



INVESTIGATION OF CYTOTOXIC EFFECTS OF EUGENIA CARYOPHYLLUS (CLOVE)

Eugenia Caryophyllus (Karanfil)'un Sitoksisitesinin Araştırılması

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ABSTRACT

Objectives: *Eugenia caryophyllus* clove has been used as a traditional medicinal herb for thousands of years. It is used as an analgesic for tooth and toothache with the effect of eugenol substance included in clove. *Eugenia caryophyllus* is used at toothpaste and some mouthwash. The aim of this study was determined of *in vitro* cytotoxic effects *Eugenia caryophyllus* on fibroblast cells.

Materials and Methods: Cytotoxic activity of *Eugenia caryophyllus* were incubated at 24 and 48 h.

Results: In the present study, toxicity of clove that is used as a pain reliever in dentistry has examined on fibroblast cells. The IC₅₀ values of *Eugenia caryophyllus* were found 57.92 ± 0.98 µg/mL in 24 h, 37.97 ± 0.74 µg/mL in 48h.

Conclusions: It is found that the acceptable level of cytotoxicity of *Eugenia caryophyllus*. There is need to further studies in this area.

Keywords: *Eugenia caryophyllus*, Cytotoxicity, Cell culture

ÖZ

Amaç: *Eugenia caryophyllus* binlerce yıldır geleneksel şifalı bitki olarak kullanılmaktadır. Karanfil içerisindeki öjenol maddesinin etkisi ile diş ve diş ağrıları için analjezik olarak kullanılır. Karanfil, diş macunu ve bazı gargaralarda kullanılmaktadır. Bu çalışmanın amacı *Eugenia caryophyllus* in vitro sitotoksik etkileri fibroblast hücre hatları üzerinde belirlemiştir.

Gereç ve Yöntem: *Eugenia caryophyllus*'un sitotoksik aktivitesi XTT testi kullanılarak değerlendirildi. *Eugenia caryophyllus* 24 ve 48 saatlerde inkübe edildi.

Bulgular: Bu çalışmada diş hekimliğinde ağrı kesici olarak kullanılan karanfil toksisitesi fibroblast hücreleri üzerinde incelenmiştir. *Eugenia caryophyllus*'un IC₅₀ değerleri 24 saat de 57,92 ± 0,98 µg / mL, 48 saatte de 37,97 ± 0,74 µg / mL olarak bulundu.

Sonuç: *Eugenia caryophyllus*'un kabul edilebilir düzeyde sitotoksitesinin olduğu bulunmuştur. Bu alanda daha ileri çalışmalara ihtiyaç vardır.

Anahtar kelimeler: *Eugenia caryophyllus*, Sitotoksitesite, Hücre kültürü

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INTRODUCTION

Nowadays, the extraction and evaluation of pure and especially main active component of medical plants and essential oils of these plants is very important both in scientific and in terms of economy.¹ The pharmacological properties of essential oil and its components are also examined and it is stated that they may be beneficial in medicine, cosmetics and industrial areas.² Essential oils are used in medicine from ancient times to the day. The use of herbal extracts for treatment of various human diseases has a long history. The abuse potential of herbal products causes serious side effects. As a result of pharmacological investigations on these medicines based on their intended use in folk medicine, some biological effects have been scientifically explained. Clove is a spice obtained from *Syzygium aromaticum* tree.³ Cloves can also be used by being kept in the mouth for a while, and also used as a spice. it keeps your breath fresh and clean. Cloves are used extensively against bad breath due to their beautiful smell. Cloves contain 15-20% volatile, 13% tannin, 10% fixed oil. Eugenol is major component of clove oil. The effect of "eugenol" contained in it is used as a local drug against mild to moderate dental and gingival pain.⁴ In addition, eugenol has antiviral and antimycotic effects. Cloves contain 15-20% volatile, 13% tannin, 10% fixed oil. Flower buds and flower stalks are dried and used. Eugenol obtained from carnauba is used as antiseptic and analgesic in modern dentistry.⁵ Its use is in the mouth chewing or their beans are boiled in water and drink. Cloves are frequently used in dentistry. The aim of this study was assessment of in vitro cytotoxic effects *Eugenia caryophyllus* on fibroblast cells.

MATERIALS AND METHODS

The Reagent

Phosphate buffer saline (PBS) tablet, trypan blue solution, dimethyl sulfoxide (DMSO), XTT assay and another chemical were purchased from Sigma (St. Louis, MO, USA). Fetal bovine

serum (FBS) was obtained from Biochrom (Berlin, Germany). Penicillin-streptomycin was purchased from Gibco (Paisley, UK) and trypsin EDTA solution from Biological Industries (Kibbutz Beit Haemek, Israel). Dulbecco's modified eagle medium (DMEM) was obtained from Lonza (Verviers, Belgium).

Eugenia caryophyllus (Clove) Oil

Eugenia caryophyllus essential oil was used in the study. *Eugenia caryophyllus* essential oil was purchased from Art de Huile.

Cell Culture

Mouse fibroblast (ATCC-CRL-6364) cells were purchased from (ATCC). Cells were grown at 37°C in a humidified incubator in 5% CO₂. All media were supplemented with 1% penicillin (100 U/mL) and streptomycin (100 µg/mL), and 10% fetal bovine serum (FBS). Cells were counted by a cell counting device (Olympus). Fibroblast cells were seeded in plates with a density of 5×10³.

Cytotoxicity Assay

Cytotoxicity was quantitatively evaluated by the 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) method. The cells were seeded in 96-well plate in growth medium then treated with different concentrations of test compounds and incubated in a humidified CO₂ atmosphere at 37°C for 24 and 48h. After the incubation, 100 µL XTT was added to each well for another 2 h incubation. The optical density values were measured at 475 nm with a microplate reader.⁶ The all cells were treated with *Eugenia caryophyllus* (Clove) (200 - 6.25 µg/mL), and incubated for 24h and 48 h. All absorbance was compared to control samples (cells without any test compound) which represented 100% viability. Cell viability was determined as in Eq1.

$$\text{Cell viability (\%)} = \frac{[OD_{\text{sample}} - OD_{\text{blank}}]}{[OD_{\text{positive control}} - OD_{\text{blank}}]} \times 100$$

(Equation 1)

OD_{blank}: dead cell with DMSO

OD_{positive control}: viable cell

Statistical analysis

Data were expressed in the form of arithmetic mean \pm standard deviation ($\bar{x} \pm SD$). Statistical analysis was performed with Sigma Plot 12.0. All determinations were computed independent triplicated ($n=3$).

RESULTS

The cytotoxic effects of *Eugenia caryophyllus* in fibroblast cells are shown in Figure 1 and 2. *Eugenia caryophyllus* were studied at two different incubation times of 24 h and 48 h. The IC₅₀ values of *Eugenia caryophyllus* (Clove) were found $57.92 \pm 0,98 \mu\text{g/mL}$ in 24 h, $37.97 \pm 0.74 \mu\text{g/mL}$ in 48h.

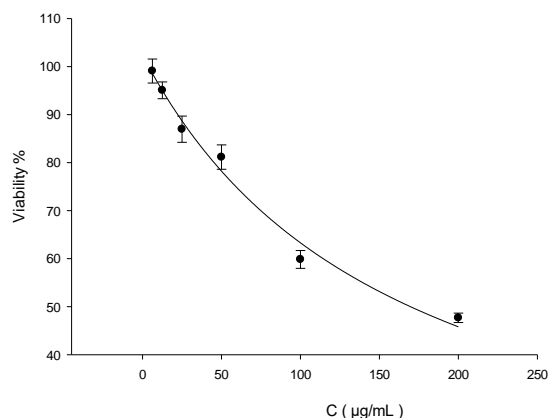


Figure 1. Cytotoxic effect of different concentrations of *Eugenia caryophyllus* in fibroblast cells for 24 h ($n=3$).

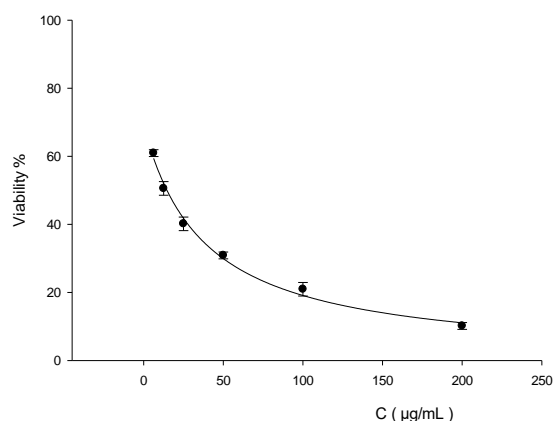


Figure 2. Cytotoxic effect of different concentrations of *Eugenia caryophyllus* in fibroblast cells for 48 h ($n=3$).

DISCUSSION

Compounds of natural product in play an essential role in human life, acting as health-protecting agents. Scientists have always been interested to active compounds of natural

sources. Natural products are an extremely important resource for medical agents. However, although a new approach to drug discovery emerges day by day, none of them is as important as the place of natural products in drug development.^{7,8}

Cloves has biological activities such as antibacterial, antifungal, insecticidal, antioxidant properties. And also, cloves have been used as flavoring and antimicrobial agents in food industries.⁹⁻¹⁰ Clove oil strengthens the immune system as it is a source of iron, calcium and omega3.¹¹ Strong biological activities of the clove oil is mostly due to the presence of eugenol in high levels. In ancient times clove oil and dry carnation seeds were used to treat infection.

Because of anti-inflammatory effect is directly used in toothache and before mucosal injection by dentists. Oral ulcers such as oral gingiva and mucosal areas are also added to the mixtures as antimicrobial and analgesic. Clove used against mouth odor are used as a natural solution in removing dental pain. It is used as a drug against dental and gingival pain of mild to moderate severity with the effect of eugenol substance contained in clove. This material is also found in toothpastes for killing bacteria. It also has pain killer and germicidal properties.¹²

According to the GC-MS results of Cloves in the studies conducted when the literature was examined; Results showed that the extracts were complex mixture of numerous compounds; many of which were present in trace amounts. The concentration of eugenol varies from 77-95%.¹³⁻¹⁴ Other studies of *Eugenia caryophyllus* was investigated for cytotoxicity; Tragoolpua and Jatisatienr stated that strains or isolates of viruses may affect the range of inhibition by *Eugenia caryophyllus*.¹⁵ Prashar *et al.* Clove oil and these components are generally recognized as 'safe', but the in-vitro study here demonstrates cytotoxic properties of both the oil and eugenol, towards human fibroblasts and endothelial cells. Clove

oil was found to be highly cytotoxic at concentrations as low as 0.03% (v/v) with up to 73% of this effect attributable to eugenol.¹⁶ Machado *et al.*, *S. aromaticum* essential oil and eugenol did not caused a significant alteration on the viability of treated mammalian cells when compared to control cells. Our results compare to literature, cytotoxicity of clove has showed lower.

CONCLUSION

The acceptable level of cytotoxicity associated with *Eugenia caryophyllus*. It is seen that the effect of many natural substances such as cloves on cytotoxicity should be examined. Such studies will help to reveal substances that can be used naturally for humanity. We will try to expand this work in our next publication. *Eugenia caryophyllus* oil may use active substance for medicine.

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