بسم الله الرحمن الرحيم

∞∞∞

تم رفع هذه الرسالة بواسطة / حسام الدين محمد مغربي

بقسم التوثيق الإلكتروني بمركز الشبكات وتكنولوجيا المعلومات دون أدنى مسئولية عن محتوى هذه الرسالة.

ملاحظات : لا يوجد

Investigation of the anti-inflammatory effect of Indoles on Ehrlich Ascites carcinoma in murine model

A Thesis submitted for the degree of Ph.D. of Science in Biochemistry

By

Ayah Zaki Mahmoud Adel El Deen Salem
M.Sc. Biochemistry -Faculty of science -Ain Shams University, 2016)

Under the Supervision of

Prof. Dr.
Shadia Abd El Hamid Fathy
Professor of Biochemistry
Faculty of Science
Ain Shams University

Prof. Dr.
Mohamed Ragaa Mohamed
Professor of Biochemistry and Molecular Biology
Ain Shams University

Prof. Dr.
Zakaria Abdel Halim Elkhayat
Research Professor of Medical Biochemistry
National Research Centre

Dr.
Sherien Mohomed El-Daly
Assistant Research Professor of Medical Biochemistry
National Research Centre

Dr.
Dalia Medhat Ahmed Ali
Assistant Research Professor of Medical Biochemistry
National Research Centre

2022

Approval sheet

Name of candidate / **Ayah Zaki Mahmoud Adel El Deen salem**

Title of thesis / **Investigation of the anti-inflammatory effect of Indoles on Ehrlich Ascites carcinoma in murine model**

This thesis has been approved for submission by

Supervisors

Prof. Dr Shadia Abd El Hamid Fathy
Professor of Biochemistry
Faculty of Science- Ain Shams University

Prof. Dr. Mohamed Ragaa Mohamed
Professor of Biochemistry
Faculty of Ain Shams University

Prof. Dr. Zakaria Abdel Halim Elkhayat
Research Professor of Medical Biochemistry
National Research Centre

Dr. Sherien Mohamed EL-Daly
Assistant Research Professor of Medical Biochemistry
National Research Centre

Dr. Dalia Medhat Ahmed Ali
Assistant Research Professor of Medical Biochemistry- National Research Centre

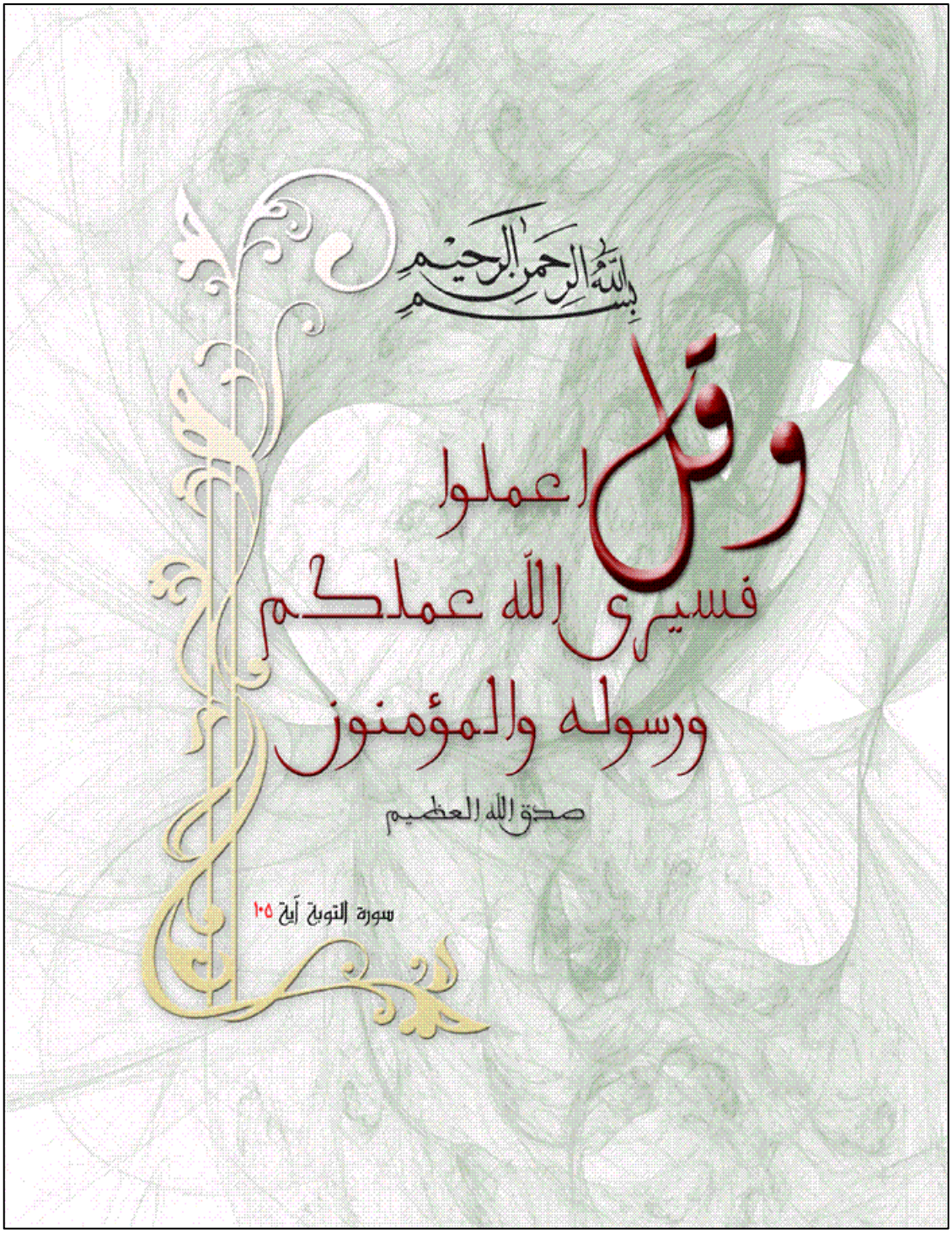
Examiners committee

Prof. Dr. Yakout Abdel Fattah Yakout El senousy
Professor of Biochemistry
Faculty of vegeterinary- Benha University

Prof. Dr. Hatem Abd El Moneam El Mezayn
Professor of Biochemistry
Faculty of Science- Helwan University

Prof. Dr shadia Abd El Hamid fathy
Professor of Biochemistry
Faculty of Science- Ain Shams University

Prof. Dr. Mohamed Ragaa Mohamed
Professor of Biochemistry
Faculty of Ain Shams University


بسم الله الرحمن الرحيم
وقل اعملوا
فسيرى الله عملكم
ورسوله والمؤمنون
صدق الله العظيم
سورة التوبة آية ١٠٥

Declaration

I declare that this thesis represents my own work which has been done after registration for the degree of PhD at Ain Shams University, and has not been previously included in a thesis or submitted to this or any other institution for a degree, diploma or other qualifications.

Ayah Zaki Mahmoud Adel Eldeen Salem

Acknowledgment

*First of all, Thanks first and last to **ALLAH**, to whom I relate any success in my life. Words stand short when they come to express my gratefulness to my supervisors.*

*I am really grateful to express my gratitude, appreciation and thanks to **Prof. Dr. Shadia Abdel Hamid Fathy**, Prof. of Biochemistry, faculty of Science, Ain Shams University for her relentless support, splendid efforts, advice and her time, attention and guidance needed to let this thesis.*

*I would like to express my profound gratitude and cordial appreciation to my eminent **Prof. Dr. Mohamed Ragaa Mohamed**, Prof. of Biochemistry and Molecular Biology, Faculty of Science, Ain Shams University, for his moral support, valuable supervision, honest help, encouragement, keep interest and guidance throughout the performance of this work, no words of praise are sufficient.*

*I am deeply grateful and deep appreciations are due to **Prof. Dr. Zakaria Abdel Halim El-Khayat,** Prof. of Medical biochemistry, Medical biochemistry department, National Research Center, for his supervision, encouragement, providing a lot of facilities, helpful discussions, directions, beneficial advice and continuous support.*

*My endless thanks to **Dr. Sherien Mohamed El-Daly**, Assi. Prof. of Medical biochemistry, Medical biochemistry department, National research center. She was supportive and taught me a lot. I greatly appreciate her help, supervision and efforts throughout the whole work, although I believe that words cannot express my gratitude*

*I would like to express my greatest thanks and appreciation to **Dr. Dalia Medhat Ahmed Ali**, Assi. Prof of Medical Biochemistry, Medical biochemistry department, National Research center, for her helpful suggestions, supervision, and continuous support. Her valuable advices helped me in this work.*

Dedication

I'd like to dedicate this work to:

My Family

My Husband &

My Sons

Thanking them for their sincere prayers, love, support and encouragement.

Abstract

Nuclear factor-κB (NF-κB) has been identified as the major link between inflammation and cancer. NF-κB controls transcriptional activation of several genes related to inflammation. Natural agents that inhibit this pathway are essential in attenuating inflammation induced by cancer and/or induced by chemotherapeutic drugs. A high intake of Brassicaceae vegetables is linked to a lower incidence of cancer, related to the breakdown of glucosinolates into bioactive indole compounds, suggesting their involvement in modulating essential pathways related to chronic diseases.

In the present study, inoculation of Ehrlich ascites carcinoma (EAC) cells in female albino mice resulted in a marked increase in packed cell volume, viable cell count, and a significant increase in the level of NF-κB, in addition to several cytokines and inflammatory biomarkers (IL-6, IL-1b, TNF-α, and NO). A significant elevation in the inflammatory-medicated miRNAs (miR-31 and miR-21) was also detected. Treatment with 5-Fluorouracil (5-FU) significantly reduces packed cell volume and the viable cell count. However, it was accompanied by a significant increase in the levels of inflammatory markers and the expression of miR-31and miR-21 compared to the untreated group. Although treatment with the glucosinolates indoles, indole-3-carbinol (I3C) and 3,3-diindolylmethane (DIM) significantly reduce the packed cell volume and the viable cell count, it was still less effective than 5-FU treatment. On the other hand, I3C and DIM significantly reduced the inflammatory response compared to both EAC inoculated untreated group and the EAC group treated with 5-FU. Moreover, their anti-inflammatory effect was modulated by a significant reduction in the inflammatory-medicated miRNAs (miR-31 and miR-21).

Our findings showed that I3C and DIM have a strong anti-inflammatory effect, implying that their use as a co-treatment with chemotherapeutic drugs could effectively improve the anti-tumor effect of chemotherapeutics.

Keywords

indole-3-carbinol; 3,3-diindolylmethane; Inflammation; 5-Fluorouracil; Ehrlich ascites carcinoma; miR-21, miR-31, NF-κB

List of Contents

List of Abbreviations

Abbr.	Full-term
DMSO	**:** Dimethyl sulfoxide
AKT	**:** Protein kinase B
Bcl-2	**:** B-cell lymphoma-2
COVID-19	**:** Coronavirus disease 2019
C-Rel	**:** Proto-oncogene C-Rel
CSF	**:** Colony stimulating factor
CV	**:** Cruciferous vegetables
DIM	**:** Diindolylmethane
DNA	**:** Deoxynucleic acid
EAC	**:** Ehrlich ascites carcinoma
EDTA	**:** Ethylene diamine tetra acetic acid
EGFR	**:** Epidermal growth factor receptor
ELISA	**:** enzyme-linked immunosorbent assay
eNOS	**:** Endothelial nitric oxide
ER	**:** Estrogen receptor
FU	**:** 5-fluorouracil
G-CSF	**:** Granulocyte colony stimulating factor
GM-CSF	**:** Granulocyte – macrophage colony stimulating factor
GPCRs	**:** G-Protein-coupled receptors
GST	**:** Glutathione -s- transferase
HGF	**:** Hepatocyte growth factor
I3C	**:** Indole-3-carbinol
IFNS	**:** Interferons
IKBα	**:** Inhibitor of nuclear factor kappa B
IL-1	**:** Interleukin-1

IL-6 : Interleukin -6
IL-8 : Interleukin -8
iNOS : inducible nitric oxide
JAK : Janus kinase
MAP : Mitogen-activated protein
MCP-1 : Monocyte chemoattractant protein-1
MDSC : Myeloid – derived suppressor
miRNA : microRNA
MPO : Myeloperoxidase
MPO : Myeloperoxidase enzymes
NF-kB : Nuclear factor kappa B
NK : Natural killer
NO : Nitric oxide
NRC : National research Centre
PBS : Phosphate buffer saline
PEITC : Phenethyl isothiocyanate
r.p.m : Round per minute
RNA : ribonucleic acid
ROMs : Reactive oxygen metabolites
ROS : Reactive oxygen species
SD : Standard deviation
SFN : Sulforaphane
SOD : Superoxide dismutase
STAT : Signal transducer and activator of transcription
TGF-β : Transforming growth factor-β
TNF-α : Tumor necrosis factor alpha
VEGF : Vascular endothelial growth factor
VEGF : Vascular endothelial growth factor

List of Tables

List of Figures

Introduction

Cancer is a growing health problem around the world - particularly with the steady rise in life expectancy, increasing urbanization and the subsequent changes in environmental conditions, including lifestyle. Cancer risk can be reduced by eliminating the identified carcinogens – or at least minizine exposure to them. It has been estimated that more than two – thirds of human cancers could be prevented through appropriate lifestyle modification ***(Bray et al., 2018).***

Chronic inflammation is implicated in the development and progression of different types of cancer. A variety of soluble factors and cellular signaling events play a crucial role in inflammation ***(David, 2021).*** The most important signaling pathway involved in the initiation and amplification of inflammatory responses is the one that leads to nuclear factor-κB (NF-κB) activation ***(Liu et al., 2017)***.

Since NF-κB regulates several genes that are activated in response to inflammation, it may play a vital role in the inflammatory response to infection and tissue injury. The transcription factors in the NF-κB family control the expression of genes that code for cytokines (like interleukin - 6 and interleukin-1b), pro-inflammatory enzymes (ex; inducible nitric oxide synthase, iNOs), and microRNAs that control tumor progression. Accordingly, developing