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ORIGINAL ARTICLE

ANTIBACTERIAL AND WOUND HEALING OF BIOSYNTHESIZED ZNNPS USING *SESAMUM INDICUM* SEEDS AGAINST MULTI DRUG RESISTANT *PEPTONIPHILUS HAREI* IN ERBIL CITY, IRAQ

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ABSTRACT

Background: Wound infections are a significant cause of death in patients, particularly those caused by Multidrug resistant (MDR) pathogens. To counter such a danger, new antibacterial agents like nanoparticles (NPs) are needed. The goal of this research was to identify a different treatment drug to fight the isolates of MDR *Peptoniphilus harei*.

Materials & methods: A total of 157 clinical specimens were obtained from various wounds and hospitals located in Erbil, Iraq**,** between July and October of 2022. 61 (38.21%) Gram positive bacteria (GPB) were found; based on 16S rRNA (PCR), sequencing, and the GenBank database, one (1.6%) of these was newly reported in Iraq. According to the findings of a sensitivity test conducted on 20 antimicrobials, *P. harei* was resistant to 14 of them. Using an albino wistar rat model, zinc nanoparticles (ZnNPs) derived from *Sesamum indicum* seeds were biosynthesized and evaluated against isolated *In vitro* antibacterial and *In vivo* wound healing.

Results: According to the current data, ZnNPs' inhibition zone has 16 mm of antibacterial activity against *P. harei*. With the standard therapies except Mebo, the recovery period with ZnNPs creams was noticeably faster than that of usual therapy. Wounds infected with *P. harei* that were treated with Zn NP, Vaseline, Fusidin, and Mebo healed in 22, 27, 28, and 16 days, respectively and the non-treated group took 33 days.

Conclusion: The recent work is a significant step towards creating innovative nanoparticles that provide better alternative uses for antibacterial medications and wound treatments.

KEY WORDS: Antimicrobial activity; *Peptoniphilus harei*; *Sesamum indicum* seeds; Wound healing; ZnNPs.

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INTRODUCTION

Wounds and skin injuries are a significant form of nosocomial infection that impacts numerous patients worldwide, it arises from a variety of causes, including burns, gunshot wounds.[1] Wound infections are a significant cause of death in patients, particularly those caused by Multidrug resistant (MDR) pathogens such as *S. aureus, Pseudomonas aeruginosa* and vancomycin-resistant Enterococci. These infections can result in healing failure and are also used as a predictor of predicted mortality.[2] It is necessary to develop a new class of antimicrobials that are easy to use, safe, and usually effective. Additionally, an efficient strategy to prevent antibiotic resistance is to use a multidisciplinary treatment approach.[3]

Nanoparticles have led to the development of particles ranging in size from 1 to 100 nanometers and demonstrated harmful effects on bacteria, affirming their suitability for diverse medical uses, such as promoting wound healing. Among these, zinc nanoparticles (ZnNPs) are the most researched, highly utilized and commercialized nanomaterials for different biomedical purposes, with a notable focus on their effectiveness in wound care.[4] ZnNPs is much safer and more environmentally friendly as compared to chemical synthesis, and is a cost-effective, excellent antibacterial, antifungal,

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anti-inflammatory properties and a low cytotoxic effect.[5] The biosynthesis of nanoparticles from plant extracts has attracted researchers, attention because their small size, large surface area, orientation, and physical properties make them suitable to be used in medical sciences.[5] According to the World Health Organization (WHO), a considerable population (80%) employs herbal remedies based on medicinal and aromatic plants to provide primary health care.[6]

Sesamum indicum has been explored for various pharmacological properties, such as antipyretic, anti-inflammatory, anti-oxidant, anti-microbial, anti-hypertensive, wound healing and anticancer properties.[7] *S. indicum* seeds-based zinc nanoparticle was effectively green synthesized– a new, simple and eco-friendly technique that avoids the usage and release of toxic chemicals is emerging in the field of nanoscience and technology.[8] Using metal nanoparticles and their oxides is a potential strategy to break the resistance of bacteria to antibiotics. In order to evaluate the antibacterial effectiveness of synthesized *S. indicum* seeds nanoparticle (ZnNPs) against the multidrug-resistant newly identified GPB, *P. harei*, *In vitro* and *In vivo* as well as wound healing were the main objectives of this work.

MATERIALS AND METHODS

Isolation and Identification

This experimental study employed an integrated in vitro-in vivo approach to evaluate the antibacterial and wound-healing efficacy of biosynthesized ZnNPs against MDR P. harei. Between July and October 2022, 157 clinical samples, including those from gunshot wounds, burns, surgeries, diabetic ulcers, and bedsores, were collected from multiple hospitals in the Erbil, Iraq area. Every specimen was immediately put into blood agar that was selective for GPB, and then incubated for 24 hours at 37 °C. GPB were identified using colonial morphology, Gram stain, and molecular identification by the PCR (16S rRNA) gene. The primers used were 27F (AGAGTTTGATCCTGGCTCAG) and 1492R (TACGGYTACCTTGTTACGACTT), followed by sequence. A web-based interface was employed to make the submission procedure easier (https://submit.ncbi.nlm.nih.gov). Every sequence has been submitted to GenBank, and all isolated bacteria are now assigned accession numbers.

Antimicrobial sensitivity test

The susceptibility of isolates to 20 antimicrobial agents-Amikacin, Amoxicillin-Clavulanic acid, Ciprofloxacin, Ampicillin, Chloramphenicol, Doxycycline, Cefotaxime, Imipenem, Gentamicin, Levofloxacin, Nalidixic acid, Meropenem, Nitrofurantoin, Rifampin, Norfloxacin, Streptomycin, Tobramycin, Tetracycline, Trimethoprim, and Vancomycin was evaluated using the Kirby Bauer disk diffusion method, following Clinical and Laboratory Standards Institute (CLSI) guidelines.[9]

Biosynthesis of ZnNPs using *S. indicum* seeds

Fresh sesamum indicum seeds were procured from local markets in Shaqlawa, Erbil, and authenticated by Salahaddin University's College of Education. Seeds were washed, dried, and ground into powder. Aqueous extract was prepared by heating 20 g of powder in 100 mL distilled water at 75°C for 45 minutes, followed by double filtration and storage at 4°C (10). For ZnNP synthesis, 100 mL of extract was added dropwise to 400 mL of 1 mM ZnO solution under continuous stirring (1000 rpm, 50°C, 2 hours). The pH was adjusted to 8–10 using NaOH, and the mixture was incubated at 37°C for 72 hours. Post-evaporation and drying at 200°C yielded sterilized ZnNP powder.[10]

Characterization of ZnNPs

ZnNPs were screened in a double-beam UV-visible spectrophotometer, Fourier Transmission Infrared Spectroscopy (FTIR), Scanning Electron Microscope (SEM), Energy Dispersive X-ray spectroscopy (EDX) and X-ray diffraction (XRD) measurements.[11]

Antibacterial activity of ZnNPs

Antibacterial efficacy against multidrug-resistant (MDR) *P. harei* was assessed via the well diffusion method. A 10 mg/mL ZnNP solution (0.1% concentration) was prepared by sonicating 0.05 g nanoparticles in 5 mL ethanol (50°C, 1 hour). Inoculated Mueller Hinton agar plates (0.5 McFarland standard) were supplemented with 150 μL ZnNP solution per well (7 mm diameter). Inhibition zones (mm) were measured after 24-hour incubation at 37°C.[12]

In vivo activity of ZnNPs

Animal care and burn generation

Female Wistar albino rats (200 ± 10 g) were acclimatized for seven days under controlled conditions (24 ± 2°C, 12-hour light/dark cycle). Anesthesia was induced using xylazine (2%) and ketamine (10%, 10+90 mg/kg). Burn wounds (4 cm^2) were generated on the dorsal region using a heated stainless-steel bar (Figure 1). Infected burns were treated with ZnNP cream (1% w/w) formulated by dissolving 0.05 g ZnNPs in DMSO (2 mL) and blending with Vaseline wax (5 g) under sterile conditions (Figure 2). Rats were divided into four groups: standard (Mebo 0.25% + Fusidin 2%), positive control (Vaseline), test (ZnNP cream), and negative control (untreated). Treatments were applied daily until wound closure.[13] The experimental procedure was approved by the ethics committee and adhered to the ethical criteria for animal experimentation set out in Directive 2010/63/EU.[14]

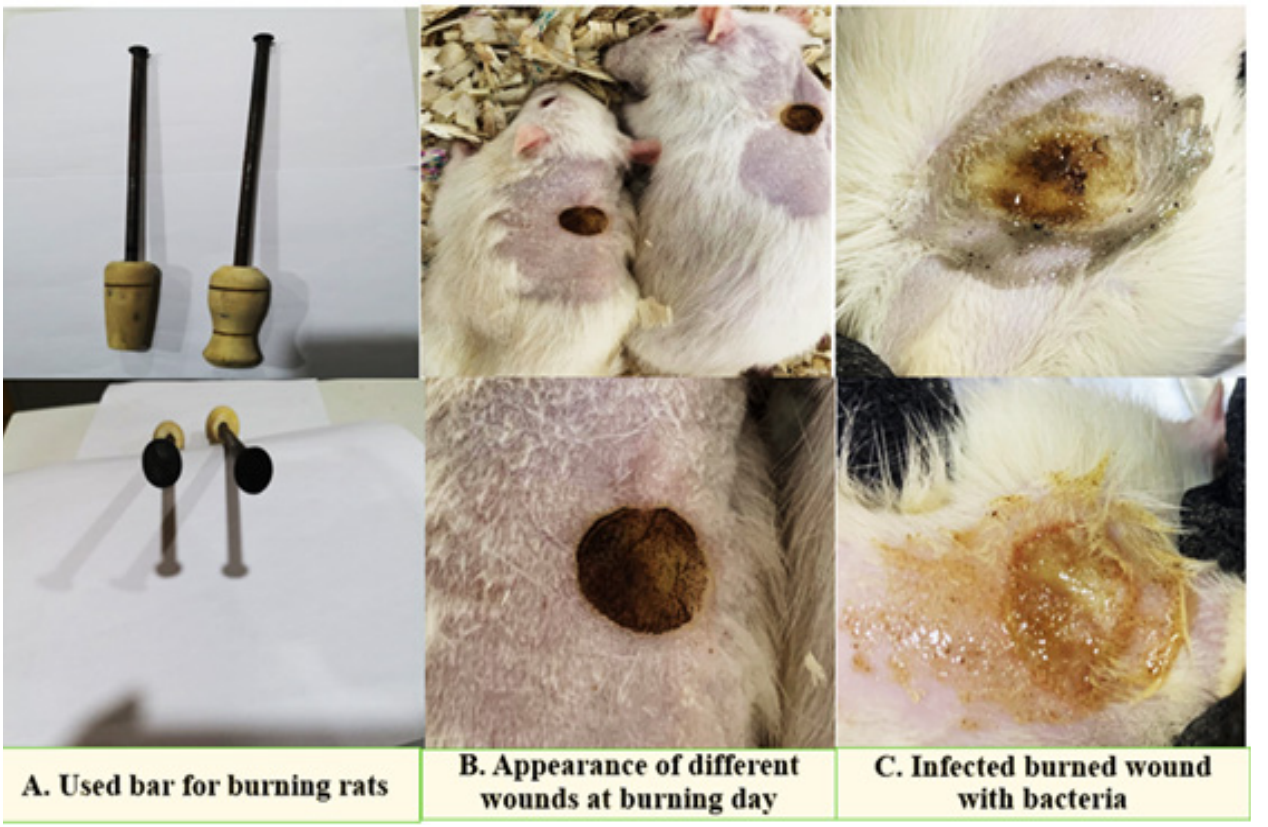


Figure 1: Burn generation

Figure 2: Prepared Zn nanoparticles cream

Oral acute toxicity

Single dose toxicity study: Acute oral toxicity testing was performed on female Wistar albino rats weighing 200 ± 10 gm to evaluate the toxicity of each nanoparticle in a living system. The test used an up-and-down pattern as defined in OECD exam Guidelines 425 (OECD, 2008). The rats had an overnight fast and a week of adaptation prior to the dosing.[15]

Dose preparation and administration

Acute oral toxicity was evaluated per OECD Guideline 425. Rats (n = 2 per dose) were fasted overnight and administered ZnNP doses (2000 or 3000 mg/kg) dissolved in ethanol and corn oil (Table 1). Behavior, ocular/skin changes, and mortality were monitored for 14 days. On day 15, euthanasia was performed using xylazine-ketamine, followed by gross pathological examination. No lethality or adverse effects were observed (Table 1).[16]

Table 1: Dose preparation and administration

Conc. mg/kg	Ethanol (mL)	Vehicle (mL)	No. of doses in 24 hours	No. of used rat	Lethality
2000	2	3	1	1	No
3000	2	3	1	1	No

RESULTS

In this study, P. harei was identified for the first time in Iraq through 16S rRNA sequencing, with the strain deposited in NCBI under accession number OQ380735. Antimicrobial susceptibility testing revealed multidrug resistance, with complete resistance to 14 out of 20 antibiotics, including ciprofloxacin, levofloxacin, tetracycline, and meropenem.

Biosynthesis of ZnNPs

Biosynthesis of ZnNPs using sesamum indicum seed extract was confirmed by a visible color transition from pale yellow to yellowish-brown (Figures 3A, 3B).

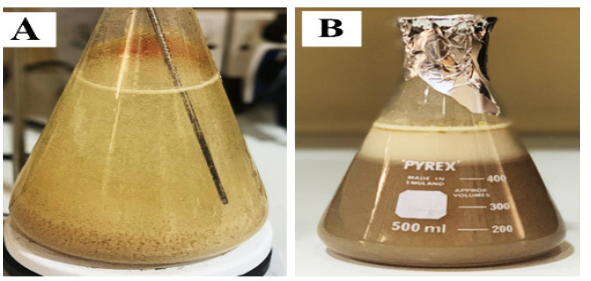


Figure 3: (A) *S. indicum seeds* extract before ZnO (B) after addition ZnO

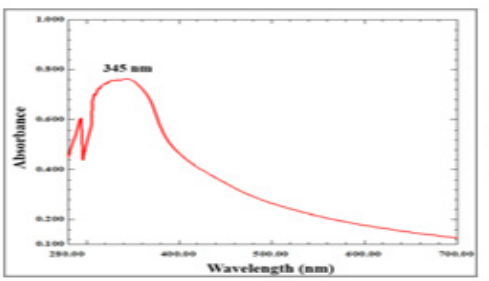
Characterization of ZnNPs from *S. indicum* seeds

UV-Visible Spectroscopy

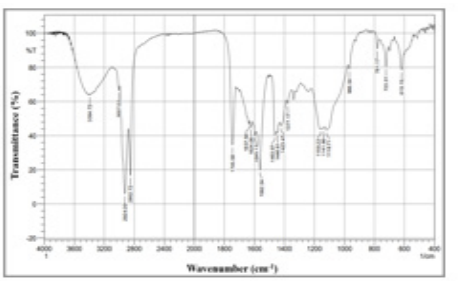
UV-Vis spectroscopy demonstrated a surface plasmon resonance peak at 345 nm (Figure 4A), indicative of ZnNP formation. FTIR analysis identified functional groups involved in nanoparticle stabilization, including C=O (1745.58 cm^{-1}), C=N (1624.06 cm^{-1}), and N-H (3394.72 cm^{-1}) bonds, suggesting biomolecule-mediated synthesis (Figure 4B) (10). XRD patterns (2θ=31.668°, 34.372°) confirmed crystalline ZnNPs with an average size of 9.07 nm (Figure 4C), while EDX revealed a prominent zinc peak at 2 keV (Figure 4D). SEM imaging further illustrated spherical nanoparticles averaging 70 nm in diameter (Figure 4).

In vitro antimicrobial activity of ZnNPs

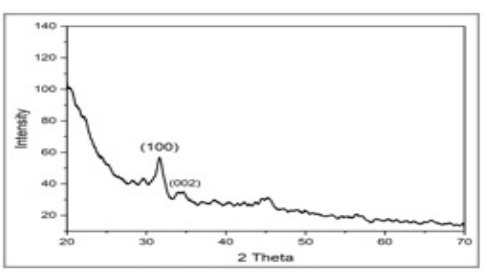
In vitro testing of 0.1% ZnNPs against P. harei showed an inhibitory zone of 16 mm (Table 2), demonstrating potent antibacterial activity. In vivo wound healing assays in rats revealed that ZnNP-treated wounds healed significantly faster (22 days) compared to vaseline (27 days), fusidin (28 days), and non-treated controls (33 days), though slower than Mebo (16 days) (Table 3, Figure 5). Acute oral toxicity studies



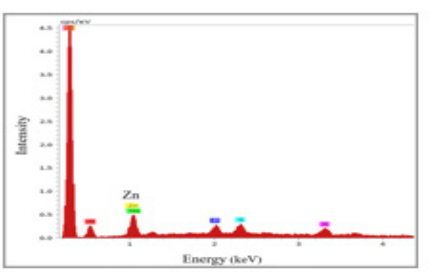
A: Uv-vis absorption spectrum of ZnNPs



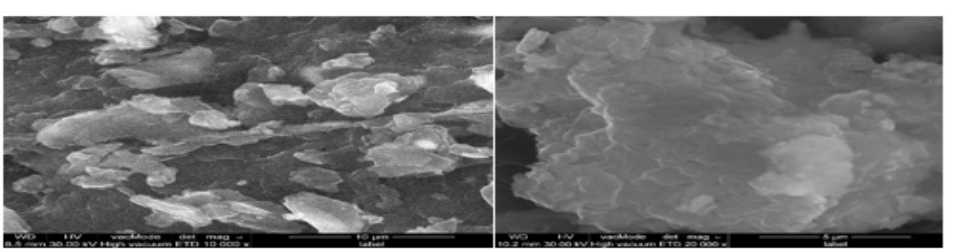
B: FTIR of ZnNPs



C: XRD of ZnNPs



D: EDX of ZnNPs



E. FE-SEM of ZnNPs

Figure 4: Characterization of ZnNPs

indicated no mortality or macroscopic organ abnormalities, suggesting low toxicity.

Table 2: Inhibition diameters of ZnNPs against isolated under the study

Nanoparticles	Zone of inhibition (mm)
	P. harei
ZnNPs	11

Table 3: Healing time of treated rat wounds under the study

Nanoparticles	Healing time (days)
	P. harei
ZnNPs	22
Vaseline (Positive Control)	27
Fusidin (Standard Control)	28
Mebo (Standard Control)	16
Non-treated (Negative Control)	33

DISCUSSION

The emergence of multidrug-resistant (MDR) bacteria in wound infections poses significant therapeutic challenges, necessitating novel treatment strategies. This study investigated MDR bacterial isolates from wound specimens in Erbil, Iraq, identifying Gram-positive bacteria (GPB) in 60 (38.21%) of cases. Molecular analysis via 16S rRNA sequencing revealed the presence of P. harei, a newly identified GPB in Iraqi bedsores.

Historically classified under Peptococcus and Peptostreptococcus, P. harei was reclassified as Peptoniphilus in 2001.[17] Its misidentification as P. asaccharolyticus through biochemical methods underscores the necessity of molecular techniques, as demonstrated by conflicting Vitek MS (99.9% confidence for P. asaccharolyticus) and PCR results (99% identity with P. harei, GenBank MZ008068.1).[18, 19] Previous studies by Murdoch et al.,[19] Wang,[20] and Parha,[21] corroborate its association with chronic wounds and purulent infections, while Song et al. reported a 23.2% prevalence in clinical specimens.[22] These findings highlight P. harei's emerging clinical relevance and diagnostic complexities.

Zinc nanoparticles (ZnNPs) biosynthesized using sesamum indicum exhibited potent antibacterial activity against MDR P. harei. Mechanisms include membrane adhesion, disruption of cellular respira-

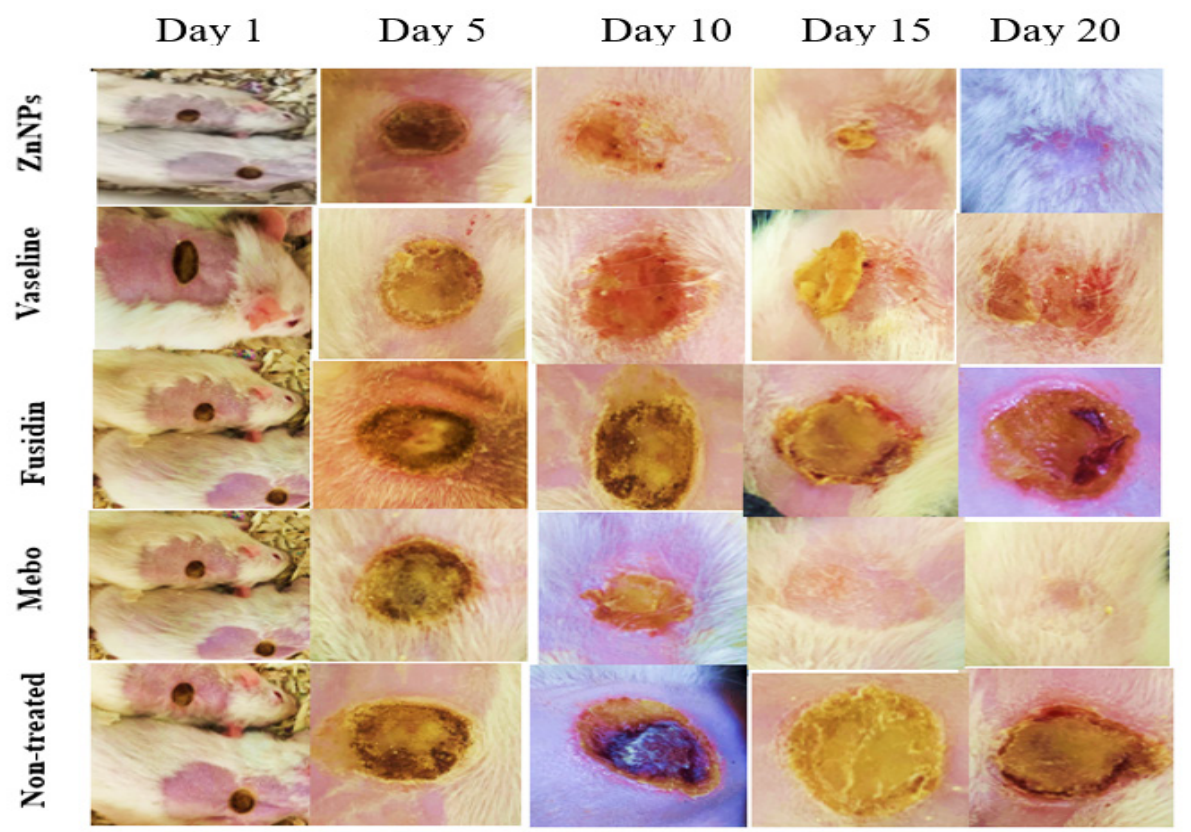


Figure 5: Wound healing on different days with ZnNPs, positive control, standard control and negative control

tion, and ROS generation, leading to DNA damage and protein denaturation.[12, 23] In wound healing assays, ZnNP-based creams significantly accelerated tissue regeneration compared to conventional treatments, aligning with studies by Wang et al.[20] and Pino et al.,[24] who observed healing within 17–22 days in animal models. The nanoparticles' dual antimicrobial and regenerative properties likely enhance epithelialization while reducing bacterial load. Topical application showed no adverse effects on skin morphology, indicating biocompatibility at tested concentrations.[25] This positions ZnNPs as a safe, cost-effective alternative to traditional antibiotics, particularly against MDR pathogens.

These findings underscore the therapeutic potential of biogenic ZnNPs in managing MDR-infected wounds. By combining targeted antimicrobial action with enhanced tissue repair, nanoparticle-based formulations address critical limitations of current therapies. Further research is warranted to elucidate molecular mechanisms and optimize clinical protocols, advancing their translation into mainstream wound care.

CONCLUCION

This study highlights ZnNPs synthesized from S. indicum seeds, demonstrating potent in vitro antibacterial activity against multidrug-resistant P. harei and accelerating in vivo excision wound healing in Wistar rats within 22 days. Characterized via advanced techniques, ZnNPs exhibited non-toxic, cost-effective synergistic effects, emphasizing their potential as topical agents for wound care and antibacterial therapies. These findings advance understanding of ZnNP-driven biological mechanisms, offering promising avenues for biomedical applications and novel therapeutic strategies.

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CONFLICT OF INTEREST
Authors declare no conflict of interest.
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None declared.

AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

Conception or Design:	MMA, PAH
Acquisition, Analysis or Interpretation of Data:	MMA, PAH, KKB
Manuscript Writing & Approval:	MMA, PAH, KKB

All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.