The Green tea Extract as a complementary therapy for improvement of therapeutic index of cancer chemotherapy "A clinical trial in hepatocellular Carcinoma patients"

Thesis Submitted to
Clinical Pharmacy Department
Faculty of Pharmacy
Ain Shams University

In partial fulfillment of the requirements
For the Master Degree in Pharmaceutical Sciences
(Clinical Pharmacy)

By

Shaimaa Fathy Mohamed Khalil
Bachelor of Pharmaceutical Science, MIU
Teaching assistant in the Department of Clinical Pharmacy and Pharmacy Practice
Faculty of Pharmacy
Misr International University (MIU)

Under the supervision of
Prof. Dr.Osama Ahmed Badary
Dr.Reda Hasan M. Tabashy
Dr.Amany Helal

Department of Clinical Pharmacy
Faculty of Pharmacy
Ain Shams University
Cairo, Egypt
2010

Supervisors

Prof. Dr.Osama Ahmed Badary

Head of the Clinical Pharmacy Department,
Ain Shams University

Dr.Reda Hasan M. Tabashy

Lecturer of Radiodiagnosis at National Cancer Institute,
Cairo University

Dr.Amany Helal

Lecturer of Medical Oncology at National Cancer Institute,
Cairo University

TO

My

Lovely

Parents

ACKNOWLEDGEMENT

My deepest gratitude and sincere appreciation to ***Prof. Dr. Osama Ahmed Badary Professor and Head of the Clinical Pharmacy Department, Ain Shams University,*** for his unlimited effort, meticulous supervision, valuable scientific discussions, and for giving generously of his time to accomplish this work.

My great thanks and appreciation to ***Dr. Reda Hassan Dabash Assistant Professor, National Cancer Institute, Cairo University,*** for his valuable advice and generous help in completing the practical part of this study.

I am greatly indebted and extremely grateful to ***Dr. Amany Helal, Assistant Professor, National Cancer Institute, Cairo University,*** for her helpful advice, supervision, and valuable guidance during this study.

My great thanks and appreciation to ***Dr. Mona Schaalan, Lecturer and Acting Head of the Clinical Pharmacy and Pharmacy Practice Department, Misr International Univerisity,*** for her valuable advice and generous help in completing the practical part of this study.

Words can never express gratitude to all staff members of ***Clinical Pharmacy Department, Ain Shams University, National Cancer Institute, Cairo University and Faculty of Pharmacy, Misr International University*** for their generous help throughout the study to be completed.

Finally, my deepest gratitude and appreciation to my family for their encouragement and support.

Contents

List of Tables

List of Figures

LIST OF ABBREVIATIONS

AF : aflatoxin

AFP : alpha-fetoprotein

ALT : alanine aminotransferase

AP-1 : activator protein-1

AST : aspartate aminotransferase

BCLCC: Barcelona-Clinic Liver Cancer Classification

CIAP : chemotherapy induced abdominal pain

CIN/V : chemotherapy induced nausea and vomiting

CIP/N : chemotherapy induced parathesia and neuropathy

CLIP : cancer for liver Italian program

CT : computed tomography

DISC : death-inducing signaling complex

DOX : Doxorubicin

EC: Epicatechin

ECG: Epicatechin-3-gallate

ECM: extracellular matrix

ECOG : Eastern Cooperative Oncology Group

EGC: Epigallocatechin

EGCG: Epigallocatechin-3-gallate

EGFR (neu/ erbB2): epidermal growth factor receptor

EPIC : European Prospective investigation into cancer and nutrition trial

ERK: Extracellular signal regulated kinase

FACT-An: Functional assessment of cancer therapy and anemia

GCL **:** glutamate-cysteine-ligase

GCLc : heavy glutamate cysteine ligase catalytic subunit

GCLm : light glutamate cysteine ligase catalytic subunit

GCS : glutamyl cysteine synthetase

GLAST: Glutamate transporter

GLT-1: Glutamate transporter

GPx : glutathione peroxidase

GSH : glutathione

GSK: glycogen synthase kinase

GS-X : glutathione S- conjugate export

GTE : green tea extract

GTPs: green tea polyphenols

HCC : Hepatocellular Carcinoma

HER2 (neu/erbB3): human epidermal growth factor receptor2

HSC : hepatic stellate cells

IAP: inhibitor of apoptosis

IGF-1: insulin like growth factor-1

IGF-1R: insulin like growth factor-1 receptor

IGFBP-3: insulin like growth factor binding protein

IH : ischemic hypoxia

JNK: Jun N-terminal kinase

LDQOL: liver disease quality of life questionnaire

LP : lipid peroxides

LT : liver transplantation

MAP : mitogen- activated protein

MAPK: mitogen-activated protein kinase

MDA : malondialdehyde

MEIA: microparticle enzyme immunoassay

MRI : magnetic resonance image

MU: methyl umbelliferone

MUP: methyl umbelliferyl phosphate

NCI-H-209: non small cell lung cancer carcinoma cell line

NF- KB: nuclear factor kappa-B

NGF : nerve growth factor

NSCLC: Non small cell lung cancer

P21/WAF1: known as cyclin-dependent kinase inhibitor 1 (CDK-interacting protein -1) is a protein located in chromosome

PAF: platelet activating factor

PARP: poly (ADP-ribose) polymerase

PE: polyphenon E

PEIT : percutaneous ethanol injection therapy

PI3K/AKT: phosphoinositide 3- kinase inhibitor / a serine theonine protein kinase, this is an intracellular signaling pathway important in apoptosis

PUFA: Poly unsaturated fatty acid

RFA : Radiofrequency ablation

ROS : reactive oxygen species

RQ: respiratory quotient

RTK: receptor tyrosine kinase

SAC: S-allyl cysteine

SCLC : small cell lung cancer

SOD: superoxide dismutase

TAC : total anti-oxidant capacity

TACE: trans-arterial chemo-embolization

TBA : thiobarbituric acid

TBARS: Thiobarbeturic acid reactive substances

TGF : transforming growth factor

ABSTRACT

Green Tea is one of the most popular beverages worldwide and its consumption has long been associated with health benefits within the complementary and alternative medicine (CAM). Most of the beneficial effects of green tea are attributed to its polyphenolic flavonoid components known as catechins. Among the beneficial physiological functions of tea catechins, its anticarcinogenic potential has recently attracted a major attention. The aim of the present study was to investigate the possible beneficial effects of Green tea extract (GTE) on cancer chemotherapeutic agents (doxorubicin & cisplatin) in hepatocellular carcinoma patients, through assessment of its enhancement of anti-tumor activity, antioxidant effect and improvement of quality of life. The antioxidant effect of green tea was investigated by measurement of serum MDA and GSH levels.

A prospective randomized controlled study was conducted in National Cancer Institute, Cairo, Egypt. Patients with primary HCC receiving chemotherapy (TACE) with performance status ≤ 2 were eligible for the study. Patients (n=32) were classified into 2 groups. In Group 1 the patients received standard chemotherapy only (Doxorubicin + Cisplatin) while in Group 2 the patients received standard chemotherapy (Doxorubicin + Cisplatin) and green tea extract 600mg/day for one month. Reevaluation of both groups was followed by assessment of tumor size by computed tomography, measurement of serum lipid peroxide and reduced GSH. A questionnaire was also performed to assess patients' quality of life. Baseline CBC, liver function tests and kidney function tests were measured for both groups and for follow up measures.

Concerning the effect of green tea on non - enzymatic oxidative stress parameters in group 1 & group 2, our results revealed that GTE caused a significant decrease in MDA and increase in glutathione levels, as well as it added to the chemotherapy's efficacy in reducing the tumor size (very minor effect). As for the effect of green tea on hemoglobin and platelets concentrations in Group 1 & Group 2, it was evident that GTE beneficially added to the improvement of bone marrow suppression resulting from chemotherapy administration, which is noticed by significantly increasing Hb and platelets count. Our results showed no significance difference between both groups concerning WBC's, while a significant difference concerning RBC's was recorded. Concerning the quality of life indicators assessed in the questionnaire, the following items showed an improvement in group 2 when compared with group 1 ($P < 0.05$): Energy level, abdominal pain, itchy Skin, faintness & dizziness, nausea /vomiting, getting enough sleep, feeling drowsy, trouble staying awake, chemotherapy induced Nausea/Vomiting, chemotherapy induced abdominal pain, and chemotherapy induced parathesia / neuropathy.

In conclusion, Green tea extract proved to play a crucial role in the attenuation of the cancer-induced oxidative stress. In addition Green tea administration improved effectively the quality of life measures of hepatocellular carcinoma patients.

Keywords: green tea, hepatocellular carcinoma, antioxidant effect, quality of life, oxidative stress.

INTRODUCTION

Hepatocellular carcinoma is a cancer arising from the liver; it is also known as primary liver cancer or hepatoma**.** Most people don't present with signs and symptoms in the early stages of primary liver cancer. When symptoms do appear, they may include: unintentional weight loss, loss of appetite, upper abdominal pain, nausea and vomiting, general weakness and fatigue, an enlarged liver (hepatomegaly), abdominal swelling, yellow discoloration of the skin and the whites of the eyes (jaundice) **(Hefaiedh et al., 2009)**.

The incidence of hepatocellular carcinoma (HCC) is rising worldwide. The number of therapeutic options for HCC has also increased, making its treatment more complex. The management of HCC requires a multidisciplinary approach including the gastroenterologist, hepatologist, oncologist, interventional radiologist and surgeon. **(Kathleen and Pratt, 2009).** The burden of hepatocellular carcinoma (HCC) has been increasing in Egypt with a doubling in the incidence rate in the past 10 years **(Anwar et al., 2008).**

A myriad of treatment options exist for HCC. The proper choice depends on tumor stage, liver function and the patient's overall functional status. Curative therapies including surgical resection, liver transplantation (LT), transarterial chemoembolization (TACE), radiofrequency ablation (RFA) and systemic chemotherapy **(Llovet et al., 2003 ;Siegel et al., 2008).**

Complementary therapy (CT) is used by an increasing number of cancer patients. The reasons cited for using CT include immunomodulation, survival prolongation, improving quality of life (QOL) and reduction of treatment-related toxic effects **(Mok et al., 2007).**Green tea (Camellia Sinensis) is one of the most

popular beverages used as complementary therapy. The major catechins are epigallocatechin-3-gallate (EGCG), epigallocate chin (EGC), epicatechin-3-gallate (ECG) and epicatechin (EC). EGCG is the most abundant catechin and may account for 50-75% of the catechins **(Yang et al., 2009).**

THE AIM OF WORK

The aim of the present study was to investigate the possible beneficial effects of Green tea extract (GTE) on cancer chemotherapeutic agents (doxorubicin & cisplatin) in hepatocellular carcinoma patients, through assessment of its efficacy on enhancing antitumor activity by decreasing tumor size, as well as, its antioxidant impact. The improvement of the quality of life via a questionnaire is also within the aim of the work.