WELCOME NOTES

Dear Colleagues,

It’s great honor to welcome you to Surabaya and 2nd International Dentisphere 2013 seminar which held on 8-9th November, 2013 at Shangrilla Hotel. My great appreciation to all the speakers from Japan, Korea, Thailand, Singapura and Indonesia, thank you for the contribution and participation and your willingness to come and share the valuable knowledge and experience. It’s been honor to us that this forum may be apart of strong role as quality control mechanism to ensure sustainability and continuous improvement of dentist.

The theme of this 2nd International Dentisphere is “Current Concept In Dentistry” This is addressed to meet our aims to provide our nation a generation of professional and skillfull dentist with continuously update knowledge. We hope this event will be increase our profesionalism to all dentist and participants.

My appreciation to the committee, for aranging this event very well. Hope the seminar will be well done accomplished tomorrow. Also, I would like to thank all sponsors who support this event. For the speaker, thank you for the contribution support of the seminar.

And for all the participants, thank you for joining the 2nd International Dentisphere, please enjoy the seminar and the events. I would like to ask for apologize if maybe in some ways we have some limitations in serving you on the event. Finally, I hope we all could get the benefit and advantage from this seminar to raise our professionalism in dentistry, in each of our ways

Sincere regards,

Dr. Dian Mulawarmanti., drg., M.S

Dean Faculty of Dentistry Hang Tuah University
WELCOME NOTES

Dear Colleagues,

It is a great pleasure for us to be the organizer of the 2\textsuperscript{nd} Dentisphere from faculty of dentistry Hang Tuah University. We extend our warmest welcome to all Participants, Speakers, and Sponsors that make this 2\textsuperscript{nd} Dentisphere to be a successful conference.

Under the theme of “Current Concept of Dentistry”, this meeting will offer a platform to learn and exchange ideas with a host of internationally and national speakers. 2\textsuperscript{nd} Dentisphere will provide participants with unique opportunities to develop their professional knowledge and skills as well as to network with another audience. I also strongly encourage you to take advantages of the presence of dental companies to keep up to date with evolving technologies of equipment and the latest dental materials. We do hope that this seminar will allow all participants to capitalize enough knowledge and experience keep in touch with issues world wide., Dental Health.

Lastly I wish to thank all Participants, Distinguish Speakers, Sponsors and all who contribute for the success of the 2\textsuperscript{nd} Dentisphere in Surabaya. Hope you not only have an event for developin our professionalism, but also you could enjoy a nice stay and have a memorable excursion on Surabaya. Thank you for your kind attention, have a nice, enjoy and fruitful discussion and God Bless You.

Sincere regards,

\textbf{Aprillia drg. Sp.KG}

Chairperson 2\textsuperscript{nd} Dentisphere
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IN VITRO CYTOTOXICITY EVALUATION OF Nannochloropsis occulata sp EXTRACT TO HUMAN GINGIVAL FIBROBLAST STEM CELLS

Syamsulina Revianti, Kristanti Parisihni
Department of Oral Biology, Faculty of Dentistry, Hang Tuah University, Surabaya – Indonesia

ABSTRACT

Background: Nannochloropsis occulata sp has many biological activities such as analgesic, anti-inflammatory, antioxidant and antibacterial properties thus potentially explored as therapeutic agent in oral disease.

Purpose: This study aims to evaluate cytotoxic effect of Nannochloropsis occulata sp extracts in human gingival fibroblast stem cells.

Methods: The study is an experimental laboratories research with post test only control group design. Nannochloropsis occulata sp extracts in concentration of 0.3125%; 0.625%; 1.25%; 2.5%; 5%; 10%; 20%, 40%; and 80% were tested its cytotoxicity on human gingival fibroblast stem cells. For the in vitro toxicity assay, serial concentration of Nannochloropsis occulata sp extracts was applied to human gingival fibroblast stem cells cultures in conditioned media. The cells (1x10^5) were cultured in 96 well plates and allowed to attach for 5 days before treatment with serial concentration of Nannochloropsis occulata sp extracts for 24 h period. Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) to purple formazan. The data concerning cell viability were statistically analyzed using two-way ANOVA test and LSD multiple comparison test at 5% significance level.

Result: Nannochloropsis occulata extracts showed toxicity in the concentration of 2.5% above and not cytotoxic in the concentrations below (p<0.05).

Conclusion: Nannochloropsis occulata sp extracts was not cytotoxic effect on human gingival fibroblast stem cells in the concentration below 2.5%.

Key word: Nannochloropsis occulata sp extracts, cytotoxicity, fibroblast gingiva stem cells

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INTRODUCTION

Microalgae, as an evolutionary form of organism, are showing an extraordinary adaptation in the ocean. Marine microalgae are a promising source of organisms that can be cultured and targeted to isolate the broad spectrum of functional metabolites. As a consequence, biochemically and ecologically significant differences have been gained a vast microalgae diversity and associated a broad spectrum of secondary metabolites. Thus, researchers are continuously mining the bioactive components from marine microalgae to determine pharmacological and medicinal values in many parts of the world. Therefore, marine microalgae have emphasized that research on their natural products are useful for the cure and for the alleviation of human diseases.

Hence, researchers have been interested in cultured marine microalgae in order to reveal the biochemical constituents of their crude extracts and to determine which components have pharmacological effects.

Microalgae such as *Nannochloropsis oculata* sp consist of many nutrients that include a rich source of protein, poly unsaturated fatty acids, carbohydrates, minerals, vitamins, pigments, and secondary metabolites. *Nannochloropsis oculata* sp as many biological activities such as analgesic, anti-inflammatory, antioxidant and antibacterial properties thus potentially explored as therapeutic agent in oral disease. Thus, its targeted for the research and revealed the health effects, for human well-being as well as for topical applications.

*Nannochloropsis oculata* sp is a unicellular marine microalgae that is an important food source and additive used in the commercial industry. In order to make these extract proper to be an agent of therapeutic, the harmful chemical or organic solvent in the extraction medium is required to be eliminated In this study, a marine microalgae species *Nannochloropsis oculata* was examined its potential medical used by screening its cytotoxicity using in vitro assays. As an attempt to explore its potential use as topical agent in oral disease *Nannochloropsis oculata* was evaluated its cytotoxicity in human gingival fibroblast stem cells.

MATERIALS AND METHODS

**Preparation of Nannochloropsis oculata sp extract.** *Nannochloropsis oculata* sp extract were obtained from Situbondo, East Java-Indonesia. The medium of RPMI 1640, Dulbecco’s Modified Eagle’s Minimum Essential Medium (DMEM), Penicilllin, Streptomycin, Amphotericin, and trypsin were obtained from Gibco (Carlsbad, CA, USA). All other chemicals were analytical or pharmaceutical grade and obtained from Sigma-Aldrich.
Chemicals (Bornem, Belgium). The *Nannochloropsis occulata* sp extract powder was collected via rotary evaporation and drying under freeze dryer. The *Nannochloropsis occulata* sp extract were collected by centrifugation (4500 rpm, 4°C, 30 min) and washed twice with deionized water. Microalgal pellet was stored in a freezer at -70°C for 24 h. The sample was then freeze-dried at -50°C at 5 m torr. The freeze-dried sample was ground to a fine powder. For the water extraction, the powdered sample (5 g) was extracted with 500 ml distilled water at 25°C for 24 h. The water extract was collected by filtering and was then concentrated by mixed with an aqueous solution.

**Stem Cells Culture.** Human gingival fibroblasts stem cells were obtained from biopsies of the attached gingival of sound permanent molar teeth of healthy persons. Informed consent based on an appropriate protocol was obtained from the donors. The biopsies were stored at 4°C for at most 24 hours in collection medium (RPMI 1640 supplemented with penicillin 100 U/mL, streptomycin 100 mg/mL, and amphotericin 2.5mg/mL) prior to amplification. The gingival tissues were cut into 1 to 2 mm³ pieces then washed three times by RPMI 1640. After that, the cut biopsies were placed into 25 cm² tissue culture flasks. The explants were incubated with culture medium consisting of DMEM 90%, 10mM HEPES, glucose (4.5 g/L), NaHCO3 (3.7 g/L), penicillin (100 U/mL), streptomycin (100 mg/mL), and amphotericin (2.5mg/mL), supplemented with 10% heat-inactivated fetal calf serum (FCS). The tissue samples were grown at 37°C in a humidified atmosphere of 10% carbon dioxide in the air. When outgrowth of cells was observed, the medium was replaced twice weekly until cells reached confluence. Cells were detached from the monolayer by a brief treatment with trypsin-EDTA (0.25% trypsin, 0.02% EDTA) and recultured in 75 cm² tissue flasks until confluent monolayer was reobtained.

**Research design.** The study is an experimental laboratories research with post test only control group design. *Nannochloropsis occulata* extracts in concentration of 0.3125%; 0.625%; 1.25%; 2.5%; 5%; 10%; 20%, 40%; and 80% were tested its cytotoxicity on human gingival fibroblast stem cells.

**MTT Cytotoxicity Test.** The MTT cytotoxicity test is tests for in vitro cytotoxicity. Cells (1 × 10^5 cells/mL) in DMEM of 50μL were seeded into 96-well plates and maintained in culture for 24 hours to form a semiconfluent monolayer. They were then exposed to the
*Nannochloropsis occulata* extracts (50 μL) over a range of 0.3125%; 0.625%; 1.25%; 2.5%; 5%; 10%; 20%; 40%; and 80% concentration. For the in vitro toxicity assay, serial concentration of *Nannochloropsis occulata* extracts was applied to human gingival fibroblast stem cells cultures in conditioned media. The cells (1x10^5) were cultured in 96 well plates and allowed to attach for 5 days before treatment with serial concentration of *Nannochloropsis occulata* extracts for 24 h period. Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) to purple formazan. After 24-hour exposure, the formazan formations were determined for each treatment concentration by ELISA reader at a wavelength of 570 nm. The relative viability of the treated cells as compared to the control cells were expressed as the % cytoviability, using the following formula:

\[
\text{% Cell viability} = \frac{A_{\text{sample}}}{A_{\text{control}}} \times 100\%
\]

Note : A sample is mean value of the measured optical density of the treated cells; A control is mean value of the measured optical density of the control cells.\(^9,10,11\)

**RESULTS**

The result of relative viability of the gingival fibroblast stem cells treated with *Nannochloropsis occulata sp* extracts serial concentration as compared to the control cells was expressed in Table 1.

**Table 1. Cytoviability percentage of human gingival fibroblasts stem cells treated by serial concentration of *Nannochloropsis occulata sp* extracts as compared to the control cells**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
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<td>Nanochloropsis 80%</td>
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<td>0.0039763</td>
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<tr>
<td>Control cell</td>
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<tr>
<td>Nanochloropsis 10%</td>
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Fig 1. Graphic of Cytoviability percentage of human gingival fibroblasts stem cells treated by serial concentration of *Nannochloropsis occulata sp* extracts as compared to the control cells

Base on data at table 1 and graphic 1, showed that increased concentration of *Nannochloropsis occulata sp* extracts concentration exposure on the cells resulted in the decreasing of cytoviability percentage of human gingival fibroblast stem cells.

Further statistical analysis by two-way ANOVA test and LSD multiple comparison test at 5% significance level was shown in table 3, which described that there was any significant influence of the treated extracts concentration on the cytoviability of human gingival fibroblast (p > 0.05). *Nannochloropsis occulata sp* extracts showed cytotoxic effect where the cells were viable less than 50% after the treatment with certain concentrations (p>0,05). *Nannochloropsis occulata sp* extracts cytotoxic effect to the human gingival fibroblast stem cells in concentration of 2,5% above where the the viable cells apperaed less than 50% after the treatment, but it showed no cytotoxic effect on the treatment of the extract in lower concentration 0,3125-2,5% (p<0,05). *Nannochloropsis occulata* extracts showed toxicity in the concentration of 2,5% above and not cytotoxic in the concentrations below (p<0,05).

**DISCUSSION**

In the field of biomaterials for therapeutic agent, it is necessary to consider aspects of security, such as elimination of cytotoxicity and other harmful effects of the material to be used. The cytotoxicity of an agent means the toxicological risks caused by a material or its extract in a cell culture. The interactions of the materials and their components with the
cells at a molecular level are responsible for tissue reactions, such as inflammation, necrosis, immunological alterations, genotoxicity, and apoptosis. During the last years, the interest of in vitro systems as an alternative to animal experiments in toxicological research has been steadily increasing.12

<table>
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<th>(I) Group</th>
<th>(J) Group</th>
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</table>
In the present study, the cytotoxicity of *Nannochloropsis occulata* sp extracts which is aimed to be used as topical application agent is analyzed using a cell culture model of primary human gingival fibroblasts stem cells. Like other tissues, normal fibroblast function is critical to maintain oral mucosal function for optimal healing. Gingival fibroblasts are chosen due to their availability and culturing characteristics.\(^8\)

Cytotoxicity test of *Nannochloropsis occulata sp* extracts was performed on human gingival fibroblast stem cells. Stem cells from human gingiva, a tissue source easily accessible from the oral cavity, namely, gingiva-derived mesenchymal stem cells (GMSCs) exhibited clonogenicity, self-renewal, and multipotent differentiation capacities. Most importantly, GMSCs were capable of immunomodulatory functions, specifically suppressed peripheral blood lymphocyte proliferation, induced expression of a wide panel of immunosuppressive factors including IL-10, IDO, inducible NO synthase (iNOS), and cyclooxygenase 2 (COX-2) in response to the inflammatory cytokine, IFN-gamma.\(^13\)

In this study we used indirect test, in which the rate of cell number) and the metabolic activity (MTT) have indicated the degree of cytotoxicity of *Nannochloropsis occulata sp* extracts. The effect of *Nannochloropsis occulata sp* extracts on human gingival fibroblast stem cells viability measured by MTT test. MTT is a yellow water-soluble tetrazolium dye which is reduced by live cells to a purple formazan product insoluble in aqueous solutions. The amount of formazan generated is directly proportional to the number of viable cells.\(^8\)

As can be seen from Table 1, *Nannochloropsis occulata sp* extracts exposure of 0.3125%; 0.625%; 1.25%; 2.5%; 5%; 10%; 20%, 40%; and 80%. during 24 hour of incubation induces the cytoviability of the gingival fibroblast stem cells to be 0.3125-80% in comparison to the control cells. It is proved that there is an increasing cytoviability on the decreasing of the extract concentration exposure. It means that more concentration exposure tends to lower the gingival fibroblast stem cells cytoviability.

Cytotoxicity testing includes numerous methods, both qualitative and quantitative. In this study we used indirect test, in which the rate of cell growth (cell number) and the metabolic activity (MTT) have indicated the degree of cytotoxicity of sea cucumber extract. Result showed cytotoxic activity on human gingival fibroblast stem cells after treated with *Nannochloropsis occulata sp* extracts start in concentration of 2.5%, shown by the cell viability less than 50% (\(p >0.05\)).

Cell culture can be used to screen for toxicity both by estimation of the basal functions of the cell or by tests on specialized cell functions. General toxicity tests, aimed
mainly at detection of the biological activity of test substances, can be carried out on many cell types, one of the common one is fibroblast cell.16

Fibroblast forms the collagen and extracellular matrix. It provides the structural scaffold to many tissues and have important role in wound healing and the formation of the main connective tissue. Fibroblast cells have the function to maintain the integrity of the connective tissue by secreting extracellular matrix precursors continuously.17,18

Recently, toxicity test have been developed and stem cells were explored regarding to some basic consideration in some advantage in the technique and result. Human stem cells are potentially attractive reagents for predictive toxicology, particularly if they can be shown to be a reliable, large-scale source of differentiated human cells. First, stem cells and their cellular derivatives could form the basis of in vitro assays that can be miniaturized and adapted to high throughput screening platforms. Use of cell-based toxicity assays during the early phases of drug development could decrease the cost of attrition at a later stage in development, and would also provide the opportunity to optimiz’e a chemical’s safety profile through targeted medicinal chemistry. Second, the use of human cells could increase the correlation between safety studies and clinical trials, an important benefit since conventional animal models of toxicity are not always predictive of human responses. Finally, stem cells that are generated from adult tissues (iPS cells) could allow models to be created from individuals with a diverse range of drug susceptibilities, resistances or disease, which could reduce the rate of adverse effects within patient subpopulations.19,20

CONCLUSION

Nannochloropsis occulata extracts was not cytotoxic effect on human gingival fibroblast stem cells in the concentration below 2,5%, can be described as a good candidate for the future therapeutic uses.

REFERENCE

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