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ORIGINAL ARTICLE

EVALUATION OF THE BIOCOMPATIBILITY OF LASER IRRADIATED PLANT EXTRACTS USED AS ADJUVANTS IN IRRIGATION AND SANITIZATION OF ROOT CANALS

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Abstract

The study aimed to evaluate the biocompatibility of some alcoholic and hydroalcoholic plant extracts, irradiated with laser beams to enhance efficacy, which was used for lavage together with the mechanical treatment of the root canals. In the study, we used seven plant extracts obtained from the Faculty of Biology, University of Bucharest, four preparations of walnut fruit (*Juglans regia*), two preparations of sage (*Salvia Officinalis*), and a preparation represented by Echinacea tincture (*Echinacea purpurea*). The biocompatibility of the seven plant extracts, treated with a 940 nm diode laser beam, in pulsed mode, at powers of 3 W and 5 W, respectively, was evaluated on ATCC (American Type Culture Collection) L929 fibroblasts, by Mosmann's tetrazolium toxicity (MTT) assay. The analysis of the extracts, from the biocompatibility point of view, highlighted the fact that irradiation influences their characteristics and their ability to react with the metabolic indicators used in the test. There was an improvement in the characteristics of the extracts following laser irradiation, so that although, initially and in some extracts there was a degree of cytotoxicity, after treatment and irradiation with a laser beam with an intensity of 5 W, they become compatible. Due to their antimicrobial properties and reduced cytotoxicity, laser beam irradiated extracts can be used as a root canal irrigator together with, or as an alternative to sodium hypochlorite.

Rezumat

Studiul a urmărit evaluarea biocompatibilității unor extracte vegetale alcoolice și hidroalcoolice, iradiate cu fascicule laser pentru potențarea eficacității, care au fost folosite pentru lavaj împreună cu tratamentul mecanic al canalelor radiculare. În cadrul studiului am utilizat 7 extracte vegetale obținute la Facultatea de Biologie, Universitatea din București, 4 preparate din fructe de nuc (*Juglans regia*), 2 preparate din salvie (*Salvia officinalis*) și un preparat reprezentat de tinctură de Echinacea (*Echinacea purpurea*). Biocompatibilitatea celor 7 extracte vegetale, tratate cu un fascicul al laserului cu diodă de 940 nm, în modul pulsat, la puteri de 3 W și respectiv 5 W, a fost evaluată pe fibroblaste ATCC (*American Type Culture Collection*) L929, prin testul MTT. Analiza extractelor, din punct de vedere al biocompatibilității, a evidențiat faptul că iradierea influențează caracteristicile acestora și capacitatea lor de a reacționa cu indicatorii metabolici utilizați în testare. S-a observat îmbunătățirea caracteristicilor extractelor consecutiv iradierii cu laser, astfel că deși inițial și la unele extracte a existat un grad de citotoxicitate, în urma tratamentului și iradierii cu un facicul laser cu intensitate de 5 W, acestea devin compatibile. Datorită proprietăților antimicrobiene și citotoxicității reduse extractele iradiate cu fascicul laser pot fi utilizate ca irigant pentru canalul radicular alături de, sau ca alternativă la hipocloritul de sodiu.

Keywords: biocompatibility, plant extracts, laser irradiation, adjuvants, root canals

Introduction

The essential conditions for the success of endodontic treatment are the elimination, by chemical-mechanical treatment, of pulp residues and microorganisms from the root canal system [3, 13]. However, all manual or rotary instrumentation systems do not act on the secondary canals, which can lead to complications in the apical periodontium due to imperfect root fillings [13]. Irrigation allows a cleaning beyond what could be obtained just by simply instrumenting the root canal [11, 23]. Practitioners should know the effects of each irrigation substance they use, the concentration at which it is effective or becomes pathogenic, so that they can use the minimum amount with maximum effect and, especially, the time it must be acted upon [16]. Then, it is very important that the different medicinal substances do not interact and do not generate precipitates inside the root canal which can no longer be removed, or their disposal is difficult, preventing the sealant from adhering to the root canal wall [27].

Endodontic treatment achieved by combining physical and chemical agents cannot ensure efficiency on the entire canalicular system, which is why other methods such as ultrasound or lasers are used [21, 28]. The use of high-power lasers in endodontics has the advantage that the laser light can reach the area inaccessible to instruments and chemicals [6, 7, 14].

Today, researchers are conducting intensive basic studies and clinical trials in laser medicine and photo-biology to develop new ways of diagnosis and treatment [17]. Progress has been made in researching the cellular effects of laser irradiation. The biological mechanisms underlying these responses differ significantly depending on the type of laser, target cells, and other experimental conditions [8]. Despite high expectations for their implementation in all areas of dentistry, the effects of lasers are still being researched for endodontic treatment, apical treatment and non-surgical periodontal treatment, due to tissue modification, detoxifying effects, and bactericidal effects [1, 21]. Due to its transmission and scattering properties, laser light can reach the area where conventional instruments can not, causing serious damage, such as alteration of intracanalicular, periapical and root surfaces, when laser beam parameters are used improperly [26].

Endodontic infections are characterized by the fact that they are polymicrobial with the mandatory predominance of anaerobic bacteria, and *Enterococcus faecalis* is dominant in persistent infections after root canal treatment, with a prevalence of 29 - 77% [10, 22]. One of the main objectives of root canal treatment is to remove bacteria, bacterial products and debris from the root canal. Most of them can be removed by the mechanical action of endodontic instruments [5]. However, in certain situations, due to the complex anatomy of the root canal system, organic residues and bacteria deposited deep inside the dentinal tubules cannot be removed even after careful mechanical instrumentation [20, 22]. Root canal irrigation plays an extremely important role in the success of endodontic treatment, and the use of irrigation solutions is essential to ensure the reduction of bacterial load and the elimination of organic tissue residues [10, 20]. Currently, in addition to many products, such as sodium hypochlorite (NaOCl), chlorhexidine gluconate, calcium hydroxide and saline solution, natural plant extracts are also used [4]. NaOCl is one of the most popular endodontic irrigants, widely used, due to its anti-bacterial properties and the ability to dissolve necrotic tissue debris [25]. During endodontic treatment, irrigants will be in contact with the pulpal and periapical tissues [15]. Although NaOCl is an effective anti-bacterial, it is harmful outside the root canal, causing damage in contact with periradicular tissues [10, 15].

It is desirable, that the chemical agents selected as endodontic irrigants, have favourable properties, such as antimicrobial activity, dissolution of organic tissue residues, debridement of the root canal system, and induction, in periapical tissues, of favourable recovery and healing reactions [2].

Plant extracts together with various antimicrobial agents can be valuable aids in combating the relatively resistant microorganisms in the endodontic system, which have been the cause of a failure, even in the treatment procedures that have followed all the necessary protocols [4, 5, 24].

Each product has different properties and thus, in numerous studies, their antimicrobial effect, chemical properties, and biocompatibility have been compared, for finding an ideal irrigation solution to be used as an adjuvant in the treatment of the root canal [4].

The aim of this *in vitro* study was to examine the potential toxicological implications of plant extracts from walnut (*Juglans regia*), sage (*Salvia officinalis*), and a laser-activated echinacea (*Echinacea purpurea*) tincture extract, and the possibility of attenuating the harmfulness outside the root canal and of potentiating some recovery and healing reactions at the level of the periapical tissues

Materials and Methods

In this study, we used seven plant extracts obtained from the Faculty of Biology, University of Bucharest, Romania, a mix of walnut fruit (*Juglans regia*), sage (*Salvia officinalis*) and an Echinacea tincture (*Echinacea purpurea*).

The sage (herb/aerial parts) was cleaned from impurities, washed and let to dry at room temperature, then chopped using a laboratory mill and mixed with the hydroethanolic solution (extract 1:5 in hydro-ethanolic solution, 96% ethylic alcohol/water:35/65 ratio). The extraction was performed by microwave with an Ethos Start D extractor (1 h at 100°C). The extracts obtained were dried using a rotary evaporator and the resulting product was reacted in DMSO (di-methylsulfoxide) at a final concentration of 20 mg/mL.

From the walnut fruits, 4 alcoholic and hydro-alcoholic extracts in methanol and ethanol (100% methanol and water:methanol 1:1 v/v, over which 5 g of plant were placed) were performed; (100% ethanol and water: ethanol 1:1 v/v over which 1 g of plant was placed) from the following components: pericarp and mesocarp (green bark); endocarp (woody part of the nut); seed coat (the skin that covers the seed); the woody walls inside the seed separating the cotyledons and lobes.

The fragments of the green peel were lyophilized before being used. In obtaining walnut fruit extracts, microwave extraction was also used.

Echinacea tincture contains 20 g of *Echinacea purpurea* and 70% v/v ethyl alcohol *per* 100 g solution.

The working solutions were coded as follows: N5 - methanol extract of freeze-dried pericarp and mesocarp of *Juglans regia*; N7 - extract in ethanol of endocarp of *Juglans regia*; N11 - methanol extract of seed coat of *Juglans regia*; N19 - extract in water:methanol of pericarp and mesocarp of *Juglans regia*; N20 - extract in water: methanol from the inner wall of the cotyledon of *Juglans regia*; S - extract in water:ethyl alcohol of *Salvia officinalis*; E - Echinacea tincture; 3W – 3 W laser beam treated extract; 5W – 5 W laser beam treated extract.

The seven extracts as analysed variants, were obtained by treating the plant extracts with a diode laser beam of 940 nm Epic Biolase, in pulse mode, at the appropriate wavelength, with a laser beam output power of 3 W and 5 W, respectively. Endodontic tips with a diameter of 400 µm and a length of 22 mm were used. The sterile, disposable tip must be checked initially and verified, to transmit the light wave correctly and concentrated at one point.

Biocompatibility of tested extracts

The biocompatibility of the compounds was evaluated on ATCC L929 fibroblasts, cultured in DMEM medium (Dulbecco's Modified Eagle Medium) supplemented with 10% foetal bovine serum. Cells were seeded at a density of 1.5 x 104 cells/well in 200 µL of culture medium, over which the test extracts were placed (1:10 dilution) and incubated at 37°C (5 % CO_2) for 24 hours.

The assessment of the biocompatibility degree (cyto-toxicity of materials) was performed by the Mosmann Tetrazolium Toxicity (MTT) test. The MTT test is a viability test, that allows the quantitative evaluation of living cells in culture. [18] The compound MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetra-zolium bromide] is permeable to living cell membranes. After metabolization of the MTT compound, iso-propanol-soluble formazan crystals are formed, resulting in a solution (purple) with an optical density that can be read at 550 nm wavelength. For the MTT test, the rest of the culture medium was removed from the 96-well L 929 cell test plate, the surface was washed with PBS, to remove any traces of foetal bovine serum which inhibits the MTT compound, an MTT solution was prepared 1 mg/mL and each sample was incubated in the presence of 1 mL MTT solution for 4 h at 37°C and 5% CO_2. In order to read the results, the formazan crystals formed were solubilized with isopropanol. The resulting solution, purple in colour, was read on a spectrophotometer at 550 nm. The colour intensity is directly proportional to the number of living cells in the sample.

For the LDH (lactate dehydrogenase) quantification, a 100 µL reaction mixture was prepared, which contained all the components of the mix equally. 50 µL of the medium was collected from the test plate in duplicate and transferred to a 96-well plate. After 100 µL was added to each sample, the plate was incubated for 15 - 20 minutes in the dark. Based on the LDH level in the culture medium, the colour intensity of the pink solution varied in direct proportion to the number of dead cells in the sample. It was read on a spectrophotometer (Flex Station 3) at a wave-length of 490 nm [5].

To consider that a particular sample is biocompatible, the optical density values for MTT tests must be higher than those of the LDH quantification test (more precisely, we say that a sample is biocompatible if the amount of viable, metabolically active cells is higher than that of dead cells).

Results and Discussion

The analysis of the extracts included in the study, from the biocompatibility point of view, showed that irradiation influences the characteristics of the extracts and their ability to react with the metabolic indicators used in the test.

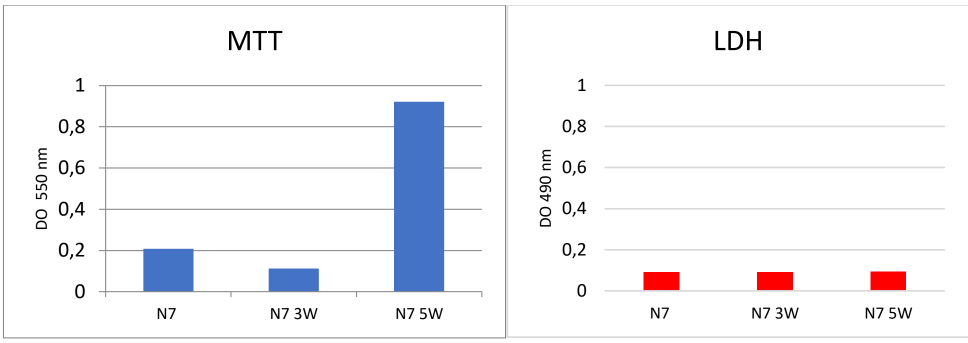

Figure 1.

MTT and LDH tests for *Juglans Regia* endocarp ethanol extract, before and after irradiation with a 5 W laser beam

For the ethanol extract from the endocarp (N7), it was observed that after irradiation with a 5 W laser beam, the number of viable cells increases considerably, the MTT value being much higher than the LDH (Figure 1).

The methanol extract from the seminal skin of *Juglans regia* (N11) after irradiation, loses its biocompatibility corresponding to the applied intensity, but does not become cytotoxic (Figure 2).

Extract in water: methanol from the wall inside the *Juglans Regia* cotyledon directed after treatment with laser beams with a power of 3 W and 5 W, respectively, shows values of optical densities for MTT tests higher than those of the LDH quantization test, proportional to the intensity of the beam used (Figure 3).

Although initially the extract in water: methanol from the pericarp and mesocarp lyophilized by *Juglans regia*, was biocompatible, the treatment with a 5 W laser beam for 30s determined the registration of a high value of LDH absorbance compared to MTT, a value that highlights the presence of a large number of dead cells (Figure 4) and the cytotoxicity of the extract.

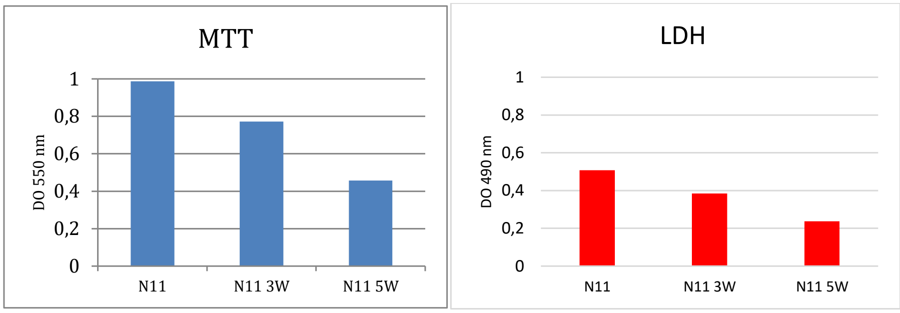

Figure 2.
MTT and LDH tests for *Juglans regia* seminal skin methanol extract, before and after irradiation with a 5 W laser beam

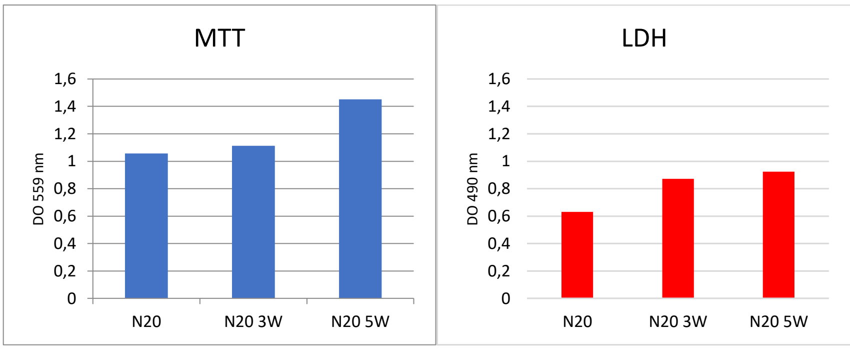

Figure 3.
MTT and LDH tests for water extract: methanol in the wall inside the *Juglans regia* cotyledon, before and after irradiation with laser beams with a power of 3 W and 5 W, respectively

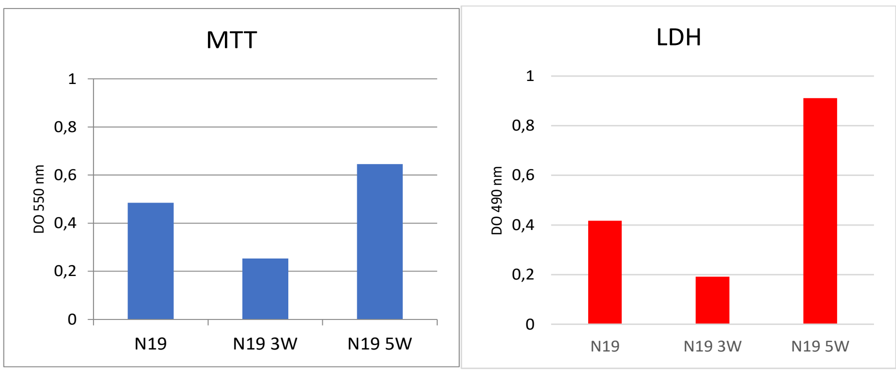

Figure 4.
MTT and LDH tests for the extract in water: methanol from pericarp and mesocarp lyophilized by *Juglans regia*, before and after irradiation with laser beams with a power of 3 W and 5 W

For the lyophilized pericarp and mesocarp methanol extract of *Juglans regia*, the improvement of the characteristics was observed, so that although initially, the extract was cytotoxic, after the treatment and application of an intensity of 5 W the values recorded for MTT exceeded the values for LDH, the extract being compatible (Figure 5).

In the case of sage extract and Echinacea tincture, an improvement in biocompatibility was observed after treatment (Figure 6 and Figure 7).

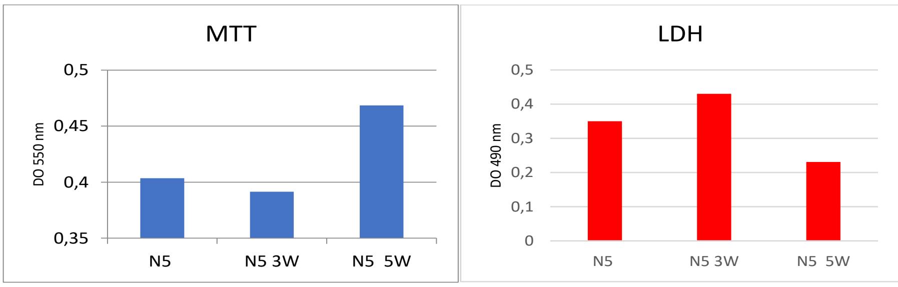

Figure 5.
MTT and LDH tests for lyophilized pericarp and mesocarp methanol extract by *Juglans regia*, before and after irradiation with 3 W and 5 W laser beams

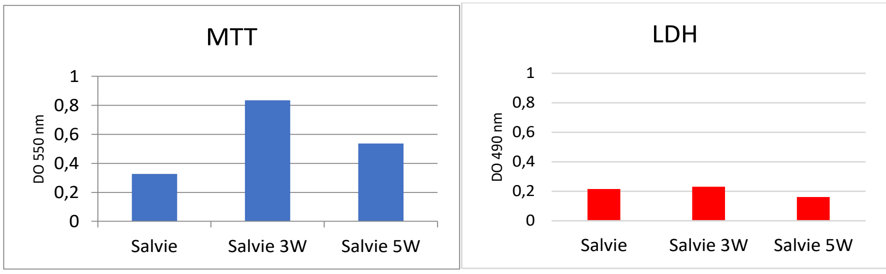

Figure 6.
MTT and LDH tests for *Salvia officinalis* extract, before and after irradiation with 3 W and 5 W laser beams

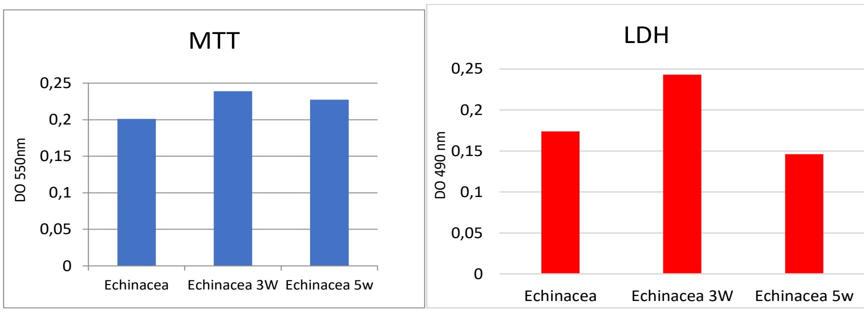

Figure 7.
MTT and LDH tests for Echinacea tincture, before and after irradiation with laser beams with a power of 3 W and 5 W

The diode laser, along with the many benefits, can also have some less positive effects still incompletely determined, so it is necessary to determine the parameters *in vitro* before the use in clinical practice [28].

Due to its good antimicrobial properties and low cytotoxicity, plant extracts treated with 5W laser beam can be used as root canal irrigants, as an alternative to sodium hypochlorite and other used irrigants [27].

The use of herbal therapies and natural products has increased significantly in many disciplines, including dentistry. Numerous plants have been investigated for their potential as an antimicrobial agent in endodontic infections. Some of these plants/plant products are propolis, *Morinda citrifolia*, *Arctium lappa*, licorice, Triphala, *Syzygium aromaticum*, *Ocimum sanctum*, green tea polyphenols, and *Cinnamomum zeylanicum*.

The potency of *Commiphora molmol* (myrrh) in order to be used as an irrigant in endodontic therapy was investigated [4, 5].

The results showed that *Commiphora molmol* (myrrh) extract has antibacterial activity against *E. faecalis* and *F. nucleatum*. Moreover, myrrh extract significantly reduced the colony-forming units of *E. faecalis* and *F. nucleatum* [14].

Licorice extract had a strong antibacterial effect against *E. faecalis* and *Streptococcus mutans*, *Salvadora persica* (miswak extract) showed antibacterial effect on *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Haemophilus influenzae*, *Streptococcus mutans*, and *Lactobacillus*. *Morinda triphala*, Citrifolia juice and green tea polyphenols showed an antibacterial effect against *E. faecalis* and in addition, propolis demonstrated antimicrobial action against *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, coccyx and Gram-positives [10, 12].

Sesquiterpenoids are responsible for the antimicrobial effects of myrrh and *Commiphora molmol* resin was beneficial for the treatment of diseases caused by microbial infections and that the toxicity of *Commiphora species* was limited, induced only by large quantities and mainly involving its volatile oil, causing allergies, nausea and decreased locomotor capacity.

Conclusions

Analysis of the extracts prepared from walnuts (*Juglans regia*), sage (*Salvia officinalis*) and Echinacea tincture (*Echinacea purpurea*), in terms of biocompatibility, showed that irradiation influences their characteristics and their ability to react with metabolic indicators used in testing.

For the ethanol extract from the endocarp (N7), it was observed that after irradiation with a 5 W beam, the number of viable cells increases considerably.

For the methanol extract from lyophilized pericarp and mesocarp by *Juglans regia*, the improvement of the characteristics was observed, so that although initially, the extract was cytotoxic, after treatment and irradiation with a laser beam with an intensity of 5 W, the extract becomes compatible.

Despite high expectations for their implementation in all areas of dentistry, the effects of lasers are still being investigated. In the near future, lasers have the potential to assert themselves as an alternative, a complement to conventional therapies.

Additional studies are recommended, both for lasers and for simple and/or laser-activated plant extracts, before use, on their efficacy as irrigants in root canals against biofilms, biocompatibility, and the ability to eradicate the dentinal smear layer.

Conflict of interest

The authors declare no conflict of interest.

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