



Genotoxicity Potential of *Thymus Vulgaris* (Thyme) on Oral Cancer Cell Line

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ABSTRACT

The aim of this study is to find the potential of genotoxic activity of *Thymus vulgaris* (thyme) on oral cancer cells. The objective is to determine the anti-carcinogenic activity of *Thymus vulgaris* (thyme) with oral cancer cell line. Genotoxicity is the destructive effect on a cell's genetic material (DNA, RNA) affecting its integrity. A substance that has the property of genotoxicity is known as a genotoxin. *Thymus vulgaris* commonly known as thyme of the botanical family Lamiaceae is known for its culinary, aromatic, cosmetic and medicinal purposes. Commonly known for its anti-microbial and anti-oxidant properties, it has shown potential for genotoxic properties. Herbs with anti-cancer property should be explored, as it can serve as a better replacement for the synthetic commercially available anticancer drugs. Genotoxicity is a property possessed by the herb which can act as an anti-cancer agent. Thus, *Thymus vulgaris* if proved to possess genotoxic property can replace the commercial drugs in treating cancer. The *Thymus vulgaris* (thyme) extract showed effective anti-cancer property against oral cancer cell lines.

Keywords: Anti-cancer, genotoxicity, oral cancer, thyme.

INTRODUCTION

One of the most common cancers in today's world is oral cancer. It is any cancerous tissue growth located in the oral cavity¹. The risk factors are tobacco and high alcohol consumption². The American Cancer Society estimated more than 35,000 new cases of cancer per year involving the oral cavity causing more than 7,000 deaths in the United States in 2009³. In Europe, the mortality rates for oral cavity squamous cell carcinoma (OCSCC) range from 29 to 40 per 100,000 inhabitants per year⁴. Treatment of head and neck carcinomas comprises surgery commonly followed by concurrent chemo- and radiation therapy for advanced tumours. However, despite the improvements which have been achieved in concurrent therapies, the overall 5-year survival rate for OCSCC remains at 50% and has not significantly improved in the past 30 years⁵. Novel tumor-specific therapies are required to be less toxic while maintaining a high degree of efficiency. As the majority of anticancer drugs are of natural origin, natural products represent a valuable source for the identification and development of novel treatment options for cancer⁶.

Mother nature has an abundance of plants and fungi that have been used as a source of medicines for ages. *Thymus vulgaris* (Thyme) is a species of flowering plant in the mint family Lamiaceae, native to southern Europe from the western Mediterranean to southern Italy. Thyme essential oil and its principle compound thymol have antimicrobial, anti-fungal^{7, 8}, antioxidant⁹ and anticancer activities^{10,11}. In general medicine, thyme is commonly used as an expectorant in upper respiratory tract infections, e.g. bronchitis and pertussis¹². In odontology,

thymol is used as the main active antiseptic ingredient in chemotherapeutic mouth-rinses against gingivitis¹³.

MATERIALS AND METHODS

Preparation of thyme extract

The thyme was collected from the herb. Only thyme of uniform size and shape, without injuries was selected. The plants were washed, wiped and cut into small pieces. They were homogenised with a clean pestle and mortar using distilled water and ethanol (10% w/v). The extracts were centrifuged at 15,000 rpm for 20 min at 4 °C and the supernatants were stored at -20 °C until further use¹⁴.

Maintenance of cell line

The vial containing KB cell lines acquired from ATCC, was removed from liquid nitrogen freezer and the vial was thawed for 2 minutes by mild agitation in a 37°C water bath. Then it was centrifuged for 10 minutes at 150 to 200g, room temperature. Supernatant was disposed and cells were cleaned with Eagle's minimum essential medium to remove residual DMSO. The cell pellet was re-suspended in 3ml of DMEM with 10% FBS. It was then incubated in a CO2 incubator (eg: phenol red) in a medium which changed color as an indicator. The culture was then kept in a growth medium with 10% fetal bovine serum, until cell line was re-established.

Treatment of oral cancer cell lines with the drug

Varying concentrations of the extract sample (125 µg, 75µg) were used on the oral cancer cell line and the cell line was incubated.



Isolation of DNA

1*10⁶ cells were incubated with 100µl of cell lysis buffer at room temperature for one hour. This was centrifuged for 15 min at 3000rpm at 4o C to sediment the cell debris. To the supernatant equal volume of phenol: chloroform: iso amyl alcohol mixture was added and mixed well. This was centrifuged at 5000rpm for 15 min. The supernatant was transferred to new tube. And the centrifugation was repeated again. To the final aqueous phase 40 µl of 3.5M ammonium acetate was added, to this ice cold isopropanol was added to precipitate the DNA. This was incubated at -20degree C for 1 hour, followed by the centrifugation at 10000 rpm for 15min. The pellet was retained and washed with 70% ethanol and stored in 20-50 µl of TE buffer. The samples were analysed in agarose gel stained with Ethidium bromide.

Genotoxicity Analysis by Agarose Gel Electrophoresis

The agarose gel has to be prepared with 1X TAE buffer and stained with 2µl of ethidium bromide. The % of agarose depends upon the molecule to be separated. DNA Samples isolated were loaded with loading dye (2 µl of loading dye is used). Electrophoresis of DNA fragments at 50 volts. Visualization of DNA fragments in the UV trans illuminator was done.

RESULTS AND DISCUSSION

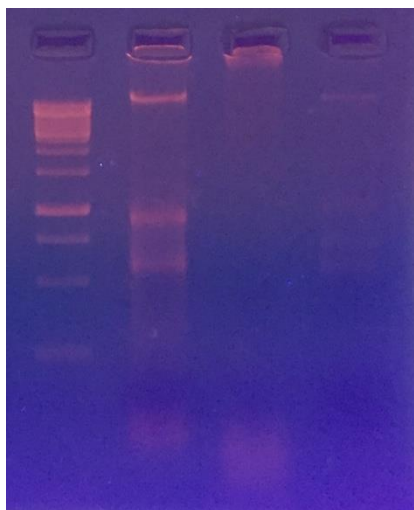


Fig 1

Lane 1 – 1kb Ladder

Lane 2 – DNA from cells treated with 125 µg sample

Lane 3 – DNA from cells treated with 75 µg sample

Lane 4 – DNA from untreated cells

DNA fragmentation is the separation or breaking of DNA strands into pieces which can be done intentionally by laboratory personnel or by cells, or can occur spontaneously. The gel picture depicts the DNA fragmentation of the KB cells incubated in the thyme extract, thus showing the genotoxicity effect of thyme extract. As the concentration of the thyme extract increases, the DNA fragmentation also increased.

Apoptosis has been characterized biochemically by the activation of a nuclear endonuclease that cleaves the DNA into multimers of 180-200 base pairs and can be visualized as an 'oligosomal ladder' by standard agarose gel electrophoresis¹⁵. This proves that thyme shows genotoxicity on the oral cancer cells by degrading its DNA. Thus, thyme has the potential to be an anti-cancerous drug.

CONCLUSION

The chemotherapeutic agents used in oncologic treatment produce deleterious side effects that augment the mortality and morbidity caused by cancer. Safer treatments are thus desperately needed. The anticancer activity of the medicinal herbs selectively targets KB cells without affecting the normal cells and inhibits their growth. From the present study, it was concluded that the extract of thyme acts against oral cancer (KB) cells which may be due to the synergetic effect of the secondary metabolites such as flavonoids present in the extract.

Thus, the anticancer activity of Thyme may be useful in the treatment of patients with oral carcinoma.

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