



Silver Oxide Nanoparticles Against Oral Protozoa Targeting *Entamoeba gingivalis* and *Trichomonas tenax* in Patients with Periapical Lesions and their Association with Interleukin-6

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Abstract

Background: Oral protozoa are measured as etiological agents around the apices of the teeth. **Objective:** The present study targeted to assess the connotation of *Entamoeba gingivalis* and *Trichomonas tenax* with the levels of salivary interleukin IL- 6 in patients with periapical lesions. The anti-parasitic potential of silver oxide nanoparticles (Ag₂O- NPs) was also investigated in relation to these protozoan species. **Methodology:** The cross-sectional study was conducted at the laboratories of the University of Misan, Iraq, and at some specialized clinics, from Nov. 2025 to April 2026. The study comprised 60 patients with clinically identified periapical lesions and 20 individuals without periapical lesions as controls. Unstimulated saliva samples were collected from all participants. The occurrence of *Entamoeba gingivalis* and *Trichomonas tenax* was assessed, salivary IL-6 levels were measured, and Ag₂O nanoparticles were synthesized, characterized, and evaluated in vitro towards the isolated protozoa. Sixty unstimulated saliva samples were collected from periapical lesion patients and 20 with no periapical lesion as controls. The prevalence of *Entamoeba gingivalis* and *Trichomonas tenax* was measured, and salivary levels of IL- 6 were determined. The physicochemical properties of Ag₂O-NPs were analysed by X -ray diffraction, Fourier transform infrared spectroscopy, field emission scanning electron microscopy, dynamic light scattering, and zeta potential analysis. The anti-parasitic potential of Ag₂O- NPs was assessed against oral parasites at 25, 50, and 100 µg/ml concentrations of the nanoparticles following 2, 6, and 24 h of incubation. **Results:** The prevalence rates of *Entamoeba gingivalis* among patients and controls were 81.7 % and 45%, respectively, while those of *Trichomonas tenax* were 70.0 % and 25.0% cases. Both protozoa presented significant relations with periapical lesions. Salivary levels of IL- 6 were significantly higher in parasite-positive patients compared to parasite-negative controls; median levels of IL-6 were 30.34 pg/ml (Positive E.g.) versus 12.02 pg/ml for those who were negative for the parasite. Likewise, for *Trichomonas tenax* positive participants, median levels of IL-6 were 33.41 pg/ml as compared to 14.50 pg /ml for *Trichomonas tenax* negative participants. Ag₂O- NPs were found to possess crystalline structures with a surface charge and polydispersity and showed significant anti-protozoan activity. At 50 and 100 µg/ml concentrations of Ag₂O- NPs, no trophozoites were observed after 24 hours of exposure. **Conclusion:** An association was observed between the presence of oral parasites and increased levels of salivary IL- 6. Ag₂O- NPs displayed significant anti-protozoan activity in vitro, [Bangladesh Journal of Infectious Diseases, June 2026;13(1):262-270]

Keywords: *Entamoeba gingivalis*; *Trichomonas tenax*; periapical lesions; interleukin-6; silver oxide nanoparticles; oral protozoa; antiprotozoal parasites activity

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Introduction

Periodontal cases remain a major public health challenge as they are highly prevalent, biologically complex, and closely linked to chronic inflammation-driven tissue destruction. The World Health Organization (WHO) estimates that oral diseases affect nearly 3.7 billion individuals around the world, and more than 1 billion cases are accounted for by severe periodontal disease alone¹. Despite advances in preventive dentistry and periodontal therapy, disease control is still hindered by delayed diagnosis, polymicrobial biofilms, recurrent inflammation, and the incomplete response of a number of patients to conventional mechanical and antimicrobial approaches. These limitations continue to drive interest in adjunctive strategies capable of acting within the hostile microenvironment of the periodontal pocket while minimizing collateral injury to host tissues^{2,3}. Even though bacterial dysbiosis remains central to current models of many body diseases, especially periodontitis, the parasitic component of the human microbiome is receiving renewed attention⁴⁻⁶. Among the best studied oral protozoa are *Entamoeba gingivalis* and *Trichomonas tenax*, both of which are frequently noticed at higher occurrences in gingivitis and periodontitis than in periodontal health. Recent literature has strengthened this association, with recent clinical studies continuing to report developed recognition rates of *Entamoeba gingivalis* and, to a lesser extent, *Trichomonas tenax* in diseased periodontal sites than in healthy controls with no problems in their oral cavity⁷.

The two listed parasites may contribute to disease through partly imbricated but biologically dissimilar pathways. *Entamoeba gingivalis* has been connected with periodontal inflammation, co-occurrence with periodontopathogenic bacteria and destructive interactions with host tissues. Investigational work has shown that *Entamoeba gingivalis* can promote oral inflammation and tissue obliteration, while a cross-sectional sequencing-based study in young adults found that its prevalence and relative abundance became high across the transition from health to gingivitis and periodontitis. In contrast, *Trichomonas tenax* has often shown a stronger relationship with more advanced periodontal breakdown, and in vitro evidence indicates that it can damage gingival epithelial barriers and stimulate inflammatory signaling such as IL-6 production⁸. Emerging nanotechnology investigate suggests that engineered and fabricated nanoparticle platforms can strengthen biological activity and optimize

localized efficiency, while advances in microenvironment-controlled systems further support the development of more precise and responsive therapeutic formulations⁹⁻¹³. Iron oxide nanoparticle preparations, for instance, have demonstrated improved effectiveness in antiparasitic and malaria vaccine applications¹⁴⁻¹⁶. Combined with such advances in anti-microbial nanomaterials, artificial intelligence is progressively being explored as a tool to hasten nanotechnology progress by supplementing the structure and performance prediction of nanoscale structures. Machine learning and deep learning models have revealed robust measurements for design recognition and automated sorting in compound biological and environmental datasets, highlighting the upcoming possible of data-driven frameworks for nanoparticle optimization and pathogen detection¹⁷⁻¹⁹.

Silver-based nanomaterials are attractive since their broad spectrum as anti-microbial platforms, high surface reactivity, and capability to affect multiple cellular processes at once. In dentistry, silver-containing nanoparticles have been investigated for suppressing oral microorganisms, enhancing antimicrobial dental supplies, and restricting infection in plaque-associated conditions²⁰. In addition, silver-based nanoparticles have previously revealed antiprotozoal efficacy in related systems, including *Entamoeba histolytica*, where silver nanoparticles reduced trophozoite viability, and earlier work combining silver and copper oxide nanoparticles demonstrated antiparasitic activity against *Entamoeba histolytica* cysts. This analogue evidence strengthens the biological plausibility of testing Ag₂O-based nanotherapeutics against oral protozoa²¹.

At present, though, the literature seems to cover a clear translational gap, but focused searches do not disclose an indexed study directly evaluating silver oxide nanoparticles against *Entamoeba gingivalis* and *Trichomonas tenax* together. Accordingly, the present study was designed to investigate the anti-protozoal potential of silver oxide nanoparticles against *Entamoeba gingivalis* and *Trichomonas tenax*. This work aims to extend current periodontal nanomedicine beyond bacteria-centered models and to provide a stronger basis for future targeted approaches towards the protozoal component of oral dysbiosis.

Methodology

Study Design: This cross-sectional research was completed at the laboratories of the University of

Misan, Iraq, and at some specialized clinics during the period from Nov. 2025 to April 2026. Eighty adult individuals of both genders, aged 20 to 60 years, were separated into two groups: patients with a periapical lesion group (60) with at least one untreated permanent tooth demonstrating pulp necrosis and a radiographically visible periapical lesion. The control group (20 participants) was deprived of clinical or radiographic evidence of a periapical lesion. Taking antiparasitic medicine in the last four weeks, uncontrolled diabetes mellitus, periodontal handling during the last three months, pregnancy or lactation, and smoking cases were excluded from the study.

Sample Collection: Unstimulated whole saliva was collected before endodontic treatment, dental cleaning, or prescription of any antimicrobial medication. Whenever possible, samples were collected between 9:00 and 12:00 in the morning. Each individual rinsed the mouth with 10 ml of sterile distilled water for approximately 30 sec. After a ten-minute resting period, saliva was allowed to gather naturally and was taken by passive drooling into a sterile graduated tube. At least 5 ml of saliva was collected. Each saliva specimen was mixed gently and immediately divided into four divisions. Investigation and inoculation into culture media was started within 30- 60 min of gathering. A drop of well-mixed saliva was transferred to a clean glass slide, covered with a coverslip, and inspected under light microscopy at 100X and 400X magnification for oral parasite discovery. Samples were also collected from periodontal pockets and from periapical lesion tissue.

Cultivation of Oral Parasites: Only fresh samples containing motile trophozoites were used for culturing. Approximately 0.5 ml of fresh saliva was inoculated into Diamond's TYM medium supplemented with 10.0% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin, 100 µg/ml streptomycin, and 2.5 µg/ml amphotericin B. The cultures were incubated at 37°C in tightly capped tubes. Fresh medium was changed every 48 h until adequate trophozoite growth was obtained. Fresh active amoeba was inoculated into Liquid Dulbecco's Modified Eagle Medium (DMEM) and incubated at approximately 37°C. To maintain *E. gingivalis* as a completely bacteria-free culture, the isolate was initially sustained as a xenic culture containing associated oral microorganisms.

Silver Oxide Nanoparticles Synthesis: Silver oxide nanoparticles were synthesized by alkaline precipitation. A 0.1 M AgNO₃ solution was

prepared by dissolving 1.36 g of silver nitrate in deionized water and adjusting the final volume to 80 mL. Separately, 0.32 g of NaOH was dissolved and diluted to 40 mL to obtain a 0.2 M solution. The AgNO₃ solution was heated to 70°C and stirred at 400 rpm. NaOH was then added dropwise, at an approximate rate of one drop every 40 seconds while maintaining the pH at 10-11. Formation of a dark brown precipitate indicated the production of Ag₂O nanoparticles (Ag₂O- NPs). After cooling, the suspension was centrifuged at 8000 rpm for 20 minutes. The precipitate was washed three times with deionized water, with centrifugation at 8000 rpm for 10 minutes after each wash. The recovered material was dried at room temperature for two days and subsequently calcined at 200°C to obtain crystalline Ag₂O nanoparticles. Concentrations of 25 µg/ ml, 50 µg/ ml, and 100 µg/ ml were prepared for the in vitro tests. The synthesized nanoparticles were characterized using X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), field emission scanning electron microscopy (FESEM) and zeta potential analysis.

In Vitro Antiparasitic Assay: Trophozoites collected during logarithmic growth were counted using a Neubauer haemocytometer. The suspension was adjusted to approximately 105 a measured volume of the trophozoite suspension was distributed into culture tubes. Ag₂O-NPs were then added to obtain the required final concentrations. Viability was examined after 2, 6 and 24 hours. Trophozoites and culture medium without nanoparticles were used as a control. Viability was initially evaluated by measuring trophozoite movement and membrane integrity. An equal volume of the cell suspension and 0.4% Trypan blue was mixed. Cells excluding the stain were considered viable, whereas blue-stained cells were considered nonviable.

IL-6 Determination: Approximately 1.5– 2 ml was centrifuged at 3000 rpm for 15 minutes. The clear supernatant was distributed into small sterile tubes and stored at -80°C. IL- 6 was quantified using a sandwich ELISA kit (Mybiosource / USA). The steps started with the addition of samples to the assigned wells, then incubation to permit IL- 6 binding, then washing to remove unbound material. Subsequently, the detection antibody and enzyme conjugate were added. After that, the chromogenic substrate was added, and the colour development was terminated using stop solution, and absorbance at 450 nm was measured.

Statistical Analysis: Data were analysed using SPSS Statistics version 24.0. Frequencies and

percentages were used for categorical data, while IL- 6 levels were expressed as mean $\pm$ SD. Group differences were tested using the chi-square test and Fisher's exact test as appropriate. Odds ratios with 95% confidence intervals were calculated, and $P < 0.05$ was considered statistically significant.

Ethical Consideration: The research method has been reviewed and approved by the Research Ethics Committee of the College of Science, University of Misan, Iraq. Informed consent has been signed by each participant before collecting the sample.

Results

A total of 80 participants were enrolled, comprising 60 patients with periapical lesions and 20 healthy controls with no periapical lesions. Among the patient group, 19 (31.7%) participants were male, and 41 (68.3%) were female. The control group included 8 (40.0%) males and 12 (60.0%) females. The study population consisted of 27(33.8%) males and 53(66.2%) females. The P- value was calculated using Pearson's chi-square test and indicates no significant difference in sex distribution between the groups (Table 1).

Detection of Oral Parasites: A higher frequency of oral parasites was revealed using the microscopic examination among periapical lesion patients in comparison to healthy controls with no periapical lesions. The oral parasite *Entamoeba gingivalis* was detected in 49 of 60 patients and in 9 of 20 controls. While *Trichomonas tenax* was recognized in 42 patients compared with 5 controls. Both parasites were significantly more frequent among patients with periapical lesions than among controls with no periapical lesions ($p < 0.001$) (Table 2).

Characterization of Ag₂O nanoparticles: X- ray diffraction (XRD) evaluation exposed a crystalline diffraction pattern across the scanned range of

8.887-79.987° 2 θ using Cu K α radiation ($\lambda = 1.5406$ Å). The dominant diffraction region was sandwiched between 32.69° and 33.34°. The principal diffraction peaks matched the cubic Ag₂O structure and were tentatively assigned to the (111), (200), (220), (311), and (222) planes, with (111) being the dominant reflection, thereby supporting the formation of crystalline silver oxide. (Figure I).

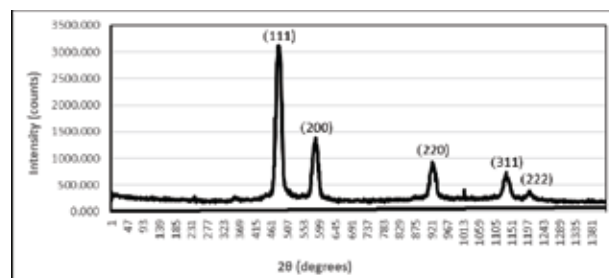


Figure I: X-ray diffraction pattern of the synthesized Ag₂O nanoparticles recorded using Cu K α radiation over a 2 θ range of 8.887–79.987°

The FTIR spectrum of the fabricated Ag₂O nanoparticles was measured over 4000-400 cm⁻¹. A broad absorption band centred at roughly 3388.9 cm⁻¹ was observed, which can be attributed to O-H stretching from surface hydroxyl groups or adsorbed moisture. The band at 1647.2 cm⁻¹ was connected with the bending vibration of adsorbed water molecules. A visible absorption feature appeared at 1375.2 cm⁻¹ probably representative of a minor quantity of residual nitrate originating from the silver nitrate precursor. Additional bands were detected at approximately 1058.9 and 883.4 cm⁻¹ and may arise from minor surface-bound or residual species. Several absorption bands were present in the lower-wavenumber region at 707.9, 657.7, 632.7, 594.1, 534.3, 486.1, and 447.5 cm⁻¹. These bands are consistent with metal-oxygen lattice

Table 1: Distribution of Participants by Study Group and Gender

Study Group	Male	Female	Total	P value
Periapical Lesion Group	19(31.7%)	41(68.3%)	60(75.0%)	0.495
Control Group	8(40.0%)	12(60.0%)	20(25.0%)	
Total	27(33.8%)	53(66.2%)	80(100.0%)	

Table 2: Frequency of Oral Parasites in Patients with Periapical Lesions and Healthy Control

Oral parasite	Case Group	Control Group	Total	P value
<i>Entamoeba gingivalis</i>	49 (81.7%)	9 (45.0%)	58 (72.5%)	0.001
<i>Trichomonas tenax</i>	42 (70.0%)	5 (25.0%)	47 (58.8%)	0.001

vibrations and support the formation of Ag-O bonds in the synthesized nanoparticles. The FTIR results complement the XRD findings and provide further evidence for the successful production of silver oxide nanoparticles (Figure II).

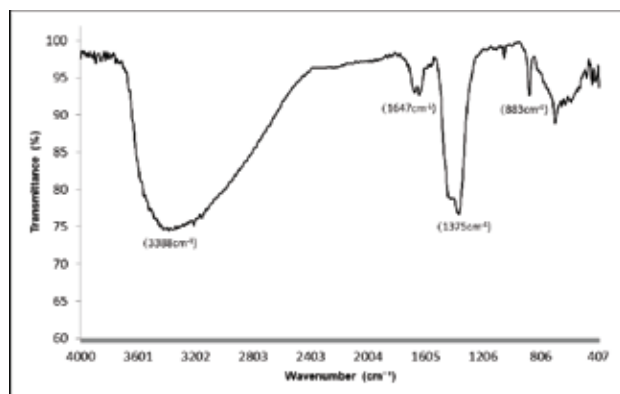


Figure II: FTIR spectrum of the synthesized Ag₂O nanoparticles recorded over the wavenumber range of 4000-400 cm⁻¹

Dynamic light scattering (DLS) analysis presented a Z-average hydrodynamic diameter of 28.0 nm and a PDI of 0.802. The high PDI indicates a broad and heterogeneous particle-size distribution, signifying the existence of particles or aggregates of particles in different sizes. The number-based distribution was dominated by smaller particles, whereas bigger particles contributed more strongly to the intensity-based distribution because light scattering intensity increases evidently with particle size (Figure III).

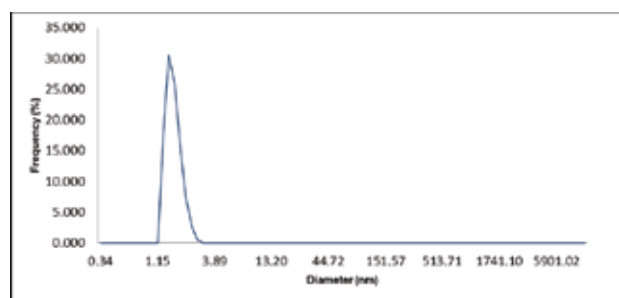


Figure III: Dynamic light scattering distributions of the synthesized Ag₂O nanoparticles according to particle number, volume, and scattered-light intensity

Zeta potential examination exhibited a solitary dominant peak in the negative region, with a mean value of -36.49 mV for Ag₂O nanoparticles. This negative surface charge suggests adequate electrostatic repulsion among particles and supports satisfactory short-term colloidal stability below the

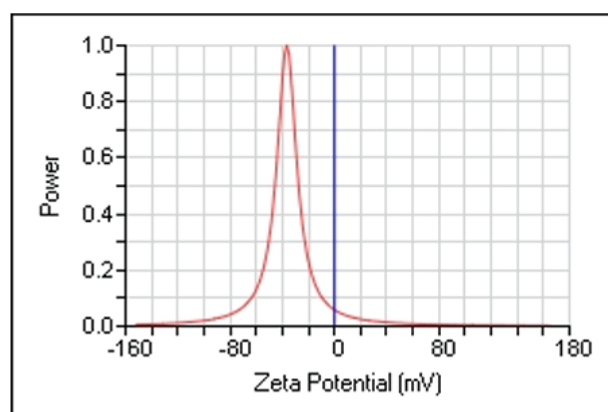


Figure IV: Zeta-potential distribution of the synthesized Ag₂O nanoparticles

FESEM examination displayed that the synthesized Ag₂O nanoparticles formed irregular spherical agglomerates with rough and porous surfaces. The greater clusters appeared to comprise minor nanoscale structural particles that were thoroughly attached. Using ImageJ, 100 nanoparticles were measured to obtain an average diameter of 82 nm. The uneven surface texture indicates the formation of nanoscale silver oxide structures (Figure V).

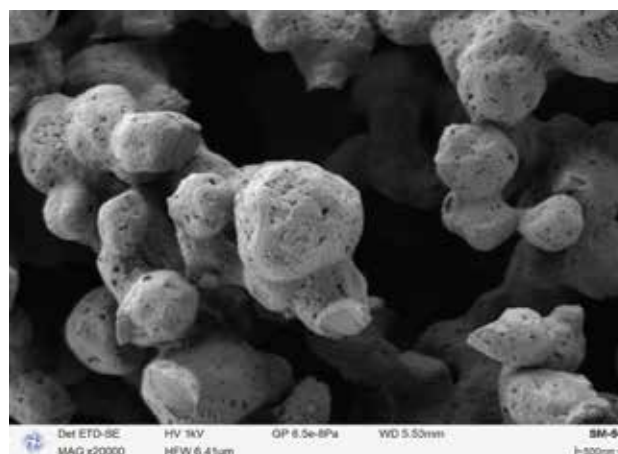


Figure V: FESEM Micrograph of the Synthesized Ag₂O nanoparticles (Scale bar = 500 nm)

The viability of *Entamoeba gingivalis* following introduction to different Ag₂O nanoparticle concentrations were recorded. Viability deteriorated progressively with increasing concentration and incubation time, with comprehensive inhibition detected at 50 and 100 µg/ml after 24 hours (Table 3).

Antiparasitic activity of Ag₂O Nanoparticles: The consequence of Ag₂O nanoparticles on the viability of *E. gingivalis* at concentrations of 25, 50 and 100 µg/ml after 2, 6 and 24 hours. Trophozoite viability reduced with increasing nanoparticle

concentration and exposure time. After 24 hours, inhibition reached 88.42 % at 25 µg /ml and 100 % at both 50 and 100 µg/ml, while the untreated control preserved the maximum viability (Figure VI).

Table 3: Viability of *Entamoeba gingivalis* Following Exposure to Ag₂O Nanoparticles

Ag ₂ O-NP Concentration	Viability		
	After 2 hr	After 6 hr	After 24 hr
Control	98.0%	98.0%	96.8%
25 µg/ml	82.3%	55.2%	11.2%
50 µg/ml	68.4%	33.9%	0.0%
100 µg/ml	44.2%	19.1%	0.0%

hr=hour

The viability of *Trichomonas tenax* after treatment with Ag₂O nanoparticles was recorded. The strongest activity happened after 24 hours, when concentrations of 50 and 100 µg/ ml produced whole inhibition, while 25 µg/ ml caused 83.48 % inhibition (Table 4).

The action of Ag₂O nanoparticles in contradiction of *Trichomonas tenax*. A concentration- and time-dependent decrease in trophozoite viability

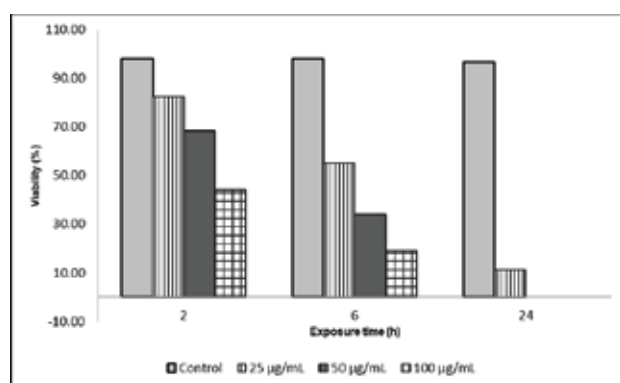


Figure VI: Effect of Ag₂O nanoparticles on the viability of *Entamoeba gingivalis* trophozoites

Table 4: Viability of *Trichomonas tenax* following exposure to Ag₂O nanoparticles

Ag ₂ O-NP concentration	Viability after 2 h, %	Viability after 6 h, %	Viability after 24 h, %
Control	94.22	94.16	94.01
25 µg/ml	88.02	61.01	15.53
50 µg/ml	71.77	42.71	0.00
100 µg/ml	53.23	25.02	0.00

Table 5: Salivary IL- 6 (pg/ ml) Concentrations According To Oral Parasite Detection

Detected parasite	Case	Mean±SD	Control	Mean±SD	Fold Raised
<i>Entamoeba gingivalis</i>	49	30.3±	9	12.0±	2.52
<i>Trichomonas tenax</i>	42	33.4±	5	14.5±	2.30

was observed. After 24 hours, inhibition rates were 83.5%, 100.0%, and 100.0% at concentrations of 25, 50, and 100 µg/ml, respectively, whereas viability continued to be highest in the untreated control (Figure VII).

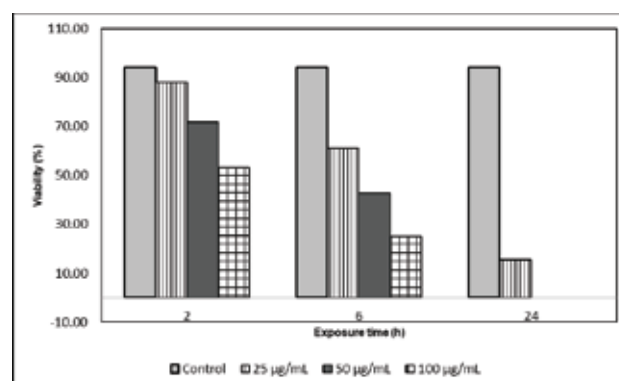


Figure VII: Effect of Ag₂O nanoparticles on the viability of *Trichomonas tenax* trophozoites

Salivary IL-6 levels according to parasite detection: Salivary IL- 6 concentrations were higher in parasite-positive patients with periapical lesions than in parasite-positive controls. Among participants positive for *Entamoeba gingivalis*, the mean IL- 6 concentration was 30.34 pg/ ml in patients compared with 12.02 pg/ ml in controls, corresponding to an approximately 2.52- fold increase. Similarly, among participants positive for *Trichomonas tenax*, mean IL- 6 was 33.41 pg/ml in patients and 14.50 pg/ ml in controls, representing an approximately 2.30- fold increase (Table 5). Associations between Oral Parasites, IL-6, and Clinical Status: Salivary IL-6 levels according to parasite detection: Both oral parasites were significantly associated with the presence of periapical lesions. Participants with periapical lesions had 5.44-fold higher odds of *Entamoeba gingivalis* detection than controls (95 % CI 1.82 to 16.31; p = 0.001). The corresponding odds of

Table 6: Associations between Oral Parasite Detection, Periapical Lesions, and Salivary IL-6

Parasite	Case (Positive/ Negative)	Control (Positive/ Negative)	Odds ratio (95% CI)	P value	Case IL-6 (pg/ml)	Control IL-6 (pg/ml)
<i>Entamoeba gingivalis</i>	49/11	9/11	5.44 (1.82-16.31)	0.001	30.34	12.02
<i>Trichomonas tenax</i>	42/18	5/15	7.00 (2.21-22.17)	0.001	33.41	14.50

Trichomonas tenax detection were 7.0 times higher in the patient group (95.0% CI 2.21 to 22.17, $p < 0.001$). Parasite-positive patients also showed higher salivary IL-6 concentrations than parasite-negative controls. IL-6 was approximately 2.52-fold higher in participants positive for *Entamoeba gingivalis* and 2.30-fold higher in those positive for *Trichomonas tenax*. These findings indicate that parasite detection was associated with both periapical disease and an increased inflammatory response (Table 6).

Discussion

The present study integrates clinical immunological, physicochemical, and experimental evidence to examine the possible involvement of *Entamoeba gingivalis* and *Trichomonas tenax* in periapical disease and to evaluate the in vitro antiparasitic potential of silver oxide nanoparticles (Ag₂O- NPs). *Entamoeba gingivalis* was detected in 81.7% of patients with periapical lesions compared with 45.0 % of controls; this difference was found to be statistically significant. On the other hand, *Trichomonas tenax* findings demonstrate robust associations between the existence of periapical lesions and colonization by both oral protozoa. Yaseen et al²² reported *Entamoeba gingivalis* in 88.9% of subjects with periodontitis compared with 47.9% of healthy participants and *Trichomonas tenax* in 25.6% of periodontitis cases and 3.2% of healthy subjects. Jiao et al²³ also detected the organism more frequently and at a greater relative abundance in diseased periodontal sites, where its presence was associated with clinical inflammation, dysbiotic bacterial communities and altered gingival crevicular cytokines. A 2025 study reported *Entamoeba gingivalis* in 80.0% of periodontitis patients and 20.0% of controls²⁴. Direct interaction between the amoeba and gingival epithelial cells has been associated with epithelial injury, inflammatory activation, and tissue destruction. It has been shown that *Trichomonas gingivalis*²⁵ and *Trichomonas tenax*²⁶ can invade inflamed gingival tissue and exert cytopathic effects; these properties could permit the organism to intensify inflammation after colonization of an already disrupted oral site rather than acting as a solitary initiating pathogen.

Salivary IL-6 concentrations were consistently higher in parasite-positive patients than in parasite-negative controls. The parallel elevation associated with both parasites supports the presence of a more active inflammatory environment in patients with periapical lesions. Within periapical disease, it may participate in immune cell recruitment, amplification of local inflammation, and regulation of pathways involved in osteoclastogenesis and bone resorption. Earlier analysis of human periapical tissue demonstrated the presence of IL-6 within inflammatory lesions, while recent microbiome research has shown relationships between the salivary microbiome of patients with apical periodontitis and salivary inflammatory mediators, including IL-6^{27,28}. The particularly high IL-6 concentration in *Trichomonas tenax* positive patients is biologically compatible with the experimental observation that this organism can induce IL-6 release from gingival epithelial cells²⁹.

In the current study, the structural analyses supported the creation of silver oxide nanomaterial. The sharpness of the dominant reflections indicates the presence of an ordered crystalline phase³⁰. The FTIR spectrum indicated the presence of several surface-associated functional groups. Similar FTIR evidence for plant-derived surface molecules has been described in recent green synthesis studies of Ag₂O- NPs. FESEM revealed rough surfaces and visible agglomeration. This morphology is compatible with the tendency of high surface energy nanoparticles to associate during synthesis, washing, or drying. DLS divergence is expected in heterogeneous suspensions because intensity-weighted DLS is strongly influenced by larger scattering species, whereas mathematical conversion to a number distribution can exaggerate the apparent contribution of small particles. Methodological studies have emphasized that DLS averages and weighting transformations can produce misleading interpretations in polydisperse nanoparticle systems³¹.

The antiparasitic action of silver-based nanoparticles may involve several simultaneous mechanisms. Proposed processes include interaction with thiol-containing proteins, impairment of respiratory or metabolic enzymes, oxidative stress and damage to nucleic acids. Ag₂O- NPs may additionally

act as a reservoir for biologically available silver ions³². The current findings are consistent with previous reports of anti-amoebic activity by silver nanomaterials. Phytosynthesized silver nanoparticles reduced the viability of *Acanthamoeba castellanii* trophozoites more effectively than the plant extracts used for their production. Tannic acid-modified silver nanoparticles have also enhanced anti-*Acanthamoeba* activity in vitro. Direct comparison is limited because those studies investigated different protozoa, nanoparticle compositions, surface coatings, concentrations and exposure periods^{33,34}. Nevertheless, they support the broader concept that silver-based nanomaterials can exert activity against free-living trophozoites. Current Ag₂O studies illustrate that biological activity and cytotoxicity vary according to synthesis method, surface chemistry, particle size and target cell type³⁵. Periapical disease creates an inflammatory and nutritionally rich oral environment in which *Entamoeba gingivalis* and *Trichomonas tenax* may become more prevalent. Once enriched, the protozoa could further disturb epithelial barriers, interact with bacterial biofilms, and stimulate inflammatory signalling, including IL-6 production. Ag₂O- NPs can markedly reduce trophozoite viability under controlled in vitro conditions, raising the possibility of their eventual use as components of locally delivered antimicrobial systems.

Conclusion

Entamoeba gingivalis and *Trichomonas tenax* were noticed more frequently in individuals with periapical lesions than in controls, and parasite-positive participants showed higher salivary IL-6 levels. These results propose an association between oral protozoal colonization, periapical disease, and local inflammatory activity, although they do not establish a direct causal relationship. The fabricated Ag₂O nanoparticles exhibited features consistent with crystalline silver oxide and demonstrated clear time- and concentration-dependent antiprotozoal activity against both organisms. Ag₂O nanoparticles may therefore represent a promising experimental antiparasitic material. Further investigations incorporating cytotoxicity assessment, molecular quantification, larger study populations and in vivo models are required before clinical use can be considered.

Acknowledgements

None.

Conflict of interest:

The authors declare that there is no conflict of interest

regarding the publication of this study.

Financial Disclosure

This study was funded by the contributing researchers.

Authors' contributions

Fawzi Hussain Abdulameer and Moumal Al-Saady contribution in methodology, sample collection, and laboratory work; Mahdi S. AL-Maryani Supervision, Formal Analysis, Writing -Review & Editing. All the authors approved the final manuscript.

Data Availability

Any questions regarding the availability of the study's supporting data should be addressed to the corresponding author, who can provide it upon justifiable request.

Ethics Approval and Consent to Participate

Ethical approval for this study was granted by the University of Misan Since this was a follow-up study, every study participant provided formal informed consent. Each method followed the appropriate rules and regulations.

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How to cite this article: AL-Maryani MS, Abdulameer FH, Al-Saady M. Silver Oxide Nanoparticles Against Oral Protozoa Targeting *Entamoeba gingivalis* and *Trichomonas tenax* in Patients with Periapical Lesions and their Association with Interleukin-6. Bangladesh J Infect Dis 2026;13(1):262-270

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Article Info

Received on: 1 April 2026

Accepted on: 12 May 2026

Published on: 1 June 2026

References

- Charles-Ayinde M, Cieplik F, Gilbert GH, Moriyama K, Wu B, Fox CH. Action needed on oral diseases within the global NCD agenda. *Journal of Dental Research*. 2025;104(12):1288-1293.
- Mushtaq E, Al-Abboodi AK. Comparative prevalence of oral parasites in orthodontic and non-orthodontic subjects. *Nigerian Journal of Parasitology*. 2023;44(2):139-143
- Mushtaq E, Al-Abboodi AK. Molecular evaluation of *Entamoeba gingivalis* and *Trichomonas tenax* isolated from orthodontic patients in Maysan Province, Iraq. *Nigerian Journal of Parasitology*. 2024;45(1):77-85
- Alsaady HAM, Al-Abboodi A, Awdaa A, Rshak M. The infestation study of *Oestrus ovis* L. 1761 in sheep of Al-Amara Region, Maysan Province, South of Iraq. *Indian Journal of Forensic Medicine & Toxicology*. 2020;14(2):1180-1187
- Alsaady HAM, Aswan AA, Al Abboodi ES. Sero-epidemiology of *Toxoplasma gondii* among men and pregnant women in

- Maysan Province, South of Iraq. *Iranian Journal of Ichthyology*. 2021;8:27-37
6. Al-Abboodi AK. Parasitological contamination of raw vegetables collected from selected local markets in Maysan Province, South of Iraq. *Nigerian Journal of Parasitology*. 2023;44(2). Page Numbr
 7. Moosazadeh M, Sabeti MA, Hashemi SM, Ghazaloo A, Mousavi T, Mahdavi S, et al. The relationship between *Entamoeba gingivalis* and *Trichomonas tenax* with periodontitis and gingivitis: a systematic review, meta-analysis, and meta-regression. *Journal of Evidence-Based Dental Practice*. 2025;25(3):102141
 8. Bao X, Wiehe R, Dommisch H, Schaefer AS. *Entamoeba gingivalis* causes oral inflammation and tissue destruction. *Journal of Dental Research*. 2020;99(5):561-567
 9. Al-Abboodi A, Tjeung R, Doran P, et al. Generation of dynamic microenvironment in a hydrogel-based microfluidic device for cell culture study. *Proceedings of the 16th International Conference on Miniaturized Systems for Chemistry and Life Sciences (MicroTAS)*. 2012:1636-1638
 10. Alhasan L, Qi A, Al-Abboodi A, Rezk A, Shilton RR, Chan PP, et al. Surface acoustic streaming in a microfluidic system for rapid multicellular tumor spheroid generation. *Micro/Nano Materials, Devices, and Systems*. 2013;8923:828-831
 11. Kim Y, Al-Saady M, Chan PPY, Shen H, Sengupta S, Fu J. Development of site-specific coating and assessments for drug-eluting ureteral stents. *Proceedings of the 2024 IEEE-EMBS Conference on Biomedical Engineering and Sciences*. 2024:448-451
 12. Kim Y, Al-Saady M, Djoulde A, Chan PP, Shen HH, Sengupta S, et al. Site-specific porous hydrogel coating and characterization for tunable drug-eluting ureteral stent. *International Journal of Pharmaceutics*. 2026:126807
 13. Al-Abboodi A, Tjeung R, Doran P, Yeo L, Friend J, Chan P. Microfluidic chip containing porous gradient for chemotaxis study. *Smart Nano-Micro Materials and Devices*. 2011;8204:219-224.
 14. Zainab JM, Sahira K, Al-Abboodi AK, Alsaady HA. Enhancing pediculicidal activity against *Pediculus humanus capitis* using iron oxide nanoparticle-based formulations of some plant extracts and acetic acid solution. *Nigerian Journal of Parasitology*. 2024;45(2):460-469
 15. Al-Abboodi A, Falih IQ, Al-Asadi M, Hussein BA, Al-Saady MAAJ, Abdullah TA, et al. Iron-oxide nanoparticles (SPIONs) enhance malaria vaccine antibody response. *Vaccine*. 2026;77:128353
 16. Sahira K, Al-Abboodi AK. Comparative assessment of malaria diagnostic techniques updates. *Nigerian Journal of Parasitology*. 2024;45(1): Page Numbr
 17. Yin IX, Udduttulla A, Xu VW, Chen KJ, Zhang MY, Chu CH. Use of antimicrobial nanoparticles for the management of dental diseases. *Nanomaterials*. 2025;15(3):209.
 18. Hassan SADH, Almaliki MNS, Hussein ZA, Albehadili HM, Rabeea Banoon S, Abboodi A, et al. Development of nanotechnology by artificial intelligence: a comprehensive review. *Journal of Nanostructures*. 2023;13(4):915-932.
 19. Rashid MK, Salman IR, Obaid AL, Hassan SADH, Al-Musawi MR, Al-Saady M. Application of machine learning in predicting sources of water pollution in the Euphrates and Tigris rivers in Iraq. *International Journal of Aquatic Biology*. 2024;12(6):581-589.
 20. Salman IR, Rasheed AA, Hassan SADH, Hussein RA, Al-Saady M. Automated aquatic biodiversity monitoring using deep learning on the Tigris River: species identification and ecosystem assessment. *International Journal of Aquatic Biology*. 2025;13(1):30-40
 21. Saad AHA, Soliman MI, Azzam AM, Mostafa AB. Antiparasitic activity of silver and copper oxide nanoparticles against *Entamoeba histolytica* and *Cryptosporidium parvum* cysts. *Journal of the Egyptian Society of Parasitology*. 2015;45(3):593-602.
 22. Yaseen A, Mahafzah A, Dababseh D, et al. Oral colonization by *Entamoeba gingivalis* and *Trichomonas tenax*: a PCR-based study in health, gingivitis, and periodontitis. *Frontiers in Cellular and Infection Microbiology*. 2021;11:782805.
 23. Jiao J, Bie M, Xu X, et al. *Entamoeba gingivalis* is associated with periodontal conditions in Chinese young patients: a cross-sectional study. *Frontiers in Cellular and Infection Microbiology*. 2022;12:1020730.
 24. Acioz M, Ak G, Bozkaya F. Investigation of *Entamoeba gingivalis* and *Trichomonas tenax* in gingivitis and periodontitis patients. *BMC Oral Health*. 2025;25:1105.
 25. Rosenfeld L, Neumann N, Bao X, Adam A, Schaefer AS. *Entamoeba gingivalis* induces gingival cell death, collagen breakdown, and host immune response via VAMP8/-3-driven exocytosis pathways. *Infection and Immunity*. 2025;93(4):e00005-25.
 26. Hong ZB, Lai YT, Chen CH, Chen YJ, Chen CC, Lin WC. *Trichomonas tenax* induces barrier defects and modulates the inflammatory cytotoxicity of gingival and pulmonary epithelial cells. *Parasite*. 2023;30:7
 27. Brekalo Pršo I, Kocjan W, Šimić H, et al. Tumor necrosis factor- α and interleukin 6 in human periapical lesions. *Mediators of Inflammation*. 2007;2007:38210
 28. Bakhsh A, Joseph S, Mannocci F, Proctor G, Moyes D, Niazi SA. Apical periodontitis microbiome association with salivary and serum inflammatory burden. *International Endodontic Journal*. 2025;58(3):504-515
 29. Chen CH, Kuo CW, Tsai CM, Hong ZB, Lai CH, Chan TY, Lin WC. Detection of trichomonads in patients with lung cancer and transcription analysis on the response of human pulmonary epithelial cells to *Trichomonas tenax* invasion. *Journal of Microbiology, Immunology and Infection*. 2024;57(4):638-646
 30. Gul F, Ullah Z, Iqbal J, et al. Ecofriendly synthesis, characterization and biological activities of *Eruca sativa*-mediated silver oxide nanoparticles. *Scientific Reports*. 2025;15:13466
 31. Farkas N, Kramar JA. Dynamic light scattering distributions by any means. *Journal of Nanoparticle Research*. 2021;23(5).
 32. Zúñiga-Miranda J, Vaca-Vega D, Vizuete K, Carrera-Pacheco SE, Gonzalez-Pastor R, Heredia-Moya J, et al. Green synthesis of silver oxide nanoparticles from *Mauritia flexuosa* fruit extract: characterization and bioactivity assessment. *Nanomaterials*. 2024;14(23):1875
 33. Borase HP, Patil CD, Sauter IP, Rott MB, Patil SV. Amoebicidal activity of phytosynthesized silver nanoparticles and their in-vitro cytotoxicity to human cells. *FEMS Microbiology Letters*. 2013;345(2):127-131.
 34. Hendiger EB, Padzik M, Żochowska A, et al. Tannic acid-modified silver nanoparticles enhance the anti-*Acanthamoeba* activity of three multipurpose contact-lens solutions without increasing their cytotoxicity. *Parasites & Vectors*. 2020;13:624.
 35. Ullah Z, Iqbal J, Gul F, Abbasi BA, Kanwal S, Elsadek MF, et al. Biogenic synthesis, characterization, and in vitro biological investigation of silver oxide nanoparticles using *Rhynchosia capitata*. *Scientific Reports*. 2024;14(1):10484.