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Comparative efficacy of green synthesized silver nanoparticles of *Azadirachta indica* and *Catharanthus roseus* on wound healing in goats

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Abstract
This experiment was undertaken on 12 clinical cases of goats presented to TVCC, PGIVAS, Akola with fresh contaminated wounds irrespective of their sex, body weight, and breed. These cases were treated with green synthesized silver nanoparticle ointments of *Azadirachta indica* and *Catharanthus roseus*. Better wound contraction with thick and well-organized collagen fibers was observed in *Azadirachta indica* group as compared to the group treated with *Catharanthus roseus*.

Keywords: Wound, *Azadirachta indica, Catharanthus roseus*, silver nanoparticle, green synthesis, goats

1. Introduction
A wound is a discontinuity of tissue integrity that results in damage and may be accompanied by loss of function. Healing of wound is a biological process which involved different phases that begin with trauma and often end by scar formation. Many ayurvedic medicinal plants have a very important role in the process of wound healing (Sengupta 2017) [1]. Phytochemicals are biologically active, naturally occurring chemical compounds present in plants, which provide health benefits (Saxena *et al.* 2013) [2]. Haphazard use of various topical applications and antibiotics to hasten the wound healing process leads to microbial drug resistance. Since the ancient period, silver utensils such as plates and glasses were used for eating and drinking purposes mainly due to the antibacterial property of the silver. Therefore nowadays researchers are shifting towards silver nanoparticles which plays an important role in medicine due to its bacteriostatic and physiochemical properties. Environmental concern leads the scientist to synthesized nanoparticles by using bacteria, fungi and plants which are known as green synthesized silver nanoparticles. The use of green synthesized silver nanoparticles is the best alternative for wound management with no side effects. These attractive green strategies are free of the shortfalls associated with conventional synthetic strategies, i.e. they are eco-friendly and offer several advantages such as rapid synthesis, high yields and importantly, the lack of costly downstream processing required producing the particles (Moodley *et al.* 2018) [3]. Keeping in view the properties and activities having with *Azadirachta indica* and *Catharanthus roseus* on wound healing, the green synthesized silver nanoparticle ointment of *Azadirachta indica* and *Catharanthus roseus* was used as a topical application for wound management in goats.

2. Materials and Methods
2.1 Plant used
Leaves of *Azadirachta indica* and *Catharanthus roseus* were used for the synthesis of green silver nanoparticles.

2.2 Preparation of extracts
Leaves of *Azadirachta indica* and *Catharanthus roseus* were collected from the local area and in and around the institute campus. The leaves were clean under tap water to remove debris, organic contents and other contaminants followed by a second wash using double distilled water these clean leaves were finely cut into small pieces and then air-dried at room temperature. 10 gm finely cut leaves of *Azadirachta indica* then added into the beaker containing 100 ml of double-distilled water and boiled for 30 minutes. The extract was then

cooled down and filtered by using Whatman filter paper no.1 and stored at 4^0C for further use (Ahmed *et al.* 2015) [4]. Similarly, 10 gm finely cut leaves of *Catharanthus roseus* were added in a flask containing 100 ml of double-distilled water and boiled it for 5 minutes before finally decanting it. The extract was filtered with Whatman filter paper no. 1 and stored at -15^0 C (Ponarulselvam *et al.* 2012) [5].

2.3 Green synthesis of silver nanoparticles

The 5 ml of plant filtrate extract of *Azadirachta indica* and *Catharanthus roseus* was treated with 95 ml aqueous 1mM silver nitrate solution in an Erlenmeyer flask respectively. This set up was incubated at room temperature in a dark chamber to minimize photo-activation of silver nitrate. A brown-yellow colour of solution formed due to the reduction of silver ions confirms the formation of silver nanoparticles.

2.4 Characterisation of Silver nanoparticles

Accurate determination of the size and concentration of silver nanoparticles was the pre-requisite for the biomedical application of nanoparticles. The samples were sent to the RUSKA Labs, Rajendranagar, Hyderabad for Transmission Electron Microscopy (TEM). The size of *Azadirachta indica* and *Catharanthus roseus* silver nanoparticles were 25 nm (Fig. 1) and 30 nm (Fig.2) respectively. The concentration of synthesized silver nanoparticles was determined in the nanometer (nm) by the standard method (Marquis *et al.* 2009) [6]. The concentration of *Azadirachta indica* silver nanoparticles (AISNP) and *Catharanthus roseus* silver nanoparticles (CRSNP) in the solution obtained was 58.84 nM/ml and 20.56 nM/ml respectively.

2.5 Preparation silver nanoparticle ointments by fusion method

0.1 Litre solution of AISNP and CRSNP was taken respectively. These solutions were mixed drop by drop in 40 gm of honey beeswax and 60 gm of liquid paraffin at 75 ^oC under constant stirring so as to evaporate the water content of silver nanoparticles and silver nanoparticles remain in the ointment base.100 gm ointment of *Azadirachta indica* and *Catharanthus roseus* contained 5.884 nM/g and 2.056 nM/g of silver nanoparticles respectively.

3. Clinical study

The present study was undertaken during the period of February to August 2019 on 12 clinical cases of goats with fresh contaminated wounds presented to the Teaching Veterinary Clinical Complex, Post Graduate Institute of Veterinary and Animal Sciences, Akola. These clinical cases were randomly divided into two equal groups viz; Group A and B irrespective of their sex, breed and body weight. Cases in group A (n=6) were treated with topical application of Silver nanoparticle ointment of *Azadirachta indica,* whereas in group B (n=6) Silver nanoparticle ointment of *Catharanthus roseus* was used. In all clinical cases wound area was shaved liberally to avoid further contamination. The wound was cleaned and washed with normal saline and application of ointment was done once in a day up to the complete wound healing.

3.1 Parameters studied

During the clinical study quality of wound healing was judged on the basis of wound contraction by using formula (Parhizkar *et al.* 2008) [7] on 0^{th}, 3^{rd}, 7^{th} and 14^{th} days. The histochemical study was undertaken on the 0^{th} day and after complete wound healing by using Van Gieson stains.

4. Statistical analysis

Statistical analysis of various parameters was carried out by applying WASP 2.0. The analysis of variance was done by using two-way factorial experiments for different parameters to know the effect of both treatment protocol and time intervals as well.

5. Results and Discussion

5.1 Wound contraction

The mean value of wound contraction in group A on 0^{th} day was 10.19±0.2 which significantly increased (p<0.01) to 11.72±0.10, 26.65±1.04 and 35.22±1.24 on 3^{rd}, 7^{th} and 14^{th} day of treatment respectively with the pooled mean of 20.94±6.04 (Table 1.). In group B the value on 0^{th} day 9.86±0.16 which significantly increased (p<0.01) to 10.58±0.16, 12.90±0.25 and 18.29± 0.29 on 3^{rd}, 7^{th} and 14^{th} day of treatment respectively with pooled mean of 12.91±1.91. (p<0.01) In both, the group's overall mean value on 0 days was 10.03±0.14 which increased significantly to 11.15 ±0.19, 19.77±2.14 and 26.76±2.62 on 3^{rd}, 7^{th} and 14^{th} day of treatment respectively (p<0.01).

Table 1: Mean ± SE values of wound contraction (%) in both the groups

Intervals	0 day	3^{rd} day	7^{th} day	14^{th} day	Pooled mean
Group A	10.19^a ± 0.2	11.72^b ±0.10	26.65^c ±1.04	35.22^d ±1.24	20.94^B ±6.04
Group B	9.86^a ± 0.16	10.58^b ±0.16	12.90^c ±0.25	18.29^d ±0.29	12.91^A ±1.91
Pooled mean	10.03^I ±0.14	11.15^{II} ± 0.19	19.77^{III}±2.14	26.76^{IV}±2.62	

Means bearing different superscript differed significantly (p<0.01).

The mean values of wound contraction increased significantly (p<0.01) with time interval as compared to pre-treatment values in both the groups. A higher percentage of wound contraction was recorded in group A as compared to group B.
The higher percentage of wound contraction in group A might be due to the angiogenesis, antibacterial, anti-inflammatory and antioxidant properties of *Azadirachta indica* [8]. The activity of angiogenesis in *Azadiractia indica* increases the blood supply to the wound which ultimately responsible for more supply of nutrients and oxygen which favors early wound contraction. Wang *et al.* (2004) [9] and Sen *et al.* (2013) [10] stated that *Catharanthus roseus* possess anti-angiogenesis and cytotoxic activity which might be responsible for delayed wound healing in group B as compared to group A.

5.2 Histochemical study

On '0' day in group, A histochemical examination revealed intense inflammatory reactions with edema and mild infiltration of macrophages along with inflammatory cells (Fig.3). However, after complete healing well-arranged thick collagen fibers deposition and neovascularization with dense mesenchymal matrix were observed (Fig.4). These findings are in accordance with Nagesh *et al*. (2015) [8] who reported proliferation of fibrocollageneous tissue and angiogenesis in wounds treated with *Azadirachta indica* leaves. Firdous and sautya (2018) [11] stated that angiogenesis is one of the property responsible for wound healing ability of medicinal plants. Shukla *et al.* (1999) [12] and habibipour *et al*. (2003) [13] revealed abundant fibroblast proliferation and collagen synthesis with neovascularisation in treated healed wounds.

In group B on '0' day intense inflammatory reactions with exudation and inflammatory cells with evidence of blood clots and round cells were observed (Fig.5). After complete healing, there were less irregularly arranged and sparsely dense collagen fibers and cornified epithelium with moderate elastic fibers, thin mesenchymal matrix deposition and absence of angiogenesis were observed. (Fig.6) Boayke *et al.* (2018) [14] observed fewer collagen fibers and absence of angiogenesis in untreated groups indicative of poor wound-healing rate, similar findings were also recorded by Agra *et al.* (2013) [15] who observed less epithelialization and less collagen formation in saline-treated groups indicating poor wound healing. Cuzzell and Stotts (1990) [16] stated that inadequate blood supply and hampered tissue perfusion leads to delayed wound healing**.**

6. Conclusion

In the light of research, it can be concluded that green synthesized silver nanoparticle ointment of *Azadirachta indica* had better wound healing ability than that of green synthesized silver nanoparticles of *Catharanthus roseus* in goats. Green synthesis of a silver nanoparticle is a cheap, rapid and eco-friendly technique and a better alternative to the other techniques used commercially.

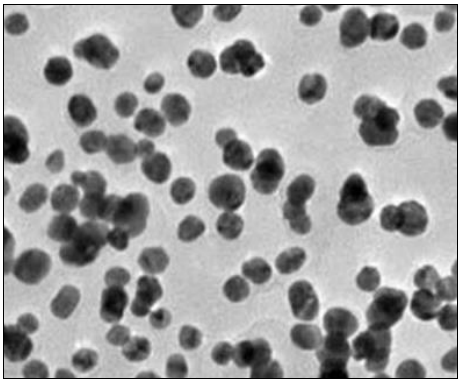

Fig 1: TEM image of silver nanoparticle of *Azadirachta indica* (25 nm).

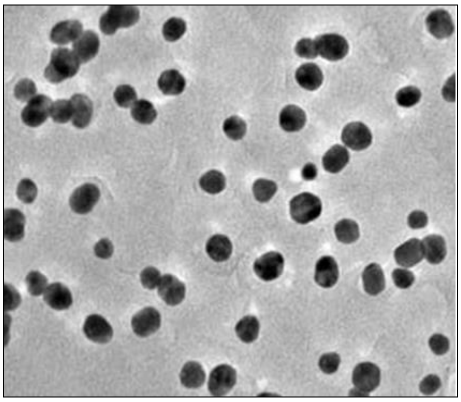

Fig 2: TEM image of silver nanoparticles of *Cathranthus roseus* (30 nm).

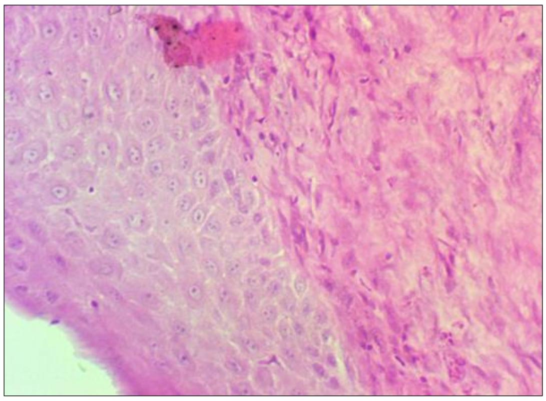

Fig 3: Inflammatory reaction with oedema and mild infiltration of inflammatory cells on 0^{th} day in group A.

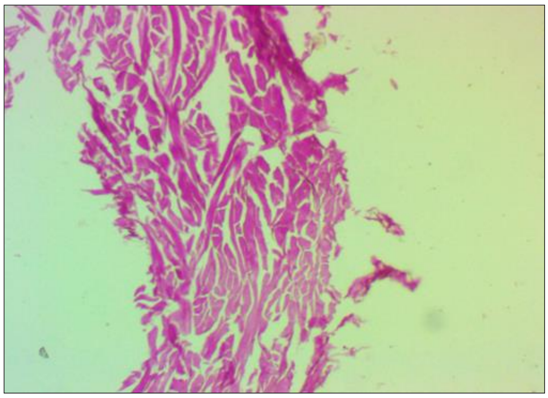

Fig 4: Well-arranged thick collagen fibers deposition with angiogenesis in group A.

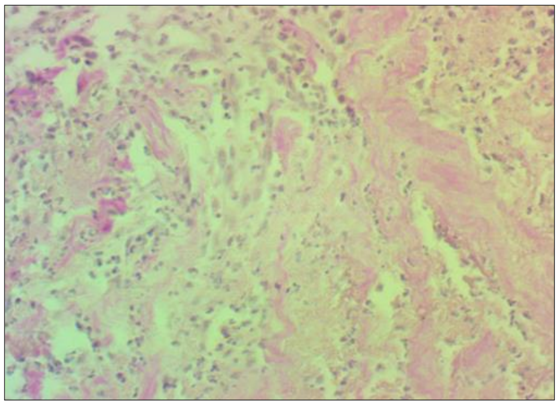

Fig 5: Intense inflammatory reaction with exudation and evidence of blood clots on 0^{th} day in group B.

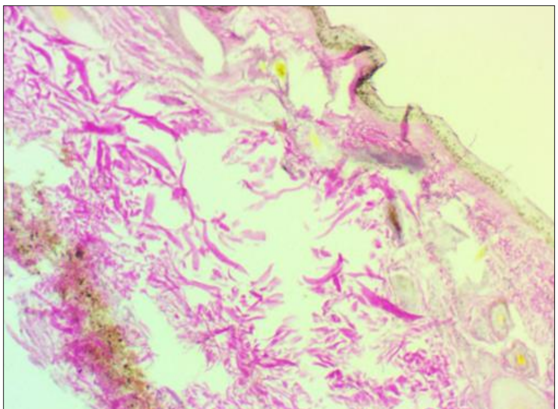

Fig 6: Irregularly arranged and sparsely dense collagen fibers after complete healing in group B.

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