Vol 11, Special Issue 1, 2019

INTERNATIONAL JOURNAL OF APPLIED PHARMACEUTICS

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

ISSN - 0975-7058

Research Article

CUMINUM CYMINUM EXTRACT REDUCES DENTAL PULP STEM CELL VIABILITY AT HIGHER CONCENTRATION

LIPUR KURNIAWATI[1], ZAAL HAQ M.[1], ANGGRAINI MARGONO[2]*, DEWA AYU NYOMAN PUTRI ARTININGSIH[2]

Department of Conservative Dentistry, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia. Email: margonodewi@yahoo.com

Received: 27 August 2018, Revised and Accepted: 07 February 2019

ABSTRACT

Objective: *Cuminum cyminum* (cumin) extract has potential antibacterial and antifungal activities and is not toxic for mouse fibroblasts. However, to our knowledge, no research exists investigating the toxicity of cumin extract on dental pulp stem cells (DPSCs). Therefore, we compared the viability of DPSCs after treatment with different doses of the cumin extract (0.1, 0.4, 0.7, and 1.0 mg/mL) at 1 h, 24 h, and 4 days.

Methods: DPSCs were gently evacuated from exfoliated third molars. Subsequently, cumin seeds were extracted by steam distillation to obtain 0.1, 0.4, 0.7, and 1.0 mg/mL concentrations. Then, the cell viability of DPSCs was analyzed using the [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay by calculating the absorbance values using a microplate reader, with the optical density (OD) as the final result.

Results: There were statistically significant differences in the viability of DPSCs ($p<0.05$) between 0.1 and 0.4, 0.7, and 1.0 mg/mL cumin extract, but there was no difference ($p\geq0.05$) in the viability of DPSCs at 1 h in each group.

Conclusion: The cumin extract at all concentrations did not affect the viability of DPSCs at 1 h. However, the cumin extract at 0.4, 0.7, and 1.0 mg/mL decreased the viability of DPSCs at 24 h and 4 days.

Keywords: Toxicity, Cell viability, *Cuminum cyminum*, Dental pulp stem cells.

© 2019 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4. 0/) DOI: http://dx.doi.org/10.22159/ijap.2019.v11s1.16015

INTRODUCTION

Plants are valuable biological natural resources that can be used as medicines because they contain a wide variety of natural chemical compounds with pharmacological effects [1]. Most materials in dentistry, especially endodontic treatments such as sodium hypochlorite and chlorhexidine, consist of highly irritating synthetic materials that are toxic to stem cells, which are the key to regeneration during treatment. To improve the biologic safety, herbal medicines that chemically have the same compounds that can support the regenerative endodontic treatment are selected [2-7].

In general, herbal medicines are considered safer than synthetic drugs. For herbal medicines to be widely used, they must meet the World Health Organization's standardization and quality control criteria to ensure its safety (toxicity) and efficacy locally and systemically [7,8]. The standardization process of herbal medicines consists of various chemical analysis methods based on biocompatibility tests on a medicinal plant extract [1,8]. Cytotoxicity is a sensitive biocompatibility test for assessing toxicity when a substance comes into contact with a particular cell culture (ISO 10993), and it is the best initial step for a biocompatibility test circuit of a substance to a cell or living tissue. A substance is considered non-toxic or biocompatible if the viability test results in a percentage of living cells ≥70% of the control [6-12].

One herb known to have antibacterial and antifungal effects is cumin [13-17]. A study on cumin extract as a root canal medicament reported it to be effective against *Enterococcus faecalis* and biocompatible with L929 fibroblasts compared to 2% chlorhexidine. To date, to our knowledge, no one has examined the toxicity of cumin extract to dental pulp stem cells (DPSCs) [11]. Therefore, the effect of cumin extract on the viability of DPSCs was examined.

METHODS

This experimental laboratory study was approved by the Ethics Committee of Universitas Indonesia, Faculty of Dentistry (Ethics No. 142/Ethical Approval FKGUI/XII/2017-Protocol 051291017). DPSCs were obtained from mature third molars from healthy patients (19–35 years old) at the Dental Hospital of Universitas Indonesia, who did not have any degenerative diseases. Cumin extract (0.1, 0.4, 0.7, and 1.0 mg/mL) was used as the test reagent at 1 h, 24 h, and 4 days.

The procedures were performed in a biohazard cabinet using sterile tools and working procedures. The complete culture medium consisted of Dulbecco's Modified Eagle's Medium (DMEM) containing penicillin–streptomycin–amphotericin (PSA) and 20% fetal bovine serum. The culture medium was filtered using a Sartorius Minisart single-use syringe with a 50 mL sterile filter with a diameter of 0.2 µm and stored in a refrigerator. This research consisted of several stages: DPSC culture, preparation of the cumin extract, flow cytometry, [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) assay, and statistical analysis.

DPSC culture

The extracted third molars (<24 h) were cleaned and immersed in 20 mL of complete culture medium or phosphate-buffered saline and PSA. Teeth were separated using a sterile mortar and pestle. Pulp tissue was obtained with an extirpation needle, minced into 2.0 mm × 2.0 mm × 1.0 mm fragments, incubated in a 15 mL tube with 2.0 mL of 3.0 mg/mL collagenase type I, and left to stand for 1 h. Subsequently, collagenase was washed by complete DMEM and centrifuged at 1000 rpm for 5 min, and the supernatant was discarded. Then, the cells were placed on a 10cm Petri dish with complete DMEM. Cells were incubated in 37°C, within 5% CO_2 until 80% confluent.

Preparation of cumin extract

Cumin extract was obtained by steam distillation. Chemical compound analyses were performed with gas chromatography–mass spectrometry. All cumin extracts were placed in a black bottle.

Flow cytometry test

Flow cytometry was performed by placing each cell in a chamber slide, followed by incubating at 37°C and 5% CO_2 and examining for CD73, CD90, and CD105.

MTT assay test

After 24 h of incubation, the cell culture medium was removed and the test material was expressed as 100 µL per well with three replications. Then, the cells were incubated at 37°C and 5% CO_2 for 4–8 h. The specimens were then divided into two groups: The control group (cells without treatment) and the test group (given cumin concentrations of 0.1, 0.4, 0.7, and 1.0 mg/mL).

As much as 100 µL of MTT was added, and the cells were then incubated at 37°C and 5% CO_2 for 4 h. The cell supernatant was removed, and the formed formazan crystals were dissolved with 70% ethanol. Optical density (OD) readings were conducted using a microplate reader with a wavelength of 560 nm. The OD of each test group was then presented against that for the control group to determine cell viability.

Statistical analysis

All data were analyzed using the Kruskal–Wallis and Mann–Whitney U-tests to compare the OD between test groups. $p<0.05$ was considered to be statistically significant.

RESULTS

The characteristics of DPSCs were seen from the flow cytometry analysis of surface antigen expression. The results were positive for CD90 (98.3%) DPSCs, CD73 (98.5%), and CD105 (71%). Thus, we concluded that the cells are DPSCs.

The test group cells were exposed to cumin extract (concentration: 0.1, 0.4, 0.7, and 1.0 mg/mL) and incubated for 1 h, 24 h, and 4 days. The MTT test and a microplate reader were used to measure the viability. OD was expressed as a percentage of the control group indicating the viability of DPSCs. Median OD and percentage cell viability are noted in Tables 1 and 2. The significance of cell viability comparing both the groups is noted in Tables 3 and 4.

The highest viability of DPSCs was at a concentration of 0.1 mg/mL (123.70±29.94) and at 1 h (102.22±6.25), whereas the lowest viability was at 0.4 mg/mL (17.84±42.22) at 4 days (19.130±46.012).

In all groups, there was a significant difference in the viability of DPSCs on the basis of the dose ($p\leq0.05$) but not time ($p\geq0.05$). Furthermore, Mann–Whitney U-test was performed to determine the significance in each group.

In the 1st h, there was no significant difference ($p<0.05$) in the viability of DPSCs in all groups. There was a significant difference between Groups 1 and 2, Groups 1 and 3, and Groups 1 and 5 at 24 h and between Groups 1 and 2, Groups 1 and 3, Groups 1 and 4, Groups 1 and 5, and Groups 2 and 3 on day 4; (Table 4).

DISCUSSION

Herein, we studied DPSC cultures. According to the International Society for Cellular Therapy, one criterion for mesenchymal stem cells is that the cells should positively express marker proteins, such as CD73, CD90, and CD105. In our study, flow cytometry was positive for CD90 (98.3%), CD73 (98.5%), and CD105 (71%). In the 1st h, there was no significant difference ($p<0.05$) in the viability of DPSCs in all groups (Table 4), showing that the cumin extract did not cause DPSC death at exposure for up to 1 h.

Table 1: Median cell viability and SD in the control and test groups based on concentration

Group	Median±SD	Minimum	Maximum
Control	100±0.00	–	–
Concentration (mg/mL)			
0.1	123.70±29.94	101.099	201.126
0.4	17.84±42.22	0.696	107.692
0.7	48.79±45.96	14.820	115.584
1.0	40.74±36.47	11.826	97.674

SD: Standard deviation

Table 2: Median value of cell viability and SD in control and test groups based on time

Group	Median±SD	Minimum	Maximum
1 h	102.22±6.25	95.604	115.385
24 h	81.481±34.569	0.696	136.522
4 days	19.130±46.012	16.739	121.087

SD: Standard deviation

Table 3: Significance of cell viability of control and test groups based on time and dose

DPSC viability groups	Significant value
Dose based (five groups)	0.000*
Time based (four groups)	0.236

*Kruskal–Wallis test; p≤0.0

At 24 h, there was a significant difference between Groups 1 and 2, Groups 1 and 3, and Groups 1 and 5. After 24 h, the number of cells decreased further, especially at concentrations of 0.4 and 1.0 mg/mL. However, at a concentration of 0.1 mg/mL, the number of cells in the test group was higher than that in the controls. The viability of DPSCs at a concentration of 0.7mg/mL did not differ significantly from that of controls.

At 4 days, there were significant differences in Groups 1 and 2, Groups 1 and 3, Groups 1 and 4, Groups 1 and 5, and Groups 2 and 3. After 4 days, the number of cells was very much reduced at the concentrations of 0.4, 0.7, and 1.0 mg/mL compared to controls, with the least number of cells at 1.0 mg/mL, whereas at 0.1 mg/mL, the number of cells was higher than that in the controls.

At 24 h, the average cell viability was reduced, except at 0.1 mg/mL. All cells treated with concentrations above 0.1 mg/mL showed increasingly low viability as time passed. In synthetic drugs, if the concentration had been higher, it would be more effective. Unlike the case with herbs, the graph of the relationship among the concentration, effectiveness, and toxicity is not linear. Therefore, finding the optimum dose of the herbal compound is necessary to determine the maximum effect with minimum toxicity.

Cuminaldehyde (chemical formula: $C_6H_{12}O$), the main component in cumin (approximately 35–63%), is a volatile compound with a major role in providing a stinging smell to cumin. It has an important antibacterial activity and has an antifungal effect because it inhibits the filament and yeast formation in fungi [17,18].

Aldehydes are electrophilic (electron-deficient species) that forms covalent bonds with nucleophilic (electron-rich) target cells. The resulting formation of these bonds may interfere with the function of the enzymes, DNA, protein structures, and other macromolecules, thus causing the inhibition of cellular processes and ultimately toxicity to cells. Potential of toxic depends on covalent formation which has formed by cell and was decised with a large of aldehyde concentration. Therefore, it can be concluded that the magnitude of the toxicity of the

Table 4: Significance of cell viability of control and test groups based on dose against time

T/C	1 and 2	1 and 3	1 and 4	1 and 5	2 and 3	2 and 4	2 and 5	3 and 4	3 and 5	4 and 5
1 h	0.487	0.487	0.487	0.487	0.827	0.275	0.827	0.275	0.827	0.127
24 h	0.037*	0.037*	0.487	0.037*	0.05	0.05	0.05	0.127	0.513	0.05
4 days	0.037*	0.034*	0.037*	0.037*	0.046*	0.05	0.05	0.268	0.825	0.127

*T/C: Time/concentration. 1, control; 2, 0.1 mg/mL; 3, 0.4 mg/mL; 4, 0.7 mg/mL; 5, 1.0 mg/mL

aldehyde is directly proportional to time and concentration. It will be more toxic to the cells if exposed longer to a higher concentration of aldehyde [19].

Cumin extract at concentrations of 0.1, 0.4, 0.7, and 1.0 mg/mL can be used for 1 h because it does not affect the viability of DPSCs. However, at 24 h and at 4 days, it can reduce the viability of DPSCs, except at the concentration of 0.1 mg/mL. From the result of this study, it is expected that, in the future, cumin extract can be an alternative irrigation material in endodontic treatment.

CONCLUSION

The cumin extract at all concentrations did not affect the viability of DPSCs at 1 h. However, the cumin extract at all concentration decreased the viability of DPSCs at 24 h and 4 days, except for the concentration of 0.1 mg/mL.

ACKNOWLEDGMENTS

This research is approved and funded by HIBAH PITTA from Directorate Research and Community Engagement Universitas Indonesia. We wish to thank Prodia Stem Cell Indonesia for their support of this study.

CONFLICTS OF INTEREST

The author reports no conflicts of interest.

REFERENCES

1. Saifudin A, Rahayu V, Teruna HY. Standardization of Natural Medicine. 1st ed. Yogyakarta: Graha Ilmu; 2011.
2. Hristov K, Gateva N, Stanimirov P. Influence of irrigants and intracanal medicaments on the vitality of stem cells from apical papilla - An overview. Int J Sci Res 2017;6:999-1002.
3. Petters OA, Peters C, Basrani B. Cleaning and shaping the root canal system. In: Hargreaves KM, Berman LH, Rotstein I, editors. Cohen Pathways of the Pulp. 11th ed. Canada: Elsevier; 2016. p. 209-78.
4. Simon S, Smith AJ. Regenerative endodontics. Br Dent J 2014;216:E13.
5. Patel AB. Dental stem cells and their potential role in regenerative dentistry. Int J Dent Heal Sci 2015;2:852-61.
6. Taheri JB, Azimi S, Rafieian N, Zanjani HA. Herbs in dentistry. Int Dent J 2011;61:287-96.
7. Aly LA. Stem cells: Sources, and regenerative therapies in dental research and practice. World J Stem Cells 2015;7:1047-53.
8. Liu WJ. Traditional Herbal Medicine Research Methods. Singapore: John Wiley and Sons, Inc.; 2011.
9. General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine World Health Organization; 2000.
10. Laredo-Naranjo MA, Carrillo-Gonzalez R, De La Garza-Ramos MA, Garza-Navarro MA, Torre-Martinez HH, Del Angel-Mosqueda C, *et al.* Antimicrobial properties and dental pulp stem cell cytotoxicity using carboxymethyl cellulose-silver nanoparticles deposited on titanium plates. Acta Biomater Odontol Scand 2016;2:60-7.
11. Abbaszadegan A, Gholami A, Ghahramani Y, Ghareghan R, Ghareghan M, Kazemi A, *et al.* Antimicrobial and cytotoxic activity of cuminum cyminum as an intracanal medicament compared to chlorhexidine gel. Iran Endod J 2016;11:44-50.
12. Service M. Assessing biocompatibility. In: Kucklick TR, editor. The Medical Device R and D Handbook. 2nd ed. Boca Raton, Florida: CRC Press Taylor and Francis Group; 2013.
13. ISO Standard 1993. Biological Evaluation of Medical Devices - Parts 1 – 2; 1993.
14. Johri RK. Cuminum cyminum and carum carvi: An update. Pharmacogn Rev 2011;5:63-72.
15. Al-Snafi AE. The pharmacological activities of *Cuminum cyminum* - A review. IOSR J Pharm 2016;6:2250-3013.
16. Kamble VA. *In vitro* anti-fungal activity of *Cuminum cyminum* (Cumin Seed) essential oil against clinical isolates of candida species. Am J Phytomed Clin Ther 2015;3:264-75.
17. Romagnoli C, Andreotti E, Maietti S, Mahendra R, Mares D. Antifungal activity of essential oil from fruits of indian *Cuminum cyminum*. Pharm Biol 2010;48:834-8.
18. Kaur D, Sharma R. An update on pharmacological properties of cumin. Int J Res Pharm Sci 2012;2:14-27.
19. LoPachin RM, Gavin T. Molecular mechanisms of aldehyde toxicity: A chemical perspective. Chem Res Toxicol 2014;27:1081-91.