

**A Study on the Possible Protective
Effect of Ginkgo Biloba Extract
Against Lomefloxacin Phototoxicity in Mice**

A thesis submitted in partial fulfillment of the requirement for the master degree in pharmaceutical sciences (pharmacology and toxicology)

By

Nancy Wagih Shehata Basta

Research assistant,
National Organization for Drug Control and Research.

Supervised By

Prof. Dr. Mohamed Raouf Hamed
Professor of Pharmacology and
Toxicology, National Organization for
Drug Control and Research.

Prof. Dr. Amani Emam Khalifa
Vice-Dean for Graduate Studies and
Research, Faculty of Pharmacy, Ain-
Shams university.

Dr. Yehia Ahmed Ismail Raslan
Assistant Professor, Department of
Pharmacology, National Organization for Drug
Control and Research.

Faculty of pharmacy, Ain Shams University, Cairo.

Examination Board Approval Sheet

Name of the candidate: Nancy WagihShehataBasta.

Title of the thesis: A Study on the Possible Protective Effect of Ginkgo Biloba Extract Against Lomefloxacin Phototoxicity in Mice.

Submitted to faculty of Pharmacy, Ain Shams University, Department of Pharmacology and Toxicology.

Approved by the committee in charge:

١. **Prof. Dr./ Mohamed Raouf Hamed**
٢. **Prof. Dr./ Laila Gamal El Din Mahran**
٣. **Prof. Dr./ Amani Emam Khalifa**
٤. **Prof. Dr./ Nahed Mohamed Ahmed Hassanin**

Date: ٢٥ / ٠٧ / ٢٠١٢

Acknowledgment

First and foremost thanks to God

I would like to express my great appreciation and infinite gratitude to **Prof. Dr. Mohamed Raouf Hamed** Professor of Pharmacology and Toxicology, National Organization for Drug Control and Research, for his great assistance, supervision, scientific guidance, and continuous help throughout the whole work.

Special gratitude and sincere thanks to **Prof. Dr. Amani Khalifa** Professor of Pharmacology and Vice-Dean for Graduate Studies and Research, Faculty of Pharmacy, Ain-Shams University, for her supervision, valuable suggestions and for her help throughout this work and during the preparation of the manuscript.

I would like to express my deepest gratitude and sincere appreciation to **Ass. Prof. Yehia Ahmed Ismail Raslan** Assistant Professor, department of pharmacology, National Organization for Drug Control and Research, for his help in performing the

statistical tests and his keen supervision during writing the manuscript.

My thanks and gratitude to the head of developmental pharmacology department, National Organization for Drug Control and Research, for his assistance to perform all the experimental work in the department and to all my colleagues in this department for their cooperation. Special thanks to **Dr. Michael Kamal Ibrahim**, department of developmental pharmacology, National Organization for Drug Control and Research, for providing help and support all over the work especially in the practical part of the thesis.

I am very grateful to **Prof. Dr. Laila Ibrahim**, head of Physical Chemistry laboratory in National Organization for Drug Control and Research, and **Dr. Rania Mohamed Youssef** for their help and support to perform the detection of zinc using the atomic absorption spectrophotometer.

I would like to express my thanks to **Prof. Dr. Adel Bakeer Kholossy**, professor of Pathology, faculty of veterinary medicine, Cairo University, for his great effort in accomplishing the part of histopathological technique.

My deepest thanks and appreciation goes to **Dr. Jackleen Raafat Awadallah**, Biochemistry Department, National Research Center, for her great help, encouragement and revising the manuscript.

Finally, but of great importance, i wish to express my deep gratefulness to my mother for her all sacrifices and patience along the way to help me, my beloved father who has been a great source of motivation and inspiration, my husband, my son and my sister who believe in me and my ability to achieve my ambitions.

Nancy Wagih Shehata

Abstract

Lomefloxacin is considered as the most phototoxic drug in quinolone antibiotics. Its phototoxic potential has been demonstrated in human being as well as in laboratory animals. The aim of the present work was to evaluate the possible protective effect of ginkgo biloba extract (GBE), against lomefloxacin-induced phototoxicity. The study was performed on female balb/c mice. Lomefloxacin 200 mg/kg was administered orally to induce phototoxicity. UVA radiation was directed to the animals' ears for 90 minutes at a dose of 21.6 J/cm². Immediately after radiation, animals were given a single oral dose of GBE where four dose levels were utilized (25, 50, 100 and 200 mg/kg). Twenty four hours after irradiation, animals were examined for morphological parameters; i.e. ear erythema, ear pinna thickness and relative ear weight to body weight, and for biochemical parameters; i.e. ear tissue level of malondialdehyde, glutathione and zinc. The utilized dose of lomefloxacin was associated with

significant phototoxic changes that were seen in all the investigated parameters. Morphologically, the utilized four dose levels of GBE showed variable, but dose-dependent, protective potentials against lomefloxacin phototoxic consequences. As regarding the biochemical changes, co-administration of each of the used four dose levels of GBE could result in a significant increase in the ear tissue level of glutathione, as compared to lomefloxacin / UV treatment. However, none of the four dose levels did cause parallel significant ear tissue level decrease in malondialdehyde or increase in zinc. The histopathological findings were reflecting those gross and biochemical ones. It is concluded that GBE could produce dose-related protection against experimentally induced phototoxicity by lomefloxacin.

Pre-requisite Post Graduate courses

Beside the work presented in this thesis, the candidate has attended the following courses:

General courses:

Courses of instrumental analysis, physical chemistry, computer skills and statistics.

Special courses:

١. Clinical pharmacology and therapeutics.
٢. Clinical toxicology.
٣. Molecular pharmacology.
٤. Selected topics in pharmacology and toxicology.
٥. Pharmacology.

She has successfully passed examination in these courses with general grade Excellent

Department of Pharmacology and Toxicology

Contents

Subject	Page
١. List of Abbreviations	I
٢. List of Tables	III
٣. List of Figures	IV
٤. Aim of Work	١
٥. Introduction	٢
• Phototoxicity	٢
• Lomefloxacin	١٥
• Ginkgo Biloba Extract	٢٥
٦. Materials and Methods	٣٩
٧. Results	٥٢
٨. Discussion	٧٢
٩. Summary	٨٧
١٠. References	٩١
١١. Arabic summary	

List of abbreviations

ANOVA: Analysis of variance.

cm: Centimeter.

CMC: Carboxymethyl cellulose.

DNA: Deoxyribonucleic acid.

e⁻: Electron.

Fig.: Figure.

FQ: Fluroquinolone.

g.: Gram.

GBE: Ginkgo Biloba Extract.

GSH: Glutathione reduced.

H⁺: Proton.

H₂O₂: Hydrogen peroxide.

IR: Infra-Red radiation.

Kg: Kilogram.

Lom: Lomefloxacin.

MDA: Malondialdehyde.

Min.: Minute.

mj: Milli Joule.

ml: Milliliter.

mm.: Millimeter.

mM: Milli mole.

mW: Milli Watt.

r.p.m.: Rotation per minute.

ROS: Reactive Oxygen Species.

s: Second.

SEM: Standard error of the mean.

SOD: Superoxide dismutase.

TBA: Thiobarbituric acid.

TBARS: Thiobarbituric acid reactive species.

UVA: Ultra-violet A radiation.

UVR: Ultra-violet radiation.

List of Tables

Table (1): Experimental Design.

Table (2a): Effect of administration of different concentrations of GBE (25, 50, 100 and 200 mg/kg) on lomefloxacin phototoxicity 190 minutes after UV radiation on ear erythema score, ear pinna thickness (edema) and relative ear weight to body wt.

Table (2b): Effect of administration of different concentrations of GBE (25, 50, 100 and 200 mg/kg) after 220 minutes of lomefloxacin administration without UV radiation on ear erythema score, ear pinna thickness(edema) and relative ear weight to body wt.

Table (3): Effect of administration of different concentration of GBE (25, 50, 100 and 200 mg/kg) on lomefloxacin phototoxicity 190 minutes after UV radiation on ear tissue glutathione, ear tissue malondialdehyde and ear tissue zinc level.

List of Figures

Figure (١): Influence of UV radiation on ear erythema score after administration of lomefloxacin (lom) ٢٠٠ mg/kg either alone or in combination with GBE (٢٥, ٥٠, ١٠٠ and ٢٠٠ mg/kg).

Fig. (٢): Influence of UV radiation on ear edma (ear pinna thickness) after administration of lomefloxacin (lom) ٢٠٠ mg/kg either alone or in combination with GBE (٢٥, ٥٠, ١٠٠ and ٢٠٠ mg/kg).

Fig. (٣): Influence of UV radiation on relative ear weight to body weight after administration of lomefloxacin (lom) ٢٠٠ mg/kg either alone or in combination with GBE (٢٥, ٥٠, ١٠٠ and ٢٠٠ mg/kg).

Fig. (٤): Influence of UV radiation on ear tissue Malondialdehyde level (MDA) after administration of lomefloxacin (lom) ٢٠٠ mg/kg either alone or in combination with GBE (٢٥, ٥٠, ١٠٠ and ٢٠٠ mg/kg).

Fig. (٥): Influence of UV radiation on ear tissue glutathione level (GSH) after administration of lomefloxacin (lom) ٢٠٠ mg/kg either alone or in combination with GBE (٢٥, ٥٠, ١٠٠ and ٢٠٠ mg/kg).

Fig. (٦): Influence of UV radiation on ear tissue zinc level (Zn) after administration of lomefloxacin (lom) ٢٠٠ mg/kg either alone or in combination with GBE (٢٠, ٥٠, ١٠٠ and ٢٠٠ mg/kg).

Fig. (٧): Ear section of balb/c mice administered the vehicle and exposed to UV radiation showing the normal histological structure of the skin from both surfaces (S) and cartilage (C) with fat (F) in central zone, (H&E stain, X٦٤).

Fig. (٨): Ear section of balb/c mice administered a single oral dose of GBE (٢٠ mg/kg) after exposure to UV radiation showing inflammatory cells infiltration (arrow) and oedema (o) in the dermis and subcutaneous tissue, (H&E stain, X٦٤).

Fig. (٩): Ear section of balb/c mice administered a single oral dose of GBE (٥٠ mg/kg) after exposure to UV radiation showing inflammatory cells infiltration (arrow) and oedema (o) in the dermis and subcutaneous tissue, (H&E stain, X٦٤).

Fig. (١٠): Ear section of balb/c mice administered a single oral dose of GBE (١٠٠ mg/kg) after exposure to UV

radiation showing few inflammatory cells infiltration (arrow) in the dermis, (H&E stain, X¹⁰⁰).

Fig. (11): Ear section of balb/c mice administered a single oral dose of GBE (200 mg/kg) after exposure to UV radiation showing intact histological structure, (H&E stain, X¹⁰⁰).

Fig. (12): Ear section of balb/c mice administered a single oral dose of lomefloxacin (200 mg/kg) and exposed to UV radiation showing focal inflammatory cells aggregation (m) in the dermis and subcutaneous tissue, (H&E stain, X¹⁰⁰).

Fig. (13): Ear section of balb/c mice administered a single oral dose of lomefloxacin (200 mg/kg) and exposed to UV radiation followed by a single oral dose of GBE (200 mg/kg) showing massive number of inflammatory cells infiltration in subcutaneous tissue between the two surfaces of the skin with oedema (o), (H&E stain, X¹⁰⁰).

Fig. (14): Ear section of balb/c mice administered a single oral dose of lomefloxacin (200 mg/kg) and exposed to UV radiation followed by a single oral dose of GBE (200 mg/kg) showing massive number of inflammatory cells