F. Abedi, and M. Y. Hanafi-Bojd, "Topical treatment of cutaneous leishmaniasis lesions using quercetin/Artemisia-capped silver nanoparticles ointment: modulation of inflammatory response," *Acta Tropica*, vol. 228, Article ID 106325, 2022.

[28] M. Hekmati, S. Hasanirad, A. Khaledi, and D. Esmaeili, "Green synthesis of silver nanoparticles using extracts of Allium rotundum l, Falcaria vulgaris Bernh, and Ferulago angulate Boiss, and their antimicrobial effects in vitro," *Gene Reports*, vol. 19, Article ID 100589, 2020.

[29] A. K. Mittal, Y. Chisti, and U. C. Banerjee, "Synthesis of metallic nanoparticles using plant extracts," *Biotechnology Advances*, vol. 31, no. 2, pp. 346–356, 2013.

[30] F. Fonseca-Silva, J. D. Inacio, M. M. Canto-Cavalheiro, and E. E. Almeida-Amaral, "Reactive oxygen species production and mitochondrial dysfunction contribute to quercetin induced death in Leishmania amazonensis," *PLoS One*, vol. 6, no. 2, Article ID e14666, 2011.

[31] A. C. Gharehpapagh, M. R. Farahpour, and S. Jafarirad, "The biological synthesis of gold/perlite nanocomposite using Urtica dioica extract and its chitosan-capped derivative for

healing wounds infected with methicillin-resistant *Staphylococcus aureus*," *International Journal of Biological Macromolecules*, vol. 183, pp. 447–456, 2021.

[32] A. Chahardoli, P. Hajmomeni, M. Ghowsi, F. Qalekhani, Y. Shokoohinia, and A. Fattahi, "Optimization of quercetin-assisted silver nanoparticles synthesis and evaluation of their hemocompatibility, antioxidant, anti-inflammatory, and antibacterial effects," *Global Challenges*, vol. 5, no. 12, Article ID 2100075, 2021.

[33] S. Pandian, S. Kunjiappan, V. Ravishankar, and V. Sundarapandian, "Synthesis of quercetin-functionalized silver nanoparticles by rapid one-pot approach," *Bio-Technologia Journal of Biotechnology Computational Biology and Bionanotechnology*, vol. 102, no. 1, 2021.

[34] A. Daemi, M. R. Farahpour, A. Oryan, S. Karimzadeh, and E. Tajer, "Topical administration of hydroethanolic extract of Lawsonia inermis (henna) accelerates excisional wound healing process by reducing tissue inflammation and amplifying glucose uptake," *The Kaohsiung Journal of Medical Sciences*, vol. 35, no. 1, pp. 24–32, 2019.

[35] R. F. Mohammad, T. Human, H. Mostafa, and A. Z. Mohammad, "Wound healing activity of flaxseed Linum usitatissimum L. in rats," *African Journal of Pharmacy and Pharmacology*, vol. 5, no. 21, pp. 2386–2389, 2011.

[36] S. P. Deshmukh, S. Patil, S. Mullani, and S. Delekar, "Silver nanoparticles as an effective disinfectant: a review," *Materials Science and Engineering: C*, vol. 97, pp. 954–965, 2019.

[37] M. Saravanan, H. Barabadi, and H. Vahidi, *Green Nanotechnology: Isolation of Bioactive Molecules and Modified Approach of Biosynthesis. Biogenic Nanoparticles for Cancer Theranostics*, Elsevier, Amsterdam, Netherlands, 2021.

[38] M. Saravanan, H. Barabadi, H. Vahidi, T. J. Webster, D. Medina-Cruz, and E. Mostafavi, "Emerging theranostic silver and gold nanobiomaterials for breast cancer: present status and future prospects," *Handbook on Nanobiomaterials for Therapeutics and Diagnostic Applications*, pp. 439–456, 2021.

[39] H. Barabadi, H. Vahidi, K. Damavandi Kamali, M. Rashedi, and M. Saravanan, "Antineoplastic biogenic silver nanomaterials to combat cervical cancer: a novel approach in cancer therapeutics," *Journal of Cluster Science*, vol. 31, no. 4, pp. 659–672, 2020.

[40] P. Mathur, S. Jha, S. Ramteke, and N. Jain, "Pharmaceutical aspects of silver nanoparticles," *Artificial Cells, Nanomedicine, and Biotechnology*, vol. 46, no. 1, pp. 115–126, 2018.

[41] H. Barabadi, H. Vahidi, M. A. Mahjoub, Z. Kosar, K. Damavandi Kamali, K. Ponmurugan et al., "Emerging antineoplastic gold nanomaterials for cervical cancer therapeutics: a systematic review," *Journal of Cluster Science*, vol. 31, no. 6, pp. 1173–1184, 2020.

[42] H. Barabadi, H. Vahidi, K. Damavandi Kamali, M. Rashedi, O. Hosseini, and M. Saravanan, "Emerging theranostic gold nanomaterials to combat colorectal cancer: a systematic review," *Journal of Cluster Science*, vol. 31, no. 4, pp. 651–658, 2020.

[43] M. Reda, A. Ashames, Z. Edis, S. Bloukh, R. Bhandare, and H. Abu Sara, "Green synthesis of potent antimicrobial silver nanoparticles using different plant extracts and their mixtures," *Processes*, vol. 7, no. 8, p. 510, 2019.

[44] A. Ahmad, Y. Wei, F. Syed, S. Khan, G. M. Khan, and K. Tahir, "Isatis tinctoria mediated synthesis of amphotericin B-bound silver nanoparticles with enhanced photoinduced antileishmanial activity: a novel green approach," *Journal of Photochemistry and Photobiology B: Biology*, vol. 161, pp. 17–24, 2016.

[45] J. K. Patra and K.-H. Baek, "Green nanobiotechnology: factors affecting synthesis and characterization techniques," *Journal of Nanomaterials*, vol. 2014, 2015.

[46] I. Ullah, G. Cosar, E. S. Abamor, M. Bagirova, Z. K. Shinwari, and A. M. Allahverdiyev, "Comparative study on the antileishmanial activities of chemically and biologically synthesized silver nanoparticles (AgNPs)," *3 Biotech*, vol. 8, no. 2, pp. 1–8, 2018.

[47] A. M. Allahverdiyev, E. S. Abamor, M. Bagirova, S. Y. Baydar, S. C. Ates, and F. Kaya, "Investigation of antileishmanial activities of Tio2@ Ag nanoparticles on biological properties of L. tropica and L. infantum parasites, in vitro," *Experimental Parasitology*, vol. 135, no. 1, pp. 55–63, 2013.

[48] A. A. Zahir, I. S. Chauhan, A. Bagavan, C. Kamaraj, G. Elango, and J. Shankar, "Green synthesis of silver and titanium dioxide nanoparticles using Euphorbia prostrata extract shows shift from apoptosis to G0/G1 arrest followed by necrotic cell death in Leishmania donovani," *Antimicrobial Agents and Chemotherapy*, vol. 59, no. 8, pp. 4782–4799, 2015.

[49] C. Banuls, M. Rocha, S. Rovira-Llopis, R. Falcon, R. Castello, and J. R Herance, "The pivotal role of nitric oxide: effects on the nervous and immune systems," *Current Pharmaceutical Design*, vol. 20, no. 29, pp. 4679–4689, 2014.

[50] S. Elcicek, M. Bagirova, and A. M. Allahverdiyev, "Generation of avirulent Leishmania parasites and induction of nitric oxide production in macrophages by using polyacrylic acid," *Experimental Parasitology*, vol. 133, no. 3, pp. 237–242, 2013.

[51] A. M. Allahverdiyev, E. S. Abamor, M. Bagirova, and M. Rafailovich, "Antimicrobial effects of TiO2 and Ag2O nanoparticles against drug-resistant bacteria and leishmania parasites," *Future Microbiology*, vol. 6, no. 8, pp. 933–940, 2011.

[52] I. L. Wong, K.-F. Chan, Y.-F. Chen, Z.-R. Lun, T. H. Chan, and L. M. Chow, "In vitro and in vivo efficacy of novel flavonoid dimers against cutaneous leishmaniasis," *Antimicrobial Agents and Chemotherapy*, vol. 58, no. 6, pp. 3379–3388, 2014.

[53] A. H. D. Cataneo, F. Tomiotto-Pellissier, M. M. Miranda-Sapla, J. P. Assolini, C. Panis, D. Kian et al., "Quercetin promotes antipromastigote effect by increasing the ROS production and anti-amastigote by upregulating Nrf2/HO-1 expression, affecting iron availability," *Biomedicine and Pharmacotherapy*, vol. 113, Article ID 108745, 2019.

[54] E. R. da Silva, C. do Carmo Maquiaveli, and P. P. Magalhães, "The leishmanicidal flavonols quercetin and quercitrin target Leishmania (Leishmania) amazonensis arginase," *Experimental Parasitology*, vol. 130, no. 3, pp. 183–188, 2012.

[55] E. Montrieux, W. H. Perera, M. García, L. Maes, P. Cos, and L. Monzote, "In vitro and in vivo activity of major constituents from Pluchea carolinensis against Leishmania amazonensis," *Parasitology Research*, vol. 113, no. 8, pp. 2925–2932, 2014.

[56] M. F. Muzitano, C. A. Falcão, E. A. Cruz, M. C. Bergonzi, A. R. Bilia, and F. F. Vincieri, "Oral metabolism and efficacy of

Kalanchoe pinnata flavonoids in a murine model of cutaneous leishmaniasis," *Planta Medica*, vol. 75, no. 04, pp. 307–311, 2009.

[57] B. Salehi, L. Machin, L. Monzote, J. Sharifi-Rad, S. M. Ezzat, and M. A. Salem, "Therapeutic potential of quercetin: new insights and perspectives for human health," *ACS Omega*, vol. 5, no. 20, pp. 11849–11872, 2020.

[58] M. A. Awad, E. M. Al Olayan, M. I. Siddiqui, N. M. Merghani, S. S. A.-l Alsaif, and A. S. Aloufi, "Antileishmanial effect of silver nanoparticles: green synthesis, characterization, in vivo and in vitro assessment," *Biomedicine and Pharmacotherapy*, vol. 137, Article ID 111294, 2021.