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

In-Vitro Study

Thesis

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Presented by

Aisha Abubaker Ali Shanan

B.D.S

Faculty of Dentistry

Tripoli University

2006

Faculty of Dentistry

Ain Shams University

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Supervisors

Prof. Mohammed Hossam Eldin El Mallahi

Professor of Oral Pathology

Ain Shams University

President of Nahda University in Beni Suef, Egypt

Dr. Shaimaa Eliwa Ghazy Eliwa

Lecturer of Oral Pathology

Faculty of Dentistry

Ain Shams University

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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: DeoxyribonucleicAcid

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

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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 campothecin[(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