B-5582
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RESEARCH ARTICLE Indian Journal of Animal Research

Evaluation of Phytosynthesized Silver Nanoparticle Coated Polyglactin 910 in Oral Mucosal Surgery of Dogs

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10.18805/IJAR.B-5582

ABSTRACT

Background: Comparative evaluation of MO-AgNP versus triclosan coated polyglactin 910 via evaluation of antibacterial activity and clinical wound healing of dogs undergoing oral mucosal surgeries.

Methods: In this randomized study, 18 dogs were divided into three groups of 06 animals each. Group I was control group in which uncoated polyglactin 910 suture material was used. In group II, triclosan coated polyglactin 910 and in group III, MO-AgNP coated polyglactin 910 was used to analyse of antibacterial activity of MO-AgNP and healing index of oral mucosa of dogs.

Result: Group III showed significant reduction in cfu/ml from 3.69±0.18 to 2.77±0.21 in comparison to group II (4.43±0.31 to 4.02± 0.39) and group I (3.70±0.17 to 3.36±0.21) on day 0 and 10 respectively. Healing index was significantly higher on day 03 (03.66±0.21) and day 10 (4.33±0.21) in group III animals in comparison to 2.50±0.22 and 2.83±0.47 in group II and 2.16±0.31 and 2.50±0.34 in group I respectively.

Key words: Colony forming unit, Dog, Healing index, MO-AgNP, Oral mucosal healing, Polyglactin 910, Triclosan.

INTRODUCTION

Applications for silver nanoparticles (AgNPs) in biomedicine are becoming more and more common (Al-Mashhadani *et al.,* 2024). Sliver nanoparticles exhibit excellent antibacterial, anti-inflammatory and angiogenic activities. The synthesis of silver nanoparticles from plant extract is gaining more attention these days. In the process of synthesizing nanoparticles, the plant extract serves as a capping and reducing agent. Silver nanoparticles have been synthesized with success using extract of *Moringa oleifera* (Asif *et al.,* 2022). Leaves of *Moringa oleifera* have a number of biological properties, including diuretic, immune-stimulating, hypotensive, anti-inflammatory, anti-ulcer, anti-hepatotoxic, anti-tumor, thyroid hormone status regulation, hypocholesterolemic, antidiabetic and antioxidant properties (Rai, 2023). The situation of antibiotic resistance is exacerbated by the widespread use of antibiotics and other antimicrobial medicines to prevent post-surgery infections (De Simone *et al*., 2014). Nanoparticles can be added to biomaterials and scaffolds to generate nanocomposite materials which can facilitate wound healing. In accordance to this, silver nanoparticle coated suture material can overcome antibiotic resistance, surgical site infections and thus can be more useful in surgical procedures requiring suturing of oral mucosa such as mandible fracture, lacerations, oral tumour excision etc. hence avoiding multiple topical applications of antibacterial gel.

MATERIALS AND METHODS

Animals

Study was conducted on 18 dogs irrespective of age, sex, breed and body weight with various oral and dental surgeries requiring suturing of oral mucosa. Dogs were randomly divided into three equal groups with six dogs in each group (Table 1).

Surgical procedure

Preparation of animal

The animal was kept off feed for 12 hours and off water for 6 hours prior to surgery.

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How to cite this article: Sharma, N., Mishra, A., Shahi, A., Gupta, N., Nayak, A., Das, B., Singh, R. and Singh, A. (2025). Evaluation of Phytosynthesized Silver Nanoparticle Coated Polyglactin 910 in Oral Mucosal Surgery of Dogs. *Indian Journal of Animal Research*. 1-6. doi: 10.18805/IJAR.B-5582.

Submitted: 10-03-2025 **Accepted:** 10-09-2025 **Online:** 09-12-2025

Anaesthetic protocol

The surgical procedure was performed under adequate general anaesthesia in all animals and local anaesthesia using 2% Lignocaine HCL as and when required. General anaesthesia was induced using Inj. atropine sulphate @ 0.04 mg/kg body weight intramuscularly, after 10 minutes Inj. xylazine hydrochloride @ 1 mg/kg body weight intramuscularly and after 10 minute Inj. ketamine hydrochloride @ 5 mg/kg body weight intramuscularly. Maintenance of anaesthesia was done by using Inj. ketamine hydrochloride intravenously as and when required.

Procedure

Animals were operated as per surgical procedure described by Fossum *et al.* (2018) for various oral affections including surgeries like gingival flap surgery, fracture repair of mandible and maxilla, tumor excision *etc.* Closure of various oral mucosal surgical wounds was done using interrupted suture pattern using uncoated, triclosan and MO-AgNP coated polyglactin 910 in group G1, G2 and G3 animals respectively.

Parameters of the study

Colony forming unit

The oral swab samples were collected on day 0 and 10. The swabs were tenfold serially diluted in 15 ml test tubes.

Table 1: Experimental design.

Group	No. of animals	Suture material
I	06	Polyglactin 910
II	06	Triclosan coated polyglactin 910
III	06	MO-AgNP coated polyglactin 910

100µl 10^{-2} to 10^{-6} serially diluted samples were inoculated on plate count agar in triplicate and uniformly spread over entire surface using a sterile L- shape spreader. The plates were incubated at 37°C for 24 hours and colonies were counted (Markey *et al.,* 2013).

Healing index (HI)

Healing index score was recorded on day 0, 3 and 10 as per Landry *et al.* (1988) with slight modification (Table 2).

RESULTS AND DISCUSSION

Colony forming unit

Oral swabs were collected on day 0 and 10 from all groups. G3 showed significant ($p \leq 0.05$) reduction in cfu/ml from (3.69±0.18) to (2.77±0.2) whereas no significant reduction was observed in G2 and G1 between day 0 and 10. Among all groups, G3 (2.77±0.2) revealed significantly ($p \leq 0.05$) lesser cfu/ml on day 10 in comparison to G2 (4.02±0.39) and G1 (3.36±0.21). No significant difference was observed between G1 and G2 (Table 3).

Likewise, Dubas *et al.* (2011) evaluated antimicrobial properties of surgical sutures and concluded that metallic silver nanoparticle coating obtained reduction in bacterial concentration. Similarly, Venema *et al.* (2011) reported no significant difference in CFUs in between triclosan coated sutures and plain sutures. Furthermore, De Simone *et al.* (2014) reported reduction in values colony forming unit for the silver treated silk suture against *Escherichia coli* and *Staphylococcus aureus* compared to untreated silk sutures and Etemadi *et al.* (2019) recorded the CFU values against *Enterococcus* and *Escherichia* coli for triclosan coated suture

Table 2: Healing index score.

Score	Grade	Description
1	Very poor	Mucosa color: ≥ 50% of mucosa red with ulceration Response of palpation: BleedingGranulation tissue: Present Incision margin: Not epithelialized, with lossof epithelium beyond incision margin Suppuration: Present
2	Poor	Mucosa color: ≥ 50% of mucosa red with focal desquamation Response to palpation: Bleeding Granulation tissue: Present Incision margin: Not epithelialized, with connective tissue exposed
3	Good	Mucosa color: ≥ 25% and >50% of mucosa red with slight whiteness without desquamation Response to palpation: No bleeding Granulation tissue: None Incision margin: No connective tissue exposed
4	Very good	Mucosa color: < 25% of mucosa red and coated Response to palpation: No bleeding Granulation tissue: None Incision margin: No connective tissue exposed
5	Excellent	Mucosa color: All mucosa pink and moist Response to palpation: No bleeding Granulation tissue: None Incision margin: No connective tissue exposed

and found no significant differences between triclosan coated and silk suture. The study concluded that triclosan coated suture had no antibacterial effect 7 days after surgery. Ramteke *et al.* (2024) also concluded that green silver nanoparticle synthesized by reducing with *Azaderichta indica* leaf extract provided significant antibacterial properties against multidrug resistance in *Staphylococcus aureus* (MDRSA) isolated from mastitic cow milk samples indicating its potential for future applications against MDRSA.

Comparable to their findings, Syukri *et al.* (2020) concluded that *Eucalyptus camaldulensis* synthesized silver nanoparticles coated silk sutures provide a strong bacteriostatic effect against *Staphylococcus aureus*. However, these coated silk sutures showed bactericidal effects against Gram negative bacteria like *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa.* The coated sutures retained antibacterial properties upto 12 weeks.

Inconsistent with present findings, Sethi *et al.* (2016) reported value of total cfu/mL for plain uncoated suture and triclosan coated suture. Significant ($p < 0.05$) difference was observed in between triclosan and plain uncoated suture.

Table 3: Mean values (±SE) of colony forming unit log10 (cfu/ml).

Groups / Days	0	10
G1	3.70±0.17	3.36^{AB}±0.21
G2	4.43±0.31	4.02^{A}±0.39
G3	3.69^{a}±0.18	2.77^{Bb}±0.21

Mean values with different superscripts between groups (A, B) and between days (a, b) varied significantly ($p \leq 0.05$).

In the current research work, Colony forming unit estimated the antibacterial activity of suture materials, postoperatively. Clinically, most oral affections revealed mixed type of bacterial growth (Gram positive and negative). Reduction in cfu/ml of MO-AgNP coated polyglactin 910 may be due to its comparable antibacterial activity with triclosan against Gram negative bacteria and its prolonged activity at site of wound over a span of 10 days. Moreover, suture dehiscence was seen in two cases of G2 which can lead to persistent infection in oral mucosa on day 10. No reduction in CFU was seen in G1 because plain or uncoated polyglactin 910 does not have any antibacterial activity of its own.

AgNPs are said to be more effective against Gram negative bacteria than Gram positive. They damage bacterial cell membranes, DNA and interact with cellular organelles and biomolecules leading to cell death as stated by Dubas *et al.* (2011) and Enab *et al.* (2014).

Mean values (±SE) of healing index at different time intervals

Mean values of healing index were significantly ($p \leq 0.05$) higher on day 03 (03.66±0.21) and day 10 (4.33±0.21) in G3 compared to G2 and G1. In G2, mean values of healing index were significantly ($p \leq 0.05$) higher from day 0 (1.50 ± 0.22) to day 10 (2.83±0.47) but no significant variation was seen from day 0 to day 03. While in G1, non-significant changes were observed from day 0 to day 03 followed by day 10 (Table 4 and Fig 1-3).

In corroboration with these findings, Syukri *et al.* (2021) evaluated biogenic silver nanoparticle (AgNPs) coated on monofilament nylon suture. Silver nanoparticles inhibited nitric oxide and improved the migration fibroblast cells *in vitro*. This research work suggested that biogenic AgNPs on non-absorbable nylon monofilament surgical sutures

Table 4: Mean values (±SE) of healing index at different time intervals.

Groups / Days	0	03	10
G1	1.50±0.22	2.16^{B}±0.31	2.50^{B}±0.34
G2	1.50^{b}±0.22	2.50^{Bab}±0.22	2.83^{Ba}±0.47
G3	1.33^{b}±0.21	3.66^{Aa}±0.21	4.33^{Aa}±0.21

Mean values with different superscript between group (A, B) and between days (a, b) varied significantly ($p \leq 0.05$) at different time intervals.

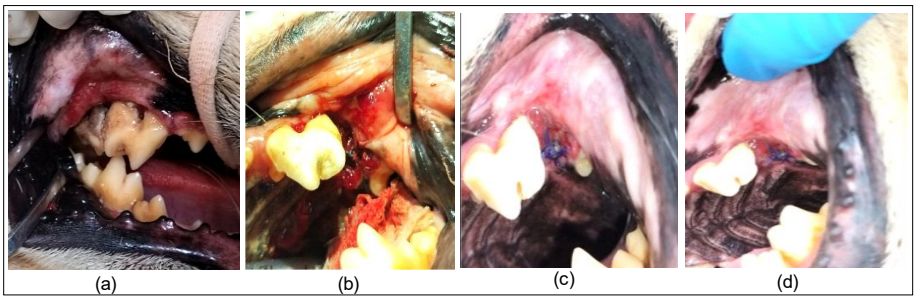

Fig 1: Healing index of Labrador Retriever in G1 showing (a) Suppuration and bleeding of mucosal epithelium on day 0 (b) Sutured mucosa using uncoated polyglactin 910 on day 0 (c) Focal desquamation on day 03 (d) Slight whiteness without desquamation and no bleeding on day.

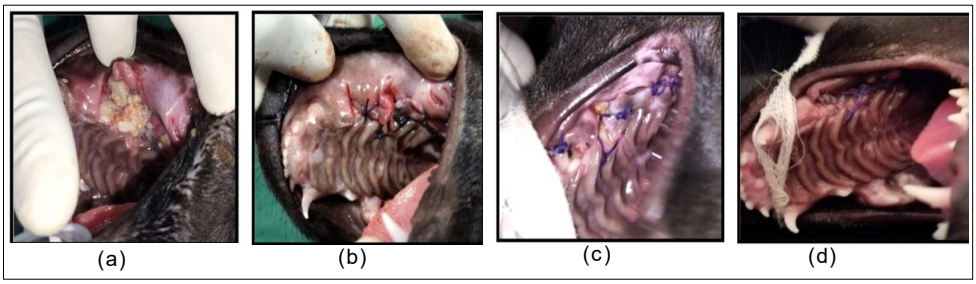

Fig 2: Healing index of non- descript in G2 showing (a) Lacerated oral mucosa exposing maxilla bone on day 0 (b) Sutured mucosa using triclosan coated polyglactin 910 on day 0 (c) Exposed connective tissue day 03 (d) Erythematous oral mucosa and no bleeding on day 10.

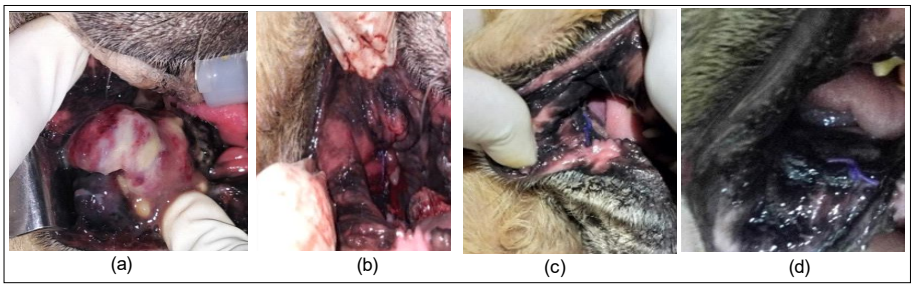

Fig 3: Healing index of Pug in G3 showing (a) Suppuration, granulation tissue and bleeding on day 0 (b) Sutured mucosa using MO-AgNP coated polyglactin 910 bleeding and loss of epithelium on day 0 (c) Erythematous oral mucosa with no bleeding tissue on day 03 (d) Normal moist pigmented oral mucosa on day 10.

Table 5: Mean values (±SE) of tota leucocyte count (TLC) at different time intervals.

TLC (thousand/µl)	G1	17.55±1.46	18.39^{A}±1.07
	G2	17.85±0.66	14.18^{B}±1.97
	G3	19.14^{a}±3.03	11.31^{Bb}±1.76

Mean values with different superscript between group (A, B) and between days (a, b) varied significantly ($p \leq 0.05$) at different time intervals.

used to surgical site infections increases the wound healing process. Likewise, Aggarwal *et al.* (2021) documented the impact of nano crystalline silver membrane dressing on early wound healing after periodontal surgery. The wound healing scores showed highly significant ($p < 0.001$) difference in between nano-crystalline silver and control group on day 07 post surgery. The study concluded that nano-crystalline silver membrane dressing promotes wound healing. Additionally, Chaganti *et al.* (2023) observed healing index on day 08 which was statistically non-significant in between triclosan coated and plain suture materials.

However, Karde *et al.* (2019) evaluated chlorhexidine, triclosan coated sutures and noncoated resorbable sutures in periodontal flap surgery. The result demonstrated that healing index at different intervals was not statistically significant in between groups.

MO-AgNP coated polyglactin 910 demonstrated superior oral mucosal healing in the current study when compared to other sutures. This could be because MO-AgNPs support wound healing through numerous mechanisms, including antimicrobial activity, anti-inflammatory effects, tissue regeneration and antioxidant protection. In general, AgNPs promote fibroblast and keratinocyte migration, which is essential for wound closure and tissue repair. Antioxidants found in moringa leaves extract aid in scavenging reactive oxygen species (ROS) generated during wound healing. Although ROS can harm cells, antioxidants shield them and promote a quicker recovery. AgNPs are more readily absorbed into the mucosa because of their small size and increased surface area. Their ability to deliver antibacterial and healing qualities directly to the wound site is hence improved as affirmed by Syukri *et al.* (2021).

However, uncoated polyglactin 910 only gives the mucosa mechanical strength and helps in tissue apposition, whereas triclosan solely possesses antibacterial qualities. Their relatively low healing score could be due to this.

Total leucocyte count (TLC)

Mean values of total leucocyte count (thousand/µl) decreased significantly ($p \leq 0.05$) in G3 dogs from day 0 (19.14±3.03) to day 10 (11.31±1.76). However, no significant reduction was found between days in G1 and G2. Between groups, G3 and G2 showed significantly lower TLC count in comparison with G1 on day 10. G3 dogs had lower TLC count than G2 on day 10 but the difference was non - significant (Table 5).

Shinde (2023) reported that mean values of total leucocyte count showed non-significant variations in periodontal diseases.

The reason for significant reduction in TLC count in G2 and G3 may be due to the antibacterial activity of triclosan and MO-AgNP against common Gram positive and negative infections. Whereas, uncoated polyglactin 910 does not have any bacteriostatic property of its own leading to persistently raised TLC values.

CONCLUSION

On the basis of present study, it is concluded that MO-AgNP coated polyglactin 910 showed significantly lower colony forming unit and total leucocyte count in comparison to triclosan coated and plain or uncoated polyglactin 910. Clinically, healing of oral mucosa was significantly better in MO-AgNP coated polyglactin 910 in comparison to triclosan coated and plain polyglactin 910.

ACKNOWLEDGEMENT

The present study was supported by College of Veterinary Science and A.H., NDVSU, Jabalpur providing all possible infrastructure, facilities and equipments for this study.

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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