STUDIA UBB CHEMIA, LXVIII, 2, 2023 (p. 85-97)
(RECOMMENDED CITATION)
DOI:10.24193/subbchem.2023.2.06

THE ANTIOXIDANT EFFECT AND CHEMICAL COMPOSITION OF TWO EXPERIMENTAL PHOTOSENSITIZERS IN INDUCED PERIODONTITIS

Ariadna PĂUN[a], Laura Monica RUSU[b,*], Mărioara MOLDOVAN[c], Sorina SAVA[b], Alexandra DREANCA[d], Cecilia BACALI[b], Mîndra Eugenia BADEA[e], Cristina BORZAN[a], Ioana BÂLDEA[f]

ABSTRACT. The aim of this study is to identify and test new natural photosensitizers used in antibacterial photodynamic therapy due to the antimicrobial and antioxidant effect they present. The experimental study was conducted on rats in which periodontal disease was induced. As treatment, photodynamic therapy was used in the presence of new developed natural photosensitizers based on oregano essential oil and curcumin extract. Malondialdehyde as well as low and oxidized glutathione levels were measured by spectrophotometry to assess oxidative stress in treated groups of rats. New photosensitizers were characterized by modern testing methods, using FTIR infrared spectroscopy, gas chromatography-mass spectrometry and UV-Vis analysis. The results obtained suggest that the prooxidant effect is mainly due to exposure to photodynamic therapy (irradiation) and leads to the antibacterial therapeutic effect that is maintained even when applying gels with antioxidant agents.

Keywords: *Oxidative stress, essential oils, photodynamic therapy*

[a] *Department of Public Health and Management, Iuliu Hatieganu University of Medicine and Pharmacy, 31 Avram Iancu str., RO-400083, Cluj-Napoca, Romania.*
[b] *Department of Prosthodontics and Dental Materials, Iuliu Hatieganu University of Medicine and Pharmacy, 31 Avram Iancu str., RO-400083, Cluj-Napoca, Romania.*
[c] *Department of Polymer Composites, Babes-Bolyai University, Institute of Chemistry Raluca Ripan, 30 Fantanele str., RO-400294, Cluj-Napoca, Romania.*
[d] *Department of Pathophysiology/Toxicology, University of Agricultural Science and Veterinary Medicine, 3-5 Manastur str., RO-400372, Cluj-Napoca, Romania.*
[e] *Department of Preventive Dental Medicine, Iuliu Hatieganu University of Medicine and Pharmacy, 31 Avram Iancu str., RO-400083, Cluj-Napoca, Romania.*
[f] *Department of Physiology, Iuliu Hatieganu University of Medicine and Pharmacy, 1 Clinicilor str., RO-400006, Cluj-Napoca, Romania.*
[*] *Corresponding author: dascalu.monica@umfcluj.ro*

INTRODUCTION

The fact that aromatic plants and spices have biologically active components is no longer a novelty. Numerous studies attest to the fact that they have antibacterial, antifungal and antioxidant properties due to their chemical compounds. These compounds began to be used on a large scale for the needs of the pharmaceutical and food industries. They have also been noted for their antioxidant capacity that can prevent oxidative stress through their complex composition and richness in bioactive molecules.

[1,2].Oxidative stress is defined as a persistent imbalance between oxidation and antioxidant, leading to damage of cellular macromolecules. Free radicals consist of reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS contain one or more unpaired oxygen electrons, such as hydroxyl (• OH) and superoxide (O2 • -) and are the target of intense research examining their chemical and physiological activity and their pathological roles in living organisms [3,4]. Inflammation caused by oxidative stress is one of the plausible ways to contribute to the development of periodontal disease. This implies that the prevention and conventional treatment of periodontal disease that focuses on the management of bacterial pathogens appear to be insufficient. In an attempt to reduce oxidative stress using antioxidant supplements, various compounds have emerged as promising preventive and therapeutically adjuvant treatments for these diseases [5].

Essential oils from medicinal or aromatic plants represent a natural source exploited with a heightened interest from the point of view of their antimicrobial and antioxidant properties.[6].

Origanum vulgare L., commonly known as oregano, is one of the most famous aromatic species, widespread throughout the Mediterranean and Asia. It is a plant traditionally used as a spice and medicinal plant, but also as a well-established source of valuable herbal medicines in modern phytotherapy. Studies have shown that oregano essential oil has an increased content of carvacrol and thymol. This fact makes it a good natural alternative against Gram-positive and Gram-negative bacterial strains, including highly resistant Gram-positive and Gram-negative bacteria, such as Streptococcus aureus, Escherichia coli, Porphyromonas aeruginosa, Klebsiella pneumoniae. The antioxidant activity of EO is attributed to the presence of various compounds in its componence [7].

Curcumin, also named Turmeric (Curcuma longa L., Zingiberaceae) is especially popular worldwide due to its attractive culinary, cosmetic and medicinal uses. This tuberous species is interesting in terms of its exploitation as a coloring and flavoring agent, as well as its many pharmacological activities. The antioxidant properties of curcumin have been widely studied. In addition to its antioxidant properties, it also has anticancer properties, anti-inflammatory, neuro- and dermoprotective, antiasthmatic or hypoglycemic [8].

Antimicrobial photodynamic therapy (aPDT) is considered a non-invasive therapeutic method successfully used nowadays in various branches of medicine. It can target periodontal pathogens without damaging the host tissues. This involves the topical application of a photosensitizer (PS) to a targeted area, which is then exposed to a light source of a certain wavelength. Singlet oxygen and free radicals are generated in the presence of light, and given the fact that they are cytotoxic for microorganisms, they will lead to their destruction or inactivation. [9,10].

The purpose of this study is to identify the chemical compounds of two experimental natural gels and to test their antioxidant effect when used with photodynamic therapy. The new photosensitizers were characterized by modern testing methods, using FTIR infrared spectroscopy and gas chromatography-mass spectrometry (GC-MS) for the physico-chemical properties. UV-Vis analysis of the experimental photosensitizers was also performed to determine the wavelength corresponding to the maximum absorption for the prepared gels.

RESULTS AND DISCUSSION

FTIR spectroscopy analysis

As seen in figure 1, the FT-IR spectra of gel C and gel O recorded with the attenuated reflection device (ATR) are complex due to the individual components and due to the overlap of different absorption bands. Although the contribution of the main components is not greater than 25% of the total amount, the components with low concentration in the essential oils (<1%) do not significantly influence the ATR-FTIR spectra. ATR-FTIR spectra of gels recorded with ATR devices show that samples of experimental photosensitizers presented absorbance in the UV-Vis range.

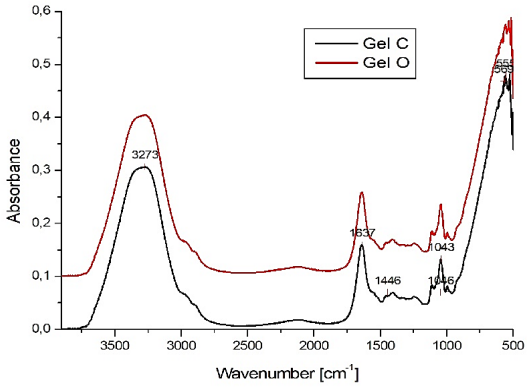

Figure 1. ATR-FTIR spectra of experimental gels

The ATR-FTIR spectra of analyzed gels show characteristic C-H bond vibrations (~2900 cm^{-1}), C=O elongation (~1700 cm^{-1}), a wide O-H bond elongation band (~3400 cm^{-1}) and C-O elongation (~1100 cm^{-1}) of terpenoid compounds from essential oils. In the FT-IR spectra of the gels, the absorption bands of monoterpenes appear around the values of 850, 1450 and 1650 cm^{-1}. The displacements of the absorption bands are due to the interactions between the various functional groups in the oils.

FTIR spectroscopy can be a technique that provides information related to essential oils by being able to identify various types of oil through the method “Principal Component Analysis for Spectroscopy App for OriginPro®”.

GC-MS analysis of experimental gels

GC-MS analysis shows the volatile compounds found in the experimental PS. Chemical composition was expressed as a percentage of total volatile compounds for each component (area %) and is presented in the table below.

Table 1. Chemical composition of the oregano-based gel

No.	Compounds	Retention Time	*Area %*
1	α-pinene	10.295	1.37
2.	2-Carena	12.120	0.48
3.	p-cimene	16.009	14.73
4.	Eucalyptol	16.178	0.34
5.	Gamma-terpinene	16.893	5.15
6.	α-terpinolene	17.739	0.38
7.	Linalool	18.315	2.34
8.	Terpinene-4-ol	21.081	0.40
9.	α-Terpineol	21.576	0.90
10.	Thymol	24.281	7.88
11.	Carvacrol	24.641	61.73
12.	Caryophyellene	27.263	3.08
13.	Humulene	28.265	0.44
14.	Caryophyllene oxide	31.808	0.26
	Total		99.48

Oregano is a very well-known aromatic plant with widespread use nowadays. The GC-MS analysis was carried out in order to identify the chemical compounds in the experimental gels. Carvacrol (CV), the main compound

highlighted in the oregano-based gel (Table 1) is a phenolic monoterpenoid, which possesses a wide range of bioactive properties, such as antimicrobial and antioxidant activity, useful for clinical applications. Essential oils have an extremely complex composition that differs depending on the type of oil considered. On the other hand, the composition of essential oils can undergo changes even within the same botanical species, depending on the geographical area, factors related to the climate and the method of extraction and purification. Because of this, the identification and characterization of essential oils is a complex problem. For this purpose, the separation of the chemical constituents and the quantitative determination by chromatographic techniques (gas chromatography or liquid chromatography) coupled with mass spectroscopy or NMR, laborious methods, are necessary.

UV-Vis analysis

As presented in figure 2, UV-Vis spectra of oregano-based gel demonstrates that the fingerprint is given by the three shoulders that give the range of the spectrum, between 200 nm and 350 nm. At 201 nm a shoulder with high absorbance is highlighted and at 281 nm a shoulder with lower absorbance.

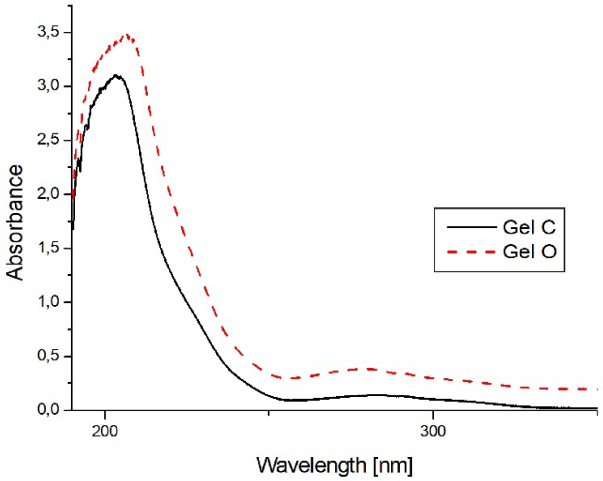

Figure 2. UV-Vis spectra of the analyzed gels (specific fingerprint in 190-900 nm range), containing details regarding the maximum absorbance at the specific peak

UV-Vis analysis of experimental gels was performed in order to determine the wavelength corresponding to maximum absorption. The obtained results demonstrate that the samples show absorption in the UV-vis spectrum, which is why they can be used in combination with low-intensity laser therapy.

Oxidative stress analysis

Following the spectrophotometric analysis, the values obtained for MDA (malondialdehyde), GSH (reduced glutathione) and GSSG (oxidized glutathione) were recorded. The results obtained are presented in Table 2.

Table 2. Data obtained from spectrophotometric analysis.

Nr.Crt.	Samples	MDA (nmol/mL)	GSH (nmol/mL)	GSSG (nmol/mL)
1	M1	1.182	7.52	0.9
2	M2	1.76908	0	0
3	M3	1.5267	8.415	0.78
4	M4	1.51396	6.535	0.94
5	O1	1.35952	11.375	1.22
6	O2	1.8954	2	0.78
7	O3	1.50732	4.265	1.02
8	O4	0.96374	4.2	1.14
9	C1	1.34198	4.55	1.04
10	C2	0.98118	3.905	1.4
11	C3	2.34766	9.03	0.92
12	C4	1.37702	10.22	0.88
13	P1	2.40432	7.6	1.1
14	P2	1.03778	8.005	0.88
15	L1	1.2562	0	0
16	L2	2.17078	2.92	1.08
17	L3	1.94022	2.31	0.92
18	L4	1.71554	5.99	0.78

M=Control group of rats; O=group of rats treated with oregano; C=group of rats treated with curcumin; P=group of rats with periodontal disease; L=group of rats treated with laser

Periodontal disease induced a statistically insignificant increase in lipid peroxidation compared to the control group. A decrease in malondialdehyde (MDA) was observed in the groups of rats treated with oregano and curcumin, while the group of rats exposed to irradiation (Laser group) had a comparative value with the group of rats with untreated periodontal disease (figure 3 left).

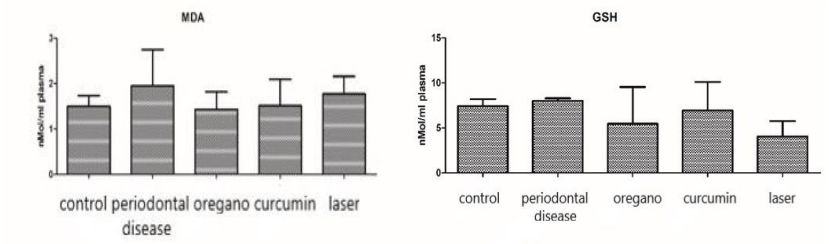

Figure 3. Left-Malondialdehyde level (MDA) in treated groups of rats; Right-rReduced glutathione (GSH) levels in treated groups of rats

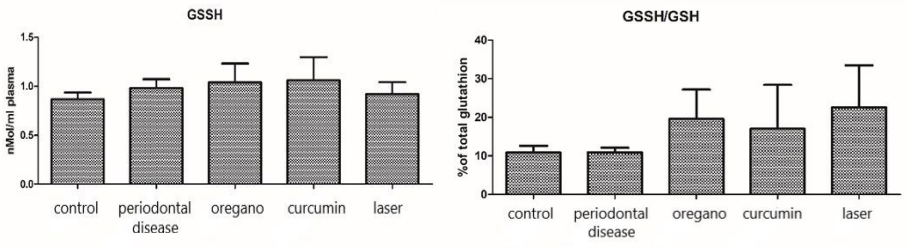

Figure 4. Left-Oxidized glutathione level (GSSH) in treated groups of rats; Right-The ratio of reduced glutathione / oxidized glutathione in treated groups of rats

Red blood cells are well equipped to handle intracellular oxidative stress, their membranes are permeable to O_2- and H_2O_2, and in this way they are important regulators of oxygen reactions occurring in their surroundings. The protective effect against reduced oxygen species - generated during the endothelial cell injury of various tissues - is attributed mainly to the glutathione metabolism of red blood cells [11].

Liquid chromatography and mass spectroscopy are more reliable and specific methods for the detection of MDA [12,13]. These methods were used to study MDA levels in serum and saliva of periodontitis patients [12, 14–16]. Significantly higher levels of MDA were found in gingival tissue of periodontitis patients compared to periodontal healthy controls [17]. MDA is a reliable marker of lipid peroxidation, which allows the evaluation of the oxidative induced damage, following different clinical and experimental scenarios [18,19].

Glutathione (GSH) is the most abundant antioxidant in aerobic cells, present in micromolar concentrations (μM) in body fluids and in millimolar concentrations (mM) in tissue. GSH is essential for protecting the brain from oxidative stress, acting as a free radical scavenger and an inhibitor of lipid peroxidation. GSH also participates in the detoxification of hydrogen peroxide

by various glutathione peroxidases. The ratio of reduced GSH to oxidized GSH (GSSG) is an indicator of cellular health, being reduced in neurodegenerative diseases like Parkinson or Alzheimer, inflammatory diseases and cancer. The GSH / GSSG ratio is an excellent way to assess potential therapeutic efficacy in maintaining cellular redox potential [20].

MDA was measured spectrophotometrically from the cell lysates to evaluate the oxidative stress induced damage.. Periodontal disease induced an increase in the lipid peroxidation compared to the control group although not statistically significant. In the groups of oregano and curcumin, a decrease in malondialdehyde (MDA) was observed, while the group exposed to irradiation (Laser group) registered an increased value compared to control similar to the group of untreated periodontal disease (figure 3-left). The data also show a protective effect of the oregano and curcumin based gels against the oxidative damage induced by the periodontal disease ($p \leq 0.02$, oregano, respectively curcumin group versus periodontal disease group).

As seen in figure 3-right, the GSH level was increased in animals with periodontal disease compared to the control group, which can be explained by the formation of ROS that stimulate the antioxidant defense mechanisms of the cells, leading to a higher antioxidant reserve of reduced glutathione. In a similar way, GSH was decreased in oregano and curcumin gels, since these gels already contain high amounts of antioxidant substances that lowered the oxidative damage as shown by the reduced MDA level. In Laser group, GSH level was reduced because it was consumed within the processes of neutralization of the free oxygen radicals induced by the therapy. However, since the level of MDA was increased, in this group, there was oxidative damage induced by the therapy, but at lower levels when compared to the untreated periodontal disease.

The oxidized GSSH level was increased in all treated groups compared to the control (figure 4-left), but these increases were not significant.

The most important parameter, GSSH / GSH ratio (figure 4-right) showed increased values in all treated groups, compared to the control group. The most discrete changes in the antioxidant balance were recorded in periodontal disease group, where the reduced GSH values were higher, as a defense against periodontal disease. This led to a reduced GSSH/GSH ratio, correlated with high MDA level, which indicates that the antioxidant defence was not effective against the prooxidant free radicals produced by the periodonatal disease, leading to oxidative damage.

In the groups treated with curcumin ($p = 0.07$) and oregano ($p = 0.054$) the GSSH/GSH ratio favored a prooxidant balance, which was mostly induced by the decrease of GSH and the light increase of the oxidized form. However, these alterations were correlated with a significantly lower MDA,

compared to periodontal disease, that shows an efficient antioxidant effect, probably thorough a different mechanism, such as increased antioxidant enzymes like superoxide dismutase and/or catalase.

Laser group ($p = 0.054$) had a prooxidant effect, with a decrease in GSH and an increase in GSSH compared to the periodontal disease group. This is correlated with the increase of MDA and shows a high level of free radicals generated by the therapy, leading to an increased oxidative induced damage, that overcame the antioxidant defense mechanisms of the cells. Photodynamic therapy is an emerging, non-invasive treatment, currently used in several fields of medicine, including dentistry, both as a diagnosis and as a treatment. PDT involves chemical agents as photosensitizers, light with a specific wavelength. The generation of singlet oxygen and reactive oxygen species in the presence of endogenous molecular oxygen has the effect of eliminating pathogenic microorganisms from bacterial, fungal or parasitic infections. PS agents are special compounds used in PDT that can be administered systemically or locally to the area requiring treatment. To have the desired effect, photosensitizers require activation with a well-defined wavelength of light, which will initiate the mechanism necessary to target and eradicate unhealthy tissue. In periodontal disease, it is used as an adjuvant to mechanical treatment in order to eliminate microbial factors. Considering the disadvantages of systemic administration of antibiotics, PDT seems a promising approach in non-surgical periodontal therapy. Many studies have concluded that periodontal disease induced in rats responded well to photodynamic therapy [21-23].

The recorded results suggest that the prooxidant effect is mainly due to exposure to photodynamic therapy (irradiation) and leads to the antibacterial therapeutic effect that is maintained even when applying gels with antioxidant agents. Interestingly, the lesions caused by periodontal disease by inducing oxidative stress were ameliorated (MDA level decreased) when using the 2 antioxidant gels. These findings support antioxidant protection while maintaining the effectiveness of photodynamic therapy.

CONCLUSIONS

A detailed analysis related to the characterization of essential oils by spectroscopy or chromatographic methods can be carried out in the following stages of the project. Analyzed by UV-Vis spectrometry, the experimental gels showed absorption in the UV range, while the volatile compounds specific to the experimental photosensitizers were determined by GC-MS analysis.

Research so far has revealed the fact that periodontal disease induces an increase in lipid prooxidation. After the application of experimental treatment, a decrease in MDA was observed in the groups treated with gels based on oregano, respectively curcumin. Also, the GSH level in treated groups was lower compared to the group of animals with periodontal disease, while the GSSH levels showed higher values in the treated groups.

Although it has certain limitations, this study can be extended for a more in-depth research of the chosen subject. All these data presented in the study indicates that the tested essential oils may be suitable as photosensitizers used in low-intensity photodynamic therapy.

EXPERIMENTAL SECTION

Gel formulation

Experimental studies were performed using two gels based on natural compounds and then used as photosensitizers in PDT. For the first gel, oregano essential oil (O, *Origanum vulgare*) (Young Living, Groningen, The Netherlands) was used and for the second gel, freshly prepared curcumin extract (C, *Curcuma longa*) from fresh turmeric root. In addition to the main natural ingredient, the gels also contain gelatin (GE-99.5%), glycerin (GY), Kaqun® water (K) (Kaqun Distribution Kft., Nagytarcsa, Hungary) and salicylic acid (AC-99%). For gelatin, glycerin and salicylic acid the provider was Sigma–Aldrich Inc., St. Louis, MO, USA. Gels were prepared from a 1:1 mixture of gelatin:glycerol and 60 ml of Kaqun® water, as well as a 0.015% salicylic acid solution.

Since curcumin has a certain limitation in water solubility, it requires oil or other synthetic material to make it water soluble. For this fact, arnica oil (PlantExtrakt, Cluj, Romania) was added to the gel with curcumin.

Experimental PS contain nanocapsules, which include an organic phase based on essential oils, with the active principle wrapped in a fine film of polycaprolactone (PLC) (Sigma-Aldrich Inc., St. Louis, MO, USA) in order to ensure controlled release of the active substance through the diffusion phenomenon.

FTIR spectroscopy analysis

The identification of the chemical constituents of the studied products was carried out by spectroscopy. FTIR spectroscopic analysis was performed on an FTIR spectrophotometer (Jasco FTIR-610, Jasco International Co., LTD.,

Tokyo, Japan) with an ATR (attenuated total reflectance) accessory equipped with a horizontal ZnSe crystal (Jasco PRO400S). To record the FTIR spectrum of the experimental gels, the gels were measured in their initial state (wet) compared to gel samples dried in an oven at a constant temperature of 30^0C.

GC-MS analysis

An Agilent device was used to determine the composition of volatile oils by GC-MS (model Agilent GC-MS Gas Chromatograph - 7890A/5975/2008) (Agilent Technologies, Inc. Europe, Waldbronn, Germany). To prepare the samples, 10 microliters of each oil were dissolved in one ml of hexane. Gas chromatographic analysis of the gels was performed by injecting a volume of 1 µl of the previously prepared samples into the inlet of the gas chromatograph, in "scan" mode, maintained isothermally at 250°C. The capillary column used was HP 5-MS type, 30 m x 0.25 mm x 0.25 µm (Phenomenex), high purity He carrier gas, with a flow rate of 1 ml/min. Applied temperature program: 40^0C for 1 min, then 5^0C/min up to 220^0C, then 20^0C/min up to 280^0C, maintained for 5 min. The identification of the compounds was carried out using the NIST L14 database.

Because the curcumin-based gel also contains arnica oil, its analysis was performed by HPLC chromatography (PU-980, Jasco International Co., LTD., Tokyo, Japan).

UV-Vis analysis

To measure the absorptive capacity of the gels, we used the UV-VIS Spectrophotometer Jasco V-750, (Able Jasco, Japan) JASCO 150 mm with integrating sphere model ILV924, with the following characteristics: wavelength range: 160-900 nm, speed of variable scanning between 10 ÷ 4000 nm/min, scanning speed at spectral preview of 8000 nm/min. The measurements were made in quartz vats, using 98% ethyl alcohol of analytical purity as an internal standard.

Experimental design

This study was performed on 25 adult male Wistar rats in which periodontal disease was induced through a ligation procedure. All procedures that involved the use of laboratory animals followed the European guidelines and rules 337, as established by the EU Directive 2010/63/EU and the Romanian law 43/2014 and were performed by an experienced practitioner. The study protocol was approved by the Research Ethics Committee of the

University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania and they were authorized by the State Veterinary Authority (auth no.52/30.03.2017). Animals were randomly assigned to 5 groups. The groups (n = 5) were assigned according to the following treatments applied locally: group 1 was left without surgical intervention representing the control group (Control, n = 5); group 2 (Periodontal disease, n = 5) received surgical intervention in order to induce periodontal disease and was left untreated; group 3 (Oregano, n = 5) was treated with oregano photosensitizer and aPDT; group 4 (Curcumin, n = 5) was treated with a curcuma photosensitizer and aPDT, and group 5 (Laser, n = 5) was treated with laser only without photosensitizer. All treatments were carried out by a specialist.

Oxidative stress analysis

Malondialdehyde (MDA) (lipid peroxidation marker) as well as low and oxidized glutathione levels were measured by spectrophotometry (Spectrophotometer PerkinElmer, Waltham, Massachusets, USA) to assess oxidative stress in treated groups of rats. Samples were taken from plasma. The data obtained were statistically interpreted using the two way test ANOVA and TTEST, GraphPad program, 2005 version for Windows (GraphPad Software, San Diego, CA, USA).

ACKNOWLEDGMENTS

This work was supported by a grant of the Ministry of Research, Innovation and Digitization, CNCS - UEFISCDI, project number PN-III-P4-PCE-2021-1140, within PNCDI III.

REFERENCES

1. M. Kačániová *et al.; Foods*, **2020**, *9*, 282
2. F. Alminderej; S. Bakari; T. I. Almundarij; M. Snoussi; K. Aouadi; A. Kadri; *Plants*, **2020**, *9*, 1534
3. T. Liu *et al; PLOS ONE*, **2015**, *100*
4. C. A. Ferreira; D. Ni; Z. T. Rosenkrans; W. Cai; *Nano Res.*, **2018**, *11*, 4955–4984
5. T. T. T. Vo; P.-M. Chu; V. P. Tuan; J. S.-L. Te; I.-T. Lee; *Antioxidants*, **2020**, *9*, 1211
6. M. J. Simirgiotis; *Metabolites*, **2020**, *10*, 414
7. A. Lombrea *et al.; Int. J. Mol. Sci.*, **2020**, *21*, 9653
8. M. D. Ibáñez; M. A. Blázquez; *Plants*, **2020**, *10*, 44
9. P. Haag; V. Steiger-Ronay; P. Schmidlin; *Int. J. Mol. Sci.*, **2015**, *16*, 27327–27338

10. L. M. Dascalu Rusu *et al.*; *Mater. Basel Switz.*,**2020**, *13*, 3012
11. I. Németh; D. Boda; *Biomed. Biochim. Acta*, **1989**, *48*, S53-57
12. F. A. Akalin; E. Işiksal; E. Baltacioğlu; N. Renda; E. Karabulut; *Arch. Oral Biol.*, **2008**, *53*, 44–52
13. E. Baltacıoğlu *et al.;J. Periodontol.*, **2014**, *85*, 1432–1441
14. C. C. Tsai *et al.*; *J. Periodontal Res.*, **2005**, *40*, 378–384
15. F. A. Akalin; E. Baltacioğlu; A. Alver; E. Karabulut; *J. Periodontol.*, **2009**, *80*, 457–467
16. D. Wei; X.-L. Zhang; Y.-Z. Wang; C.-X. Yang; G. Chen; *Aust. Dent. J.*,**2010**, *55*, 70–78
17. Y. Wang; O. Andrukhov; X. Rausch-Fan; *Front. Physiol.*, **2017**, *8*, 910
18. I.Baldea; D.E. Olteanu; A.G. Filip; M. Cenariu; D. Dudea; A. Tofan; C. Alb; M. Moldovan; *Clin Oral Invest*, **2017**, *21*, 1315–1326
19. A. Clichici; G.A. Filip; M. Achim; I. Baldea; C. Cristea; G. Melinte; O. Pana; L.B. Tudoran; D. Dudea; R. Stefan; *Materials,* **2022**, *15(24),* 9060
20. J. B. Owen; D. A. Butterfield; Measurement of Oxidized/Reduced Glutathione Ratio. In *Protein Misfolding and Cellular Stress in Disease and Aging*; P. Bross, N. Gregersen, Eds. Totowa, NJ: Humana Press, **2010**, 269–277
21. E. J. Prażmo; M. Kwaśny; M. Łapiński; A. Mielczarek; *Adv. Clin. Exp. Med. Off,* **2016,** *25*, 799–807
22. A. Stájer; S. Kajári; M. Gajdács; A. Musah-Eroje; Z. Baráth; *Dent. J.*, **2020**, *8*, 43
23. L. M. Dascalu Rusu, M. Moldovan, C. Sarosi, S. Sava, A. Dreanca, C. Repciuc, R. Purdoiu, A. Nagy, M.E. Badea, A.G. Paun*; Gels Basel Switz.*, **2022**, *8(2)*, 134.