Wound healing properties of green (using *Lawsonia inermis* leaf extract) and chemically synthesized ZnO nanoparticles in albino rats

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Abstract

Wound healing is one of the utmost medical issues in human and veterinary medicine, which explains the urgent need for developing new agents that possess wound healing activities. The present study aimed to assess the effectiveness of green and chemical zinc oxide nanoparticles (ZnO-NPs) for wound healing. ZnO-NPs (green using *Lawsonia inermis* leaf extract and chemical) were synthesized and characterized by X-ray powder diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, and high-resolution transmission electron microscopy (HRTEM). The gels containing the nanomaterials were prepared and inspected. Forty-five albino rats were divided into three groups, the control group was treated with normal saline 0.9%, and the other two groups were treated with gels containing green or chemical ZnO-NPs, respectively. On the 3rd, 7th, 14th, and 21st days post-treatment (PT), the wounds were clinicopathologically examined. Both nanomaterials have good crystallinity and high purity, but green ZnO-NPs have a longer nanowire length and diameter than chemical ZnO-NPs. The formed gels were highly viscous with a pH of 6.5 to 7. The treated groups with ZnO-NP gels showed clinical improvement, as decreased wound surface area (WSA) percent (WSA%), increased wound contraction percent (WC%), and reduced healing time ($p < 0.05$) when compared with the control group. The histological scoring showed that the epithelialization score was significantly higher at the 21st day post-treatment in the treated groups than in the control group ($p < 0.05$), but the vasculature, necrosis, connective tissue formation, and collagen synthesis scores were mostly similar. The green and chemical ZnO-NP gels showed promising wound healing properties; however, the *L. inermis*–mediated ZnO-NPs were more effective.

Keywords ZnO nanoparticle · Characterization · Green synthesis · *Lawsonia inermis* · Gel · Wound healing
**Introduction**

Skin is the largest organ in the human and animal bodies, and it acts as a resistant insulating layer across the entire body to protect against extreme conditions and infections (George Broughton et al. 2006). Skin is subject to lose its normal continuity and anatomy as well as physiology in the form of a wound by various causes such as physical, chemical, thermal, and biological causes. Wound healing is a complicated and diverse recovery process that involves re-epithelialization and restoration of tensile strength to restore injured cells to their natural state (Naraginti et al. 2016).

The skin is a vital multifunctional organ, and to restore the normal skin anatomy and functions, the wound healing must be rapid and free of complications (Karodi et al. 2009). The ability of the skin to heal wounds quickly and effectively is critical for maintaining natural healthy skin (Christian et al. 2006). Nanotechnology, a rapidly growing and challenging research area, has piqued scientists’ interest in a variety of biomedical applications (Mohamed and Safwat 2013; Dawood et al. 2019a, b; Akbar et al. 2020; El-Sayed and Kamel 2020a; Bhat-tacharya et al. 2021; Kabir et al. 2021). Zinc oxide nanoparticles (ZnO-NPs) are one of the most commonly used metal oxide nanoparticles for biomedical purposes such as wound healing, antimicrobial, and anti-inflammatory properties, due to its unique physicochemical characteristics (Gunalan et al. 2012; Varghese and George 2015; Abdel-Daim et al. 2019), and improvement of the growth rate, immune, and reproductive status of farm animals and birds (El-Sayed and Kamel 2020b).

In recent years, ZnO-NPs have been studied for use in a variety of skin therapies because they can improve the healing of wounds and reduce inflammation at the wound site, but the mechanisms behind these improvements remain unknown (Rayyif et al. 2021).

Zinc-based formulations have also been demonstrated to enhance wound healing and bacterial infection control at the tissue site. They also helped in the development of granulation tissue at the wound site (Jamil et al. 2021).

Green nanoparticle synthesis is appealing as a potential alternative to chemical and physical methods. Plant-mediated nanoparticle synthesis is an environmentally friendly, cost-effective, and safe method (Gan and Li 2012; Kouvaris et al. 2012; El-Seedi et al. 2019; Akbar et al. 2020). *Lawsonia inermis* (henna) is a well-known plant that has been studied in herbal medicine. Extract of leaves has shown various biological activities and was used as a wound dressing as it accelerated wound healing and because of its antimicrobial properties (Nayak et al. 2007; Towfik et al. 2015; Rekik et al. 2019).

This study aimed to investigate the wound healing properties of green (using methanolic extract of *Lawsonia inermis* leaves) and chemically synthesized ZnO-NPs in cutaneous wounds of albino rats. Both nanomaterials were physicochemically characterized. Clinical and histological evaluations were used to assess their effectiveness in wound healing.

**Materials and methods**

**Chemicals and reagents**

Methanol was purchased from Lab-Scan Analytical Sciences, Poland. Activated charcoal powder was purchased from ADWIA Company, Egypt. Potassium dichromate of analytical reagent (AR) grade was used without any further purification. Zinc nitrate hexahydrate (Zn(NO$_3$)$_2$·6H$_2$O; 99%) was obtained from Sigma-Aldrich (USA). Diethyl ether and Carbopol 940® were purchased from Loba Chemie Company, India. All other chemicals and solvents were of analytical grade.

**Plant material**

Commercial dried leaves of *Lawsonia inermis* (henna) were purchased from a local market in Aswan City (latitude 24°5′15″ N; longitude 32°53′56″ E), the southern part of Egypt, and were identified by the Department of Botany, Faculty of Science, South Valley University. Leaves were screened, and the good ones were selected to be ground into a fine powder using an electric blender.

**Extract preparation**

Dried leaves of *Lawsonia inermis* were soaked in 80% methanol at a 1:10 ratio in an Erlenmeyer flask covered with aluminum foil for 24 h, with periodic agitation. Activated charcoal was added to the extract and left for 15 min for chemical depigmentation. Then, the extract was filtrated by Whatman filter paper no. 1 and the filtrate was vacuum dried in a rotary evaporator (Heidolph, Germany) at 45 °C. The extract was stored at 4 °C till the preparation of green ZnO-NPs (Table 1).

**Table 1** Botanical information and percentage yields of the leaf extract

| Plant species | Family | Local name | Plant part used | Extract pH | Extract yield (%) |
|---------------|-------|------------|-----------------|------------|-------------------|
| *Lawsonia inermis* | Lythraceae | Henna | Leaves | 5.6 | 5 |
Synthesis of nanoparticles

Zinc oxide nanoparticles were prepared via henna extract by dissolving zinc nitrate hexahydrate (0.1 M) in 40 ml of distilled water. Then, 20 ml of henna extract solution was added with continuous stirring at 150 °C for 2–3 h until completely dissolved. The resultant mixture was cooled at room temperature, and the supernatant was discarded. The resultant solid product (pale white in color) was centrifuged twice at 6000 rpm for 10 min. Finally, the solid precipitate was dried at 60 °C for about 8 h and used for further studies. Zinc oxide nanoparticles were chemically prepared by placing zinc nitrate hexahydrate (0.1 M, Zn(NO₃)₂·6H₂O) in a water bath at 60 °C, then 0.4 M of sodium hydroxide was dropped under gentle stirring. The mixture was sealed under heating and stirring for 2 h. Then, the precipitation was separated and washed several times. Finally, the products were dried using a hot air oven at 60 °C for about 8 h.

Characterization of nanoparticles

To investigate the physicochemical properties of the prepared green and chemically synthesized samples, 1 g from each sample was sent to the Egyptian Petroleum Research Institution (EPRI) for characterization. XRD pattern was used to investigate nanostructural properties and purity of the prepared ZnO nanoparticles using a PANalytical X’Pert Pro diffractometer operated with CuKa radiation (λ = 1.54060 nm) and X-ray radiation (X-ray generator current and voltage set at 40 mA and 40 kV). The diffractograms were recorded in the 2θ range of 4–90 with a 2θ step size of 0.02 and a step time of 0.7 s. FTIR analysis was used to investigate the chemical composition of the prepared nanoparticles. It was performed by the Nicolet iS10 model (USA) infrared spectrophotometer in the range of wavenumber of 4000–400 cm⁻¹. The structural morphology of prepared nanoparticles was observed by transmission electron microscopy (TEM) using high-resolution JEOL 2100FTEM (Japan) at 200 kV.

Preparation of gels

Green or chemical ZnO-NPs (200 mg) were dissolved in 20 ml of 1% acetic acid and placed on a hot plate magnetic stirrer then cooled. Distilled water (100 ml) was poured into a mixer, and 1 g Carbopol 940® was slowly added, followed by the addition of the studied nanomaterial solutions until the formation of the gel with a concentration of 0.2%, which was then stored at −20 °C for further use.

Characters of gels

The gels were visually inspected for their physical properties, such as color, transparency, homogeneity, and pH. Skin irritation was evaluated by topical application of 2 g gel on the back of the hand for observation of any lesions, irritation, or redness according to Kumar (2017). It is important to measure the gel capacity to absorb the wound exudate before biological or wound healing applications. Each nanoparticle’s gel (10 g) was weighed and placed in 10 ml distilled water, and the weight of gel was measured after the 1st, 2nd, and 3rd hours. The water absorption capacity of the gel was determined in triplicate and calculated according to Tvl et al. (2010) using the following formula:

% Water absorption capacity = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100

Animals and experimental design

Forty-five adult female albino rats (weighted 180 ± 200 g, 8–12 weeks old) were purchased from Abou Rewash Farm, Giza, Egypt, and housed for 1 week with standard environmental conditions, constant temperature (22–25 °C), and relative humidity (45–60%) with natural light/dark cycle before carrying out the experiment. They were kept in plastic cages with wire mesh covers, and they received a standard pellet diet and water ad libitum throughout the experimental period.

Animals were divided into three groups (15 rats each). Group 1 was kept as a control and their cutaneous wounds were treated with 0.9% saline solution. Groups 2 and 3 were treated topically with green and chemical ZnO-NP gels, respectively. All treatments were applied every other day for 21 days. The animals were maintained and used in accordance with the guidelines of the Animal Ethics Committee in the Faculty of Veterinary Medicine at South Valley University, Qena, Egypt (approval no. VM-2019–0017).

Wound healing studies

Wound creation

The animals were anesthetized using diethyl ether. The dorsal region of each rat was shaved with electrical clipper and cleaned with 70% ethyl alcohol. In the mid-region between the infrascapular line and the base of the tail, a circular area of full-thickness skin was removed with 8-mm-diameter punch biopsy.
The tension of skin was kept constant during the wounding. The day of wound creation was designated as 0 day. Gel was applied topically after hemostasis using gauze swabs.

Clinical evaluations

The wounds were examined clinicopathologically and photographed at the 3rd, 7th, 14th, and 21st days post-treatment (PT). The diameter of the wounds was measured by using a digital caliper. The healing time, WSA, WSA%, and WC% were evaluated using the following equations:

Healing time = (date of healing (DH) − date of wounding (DW)) (Ramos and Miranda 2007).

WSA = πr^2 (cm²) (Zaid et al. 2017).

where π (Pi) is a mathematical constant (22/7) and r^2 is the radius squared.

WSA% = \frac{Wound surface area x 100}{0.50} %

WC% = 100% − WSA%

Histopathological studies

After euthanasia of rats by a high dose of diethyl ether, specimens from skin wounds with surrounding normal skin (about 1.5 cm) on the 3rd, 7th, 14th, and 21st days PT were harvested and fixed in 10% buffered formalin and dehydrated using upgraded concentrations of ethyl alcohol. The tissue specimens were embedded in paraffin wax of 58–60 °C melting points. Sections about 5 µm thickness were prepared and stained with hematoxylin and eosin according to Bancroft and Gamble (2008) and stained with Morrison Crossman stain to detect the collagen fibers (Kim et al. 2013). Also, neovascularization, epithelialization, collagen production, and necrosis were evaluated qualitatively (Abbaszadeh et al. 2019).

Statistical analysis

The results were presented as mean values ± standard deviations (SD). One-way analysis of variance (ANOVA) was used to statistically analyze the obtained data using computerized SPSS (SPSS Statistics, version 16) software. A value of p ≤ 0.05 is considered statistically significant.

Results and discussion

Characterization of nanoparticles

Characterization of nanoparticles provides essential information about their size, shape, surface charge, surface area, and dispersity (Jayaseelan et al. 2012). The XRD patterns of the prepared ZnO-NP samples by two methods are shown in Fig. 1. All detectable peaks can be indexed as the ZnO-NP hexagonal wurtzite-type structure in the standard data (JCPDS, 36–1451) (Ghamsari et al. 2019). The synthesized ZnO-NPs have good crystallinity and are of high purity. The FTIR spectra revealed the asymmetric molecular stretching, vibration, and rotation of chemical bonds when exposed to the designated wavelengths of light. As shown in Fig. 2, the FTIR spectra of the prepared green/chemical ZnO-NPs showed that the main absorption bands at 3500 cm⁻¹, 2354 cm⁻¹, 1720 cm⁻¹, 1514 cm⁻¹, and 1220 cm⁻¹, which correspond to O–H mode, CO₂ mode, O–H mode, asymmetric C=O stretching mode, and symmetric C=O stretching mode, respectively. The intense broadband in the vicinity of 418 cm⁻¹ is the characteristic absorption peak of

Fig. 1 XRD for the prepared ZnO nanoparticles

![XRD for the prepared ZnO nanoparticles](image-url)
Zn–O bond and also authenticates the presence of ZnO; the same results were obtained by Kwon et al. (2002), Silva and Zaniquelli (2002), Li et al. (2004), Kim et al. (2005), Liufu et al. (2005), and Labuayai et al. (2009). The TEM as shown in Fig. 3 revealed that ZnO-NPs consist of nanowires as the prepared ZnO-NPs and TEM images confirmed the hexagonal structure of the synthesized ZnO-NPs. Green ZnO-NPs have wire length and wire diameter more than the obtained ZnO-NPs by chemical method. It is well documented that decreasing particle size increases their functionality as antimicrobial and anticancer agents due to the larger surface-to-volume ratio (Selim et al. 2020). The phase and purity of the prepared samples is unaffected by the preparation methods, indicating that the green method using *Lawsonia inermis* leaves is an effective method for the preparation of ZnO-NPs, which in turn confirms that green technology is superior to physical and chemical methods for producing nanoparticles (Rosi and Mirkin 2005; Gnanajobitha et al. 2013; Vidya et al. 2013; Parveen et al. 2016). Our study has confirmed that the synthesized green ZnO-NPs possesses a smaller particle size and enhanced the wound healing activity due to their large surface area-to-volume ratio and surface

![Fig. 2 FTIR spectroscopy for the prepared ZnO nanoparticles](image1)

![Fig. 3 HRTEM images of ZnO-NPs prepared with A chemical and B green methods](image2)
reactivity when compared to the synthesized chemical ZnO-NPs (Gunalan et al. 2012).

**Characters of gels**

Green and chemically synthesized ZnO-NP gels are translucent in color as shown in Fig. 4. The gels were highly viscous and homogenous and stable and spread out easily on the skin surface. The gels’ pH was 6.5 to 7, so there is no detectable itching or hypersensitivity reaction. The level of water absorption capacity % of gels containing nanoparticles at the 1st, 2nd, and 3rd hours is shown in Fig. 5. The potential of green ZnO-NP gel to absorb water is higher than that of chemically synthesized ZnO-NP gel at the different periods of the experiment. Carboxymethyl cellulose (CMC) hydrogels loaded with a bioactive agent have been reported to accelerate the healing process and improve the tissue quality of burn wounds (Singh et al. 2017). It maintains a moist environment within the wound site as a vehicle for topical administration. Maximum swelling of Carboxerm 940® was observed in simulated wound fluid (Singh et al. 2017). Therefore, we used Carbopol 940® as a vehicle for our studied nanomaterials. Our results agreed with Morgado et al. (2015), who reported that the ideal wound dressing should be able to absorb the exudate from the wound bed where water absorption or hydrophilicity allows the penetration of nutrients, cells, and bioactive molecules.

![Fig. 4 Photographs of the macroscopic appearance of A chemical ZnO nanogel (transparent in color) and B green ZnO nanogel (transparent in color)](image)

![Fig. 5 The mean± SD of water absorption capacity % of nanoparticles](image)

**Water absorption capacity %**

![Bar chart showing water absorption capacity % of nanoparticles over time](image)
Clinical picture of the wounds

Grossly as in Fig. 6, swelling and hyperemia of the wound edges were observed in day 0 of wounding. On the 3rd day PT, the wounds of rats in both ZnO-NP–treated groups lost their rounded shapes and were covered with moist reddish brown scabs, and their wound contraction increased with less exudate formation. In contrast, the wounds of rats in the control group were covered with moist scabs, and there was clearly noticeable yellowish exudate and edema through the wound edges. On the 7th day PT, the wounds of rats in both ZnO-NP–treated groups were covered with thin dry scabs, and the wound size of the green ZnO-NP–treated group was non-significantly narrower than that of the chemical ZnO-NP–treated group. The wounds of the control group were covered with thick moist scab with less contraction when significantly compared with both ZnO-NP–treated groups. On the 14th day PT, the wounds were covered with small and dry scab in individual rats of both ZnO-NP–treated groups. The wounds of rats of the control group were covered with a

Fig. 6 Photographs showing the stages of wound healing of the control and treated groups (green and chemical ZnO-NPs) at the 3rd, 7th, 14th, and 21st days PT

![Photographs showing the stages of wound healing of the control and treated groups](image)

Fig. 7 The mean ± SD of wound surface area (WSA) for the control and treated groups. *Significant changes when compared with the control group when p is ≤0.05
relatively thick scab. On the 21st day PT, the scab sloughed from wounds of rats in both ZnO-NP–treated groups leaving scar tissue, which was relatively clearer in rats of chemical ZnO-NP–treated group than that of the green ZnO-NP–treated group. Meanwhile, the control group showed marked red scar.

WSA, WSA%, and WC% are shown in Fig. 7, Fig. 8, and Fig. 9, respectively. At the 7th day PT, WSA and WSA% significantly decreased in both ZnO-NP–treated groups, compared with the control group. While at the 7th day PT, WC% significantly increased in both ZnO-NP–treated groups compared with the control group. Furthermore, at the 3rd, 14th, and 21st days PT, WSA, WSA%, and WC% showed no significance between the treated groups and the control group when $p$ was $\leq 0.05$. As shown in Fig. 10, the healing time of wounds is significantly decreased ($p \leq 0.05$) in both ZnO-NP–treated groups compared with the control group.

**Histopathological findings**

At the 3rd day PT, the wound gap of the control group showed large blood clots, necrotic tissue, fibroblast

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**Fig. 8** The mean ± SD of wound surface area percent for the control and treated groups. *Significant changes when compared with the control group when $p \leq 0.05$

**Fig. 9** The mean ± SD of wound contraction percent for the control and treated groups. *Significant changes when compared with the control group when $p \leq 0.05$
proliferation under the scabs, edema, and abundant neutrophil infiltration; however, at the 7th day PT, they showed fibroblast proliferation and abundant cell infiltration, mainly macrophage with newly formed blood vessels perpendicular on the wound edge. At the 14th day PT, the wound gap showed fibroblast proliferation with collagen-rich fiber production associated with extensive inflammatory cell infiltration. Moreover, at the 21st day PT, the wounds showed granulation tissue formation with inflammatory cell infiltration as shown in Fig. 11. At the 3rd day PT, the wound gap of the green ZnO-NP–treated group revealed necrotic tissues with a little blood clot, edema, and heavy inflammatory cell infiltrations, mainly neutrophils. Meanwhile, they showed predominant fibroblast proliferation with collagenous fiber production, high cellular macrophages, and lymphocyte infiltration with newly formed blood vessel at the 7th day PT. At the 14th day PT, the wounds showed noticeable re-epithelialization of the granulation tissue with less inflammatory cell infiltration and abundant collagen fibers. At the 21st day PT, the wounds showed complete re-epithelialization and intact epidermis with minimal inflammatory cell infiltration, newly formed blood vessel, and mature scar formation as shown in Fig. 11. At the 3rd day PT, the wound gaps of the chemical ZnO-NP–treated group were filled with extravasation of RBCs, edema, and aggregation of neutrophils. At the 7th day PT, wounds gaps were filled with granulation tissue

![Healing time](image)

**Fig. 10** The mean±SD of healing time of full-thickness skin wounds in the control and treated groups. *Significant changes when compared with the control group when *p* is ≤0.05

![Histopathological photos](image)

**Fig. 11** Histopathological photos of the control and treated groups at the 3rd, 7th, 14th, and 21st days post-treatment (H&E, bar = 200 µm)
with hemorrhage and aggregation of neutrophils and eosinophils. At the 14th day PT, the wound gaps were decreased in size with complete re-epithelialization, fibroblast proliferation, and collagen fiber production with fewer inflammatory cells. At the 21st day PT, marked epithelialization was observed with heavily aggregated collagen fiber production. Also, minimal fibroblasts and infiltration of neutrophils and macrophages were observed as shown in Fig. 11.

**Wound healing histological scoring**

The wound healing scores (epithelialization, vasculature, necrosis, connective tissue formation, and collagen synthesis) were qualitatively assessed in all animals as shown in Fig. 12. The epithelialization did not change significantly at the 3rd, 7th, and 14th days PT in both ZnO-NP–treated groups as compared to that in the control group but increased significantly (p ≤ 0.05) at the 21st day PT. The vasculature, necrosis, connective tissue formation, and collagen synthesis which were observed at the 3rd, 7th, 14th, and 21st days PT not significantly changed in both ZnO-NP–treated groups compared to the control group.

*Lawsonia inermis* is one of the plants that accelerates the wound healing process by reducing the epithelialization period and increasing the wound contraction percent (Yassine et al. 2020). The topical application of *L. inermis* rapidly initiates the inflammatory process by enhancing higher inflammatory cell infiltration and subsequently reduces the inflammatory phase by inhibiting monocyte-to-macrophage differentiation (Daemi et al. 2019). It was reported that extracts of *Lawsonia inermis* leaves contain flavonoids, alkaloids, and terpenoids, which accelerate the phenomenon of wound healing by their astringent and antibacterial activities (James and Friday 2010; Bapat and Mhapasekar 2014), prevention of cell necrosis, improvement of angiogenesis (Fikru et al. 2012), inhibition of prostaglandin synthesis (Jain et al. 2011), and modulation of cytokine expression during the inflammation phase (Antunes-Ricardo et al. 2015). *L. inermis* methanolic extracts showed antibacterial effects against different bacteria (Elansary et al. 2020; Nigussie et al. 2021); i.e., *L. inermis* inhibits the growth of gram-positive and gram-negative bacteria (Pasandi Pour and Farahbakhsh 2020). *L. inermis* has strong antifungal and antioxidant activities (Elansary et al. 2020). ZnO-NPs accelerate the collagen synthesis and wound contraction with a relatively reduced scar (Saremi et al. 2016) and regulate the endogenous growth factors and insulin-like growth factor I, which may increase epithelialization (Ågren et al. 1991;
Conclusions

We have synthesized ZnO-NPs by green and chemical methods for evaluation of their efficacy in cutaneous wound healing. The treated cutaneous wounds showed shrinkage in the wound area and enhanced skin elasticity, blood clots, and a positive effect on skin repairing. It was shown that the treated wounds had faster healing, complete re-epithelialization, increased collagen deposition, and reduced inflammatory cell infiltration compared to the control. However, after the evaluation of the obtained results, it was obvious that the treated wounds with the green synthesis of ZnO-NPs by methanolic extract of Lawsonia inermis leaves showed promising wound healing efficacy with an undetectable scar compared to chemically synthesized ZnO-NPs. Further studies are needed to assure the use of synthesized green ZnO-NPs for cutaneous wound healing.

Limitation of the study

The study aims to evaluate the in vivo wound healing activity of the studied nanoparticles in albino rats from the surgical point of view. However, the characterization of gel, such as rheological and degradation properties, is missed in this study. The in vitro studies, such as biocompatibility, toxicity, and immunological studies, are needed before commercial use.

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Author contribution ASS and AAA designed the study design, AAM carried out the experimental procedures and statistical analysis. KhE prepared the nanoparticles, MAM performed the interpretation of the pathological samples, and AAS prepared the studied gels and helped in their evaluation tests and wrote and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data availability The datasets used and analyzed in this research are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate The animals were maintained and used in accordance with the guidelines of the Animal Ethics Committee in the Faculty of Veterinary Medicine at South Valley University, Qena, Egypt (approval no. VM-2019–0017).

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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