Original Article

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The Effect of *Ocimum sanctum* Oil on Human Squamous Cell Carcinoma (HSC-4) *In Vitro*

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Objectives: This study aimed to investigate the effect of oil from *Ocimum sanctum* on cytotoxicity and cell migration of human squamous cell carcinoma.

Materials and methods: The concentrations of *Ocimum sanctum* oil used in this study were 0.02%, 0.002%, 0.0002% and 0.00002% (v/v). Human squamous cell carcinoma (HSC-4) at a concentration of $1x10^5$ cells/ml was seeded and cultured in 96-well plate. The percentage of cell viability was analyzed by MTT assay and the migration areas were measured using Image-Pro Plus version 7.

Results: It was found that the percentages of human squamous cell carcinoma viability after exposure to 0.02, 0.002, 0.0002 and 0.00002% (v/v) of *O. sanctum* were 94.44±4.216, 95.13±3.061, 105.55±3.14 and 107.63±3.54, respectively (Table1). Regarding cell migration, all concentrations of *O. sanctum* could inhibit HSC-4 migration.

Conclusion: *O. sanctum* oil at concentration of 0.02, 0.002, 0.0002 and 0.00002% (v/v) had no cytotoxic activity on HSC-4 whereas inhibitory effect on cell migration was observed. Our data could be basic knowledge for the benefit of using this oil in oral cancer prevention or treatment. Further studies are still needed to clarify mechanisms of actions of this oil.

Keywords: Cytotoxicity, Migration, *Ocimum sanctum*, Squamous cell carcinoma

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Introduction

Oral cancer is one of the highly prevalent cancers worldwide and a leading cause of mortality in certain regions like South-Central Asia. [1] From a report of the World Health Organization, in some Asian-Pacific countries, the incidence of oral cancer (cancer of the lip and oral cavity) is within the top three of all cancers. [2] In Thailand, cancer of the oral cavity is a serious health problem. The incidence is highest in the southern region of the country, where oral cancer is the second leading cancer in males and the sixth in females. Based on this evidence, many researchers, therefore, paid attention to the human squamous cell carcinoma and also the methods to prevent or cure the disease. [3]

Ocimum Sanctum Linn (Tulsi or Holy Basil), belonging to the family Labiatae, is cultivated abundantly throughout the tropical and semitropical region of India and other Asian countries. *O. Sanctum* contains many kinds of eugenol [4], urosolic acid, alkaloids, flavonoids, tannins, and carbohydrates. [4] Several pharmacological properties of *O. Sanctum* oil have been reported to have anti-fungal activity [5-7], anti-bacterial activity [8], anti-caries activity [9], anti-viral activity [10], and anti-cancer effect. [11] The previous study of Buddhadev *et al*. revealed that the ethanolic extract of *O. sanctum* mediated a significant reduction in Sarcoma-180 tumor cell size and an increase lifespan of mice with this tumor. [12] However, no information regarding its effect on human squamous cell carcinoma exists. Therefore, the aim of this study was to investigate

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the effect of *O. sanctum* oil on human squamous cell carcinoma (HSC-4).

Material and Methods

Cell Culture

Human squamous cell carcinoma (HSC-4) was obtained from Dr. Yohko Kohno Department of Oral Pathology, School of Dentistry, Showa University, Japan. The cells were seeded and cultured in Dulbecco's modified Eagle's medium (DMEM), (Gibco, USA) supplemented with 10% fetal bovine serum (FBS) (Hyclone, UK) in humidified atmosphere of 95% air and 5% CO_2 at 37°C.

Ocimum sanctum oil

A 0.2 ml of *O.sanctum* oil purchased from Botanic Essence, Bangkok, Thailand was dissolved in 1 ml of dimethyl sulfoxide (DMSO). The solution of the oil was then added to DMEM to provide *O. sanctum* oil extract at concentrations of 0.00002%, 0.0002%, 0.002% and 0.02% (v/v). The extracts were prepared freshly for each experiment.

MTT Cytotoxicity test

HSC-4 cells were seeded in 96-well plate (Costar, Conning life Science, USA) at $1x10^4$ cells/ml (100 µl per well) and incubated at 37°C, 5% CO_2 in 95% relative humidity for 24 hours. Afterwards, the culture medium was removed and 100 µl of each concentration of *O. sanctum* extract in DMEM was added to each well. The 0.1% ZDEC polyurethane film (RM-A)[4] was used as a positive control whereas Thermanox® Plastic coverslip was a negative control. The toxicity to the human squamous cell carcinoma was evaluated using MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide, thiazolyl blue) assay. After incubation at 37°C for 2 hours, MTT solution was discarded and each well plate was washed with 100 µl of phosphate buffered saline (PBS) solution. Then, 100 µl of isopropanol was added to each well and continuously shaked for 30 minutes to dissolve formazan out from the cells. The amount of viable cells was directly proportional to the value of the formazan product created which was evaluated by microtiter plate reader (EPOCH, Bio-Tek Instrument, USA) at 570 nm. The test was repeated three times. The percentage of cell viability was calculated by the following equation [14-15] and statistical analysis was done by Kruskal-Wallis test using SPSS version 18.0

$$\% \text{ cell viability} = \frac{OD_{Sample} \times 100}{OD_{Control}}$$

OD_{Sample} = the absorbance at 570 nm of sample

$OD_{Control}$ = the absorbance at 570 nm of control

Migration assay

The human squamous cell carcinoma at a concentration of $1.5x10^5$ cells/well was prepared in 24-well plate and maintained in CO_2 incubator for 24 hours until establishing a monolayer. Then, the cells were scratched with blue pipette tip and 500 µl of *O. sanctum* oil at concentrations of 0.02%, 0.002%, 0.0002% and 0.00002% (v/v) were added to each well and incubated for 24 hours. The 10% FBS in DMEM was used as a control. After incubation, the plate was rinsed with PBS, cells were fixed 100% methanol for 2 minutes and stained with 0.4% toluidine blue. The migration areas were measured by using Image-Pro Plus version 7 compared with that of control.

Result

MTT Cytotoxicity test

The result showed that the percentages of human squamous cell carcinoma viability after exposure to 0.02, 0.002, 0.0002 and 0.00002% *O. sanctum* were 94.44±4.216, 95.13±3.061, 105.55±3.14 and 107.63±3.54, respectively (Table 1). No cytotoxicity was observed after exposure to all concentrations of *O. sanctum.*

Migration assay

It was found that all concentrations of *O. sanctum* could inhibit HSC-4 migration (0% migration was observed) while 60% migration was examined in a control (Figure 1).

Discussion

Basil or Kra-proa in Thai, with a scientific name of *O. sanctum*, is commonly used for medical treatment in the form of an essential oil. In this study, *O. sanctum* oil was used to evaluate the toxicity and migration of HSC-4 cells. It was demonstrated that the *O. sanctum* oil presented more than 90% of cell viability and exhibited inhibitory effect on cell migration. This was in contrast to the result of shivpuje *et al*. [13] who noticed a significant cytotoxic activity of *O. sanctum* extract on oral epidermal carcinoma cell line. The difference may be due to many factors such as the climate, season and geographic area of harvested herbs as well as the method of extraction. *O. sanctum* has been reported to consist of a large number of phytochemicals and volatile oils (eugenol, eugenol methylether, carvacrol, rosmarinic acid, ursolic acid, linalool and vicenin-2). Even though the mechanisms of action of *O. sanctum* oil have not been documented, several chemical compounds may play role in these effects.

Table 1 The percentages of cell viability of human squamous cell carcinoma after treated with various concentrations of *O. sanctum* oil

O. sanctum % (v/v)	% cell viability mean ± SD
0.02	94.44±4.22*
0.002	95.13±3.06
0.0002	105.55±3.14
0.00002	107.63±3.54
negative Control	94.24±1.55
positive Control	18.48±1.34

* Significant difference, Kruskal-Wallis test (p-value < 0.05)
negative control : Thermanox® Plastic coverslip
positive control : 0.1% ZDEC polyurethane film

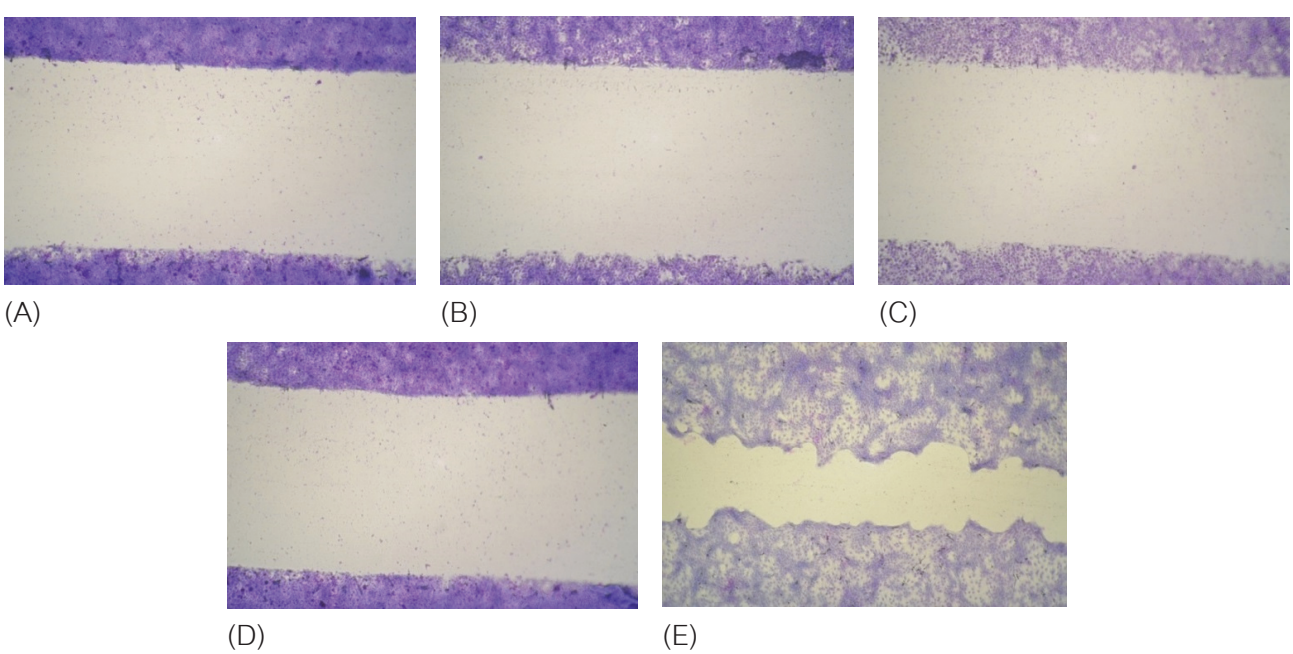


Figure 1 The HSC-4 cell migration after treated with various concentrations of *O. sanctum* oil: (A) 0.02%, (B) 0.002%, (C) 0.0002%, (D) 0.00002% and (E) control

Conclusion

O. sanctum oil at concentrations of 0.02%, 0.002%, 0.0002% and 0.00002% (v/v had no cytotoxic activity on HSC-4 whereas inhibitory effect on cell migration was observed. Our study was the preliminary experiment to give basic information for the beneficial effect of *O. sanctum* oil in cancer prevention or treatment. Further studies are required to confirm the effect of *O. sanctum* on oral cancer cells and the mechanisms of its action.

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