

**“Effect of Eucalyptus Oil and 5-Fluorouracil on Head
and Neck Squamous Cell Carcinoma Cell Line”**

In-Vitro Study

Thesis

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Table of Contents

List of Abbreviations	I
List of Tables	IV
List of Figures	VI
Introduction	1
Review of Literature	4
Oral carcinogenesis	5
prognosis	6
Oral cancer treatment	6
Apoptosis	8
Apoptotic pathway	9
Initiation phase.....	10
Extrinsic or Receptor-Mediated Pathway	10
Intrinsic or Mitochondrial Pathway	11
Perforin/Granzyme Pathway.....	11
Execution Phase	12
Apoptosis and cancer	12
Caspases	13
Apoptosis and cancer treatment	14
5-Fluorouracil(5-FU)	14
Mechanism of action of 5-Fluorouracil	16

Inhibition of thymidylate synthase (TS)	17
Misincorporation into DNA and RNA.....	18
Inactivation of 5-FU.....	18
5-FU Resistance	18
Reversal resistance of 5-FU	21
Modulation of 5-FU	22
Natural Products as Potential Anticancer Agents	23
Mechanism of action of eucalyptus oil	26
Safety of eucalyptus oil.....	28
Cancer cell line	29
The Enzyme Linked Immunosorbent Assay technique (ELISA)	30
Aim of the Study	31
Material and Methods	32
Results	48
Discussion	63
Conclusions	74
Recommendations	75
Summary	76
References	79
Arabic Summary	1

List of Abbreviations

5-FU: 5-Fluorouracil

5-FUMP: 5-Fluorouridine monophosphate

5-FUTP: 5-Fluorouridine triphosphate

5-FdUMP: 5- Fluorodeoxyuridine monophosphate

μM : Micromolar

ANOVA: Analysis of Variance

ATP: Adenosine triphosphate

Apaf-1: Apoptotic protease-activating factor 1

AIF: Apoptosis Inducing Factor

ATCC: American Type Culture Collection

Bcl-2: B-cell lymphoma

BAX: Bcl-2- Associated X protein

BAK: Bcl-2 homologous antagonist/killer

BID: BH3 Interacting Domain Death Agonist

Caspases: Cysteine-aspartic proteases

CH₂THF: 5, 10-Methylenetetrahydrofolate

dTMP: Deoxy-thymidine monophosphate

DUMP: Deoxy uridine monophosphate

DNA: Deoxyribonucleic Acid

DISC: Death-inducing signaling complex

DPD: Dihydropyrimidine dehydrogenase

- DTMP:** Deoxythymidine monophosphate
- DTTP:** Deoxythymidine triphosphate
- DNTP:** Deoxynucleotide pool
- DUTP:** Deoxyuridine triphosphate
- DHFU:** Dihydrofluorouracil
- ELISA:** Enzyme-linked immunosorbent assay
- FdUMP:** Fluorodeoxy Uridine Monophosphate
- FBS:** Fetal Bovine Serum
- FASL:** FAS Ligand
- FADD:** Fas-associated death domain
- FUPA:** α -fluoro- β -ureidopropionic acid
- FDA:** Food and Drug Administration
- FLD:** Fold
- HBV:** Hpatitis B Virus
- HCV:** Hpatitis C Virus
- HPV:** Human Papilloma Virus
- HNSCC:** Head and Neck Squamous Cell Carcinoma
- Hela:** Human cancer cell line taken from Henrietta Lacks.
- IAP:** Inhibitor of apoptosis
- IC50:** Inhibitory Concentration 50%
- IFNs:** Interferons
- LV:** Leucovorin

MTX: Methotrexate

m RNA: Messenger RNA

MTT: Methyl Thiazol Tetrazolium

MCF-7: Michigan Cancer Foundation-7

NO: Nitric Oxide

NOs: Nitric Oxide synthase

OSCC: Oral Squamous Cell Carcinoma

OD: Optical Density

RNA: Ribonucleic acid

r RNA: Ribosomal RNA

ROS: Reactive Oxygen Species

TS: Thymidylate Synthetase

TP53: Tumor Protein p53

TRAIL: TNF-related apoptosis-inducing ligand

UTP: Uridine triphosphate

WHO: World Health Organization

List of Tables

Table 1: Absorbance mean and corresponding percentage viability of MTT assay.	38
Table 2: Details of groups and subgroups used in the study.....	39
Table 3: The mean viability percentage of SCC-15 cells treated with different doses of 5-FU over different time intervals.	48
Table 4: The mean viability percentage of SCC-15 cells treated with different doses of eucalyptus oil over different time intervals.	50
Table 5: The FLD changes in the level of caspase-3 of SCC-15 cells treated with different doses of 5-FU over different time intervals..	51
Table 6: The FLD changes in the level of caspase-3 of SCC-15 cells treated with different doses of eucalyptus oil over different time intervals.....	52
Table 7: Comparison of the mean viability percentage between different 5-FU doses and control at T1 and T2 durations.....	54
Table 8: Comparison of the mean viability percentage between different Eucalyptus oil doses and control at T1 and T2 durations.	55
Table 9: Comparison of the mean viability percentage between the different 5-FU doses at T1 and T2 time duration	56
Table 10: Comparison of the mean viability percentage between different Eucalyptus oil doses at T1 and T2 durations	56
Table 11: Comparison of SCC-15 cells viability between Eucalyptus oil and 5-FU doses at T1 and T2.....	57

Table 12: Comparison of FLD between different 5-FU doses and control at T1 and T2 durations..... 59

Table 13: Comparison of FLD between different Eucalyptus doses and control at T1 and T2..... 60

Table 14: Comparison of FLD change of caspase -3 between 24 hour and 48 hour for the different 5-FU doses..... 61

Table 15: Comparison of FLD change of caspase-3 between T1 and T2 for the different Eucalyptus oil doses..... 61

Table 16: Comparison of FLD change between Eucalyptus oil and 5-FU at T1 and T2 durations..... 62

List of Figures

Figure 1: Different apoptotic pathways	9
Figure 2: Structure of 5-FU and uracil.....	15
Figure 3: Metabolism of 5-FU to active products FdUMP, FdUTP and FUTP	16
Figure 4: Inhibition of TS by 5-FU metabolite FdUMP	17
Figure 5: Culture flask	44
Figure 6: Inverted phase contrast microscope	44
Figure 7: 96-well tissue culture plate.....	45
Figure 8: ELISA reader.....	45
Figure 9: Human caspase-3 active assay sumarry	46
Figure 10: Standard curve blotted in a 4-parameter equation curves to estimate the Caspase-3 activity level	47
Figure 11: A bar chart representing differences in the mean viability percentage between different 5-FU doses at both durations	49
Figure 12: A bar chart representing differences in the mean viability percentage between different Eucalyptus oil doses at both durations.	50
Figure 13: A bar chart representing FLD change of different 5-FU doses in both durations.....	51
Figure 14: A bar chart representing FLD changes of different Eucalyptus oil doses in both durations.....	53
Figure 15: A bar chart representing the difference in cell viability percentage of the different 5-FU and eucalyptus oil doses at both durations.....	58

Introduction

Introduction

Oral cancer is a very aggressive malignant tumor which is considered the sixth most common malignancy worldwide. The exact cause of oral cancer remains unknown, there are many factors responsible for occurrence of this type of malignancy, including tobacco products, alcohol consumption, fungal infection, viral infection, nutritional deficiency, syphilis and genetic factor⁽¹⁾.

Among head and neck cancer oral squamous cell carcinoma is considered the most common malignancy with high recurrence rate. Recently the incidence of oral squamous cell carcinoma has been increased. Every year more than 200,000 new cases of oral cancer are reported worldwide⁽²⁾. There are many treatment modalities available for oral squamous cell carcinoma such as radiotherapy, chemotherapy, molecular targeted therapy and surgery⁽³⁾.

Chemotherapy stills the main treatment for malignancy to improve the survival rate and quality of life for patients. 5-fluorouracil considered as a standard chemotherapy regimen in cases of recurrent or metastatic head and neck squamous cell carcinoma⁽⁴⁾. However, the resistance of malignant cells to chemotherapy remains a major obstacle in cancer treatment. Drug resistance occurs due to prolonged use of chemotherapy by many mechanisms such as gene mutation, DNA methylation and histone modification⁽⁵⁾. So, the patients are gradually showing resistance to widely used chemotherapeutic drugs, such as 5-fluorouracil, doxorubicin, cisplatin and camptothecin⁽⁶⁾.

Recently, exploring natural products with anticancer properties become a very growing area of study to open the gate for new treatment modalities. Essential oils considered as important sources

of natural products, there are many essential oils that have anticancer properties and one of them is eucalyptus essential oil which is extracted from eucalyptus tree. Eucalyptus is native to Australia, then after the world wars it has been spread all over the world because of its medical uses⁽⁷⁾. Traditionally, eucalyptus oil have many medical uses, it is used to alleviate respiratory tract infection, rheumatoid arthritis, cough, muscle pain, tuberculosis, sinusitis and cancer. Moreover, it is used topically for ulcers, burns and wound healing⁽⁸⁾.

Eucalyptus oil consists of many chemicals that have anticancer effect, especially alpha-pinene and beta-pinene. Many other chemicals present in eucalyptus oil are still under study. According to previous studies eucalyptus extract showed strong cytotoxic effect against some human cancer cell lines⁽⁹⁾.

Apoptosis which is a programmed cell death can occur in both physiological and pathological conditions. In apoptosis there are many biochemical changes which are noted including; caspases activation, breakdown of DNA and protein, and phagocytosis. Apoptosis has important role in cancer pathogenesis and cancer therapy⁽¹⁰⁾. Caspases enzymes are considered the engine of apoptosis. Caspases are a family of intracellular cysteine proteases that activated as apoptosis occurred⁽¹¹⁾. Caspases not only play an important role during programmed cell death but some of them regulate inflammatory response⁽¹²⁾.

Caspases can be classified into three functional groups: initiator caspases (caspase 2, 8, 9 and 10), executioner caspases (caspase 3, 6 and 7), and inflammatory caspases (caspase 1, 4, 5, 11 and 12). Initiator caspases start the apoptosis signal while the executioner