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Original Article

Synergistic Effects of Cold Atmospheric Multiple Plasma Jet and Amphotericin B on *Leishmania major*: An In-Vitro Study

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Abstract

Background: This study aimed to assess the *In vitro* effects of Cold Atmospheric Multiple Plasma Jet (CAMPJ) on *Leishmania major*.

Methods: A plasma jet device was designed in the Department of Chemical Engineering, Shiraz University, Shiraz, Iran, incorporating a high-purity air supply, an air flow controller, a DC power supply, a 9-10 Farad capacitor, an oscilloscope, and a cold plasma reactor. The CAMPJ was applied to *L. major* promastigotes and amastigotes under various plasma conditions, including different flow rates, voltages, and exposure times. The effectiveness of CAMPJ was compared to amphotericin B and a combination of both therapies. Viability and cytotoxicity were assessed on *L. major* and macrophage cell lines, using MAT assay, while apoptosis was quantified through flow cytometry.

Results: The optimal experimental conditions were identified as 2 million *L. major* promastigotes in 500 µL of culture, a flow rate of 500 mL/min, a voltage of 8.6 kV, a distance of 1 cm, and an exposure time of 15 minutes. The CAMPJ showed limited cytotoxicity to macrophage cells. The CAMPJ treatment significantly reduced the viability of *L. major* and induced apoptosis. CAMPJ-amphotericin B combination treatment significantly increased the treatment efficacy, when compared with the CAMPJ alone or negative controls.

Conclusion: CAMPJ, alone or in combination with amphotericin B, effectively induces apoptosis in *L. major*. CAMPJ might be a promising alternative or adjunct therapy for cutaneous leishmaniasis, warranting further investigation in animal models and clinical settings.

Introduction

Leishmaniasis includes a spectrum of diseases caused by protozoan parasites from over 20 distinct *Leishmania* species (1). These parasites are transmitted to humans through the bite of an infected female phlebotomine sandfly (2). The clinical presentations of this condition vary based on the localization of the parasite within mammalian tissues, including cutaneous, diffuse cutaneous, mucocutaneous, visceral, post-kala-azar dermal leishmaniasis (PKDL), and recidivans form (3). Cutaneous leishmaniasis (CL) is particularly significant due to its impact on human health, social and economic consequences, and distinctive epidemiological features globally. This condition can cause disfiguring skin lesions, psychological distress, and functional impairment, affecting the well-being of afflicted individuals. Moreover, the disease can lead to significant healthcare costs and productivity losses, especially in endemic regions (4, 5).

Leishmaniasis are endemic in tropical and subtropical regions. This neglected disease is prevalent in 99 countries, posing a risk of 12 million cases and resulting in 20,000–40,000 fatalities annually, according to the WHO (6). Currently, approximately 90% of CL cases are concentrated in eight countries, predominantly in Asian and South American regions (7). In the Middle East, over 70–75% of all CL cases are recorded in six countries, including Afghanistan, Algeria, Brazil, Colombia, Iran, and Syria (3).

The disease has been documented in all 31 provinces of Iran, with the highest and lowest incidence rates identified in the western and northwest regions of the country, respectively (8). Among the various clinical manifestations of these infections, CL is considered one of the most crucial endemic vector-borne diseases in Iran, with nearly 15,000–20,000 new cases reported annually (3, 7). Conventional therapy for leishmaniasis relies on chemical medications, which are limited by issues such as toxicity, high manufacturing costs, reduced efficacy, administration challenges, and the development of resistant strains (9).

Currently, the predominant treatment modalities for leishmaniasis include the use of pentavalent antimony, amphotericin B, pentamidine, miltefosine, and paromomycin (10, 11)

Inadequate or inconsistent chemotherapeutic administration can lead to increased drug resistance in leishmaniasis, resulting in a poor response to standard treatment in individuals with CL (11). Due to prolonged usage, the prevalence of adverse effects, suboptimal efficacy rates, and escalating resistance observed with standard medications, significant efforts have been directed toward exploring alternative systemic or localized therapeutic approaches. Collaboration across diverse research domains is imperative for the advancement of scientific knowledge. A recent addition to these interdisciplinary studies is the field of plasma medicine. The development of non-thermal plasma devices operating at atmospheric pressure has facilitated the exploration of interactions between plasma and biological surfaces (12). Regarded as a convergence of medical sciences, bioengineering, and plasma physics, plasma medicine has emerged globally with therapeutic objectives (13). Plasma, often referred to as the fourth state of matter, is a gas that is partially or fully ionized. Cold plasma manifests at around ambient temperature and consists of low-temperature ions along with highly energetic free electrons (14).

This ionized gas is characterized by a cold amalgamation of free radicals and charged particles, exerting no significant thermal impact on the material upon which it acts (15). Non-thermal atmospheric plasma (NTAP) therapy technologies have been under investigation for almost two decades, leading to the

development of various medical applications (16). Atmospheric cold plasma has been effectively employed for bacterial inactivation, wound healing, blood coagulation, and the sterilization of both living and non-living surfaces (17-21). Furthermore, its applications extend to dentistry and oncology (22).

Cold plasma has demonstrated efficacy in deactivating the pathogens responsible for onychomycosis (fungal infection) (23). The germicidal properties of cold plasma are primarily attributed to reactive oxygen and nitrogen particles generated by the surrounding gases (24). In general, the plasma particles generated consist of ions, electrons, or neutrals known as Reactive Oxygen/Nitrogen Species (ROS/RNS) (25). Stable compounds such as hydrogen peroxide, ozone, and nitrogen oxides are also formed. (26).

Given the significance of atmospheric cold plasma in generating ROS and NO, as well as its antimicrobial properties, the current study was conducted to assess the *In vitro* effects of CAMPJ alone or in combination with Amphotericin B on CL induced by *L. major*.

Materials and Methods

The study was approved by the Ethics Committee of Shiraz University of Medical Sciences (Ref. No. IR.SUMS.REC.1402.504)

General structure of multiple jet plasma reactor

This device was designed and built in the Department of Chemical Engineering, Shiraz University, Shiraz, Iran in 2020. The plasma jet production device includes several components: a high-purity air capsule serving as the air supply source, an air flow controller from the capsule, a direct current power supply for high voltage, and a capacitor with a special capacity of 9-10 Farads for transient load intensity measurement. An oscilloscope is used to observe the waveform of signals, displaying the amplitude in a two-dimensional graph with time on the horizontal axis and voltage on the vertical axis. The reactor produces cold atmospheric plasma jets. The reactor consists of a central steel electrode with a diameter of 8 mm and a length of 159 mm, connected to the power supply. It is surrounded by 14 quartz tubes with an outer diameter of 2 mm and a length of 100 mm. These quartz tubes are covered by a copper sheet that acts as the external electrode, which is protected by another transparent and flexible tube. This setup ensures the external electrode is firmly in place and forms the gas entry chamber inside the quartz microtube (Fig. 1) (15, 27).

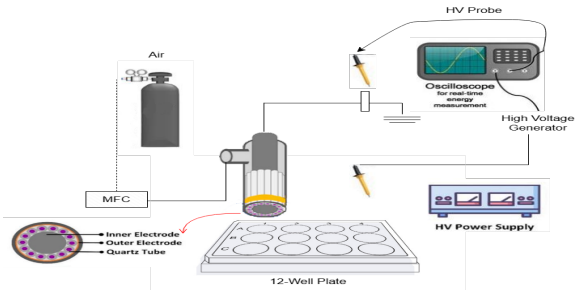

Fig. 1: *Schematic of the experimental setup (Original)*

L. major culture

L. major (MRHO/IR/75/ER) promastigotes from Shiraz University of Medical Sciences were thawed and cultured in RPMI-1640 medium with 10% heat-inactivated FBS and antibiotics at 25°C (28-29).

RAW 264.7 cell line culture

These cells were cultured in RPMI 1640 medium supplemented with 10% FBS at 37°C in a 5% CO_2 environment. The RAW 264.7 cell line originally derived from a male mouse

tumor function as a macrophage cell line (30-32).

Examining the cytotoxic effects of CAMPJ on a RAW macrophage cell line

RAW264.7 cells were cultured for 24 hours, then treated with CAMPJ (500 mL/min flow rate, 8.6 kV, 15 minutes) or left untreated as controls. Cytotoxicity was evaluated after 24 hours using the MTT assay.

Effects of CAMPJ on L. major promastigotes

Promastigote treatments—including parasite load, culture volume, and plasma parameters—were optimized to 2 × 10^6 cells in 500 µL, 500 mL/min flow, 8.6 kV, 1 cm distance, and 15 min exposure. In 12-well plates, 500 µL suspensions were then treated with CAMPJ (500 mL/min, 6.8 W, 1 cm, 15 min), amphotericin B (0.625–5 µg/mL), or their combination, alongside untreated, air-only, and blank controls. After 24 h at 25 ± 1 °C, cell viability was measured by MTT (100 µL of 0.5 mg/mL, 4 h, DMSO solubilization; absorbance at 570 nm), performed in triplicate for mean ± SD.

Quantitative determination of promastigote apoptosis using flow cytometry method

Apoptosis in CAMPJ-treated *L. major* promastigotes was measured by Annexin V/PI staining and flow cytometry. One million cells in 0.5 mL medium were centrifuged, washed three times with PBS, then resuspended in binding buffer. Samples were stained with 5 µl Annexin V and 2.5 µl propidium iodide for 15 minutes in the dark, topped up with buffer, and analyzed on a BD FACS machine. FlowJo software was used to calculate the percentage of apoptotic cells and generate histograms.

Effects of CAMPJ on L. major intracellular amastigotes

RAW264.7 cells (2×10^5) were scraped, washed, and seeded onto coverslips, then incubated at 37 °C/5% CO_2. After 24 h, they were infected with *L. major* promastigotes (10:1) for another 24 h. Wells were washed, fresh medium added, and treated with CAMPJ (500 mL/min, 6.8 W, 1 cm, 15 min), amphotericin B (0.625–5 µg/mL), their combination, or left untreated. Coverslips were Giemsa-stained, infection in ≥100 macrophages were counted by microscopy, and all experiments were performed in triplicate.

Statistical Analysis

A one-way analysis of variance (ANOVA) was used to compare multiple groups, followed by Tukey's post hoc test. When all groups were evaluated against a single control group, Dunnett's post hoc test was applied. A *P*-value of less than 0.05 was considered statistically significant.

Results

Evaluation of the cytotoxicity of CAMPJ on macrophages cell line

The cytotoxic effects of CAMPJ on the RAW 264.7 macrophage cell line were evaluated using the MTT assay. Dunnett's test indicated no statistically significant difference in macrophage viability between the CAMPJ-treated group and the control group.

Effects of CAMJ on L. major promastigotes

Different concentrations of amphotericin B (0.625, 1.25, 2.5, 5 µg/mL), CAMPJ, and the combined treatment of amphotericin B with CAMPJ showed a statistically significant difference compared to the negative control group. Based on Tukey's post hoc test, the measurement of promastigote viability revealed a significant decrease in optical density (OD) by the MTT method ($P<0.05$), indicating significant anti-leishmanial activity of CAMPJ. Moreover, there was a statistically significant difference between CAMPJ alone, different concentrations of amphotericin B, and the combined treatment of amphotericin B with CAMPJ. The lowest viability rate was observed with the combined treatment of amphotericin B and CAMPJ at concentrations of 1.25, 2.5, and 5 µg/mL. Fig. 2 shows the viability percentage of *L. major* promastigotes in

various groups, treated with CAMPJ, amphotericin B, and a combination of both.

Fig. 2: Viability percentage of *L. major* promastigotes in various groups, treated with CAMPJ, amphotericin B, and a combination of both with different concentrations after 24 hours. P: Plasma treatment, Amp: Treated with amphotericin B, P+Amp: combination of plasma and amphotericin B treatment, 500A: Only air treated at 500 flow rates, Control-: Untreated. The average number of amastigotes in 100 macrophages has been calculated

Effect of CAMPJ on apoptosis of L. major promastigotes evaluated by the flow cytometry

Promastigotes of *L. major* were allocated to five groups: CAMPJ alone, amphotericin B at 0.625 µg/mL, their combination at 0.625 µg/mL, amphotericin B at IC_{50}, and an untreated control. Flow cytometry (Fig. 3) revealed apoptosis rates of 85% (CAMPJ), 14% (amphotericin B 0.625 µg/mL), 98% (combination), 48.2% (IC_{50}), and 3.85% (control). All treatments induced significantly higher apoptosis than control. The CAMPJ–amphotericin B combination achieved the highest apoptotic effect.

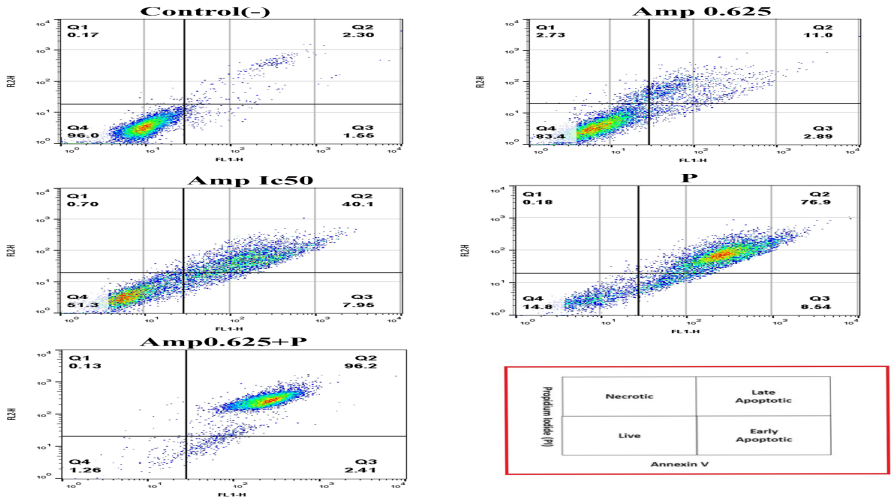

Fig. 3: CAMPJ and combined treatment of CAMPJ-amphotericin B induces apoptosis in *L. major* promastigotes, with cell death analyzed using the Annexin V/PI flow cytometry assay. P: plasma treatment, Amp: treated with amphotericin B, P+Amp: Combination of plasma and amphotericin B treatment

Effects of CAMPJ on L. major amastigotes

The results indicate that both CAMPJ and the combined treatment of amphotericin B with CAMPJ significantly reduced the number of infected macrophage cells and intracellular amastigotes compared to the negative control group ($P < 0.05$). Figs. 4 and 5 illustrate the number of amastigotes and the percentage of infected macrophages. As shown in Figure 3, the combination of amphotericin B and CAMPJ is more effective in reducing the

number of amastigotes within macrophages than plasma treatment alone. The amphotericin B-CAMPJ combination demonstrated the lowest viability rates for amastigotes and infected macrophages at concentrations of 2.5 and 5 µg/mL.

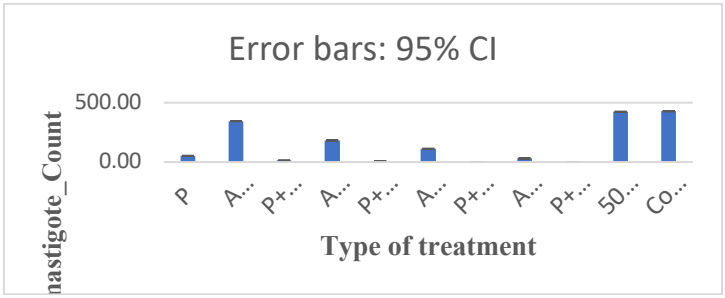

Fig. 4: The number of amastigotes in macrophages across various groups after 24 hours of treatment with CAMPJ, amphotericin B, and a combination of both at different concentrations. The groups are as follows: P: Plasma treatment, Amp: Treated with amphotericin B, P+Amp: Combination of Plasma and amphotericin B treatment, 500A: Only air treated at a 500-flow rate, and Control-: Untreated

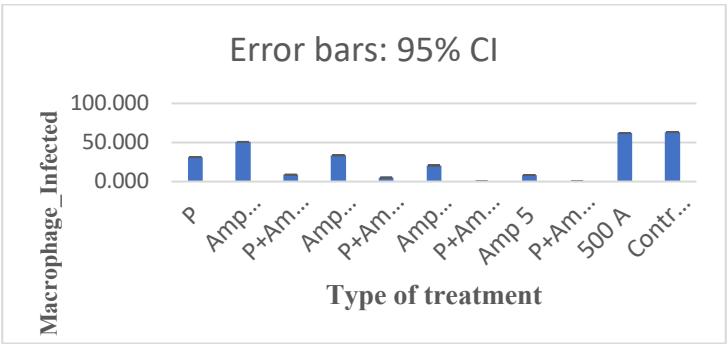

Fig. 5: The percentage of infected macrophages in various groups treated with CAMPJ, amphotericin B, and a combination of both at different concentrations after 24 hours is presented. The groups are defined as follows: P for plasma treatment, Amp for treatment with amphotericin B, P+Amp for the combination of plasma and amphotericin B treatment, 500A for air treatment at a 500-flow rate, and Control- for the untreated group

Discussion

Unresponsiveness of *Leishmania* parasite to the currently available drugs necessitates the exploration of novel therapies. In the current study, the combination of CAMPJ and amphotericin B has shown a synergistic effect in decreasing the viability of promastigotes and macrophage infection rates, aligning with findings that suggest the enhancement of leishmanicidal activity through plasma-mediated strategies (18).

Cold plasma has been investigated for its germicidal properties against pathogens such as *Escherichia coli*, *Acinetobacter baumannii* (33-35), and fungal infections like onychomycosis (18, 36, 37). In the context of *Leishmania*, the current study introduces the novel use of CAMPJ specifically targeting *L. major*, demonstrating that it induces apoptosis in promastigotes. This finding is consistent with similar plasma applications in microbial inactivation (38).

Cold plasma therapy is known to generate reactive oxygen and nitrogen species (ROS and RNS), which disrupt cellular processes. The results presented here demonstrated that CAMPJ exposure significantly decreases promastigote viability and triggers apoptosis.

These effects are parallel to those observed in previous studies on plasma treatment in cancer therapy, where plasma-generated ROS induced apoptosis in melanoma and lung cancer cells (39). The significant reduction in *Leishmania* parasite viability in this study suggests that CAMPJ may invoke similar oxidative stress mechanisms as in other pathogens, destabilizing cellular membranes and leading to cell death (25).

In this study, macrophages treated with CAMPJ exhibited limited cytotoxicity. This finding is consistent with previous plasma studies, where non-thermal plasma selectively affected pathogens while sparing mammalian cells (40, 41). Support from this notion comes from studies demonstrating cold plasma's capacity to treat non-surface infections while preserving surrounding tissues (21). The localized and controlled application of CAMPJ in this study minimizes damage to macrophages while effectively targeting the *Leishmania* parasites. This adds further credibility to CAMPJ's potential for treating intracellular parasitic infections without adverse effects on the host cells.

The synergistic effects of combining CAMPJ with amphotericin B, as demonstrated in this study, represent a promising development. While amphotericin B is a longstanding treatment for leishmaniasis, it often requires high doses to be effective, leading to severe side effects. The combination of CAMPJ and lower concentrations of amphotericin B resulted in a statistically significant reduction in parasite viability. Similar findings have been reported where cold plasma treatments enhanced the efficacy of chemotherapeutic agents against resistant cancer cells. (42).

Further studies are required to elucidate the precise molecular mechanisms behind this synergy, although preliminary data suggests enhanced permeability of parasite cell walls and increased oxidative stress as potential factors.

A critical aspect of plasma therapy is optimizing treatment parameters for maximum efficacy. The results here highlight the importance of carefully controlling variables such as flow rate, voltage, treatment time, and plasma-electrode distance. This optimization ensured that CAMPJ was applied in its most effective form without causing unnecessary damage to host tissues. Previous studies have also emphasized the importance of parameter optimization in plasma therapy for cancer treatments. (39). The results also mirror previous findings from wound-healing studies, where the optimization of cold plasma parameters was crucial for successful bacterial inactivation without harming the underlying tissues (22, 43). Such precision makes CAMPJ a viable option for clinical treatments, where non-invasive and targeted therapies are increasingly important. Applying CAMPJ in this study is a stepping stone towards its potential use in clinical settings. The reduced viability of *L. major* promastigotes and amastigotes, combined with limited cytotoxicity in macrophages, indicates a promising future for plasma-based therapies (43, 44). Given the promising findings in *In vitro* study, the next logical steps involve in vivo models to validate the safety and efficacy of CAMPJ in animal models.

Conclusion

Findings of the current study provide valuable insights into the future of leishmaniasis treatment. By leveraging the non-thermal, antimicrobial properties of cold atmospheric plasma, this therapy offers a promising alternative to traditional chemical treatments. The results demonstrated significant reductions in promastigote and amastigote viability while maintaining host cell safety. The combination of CAMPJ with amphotericin B exhibits a synergistic effect, potentially allowing for lower drug doses and reducing the toxicity associated with current treatments. CAMPJ's anti-leishmanial effects pave the way for further investigation into its application in other infectious diseases, particularly those where drug resistance is a growing concern. This study

lays the groundwork for future clinical applications in animal models and human cases, emphasizing the need for continued research into plasma therapies for parasitic diseases.

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Competing interests

The authors declare no competing interests.

References

1. Antinori S, Schifanella L, Corbellino M. Leishmaniasis: New insights from an old and neglected disease. Eur J Clin Microbiol Infect Dis. 2012;31:109-118.
2. Pereira S, Pita-Pereira D, Araujo-Pereira T, et al. First molecular detection of *Leishmania infantum* in sergentomyia minuta (diptera, psychodidae) in Alentejo, southern Portugal. Acta Trop. 2017;174:45-48.
3. Sabzevari S, Teshnizi SH, Shokri A, Bahrami F, Kouhestani F. Cutaneous leishmaniasis in Iran: A systematic review and meta-analysis. Microb Pathog. 2021;152:104721.
4. Borba JV, Silva AC, Ramos PI, et al. Unveiling the kinomes of *Leishmania infantum* and *L. braziliensis* empowers the discovery of new kinase targets and antileishmanial compounds. Comput Struct Biotechnol J. 2019;17:352-361.
5. Fata A, Moghaddas E, Rezee A, Abdali A, Jarahi L, Shamsian A. Epidemiological study of cutaneous leishmaniasis and identification of etiological species. J Mazandaran Univ Med Sci. 2018;27:123-131.
6. Almeida-Souza F, Calabrese KDS, Abreu-Silva AL, Cardoso F. *Leishmania parasites: Epidemiology, immunopathology and hosts*. BoD–Books on Demand; 2024.
7. Hajjaran H, Saberi R, Borjian A, et al. The geographical distribution of human cutaneous and visceral leishmania species identified by molecular methods in Iran: A systematic review with meta-analysis. Front Public Health. 2021;9:661674.
8. Norouzinezhad F, Ghaffari F, Norouzinejad A, et al. Cutaneous leishmaniasis in Iran: Results from an epidemiological study in urban and rural provinces. Asian Pac J Trop Biomed. 2016;6:614-619.
9. Sundar S, Singh B. Emerging therapeutic targets for treatment of leishmaniasis. Expert Opin Ther Targets. 2018;22(6):467-486.
10. World Health Organization. Global leishmaniasis update, 2006-2015: A turning point in leishmaniasis surveillance. Wkly Epidemiol Rec. 2017;92:557-565.
11. Madusanka RK, Silva H, Karunaweera ND. Treatment of Cutaneous Leishmaniasis and Insights into Species-Specific Responses: A Narrative Review. Infect Dis Ther. 2022 Apr;11(2):695-711.
12. Kong MG, Kroesen G, Morfill G, et al. Plasma medicine: An introductory review. New J Phys. 2009;11:115012.
13. Yazdani Z, Pasandi MS, Golpour M, et al. Effect of cold atmospheric plasma on changing of biomolecular structures involved in apoptosis pathways of melanoma cancer. Skin Res Technol. 2024;30(1):e13544.
14. Moldgy A, Nayak G, Aboubakr HA, et al. Inactivation of virus and bacteria using cold atmospheric pressure air plasmas and the role of reactive nitrogen species. J Phys D App Phys. 2020;53:434004.
15. Kazemi M, Taghvaei H. A novel multi array dielectric barrier discharge plasma gas diffuser for wastewater treatment: The role of reactive species. Sep Purif Technol. 2021;260:118236.

16. Bulson JM, Liveris D, Derkatch I, et al. Non-thermal atmospheric plasma treatment of onychomycosis in an *In vitro* human nail model. Mycoses. 2020;63:225-232.
17. Colagar AH, Alavi O, Sohbatzadeh F. Lipid peroxidation of *Escherichia coli* by triplet non-thermal atmospheric pressure plasma jet. Chiang Mai J Sci. 2017;44:678-687.
18. Att RR, Al Haideri H, Murbat HH. Influence of cold atmospheric plasma on *Acinetobacter baumannii*. Baghdad Sci J. 2019; 16:151-161.
19. Ali AH, Murbet HH, ALhmeedi IA, et al. Studying the effect of physical plasma on the blood using digital image processing. IJCSMC. 2015;4
20. Heslin C, Boehm D, Milosavljevic V, et al. Quantitative assessment of blood coagulation by cold atmospheric plasma. Plasma Med. 2014; 4(1-4): 153-163
21. Laroussi M. Plasma medicine: A brief introduction. Plasma. 2018;1:47-60.
22. Hoffmann C, Berganza C, Zhang J. Cold atmospheric plasma: Methods of production and application in dentistry and oncology. Med Gas Res. 2013;3:21.
23. Shemer A, Daniel R, Kassem R, Geffen Y, Galili E. Cold sub-atmospheric and atmospheric pressure plasma for the treatment of *Trichophyton rubrum* onychomycosis: An in-vitro study. Dermatol Ther. 2020;33:e14084.
24. Zhao YM, Ojha S, Burgess C, Sun DW, Tiwari B. Inactivation efficacy and mechanisms of plasma activated water on bacteria in planktonic state. J Appl Microbiol. 2020;129:1248-1260.
25. Bekeschus S, Seebauer C, Wende K, Schmidt A. Physical plasma and leukocytes - immune or reactive? Biol Chem. 2018, 19;400(1):63-75.
26. Lux J, Dobiáš R, Kuklová I, et al. Inactivation of dermatophytes causing onychomycosis and its therapy using non-thermal plasma. J Fungi (Basel). 2020;6:214.
27. Tanzooei AM, Karimi J, Taghvaei H. Exploring non-thermal plasma technology for microalgae removal. Ecotoxicol Environ Saf. 2024;286:117127.
28. Esmaeilifallah M, Khanahmad H, Ghayour Z, et al. Evaluation of the antileishmanial effect of polyclonal antibodies and cationic antimicrobial peptides. Pathog Glob Health. 2023;117(4):366-380.
29. Rahnama V, Motazedian MH, Mohammadi-Samani S, et al. Artemether-loaded nanostructured lipid carriers: preparation, characterization, and evaluation of *In vitro* effect on *Leishmania major*. Res Pharm Sci. 2021;16(6):623-633.
30. Khalili S, Mohebali M, Ebrahimzadeh E, et al. Antimicrobial activity of an antimicrobial peptide against amastigote forms of *Leishmania major*. Vet Res Forum. 2018;9(4):323-328.
31. Nadaes NR, Silva da Costa L, Santana RC, et al. DH82 Canine and RAW264.7 Murine Macrophage Cell Lines Display Distinct Activation Profiles Upon Interaction With *Leishmania infantum* and *Leishmania amazonensis*. Front Cell Infect Microbiol. 2020;10:247.
32. Alanyalı FS, Algı O. Cytotoxic and Apoptotic Impacts of Ceranib-2 on RAW 264.7 Macrophage Cells. Anticancer Agents Med Chem. 2023;23(20):2183-2188.
33. Flynn PB, Graham WG, Gilmore BF. *Acinetobacter baumannii* biofilm biomass mediates tolerance to cold plasma. Lett Appl Microbiol. 2019;68:344-349.
34. Svarnas P, Spiliopoulou A, Koutsoukos PG, et al. *Acinetobacter baumannii* deactivation by means of dbd-based helium plasma jet. Plasma. 2019;2:77-90.
35. Daeschlein G, Scholz S, Emmert S, et al. Plasma medicine in dermatology: Basic antimicrobial efficacy testing as prerequisite to clinical plasma therapy. Plasma Med. 2012;2:33-69.
36. Lokajová E, Julák J, Khun J, et al. Inactivation of dermatophytes causing onychomycosis using non-thermal plasma as a prerequisite for therapy. J Fungi. 2021;7:715.
37. Daeschlein G, Scholz S, von Woedtke T, et al. *In vitro* killing of clinical fungal strains by low-temperature atmospheric-pressure plasma jet. IEEE Trans Plasma Sci. 2010;39:815-821.
38. Julák J, Scholtz V. The potential for use of non-thermal plasma in microbiology and

medicine. Epidemiol Mikrobiol Imunol. 2020;69(1):29-37.
39. Fridman G, Shereshevsky A, Jost MM, et al. Floating electrode dielectric barrier discharge plasma in air promoting apoptotic behavior in melanoma skin cancer cell lines. Plasma Chem Plasma Process. 2007;27:163-176.
40. Lin AG, Xiang B, Merlino DJ, Baybutt TR, et al. Non-thermal plasma induces immunogenic cell death in vivo in murine ct26 colorectal tumors. Oncoimmunology. 2018;7:e1484978.
41. Moszczyńska J, Roszek K, Wiśniewski M. Non-thermal plasma application in medicine—focus on reactive species involvement. Int J Mol Sci. 2023;24:12667.
42. Panngom K, Baik K, Nam M, et al. Preferential killing of human lung cancer cell lines with mitochondrial dysfunction by nonthermal dielectric barrier discharge plasma. Cell Death Dis. 2013;4:e642.
43. Isbary G, Morfill G, Schmidt H, et al. A first prospective randomized controlled trial to decrease bacterial load using cold atmospheric argon plasma on chronic wounds in patients. Br J Dermatol. 2010;163:78-82.
44. Scholtz V, Pazlarova J, Souskova H, et al. Nonthermal plasma—a tool for decontamination and disinfection. Biotechnol Adv. 2015; 33(6 Pt 2):1108-19.