Received: 3 January 2023 | Revised: 8 March 2023 | Accepted: 9 March 2023

DOI: 10.1002/jbio.202300003

RESEARCH ARTICLE

JOURNAL OF BIOPHOTONICS

Can photobiomodulation restore anosmia and ageusia induced by COVID-19? A pilot clinical study

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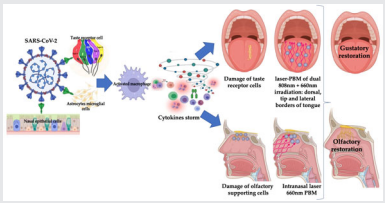
Funding information
Financier of Studies and Projects, Grant/Award Number: 01.13.0430-00; São Paulo Research Foundation and the partnership with MMOptics and INOVA Institute, Grant/Award Numbers: 2013/14001-9, 2013/07276-1

Abstract



Along with other COVID-19 clinical manifestations, management of both olfactory and gustatory dysfunction have drawn a considerable attention. Photobiomodulation (PBM) has emerged to be a possible effective therapy in restoring taste and smell functionality, but the evidence is scarce. Hence, the present pilot study is aimed to evaluate the effectiveness of intranasal and intraoral PBM administrations in management of anosmia and ageusia respectively. Twenty Caucasian subjects who diagnosed with anosmia and ageusia were recruited. Visual analogue scale was utilised to evaluate patients' self-reported for both olfactory and gustatory functionality. The laser-PBM parameters and treatment protocols for anosmia and ageusia were as follows respectively: 660 nm, 100 mW, two points intranasally, 60 J/session, 12 sessions; dual wavelengths (660 nm and 808 nm), 100 mW, three points intraorally, 216 J/session, 12 sessions. Our results showed a significant functionality improvement of both olfactory and gustatory functionality. Extensive studies with large data and long-term follow-up period are warranted.

KEYWORDS
COVID-19 disease, gustatory perception, low-level laser therapy, molecular mechanism, olfactory perception, oxidative stress, photobiomodulation, SARS-CoV-2

Vitor Hugo Panhoca and Reem Hanna contributed equally to the work.

1 | INTRODUCTION

The effects of SARS-CoV-2 on peripheral nervous system (PNS) including; anosmia (loss sense of smell), ageusia (loss sense of taste) and nerve pain have been well-documented in literature [1–3]. A Korean study conducted by Lee et al. [4] observed that anosmia and ageusia were reported in 15.3% of a total of 3191 patients at early-stage of COVID-19 disease, while the majority of them had asymptomatic to mild disease severity [4].

1.1 | SARS-CoV-2 pathogenesis in relation to olfactory and gustatory dysfunction

The pathophysiological mechanism that led to olfactory and gustatory dysfunction induced by SARS-CoV-2 remains unclear. The smell and taste senses are intertwined in the brain.

A critical review conducted by Hanna et al. [5] showed that SARS-CoV-2 can directly invade the olfactory supporting cells (OSC) (microvillar, sustentacular, globular basal) that nourish the main olfactory sensory neurons cells and indirectly can invade the olfactory bulbs. Interestingly, OSC have a high expression to angiotensin-converting enzyme 2 resulting in local inflammation, an early apoptosis of olfactory epithelial cells, changes in olfactory cilia and odour transmission, inducing damage to their ability to maintain homeostasis of the main olfactory neuron cells.

The majority of patients with COVID-19 disease regain their smell and taste functionality (transient symptoms) within 60 days of recovering from COVID-19, whereas the others are not due to a significant damage of the main olfactory neurone cells [6–9].

1.2 | Photobiomodulation molecular mechanism in restoring Gustrory and olfactory functionality

The standard treatment care in restoring smell and taste senses is either; pharmacotherapy or palliative care where its effectiveness remains questionable. Hence, PBM therapy has emerged to be an alternative non-invasive photobioenergetic treatment modality in alleviating pain, reducing inflammation, modulating immune response and healing injured tissues [10–14]. In this context, PBM can act as an antioxidant-neuromodulator-homeostatic tool in reducing oxidative stress (OS), increasing adenosine triphosphate (ATP) synthesis and enhancing nitric oxide (NO) production of injured peripheral neurons, resulting in upregulating the host immune response. Additionally, NO interaction with reactive oxygen species (ROS) and nitrogen facilitates antimicrobial molecular species production, including SASR-CoV-2 virus by disrupting RNA replication [15–17].

A review conducted by Hanna et al. [10] emphasised on PBM importance in regulating SARS-CoV-2 cytokines storm by reducing inflammatory mediators (prostaglandin E2, leucocytes and TNF-α), regulating the proinflammatory cytokines (IL-1, 6 and 8) and increasing anti-inflammatory mediators (IL-10).

Moreover, a recent review conducted by Kitchen et al. [18] highlighted the efficacy of 1068 nm PBM therapy on cytoprotection, NO release, inflammation changes, blood flow improvement and heat shock proteins (Hsp70) regulation alongside Hsp90 and Hsp27. This was well-documented on rat and mouse microglial cells through Toll-like receptor 4 (TLR-4) activation [18].

1.3 | Current literature and rationale in conducting the present study

The authors searched the available scientific literature to collect data on the effectiveness of PBM in restoring both olfactory and gustatory functionality-induced by COVID-19 disease.

A case series conducted by Soares et al. [19] utilised 660 nm (red) intranasal laser-PBM therapy with the following treatment protocols interval in the management of anosmia-induced by COVID-19: 10 sessions, twice a week with a 48-h time interval; 5 sessions, twice a week with a 48-h time interval; 10 sessions with a 24-h time interval. Despite each patient's findings varied, the majority claimed olfactory improvement. Therefore, the authors suggested further studies to determine the optimal dosimetry and treatment protocol are warranted. These findings are coincided with the findings of a study conducted by Souza et al. [20] study utilised 808 nm near infrared (NIR) laser-PBM in the management of both ageusia and anosmia-induced by COVID-19 where a total of 10 sessions was implemented and deemed to be effective.

In the line of the above notes, despite PBM therapeutic benefits in restoring gustatory and olfactory functionality, the evidence is scarce due to a lack of consistency in the laser dosimetry and treatment protocols. Hence, the present pilot study is aimed to evaluate the effectiveness of intranasal and intraoral administrations of PBM in the management of both ageusia and anosmia in patient with COVID-19. Whereas, the study's objectives were as follow: (1) to evaluate the percentage of complete recovery and prognosis timeframe; (2) to introduce a standardised preliminary laser dosimetry and treatment protocols; (3) to understand the current PBM molecular mechanisms in restoring anosmia and ageusia functionality.

2 | MATERIALS AND METHODS

2.1 | Study design

A pilot study was conducted between 1 September and 26 October 2022 to evaluate PBM effectiveness in restoring both anosmia and ageusia-induced by COVID-19 sequel.

A unique combination of letters and numbers was used to identify the 20 recruited research subjects. Also known as a unique study code. All the data were collected and stored on Microsoft Excel Spreadsheet.

The clinician who performed the laser treatment is experienced in the field. The study was approved through a project presented to the Holy House of Mercy of São Carlos (São Carlos-SP-Brazil), approval n°: 5.615.863/2022. The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (Ethics Committee) of Holy House of Mercy of São Carlos (n°: 5.615.863/2022).

An informed written consent was obtained from the all the recruited subjects, after full explanation of the proposed PBM treatments, as well for scientific publication including photos.

2.1.1 | Patient cohort

Twenty Caucasian subjects (10 females and 10 males) with an age ranged between 26- and 60-year-old presented to the Outpatient clinic at Holy House of Mercy of São Carlos (São Carlos, SP, Brazil) in partnership with the Centre for Innovation and Technology in the Health Area (CITESC, São Carlos, SP, Brazil) for treatment of post-COVID-19 sequel on 1 September 2022. All the recruited subjects reported a change in both smell and taste senses apart from one patient who had only anosmia symptoms.

All the subjects' COVID-19 treatment was not involved hospitalisation. However, their main complaint initially in the anamnesis was confusion without both taste and smell senses or one of them, which started when the virus symptoms commenced and continued until the anamnesis without any improvement.

TABLE 1 The symptoms' severity and duration, subjects' co-morbidity and demographic characteristics.

Patient ID	Sex	Age	Severity of ageusia's symptoms	Severity of anosmia's symptoms	Symptoms duration (month)	Past medical history and co-morbidity
AMF1	F	34	Severe	Severe	25	N/A
AMCCM2	F	33	Severe	Moderate	31	N/A
APAA3	F	40	Moderate	Severe	22	N/A
MLFO4	F	56	Severe	Severe	13	Hypothyroidism, insomnia
DFS5	M	36	Severe	moderate	19	N/A
CMN6	F	45	Moderate	Moderate	6	lactose intolerance
LCC7	F	58	Moderate	Mild	16	N/A
ALA8	M	60	Mild	Severe	11	High level cholesterol
JYMS9	F	26	Severe	Severe	25	N/A
LH10	M	33	Severe	Moderate	21	N/A
EOAO11	M	59	Moderate	Moderate	3	Anaemia
JENW12	M	60	Severe	Severe	7	High level cholesterol
VFP13	M	46	Moderate	Severe	17	N/A
MB14	M	56	Severe	Severe	15	N/A
MAPM15	M	42	Moderate	Mild	6	generalized anxiety syndrome
APL16	F	39	Normal	Moderate	30	N/A
JER17	M	60	Moderate	Moderate	17	Hypothyroidism, depression, history of prostate cancer, varicose veins.
NM18	M	43	Moderate	Moderate	23	N/A
RAS19	F	29	Moderate	Moderate	20	Tinnitus, depression
SFS20	F	28	Moderate	Severe	17	N/A

Abbreviations: ID, identification; F, female; M, male; N/A, not applicable.

Table 1 shows subjects' demographic characteristics and co-morbidity, as well symptoms' duration and severity based on subjects' self-reported scores on modified intensity visual analogue scale (VAS) [20]. Eighty percent of the subjects had moderate–severe intensity of anosmia and ageusia's symptoms of a duration between 6 and 30 months.

2.1.2 | Population (P), intervention (I), comparison (C), and outcome (O)—PICO

P: patients ≥18-year-old diagnosed with loss of smell and taste induced by COVID-19 disease.

I: 808 nm-laser PBM for anosmia and dual wavelength: 660 and 808 nm laser PBM for ageusia.

C: not applicable.

O: patient-self-reported improvement and restoration of gustatory and olfactory functionality.

2.1.3 | Eligibility criteria

Inclusion criteria

1. Adult ≥18-year-old of both genders diagnosed clinically with loss of both smell and taste or only one sense induced by COVID-19 disease.
2. Subjects with symptoms duration ≥3 months
3. Subjects with any degree of severity of sense loss.
4. Subjects who had never had laser therapy prior to study enrolment.
5. Subjects with controlled systematic co-morbidity.
6. Subjects who committed to the treatment.

Exclusion criteria

1. Subjects who were hospitalised as COVID-19 complications.
2. Subjects had an alteration of both smell and taste senses not related to COVID-19.
3. Subjects underwent standard treatment care for both smell and taste loss.
4. Subjects who had flue or any other sources of infection.

2.1.4 | Photobiomodulation dosimetry and treatment protocol

PBM protocol for anosmia

PBM therapy with laser low potency was performed with the Recover® device (MMOptics, São Carlos, Brazil) for anosmia (Figure 1A). The laser beam was placed underneath each nostril parallel to the inner wall of the nostril and the photonic energy of 660 nm directly irradiated the olfactory supporting cells and olfactory bulb (Figure 1B).

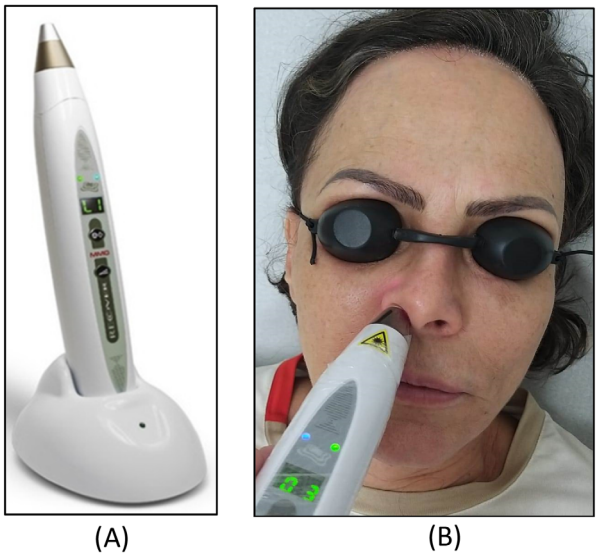


FIGURE 1 The laser device that utilised for anosmia management and its application in the nostril. (A) Description of laser device emitting 660 nm; (B) the direction of the laser beam in the right nostril, irradiating directly the olfactory supporting cell and olfactory bulb.

All the data was collected by specialist nurse who was not involved in the study and stored on Microsoft Excel Spreadsheet. Table 2 shows device description, laser parameters and treatment protocols for anosmia.

PBM protocol for ageusia

The Vacumlaser® device was originally developed for simultaneous application of laser and vacuotherapy. However, in the treatment of ageusia, as shown in Figure 2A, we program this device so that it does not carry out vacuotherapy and only applies laser.

The taste alteration application protocol was performed using the Vacumlaser® device without suction cup (Figure 2A), emitting dual wavelengths of six laser beams; three laser beams red (680 nm) and three laser beams NIR (808 nm) irradiating the dorsal surface and lateral borders of the tongue for 2 min for each area (three affected spots in total) (Figure 2B).

Table 3 shows the device description, laser parameters and treatment protocols for ageusia management. For each spot evaluation, the power output was equal to 100 mW applied for 2 min where the total energy was 12 J per spot. As we have six spots (three laser beams for 660 nm and three laser beams for 808 nm) applied to three areas of the tongue, we multiplied 12 J × 6

TABLE 2 The device specifications, laser parameters and treatment protocols for anosmia management.

Device information	Manufacturer	MMOptics, São Carlos, Brazil
	Model identifier	Duo laser®
	Number of emitters	1
	Light source	Laser
	Beam delivery system	Plastic optical fiber
Irradiation parameters	Wavelength	808 nm
	Spectral bandwidth	2.5 nm
	Operating mode	Continuous emission mode (CW)
	Beam profile	Multimodal (elliptical)
	Power output	100 mW
	Irradiance	3.33 W/cm^2
	Total irradiant energy	60 J per session
	Beam spot size at target	Average 0.2 cm^2
Treatment parameters	Number of irradiated points	2 points (one point for each nostril)
	Irradiated area	0.03 cm^2 per spot
	Irradiation time	2 min per spot
	Application technique	Spotting
	Number treatment sessions	12
	Treatment frequency	Three times per week based on 48 h time interval between each session
	Speed of movement	Static

spots × 3 areas of the tongue per session. Therefore, the total energy per applied per session was 216 J. The Vacumlaser® gauntlet containing cluster with an output of six laser spots was placed directly on the surface of the tongue mucosa. Irradiance was calculated considering power output of 100 mW and irradiated area of 0.0177 cm^2 per spot. Therefore, the irradiance value was 5.65 W/cm^2 per spot (emitters).

We chose dual wavelengths emitting 660 nm and 808 nm in order to target the superficial and deep-seated dorsal taste papillae respectively. The large circumvallate papillae reside at posterior 1/3 of tongue and contain hundreds of taste buds. These papillae are so large they are visible to the naked eye, whereas the filiform papillae are arranged at the tip, dorsal and lateral surfaces of the tongue. These papillae are covered by keratinized stratified epithelium where the thickness of the stratum corneum is 32.08 ± 1.29 μm [21].

At initial assessment (T0), the mean score of 20 patients' self-reported was "3.8" on the T-VAS, indicating a complete loss of taste. All the data was collected by specialist nurse who was not involved in the study and stored on Microsoft Excel Spreadsheet.

2.2 | Tools of outcome assessment

2.2.1 | Modified intensity visual analogue scale

VAS scale is a psychometric tool utilised to assess patient's self-reported smell and taste perception; prior commencing the PBM treatment (T0), after each treatment session (T1–T12). Smell-VAS (S-VAS) and taste-VAS (T-VAS) [20] were designed with 10 descriptions on a horizontal line from 0 to 10, where "0" represents a complete absence of smell and taste and "10" indicates normal/full recovery of olfactory and gustatory functionalities respectively. The patient was made familiar with both VAS tools, assessing qualitative outcomes assessments.

2.2.2 | Caffeine odour identification test

Caffeine aroma has beneficial effects on odour threshold to identify olfactory alteration [22]. Hence, coffee being used as the barometer for a kind of sniff-test and sipping-test identifying the taste and smell alteration respectively. It is a

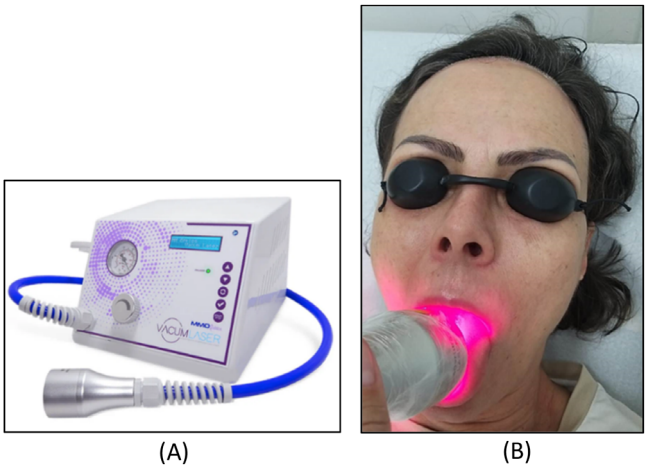


FIGURE 2 The laser device that was utilised for ageusia management and its application in the oral cavity. (A) Description of laser device emitting dual wavelengths of 660 nm (three laser beams) and 808 nm (three laser beams); (B) the direction of the laser beam of 660 and 808 nm photonic energy at 90° and in contact with tongue dorsal gustatory receptors (clusters specialized epithelial cells), irradiating all of anterior 2/3 of dorsal tongue, tip and posterior 1/3 tongue in one point of application, targeting the sweet, salty and bitter buds' receptors respectively. The remaining two points of applications were; one for each lateral border of the tongue, targeting the sour buds' receptors. The sweet and salty bud receptors are grounded within fungiform and filiform, whereas the bitter and sour are grounded within the circumvallate and foliate papillae respectively.

diagnostic and assessment tool for olfactory and gustrory functionality evaluation [23, 24].

An odour molecule enters the nose and lands on a special type of tissue called "olfactory epithelium" filled with neurons, picking up the odour molecule and transport it through the olfactory bulb to the brain for odour interpretation. The neurons are guided on this journey from the nose to the brain by the OSC [25, 26].

Based on the above-mentioned notes, all the 20 subjects' self-reported perceptions of smell and taste were evaluated, using coffee for sniff-test and sip-test respectively prior to PBM treatment (T0) and at each PBM treatment session (T1–T12).

2.3 | Statistical analysis

For data analysis between treatment sessions (T0–T12), the Kolmogorov–Smirnov normality test and subsequent one-way ANOVA analysis were performed, using the Tukey–Kramer test for multiple variables, aiming for p-value <0.05 as statistical significance. InStat 3.0 for Windows software was used.

3 | RESULTS

3.1 | Subjects' self-reported smell and taste perception

Table 4 highlights the scores of subjects' self-reported smell and taste perceptions over the timepoints of PBM treatment (T1–T12) compared with the baseline scores (T0).

The mean score of all the subjects for each sense at all PBM treatments timepoints compared with the T0 showed a gradual improvement in the olfactory and gustatory functionality (Table 4). The mean score for anosmia at T0 was "3.7", whereas at T12 was "8.1" on S-VAS. Interestingly, the means score for ageusia at T0 was "3.8", whereas at T12 was "8.2". Hence, it is noteworthy to underline the close relationship between the mean scores of anosmia and ageusia recovery over the duration of PBM timepoints for all the subjects (Table 4).

3.2 | Progression of patients' self-reported anosmia outcome on VAS

In Figure 3, the mean values of anosmia improvement were based on 20 patients' self-reported scores of the 12 PBM treatment sessions. It shows a continuous and sustainable improvement being statistically significant after T7. Hence, we observed statistically significant improvement in olfactory functionality at the following treatment timepoints compared with T0: T0 versus T7 ($p < 0.05$), T0 versus T8 ($p < 0.01$), T0 versus T9 ($p < 0.001$), T0 versus T10 ($p < 0.001$), T0 versus T11 ($p < 0.001$) and T0 versus T12 ($p < 0.001$).

3.3 | Distribution of anosmia improvement in percentage

Interestingly, we observed that 100% of the 20 patients showed an olfactory improvement above 50% (Figure 4).

3.4 | Progression of Patients' self-reported ageusia outcome on VAS

In Figure 5, the mean values of ageusia improvement were based on 20 patients' self-reported scores of the 12 PBM treatment sessions. It shows a continuous and sustainable improvement being statistically significant after T8. Hence, we observed statistically significant improvement in gustatory functionality at the following treatment timepoints compared with T0: T0 versus T8 ($p < 0.01$), T0 versus T9

TABLE 3 The device specifications, laser parameters and treatment protocols for ageusia management.

Device information	Manufacturer	MMOptics, São Carlos, Brazil
	Model identifier	Vacum laser®
	Number of emitters	6 (3 laser beams for 660 nm and 3 laser beams for 808 nm)
	Light source	Laser
	Beam delivery system	Direct (no means of delivery)
Irradiation parameters	Wavelength	Dual wavelength: 660 and 808 nm
	Spectral bandwidth	2.0 nm and 2.5 nm
	Operating mode	Continuous emission mode (CW)
	Beam profile	Unknown (not Gaussian)
	Power output	100 mW
	Irradiance	5.65 W/cm^2 per spot (emitters)
	Total irradiated energy	216 J per session
	Beam spot size at target	An average of 0.03 cm^2
Treatment parameters	Number of irradiated points	Three points (one spot included anterior 2/3, tip and posterior 1/3 tongue; one spot for right and left lateral border of tongue)
	Irradiated area	0.0177 cm^2 per spot
	Irradiation time	2 min (120 s) per spot
	Application technique	spotting
	Number treatment sessions	12
	Treatment frequency	Three times per week based on 48 h time interval between each session
	Speed of movement	Static

($p < 0.01$), T0 versus T10 ($p < 0.001$), T0 versus T11 ($p < 0.001$) and T0 versus T12 ($p < 0.001$).

3.5 | Distribution of gustatory improvement in percentage

Interestingly, we observed 100% of the patients showed an improvement in gustatory functionality above 50% (Figure 6).

3.6 | Linear relationship between the variables graphically and statistically

Figure 7 shows gradual and concurrent functionality improvement of both the smell and taste over the period of PBM timepoints (T1–T12) compared with T0 (pre-treatment).

At T8, a significant improvement in the symptoms compared with T0, where both smell and taste perception scores were "6.6" on S-VAS and T-VAS respectively, indicating a substantial improvement, which continued to improve over the remaining four sessions of treatment. The subjects had a full regain of olfactory and gustatory functionality observed at T12 (end-treatment) where the mean score of patient's self-reporting was "8.1". This indicates a positive linear relationship between the two-variable expressed graphically (Figure 7) and statistically (Figures 3 and 5).

It is noteworthy that all the subjects were discharged from our care at end of October 2022 and none of them reported back to our care. Hence, we trust that patients' olfactory and gustatory functionality have been well maintained.

4 | DISCUSSION

Our study utilised PBM-laser 660 nm and dual wavelengths of red and NIR (680 nm and 808 nm) in restoring

TABLE 4 The recruited subjects' self-reported scores of their taste and smell perception on VAS at initial baseline (T0) and during the 12 treatment sessions from T1 to T12.

Patient ID	Condition	T0	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
AMF1	Anosmia	1	1	2	3	4	4	5	6	7	7	8	8	9
	Ageusia	2	3	4	5	6	6	7	7	8	8	8	8	9
AMCCM2	Anosmia	4	5	6	7	8	9	9	9	10	10	10	10	10
	Ageusia	4	5	6	7	8	9	9	9	10	10	10	10	10
APAA3	Anosmia	3	4	4	5	5	5	6	6	6	7	8	8	9
	Ageusia	2	2	3	3	3	3	4	4	5	6	6	8	9
MLFO4	Anosmia	1	1	2	2	3	3	4	4	4	5	6	8	10
	Ageusia	0	0	1	1	2	2	3	3	4	4	6	8	10
DFS5	Anosmia	4	4	5	5	6	6	7	7	7	7	7	7	7
	Ageusia	4	4	5	5	6	6	7	7	7	7	7	7	7
CMN6	Anosmia	4	4	5	5	6	6	6	7	7	7	8	8	8
	Ageusia	4	4	5	5	6	7	8	8	8	8	8	8	8
LCC7	Anosmia	7	7	7	7	8	8	8	8	9	9	9	10	10
	Ageusia	7	7	7	7	8	8	8	8	9	9	9	10	10
ALA8	Anosmia	3	3	4	4	5	5	5	5	6	6	6	6	6
	Ageusia	3	3	4	4	5	5	5	6	7	7	7	7	7
JYMS9	Anosmia	0	0	1	1	1	2	2	3	3	4	4	5	5
	Ageusia	0	0	1	1	1	2	2	3	3	4	4	5	5
LH10	Anosmia	5	5	6	6	6	6	7	7	7	7	8	8	8
	Ageusia	5	5	6	6	6	6	7	7	7	7	8	8	9
EOAO11	Anosmia	6	6	7	7	7	8	9	10	10	10	10	10	10
	Ageusia	1	1	2	2	3	3	3	4	5	6	7	8	8
JENW12	Anosmia	0	0	1	1	2	2	3	3	4	4	5	5	6
	Ageusia	5	5	5	5	5	5	5	5	5	5	6	6	6
VFP13	Anosmia	2	2	2	2	3	3	3	3	4	4	4	4	5
	Ageusia	3	3	3	3	3	3	3	3	4	4	4	4	5
MB14	Anosmia	3	3	3	4	4	4	5	5	5	6	7	7	8
	Ageusia	6	6	6	6	6	6	6	6	6	7	8	8	9
MAPM15	Anosmia	8	8	8	9	9	9	9	9	9	9	9	10	10
	Ageusia	10	10	10	10	10	10	10	10	10	10	10	10	10
APL16	Anosmia	6	6	6	7	7	7	7	7	7	8	9	9	9
	Ageusia	6	6	6	7	7	7	7	7	8	8	9	9	9
JER17	Anosmia	6	6	6	6	6	6	7	7	7	7	8	8	8
	Ageusia	5	5	5	6	6	6	6	7	7	7	8	8	9
NM18	Anosmia	6	6	6	7	7	7	7	8	8	8	9	9	9
	Ageusia	6	6	6	7	7	7	7	8	8	8	8	8	9
RAS19	Anosmia	5	5	6	6	7	7	7	8	8	8	8	9	9
	Ageusia	4	4	4	5	5	5	6	7	7	7	7	7	7
SFS20	Anosmia	0	0	1	1	2	2	3	3	4	5	5	6	6
	Ageusia	0	0	1	1	2	2	3	3	4	5	5	6	7
Mean	Anosmia	3.7	3.8	4.4	4.8	5.3	5.5	6	6.3	6.6	6.9	7.4	7.8	8.1
	Ageusia	3.8	4.0	4.5	4.8	5.3	5.4	5.8	6.1	6.6	6.9	7.3	7.7	8.2

Note: At the end of the table, the mean of the subjects' scores is shown for T0 and for the 12 treatment sessions (T1–T12).

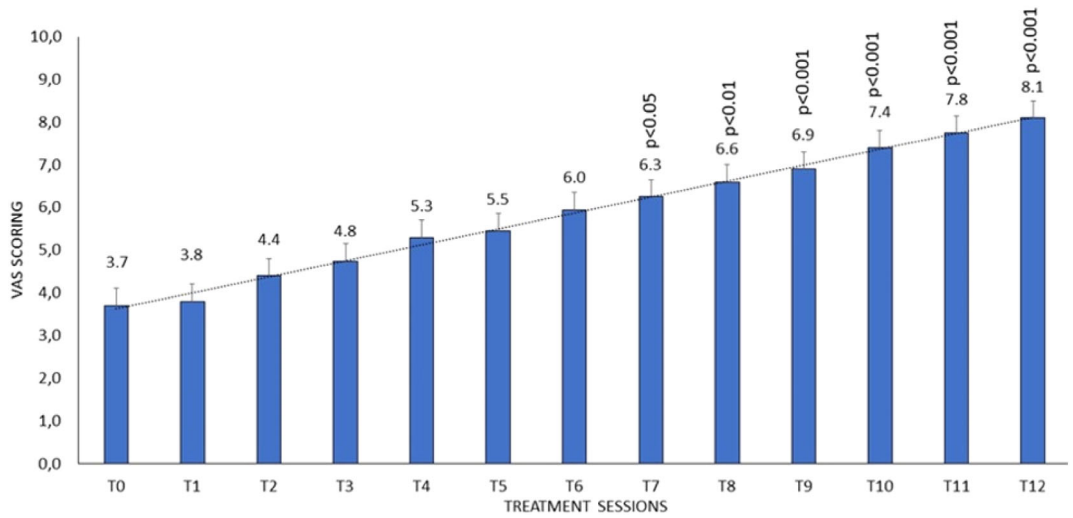


FIGURE 3 The mean score of anosmia progression of 20 patients' self-reported on S-VAS. Kolmogorov–Smirnov normality test was performed with subsequent one-way ANOVA using Tukey–Kramer post-hoc test, for $p < 0.05$. Statistically significant improvement in smell perception observed at T7 and sustained over the period between T8 and T12 compared with T0 (T0 × T7 = $p < 0.05$, T0 × T8 = $p < 0.01$, T0 × T9 = $p < 0.001$, T0 × T10 = $p < 0.001$, T0 × T11 = $p < 0.001$ and T0 × T12 = $p < 0.001$).

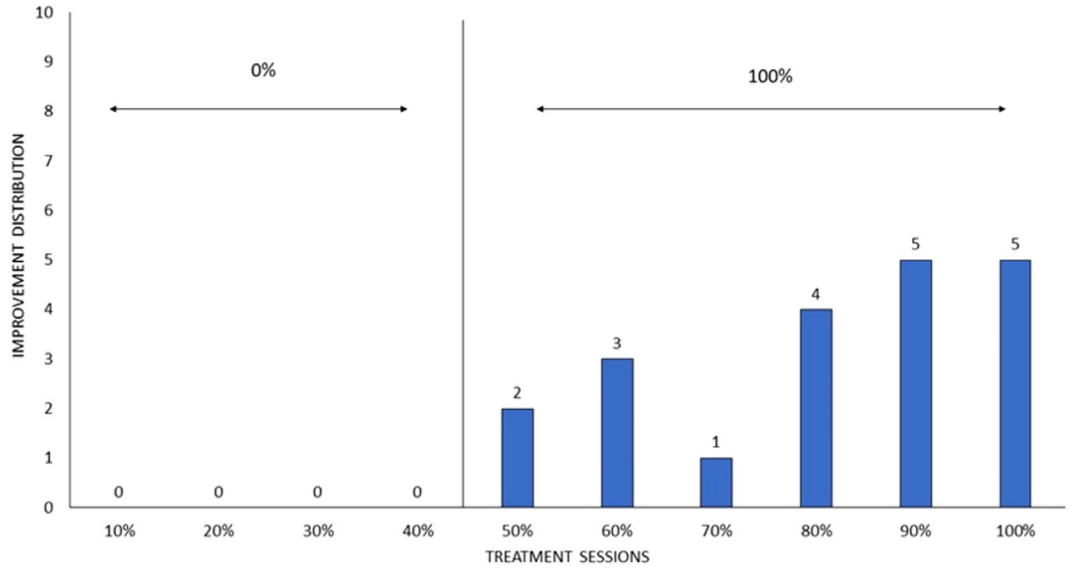


FIGURE 4 The separation into percentage ranges of olfactory improvement of the 20 recruited subjected affected with anosmia after 12 treatment sessions. One hundred percent of the patients had at least 50% improvement in olfactory functionality.

olfactory and gustatory respectively showed to be safe and effective with positive results.

The mean scoring of patient's self-reported perception for smell was "3.7" on T-VAS at T0 (pre-treatment) compared with "8.1" at T12, whereas for the mean score for taste perception was "3.8" on T-VAS at T0 and "8.2" at T12. This significantly indicates that the patient needed at least 10 sessions of PBM therapy, in order achieve a substantial regain of olfactory and gustrory functionality. This coincides with the other reported studies [19, 20]. Nevertheless, in the present pilot study a total of 12 treatment sessions was based on three-time a week with 48-h time interval was required in order to regain a full recovery of both senses. Controversially, de Souza et al. [20] showed that 10 sessions of PBM therapy was sufficient to achieve optimal olfactory and gustatory results. Whereas, a case series study of 14 patients conducted by Soares et al. [19] utilised 660 nm laser-PBM intranasally at energy of 18 J with the following different treatment protocols to obtain optimal results to improve anosmia-induced by COVID-19: 10 sessions, twice a week with 48-h time interval; 5 sessions, twice a week with

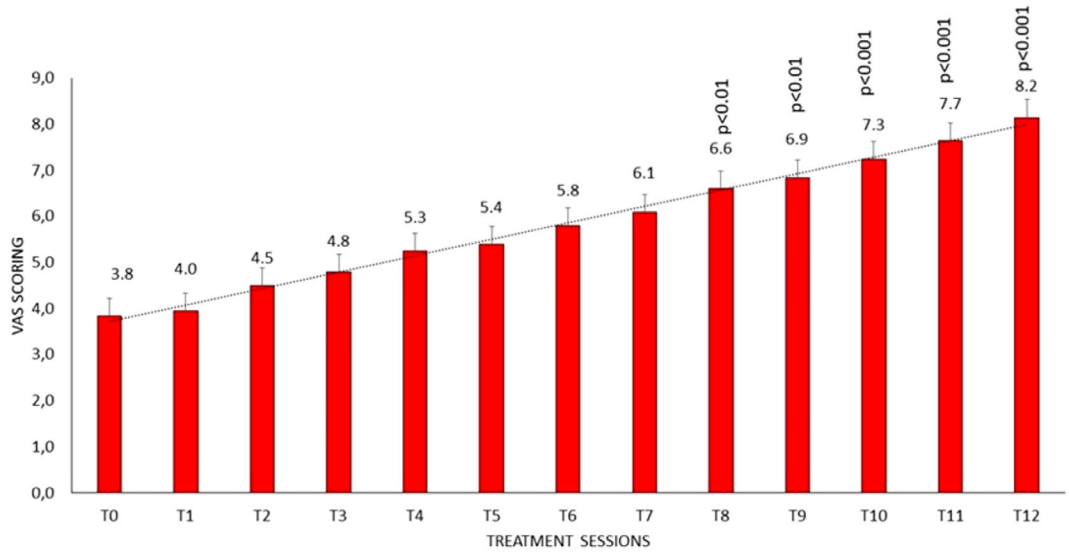


FIGURE 5 The mean score of ageusia progression of the 20 patients' self-reported on T-VAS. Kolmogorov–Smirnov normality test was performed with subsequent one-way ANOVA using Tukey–Kramer post-hoc test, for $p < 0.05$. Statistically significant improvement in taste perception observed at T8 and sustained over the period between T9 and T12 compared to T0 (T0 × T8 = $p < 0.01$, T0 × T9 = $p < 0.01$, T0 × T10 = $p < 0.001$, T0 × T11 = $p < 0.001$ and T0 × T12 = $p < 0.001$).

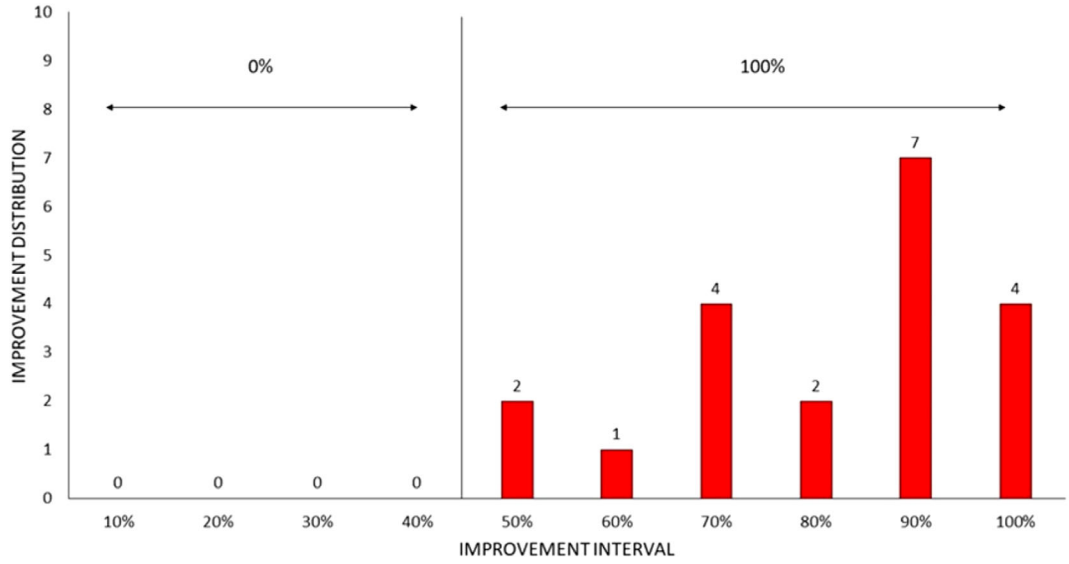


FIGURE 6 The separation into percentage ranges of gustatory improvement of the 20 recruited subjected affected with ageusia after 12 treatment sessions. One hundred percent of the patients had at least 50% improvement in gustatory functionality.

48-h time interval; 10 sessions with 24-h time interval (every day). They concluded that 660 nm laser-PBM has improved anosmia in all the 14 patients who were grouped in different treatment protocols, indicating that each subject responded to PBM therapy differently with positive outcomes. This can lead to propose that a minimal of 10 treatment sessions are required to achieve positive response.

From biological standpoint, an in vitro study conducted by Hanna et al. showed that a minimal time interval of 48 h between each laser-PBM application is necessary to achieve biostimulatory effects on cells' proliferation and differentiation [27]. In this context, we employed our treatment protocol. In terms of the therapeutic power out, 100 mW for at least 2 min showed to exert a significant improvement in olfactory and gustrory functionality [19, 20]. This coincided with the findings of the present pilot study.

Moreover, our results showed that patient's self-reported scores for both the taste and smell are more less

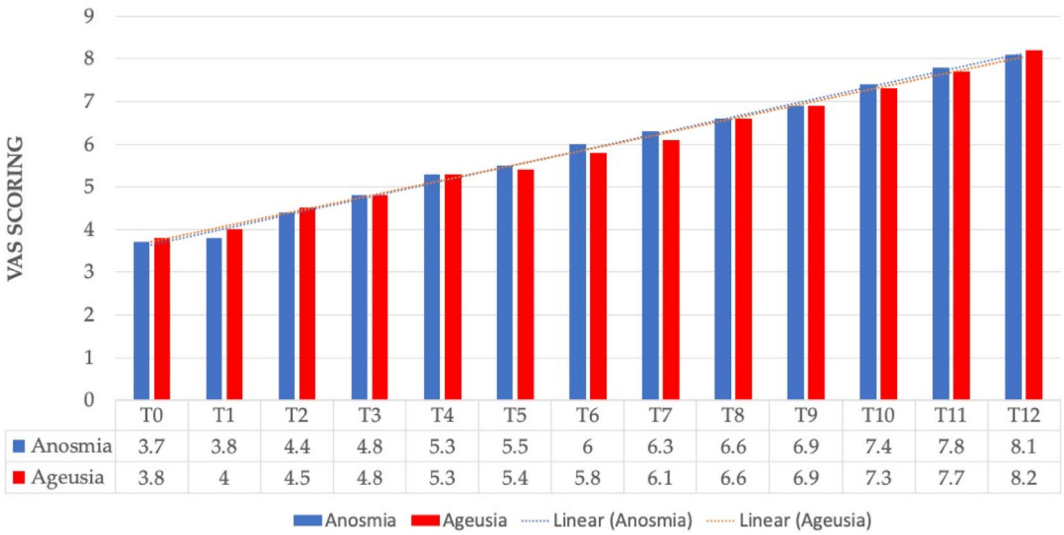


FIGURE 7 The progression of patient self-reporting outcomes on VAS (S-VAS and T-VAS) for both anosmia and ageusia pre-treatment (T0), after each treatment session from T1 to T12. The VAS scoring (T-VAS and S-VAS) represents a description of "0" represents a complete loss of olfactory and gustatory functionally, whereas a description of "10" represents a normal complete regain of functionality for both senses. The linear trendlines for both anosmia and ageusia's mean scoring show a significant improvement over the treatment timepoints.

similar. This scientifically reflects on both the taste buds of the tongue identifying the taste and the nerves in the nose identifying the smell are communicated and intertwined in our brain, where the information is integrated and the flavours are recognised and identified [28]. This was well-represented in our results where a positive linear relationship between the two variables (anosmia and ageusia) observed statistically (Figures 3 and 5) and graphically (Figure 7).

Cytokines storms and OS are evidently raised in patients with COVID-19 [5]. On this note, several studies showed that PBM can improve injured tissue by regulating OS, reducing the inflammatory cytokines such as; IL-6 and TNF-α and increasing IL-10 [10, 29, 30]. Hence, it is important to appreciate that PBM acts as an immunomodulator by inducing antioxidant and anti-inflammatory effects and regulating COVID-19 cytokines storm [31–33]. Hence, we can postulate that administration of PBM in our study targeting the tongue taste buds receptors intraorally and the OSC intranasally has induced an increase in antiapoptotic proteins' expression, a decrease in apoptosis-related markers and upregulate the inflammatory cytokines [19, 20].

Furthermore, PBM enhances the ATP synthesis, increases glucose consumption by cells, promotes cell proliferation and differentiation and increases blood circulation in the salivary glands [34]. Hence, we can hypothesise that PBM can promote molecular metabolic homeostasis that is necessary in restoring both the olfactory and gustatory antioxidant systems. Additionally, we can consider that the inflammatory modulation effect of laser-PBM photonic energy of red and NIR allows the cells of the inflamed and swollen nerve terminals to return to their normal physiological state. This occurs by permitting the molecules that are responsible for triggering electrical stimuli in these terminals to penetrate the cell membrane of the nerve terminals and trigger stimulus sensory action in a normal way. Hence, the findings of the present pilot study are in agreement with the previous studies [19, 20] postulated PBM clinical effects on anosmia and ageusia by modulating the local inflammatory processes, which result in promoting angiogenesis and improving tissue vascularisation [35].

The limitations of our research pilot are related to a lack of the objective quantitative assessment tools to measure patient's perception of both smell and taste senses. and PBM direct effects on the sensory cells of both olfactory and gustatory systems. Also, there was a lack of a control group. Moreover, the authors suggest that saliva analysis as a quantitative biomarker assessment tool is crucial to evaluate OS and ROS levels prior and at each PBM treatment session. Hence, further extensive studies to validate PBM as an antioxidant treatment strategy are warranted.

5 | CONCLUSIONS AND FUTURE PERSPECTIVES

Our clinical pilot study showed that PBM therapy can act as an immunomodulator in restoring anosmia and ageusia and promoting full regain of the smell and taste respectively.

Our positive results showed that PBM protocol of 12 treatment sessions can restore olfactory and gustatory-induced by COVID-19. Also, our results implied PBM synergetic effects on linear relationship between anosmia and ageusia improvement, suggesting their antioxidants system interlink.

Our results and suggestions can pave the roadmap for further studies with large data and controlled arm based on long follow-up timepoints to validate our PBM dosimetry and treatment protocols and sustainability over long follow-up period. Moreover, an advance understanding of the molecular activities occurring within cell after PBM laser irradiation may result in introducing several novel PBM; clinical applications in COVID-19-induced oral manifestations.

AUTHOR CONTRIBUTIONS

Conceptualization, Vitor Hugo Panhoca; methodology, Vitor Hugo Panhoca and Reem Hanna; software, Antonio Eduardo de Aquino Junior; validation, Vitor Hugo Panhoca and Reem Hanna; formal analysis, Vitor Hugo Panhoca and Reem Hanna; investigation, Laís Tatiane Ferreira; Viviane Brocca de Souza; Simone Aparecida Ferreira; Gabriely Simão; and Vitor Hugo Panhoca; resources, Reem Hanna; data curation, Reem Hanna; writing-original draft preparation, Reem Hanna and Vitor Hugo Panhoca; writing-review and editing, Reem Hanna and Vitor Hugo Panhoca; visualization, Reem Hanna; supervision, Reem Hanna and Vitor Hugo Panhoca; project administration, Vanderlei Salvador Bagnato; funding acquisition, Vanderlei Salvador Bagnato. All authors have read and agreed to the published version of the manuscript.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the financial support received from the Financier of Studies and Projects (FINEP; Grant No. 01.13.0430-00), São Paulo Research Foundation (FAPESP; Grant Nos. 2013/14001-9 and 2013/07276-1; CEPOF—CEPID Program), and the partnership with MMOptics and INOVA Institute.

FUNDING INFORMATION

This research was funded by Financier of Studies and Projects (FINEP; Grant No. 01.13.0430-00), São Paulo Research Foundation (FAPESP; Grant Nos. 2013/14001-9 and 2013/07276-1; CEPOF-CEPID Program) and the partnership with MMOptics and INOVA Institute.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All the data are available in the text.

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How to cite this article: V. H. Panhoca, L. T. Ferreira, V. B. de Souza, S. A. Ferreira, G. Simão, A. E. de Aquino Junior, V. S. Bagnato, R. Hanna, *J. Biophotonics* **2023**, e202300003. https://doi.org/10.1002/jbio.202300003