Faculty of Pharmacy

Ain Shams University

Pharmacology and Toxicology Department



"The potential curative effect of galangin against experimentally induced ulcerative colitis"

A thesis submitted for partial fulfillment of the requirements of the Master's Degree in Pharmaceutical Sciences (Pharmacology and Toxicology)

By

Samar Hosni Gerges Nesr

B.Sc. Pharmacy (2014), Faculty of Pharmacy, Ain Shams University

Under the supervision of

Prof. Ebtehal El-Demerdash Zaki

Professor and Head of Pharmacology and Toxicology Department, Faculty of Pharmacy, Ain Shams University

Dr. Doaa Ahmed Elsherbiny

Lecturer, Pharmacology and Toxicology Department, Faculty of Pharmacy, Ain Shams University

Dr. Mai Fathy Tolba

Lecturer, Pharmacology and Toxicology Department, Faculty of Pharmacy, Ain Shams University

Acknowledgements

At the beginning, I wish to express my sincere thanks and gratitude to my supervisors; they were exemplary in their roles as researchers, mentors, and teachers.

I wish to express my appreciation and gratitude to **Prof. Ebtehal El-Demerdash,** Professor and Head of Pharmacology and Toxicology Department, Faculty of Pharmacy, Ain Shams University, for being very generous with her knowledge. She has made this work possible by her continuous guidance, support, and precious advices throughout the whole study including practical work and thesis writing.

I am deeply thankful to **Dr. Doaa Ahmed Elsherbiny**, Lecturer of Pharmacology and Toxicology, Faculty of Pharmacy, Ain Shams University, for her continuous help and support in all aspects, and for her tremendous efforts in reviewing the thesis. She never stopped supporting and guiding me throughout the whole study.

Most heartfelt thanks are due to **Dr. Mai Fathy Tolba**, Lecturer of Pharmacology and Toxicology, Faculty of Pharmacy, Ain Shams University, for choosing the research point and for being very helpful, supportive, and kind. I am also very grateful for her great efforts in the practical work and in reviewing the thesis.

I would like to thank **Dr. Adel Bakir**, Professor of Pathology, Faculty of Veterinary Medicine, Cairo University, for his sincere help in histopathology, and **Dr. Ahmed Erfan**, Head of Biotechnology Unit, Animal Health Research Institute, for his kind help in real-time PCR.

I would like to thank all my Professors and colleagues at the Pharmacology and Toxicology Department, Faculty of Pharmacy, Ain Shams University, for their kind and sincere support throughout my study.

Finally, I would like to thank my family: my father, my mother, and my brother for giving me all the love and wholehearted support to continue. Without their unconditional love and understanding, I could hardly endure the hard times throughout my study.

Bamar Hosni

Contents

Subject	Page
List of Abbreviations	I
List of Tables	IV
List of Figures	VI
Abstract	1
Introduction	
1. Inflammatory bowel diseases	2
2. Pathogenesis of inflammatory bowel diseases	5
3. Risk of colorectal cancer	23
4. Experimental models of inflammatory bowel diseases	24
5. Management of inflammatory bowel diseases	25
6. Propolis	30
7. Galangin	30
8. Sulfasalazine	38
Aim of the Work	43
Material and Methods	
(A) Experimental design	45
(B) Material	50
(C) Methods	54
Results	77
Discussion	125
Summary and Conclusion	137
References	142
Arabic Summary	Í

List of Abbreviations

Abbreviation	Meaning
5-ASA	5-Aminosalicylic acid
ANOVA	Analysis of variance
Anti-TNF	Anti-tumor necrosis factor
B(a)P	Benzo(a)pyrene
CARD9	Caspase recruitment domain-containing protein 9
CAT	Catalase
CD	Crohn's disease
Con A	Concavalin A
COX	Cyclooxygenase
COX-2	Cyclooxygenase-2
CRC	Colorectal cancer
DAI	Disease activity index
DAMPs	Damage-associated molecular patterns
DCs	Dendritic cells
DSS	Dextran sulfate sodium
DTT	Dithiothreitol
ELISA	Enzyme-linked immunosorbent assay
ERK	Extracellular signal-regulated kinases
GIT	Gastrointestinal tract
GPx	Glutathione peroxidase
GSH	Reduced glutathione
H & E	Hematoxylin and eosin
HMGB1	High mobility group box protein 1
HRP	Horseradish peroxidase
HSV-1	Herpes simplex virus type 1
IBDs/IBD	Inflammatory bowel diseases
IECs	Intestinal epithelial cells
IL-1	Interleukin 1

IL-1β	Interleukin 1 beta
IL-6	Interleukin 6
IL-8	Interleukin 8
IL-13	Interleukin 13
IL-17	Interleukin 17
IL-23	Interleukin 23
iNOS	Inducible nitric oxide synthase
IRAKs	Interleukin 1 receptor-associated kinases
IV	Intravenous
ІкВ	Inhibitor kappa B
JNK	c-jun N-terminal kinase
LDH	Lactate dehydrogenase
LH	Lipid hydroperoxides
LOX	Lipoxygenase
LPS	Lipopolysaccharides
MAPKs	Mitogen-activated protein kinases
MBL	Metallo-beta-lactamase
MDA	Malondialdehyde
MDP	Muramyl dipeptide
MIC	Minimum inhibitory concentration
MyD88	Myeloid differentiation primary response protein 88
Na CMC	Sodium carboxymethyl cellulose
NADH	Nicotinamide adenine dinucleotide
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B
	cells
NLRs	NOD-like receptors
NOD2	Nucleotide-binding oligomerization domain-containing
	protein 2
NSAIDs	Non-steroidal anti-inflammatory drugs
OCPs	Oral contraceptives
OD	Optical density
OVA	Ovalbumin
PAMPs	Pathogen-associated molecular patterns

PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PI3K	Phosphoinositide 3-kinase
PIC	Protease inhibitor cocktail
PRRs	Pattern recognition receptors
RANKL	Receptor activator of nuclear factor kappa B ligand
ROS	Reactive oxygen species
SD	Standard deviation
SOD	Superoxide dismutase
SP	Streptavidin-peroxidase
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
TBS	Tris buffered saline
TGF-β	Transforming growth factor beta
TLR4	Toll-like receptor 4
TLRs	Toll-like receptors
TNF-α	Tumor necrosis factor alpha
Treg	Regulatory T cells
UC	Ulcerative colitis

List of Tables

Table (1): Drugs, chemicals, kits, antibodies, and equipment used in the study
Table (2): Primer sequences of the investigated genes
Table (3): Cycling conditions for SYBR green real-time PCR according to Quantitect SYBR green PCR kit
Table (4): Daily disease activity index scores of the five experimental groups
Table (5): The severity of histopathological alterations in the five experimental groups after the dose-selection study
Table (6): The effect of treatment with different doses of galangin (20, 40, and 80 mg/Kg) on NF-κB protein abundance in DSS-induced mice86
Table (7): The effect of treatment with different doses of galangin (20, 40, and 80 mg/Kg) on iNOS protein abundance in DSS-induced mice90
Table (8): The percentage of body weight change in the five experimental groups at the end of the mechanistic study
Table (9): The severity of histopathological alterations in the five experimental groups after the mechanistic study
Table (10): The effect of treatment with galangin, sulfasalazine, and a combination of both on serum LDH activities in DSS-induced mice102
Table (11): The effect of treatment with galangin, sulfasalazine, and a combination of both on relative TLR4 mRNA expression levels in DSS-induced mice
Table (12): The effect of treatment with galangin, sulfasalazine, and a combination of both on NF-κB p65 nuclear concentrations in DSS-induced mice

Table (13): The effect of treatment with galangin, sulfasalazine, and a combination of both on tissue IL-6 concentrations in DSS-induced mice111	
Table (14): The effect of treatment with galangin, sulfasalazine, and a combination of both on tissue TNF-α concentrations in DSS-induced mice	
Table (15): The effect of treatment with galangin, sulfasalazine, and a combination of both on relative HMGB1 mRNA expression levels in DSS-induced mice	
Table (16): The effect of treatment with galangin, sulfasalazine, and a combination of both on tissue GSH concentrations in DSS-induced mice	
Table (17): The effect of treatment with galangin, sulfasalazine, and a combination of both on tissue MDA concentrations in DSS-induced mice	

List of Figures

Figure 1: Factors involved in IBD pathogenesis
Figure 2: The intestinal epithelial barrier in normal and IBD states15
Figure 3: Signaling through all TLRs finally leads to the activation and nuclear translocation of NF-κB and MAPKs
Figure 5: Chemical structure of galangin
Figure 6: Chemical structure of sulfasalazine
Figure 7: Experimental design of the dose-selection study
Figure 8: Experimental design of the mechanistic study
Figure 9: Standard calibration curve of NF-κB p6567
Figure 10: Standard calibration curve of IL-670
Figure 11: Standard calibration curve of TNF-α73
Figure 12 (A,B): Disease activity index scores of the five experimental groups.
12A: Data are represented as median and interquartile range79
12B: Data are represented as median, error bars are omitted for clarity79
Figure 13: Disease activity index scores of the five experimental groups on the last day of the dose-selection study80
Figure 14: Histopathological findings in the five experimental groups after the dose-selection study
Figure 15: The effect of treatment with different doses of galangin (20, 40, and 80 mg/kg) on NF-kB protein abundance

Figure 16: Immunohistochemical staining images of NF-κB88
Figure 17: The effect of treatment with different doses of galangin (20, 40, and 80 mg/kg) on iNOS protein abundance
Figure 18: Immunohistochemical staining images of iNOS92
Figure 19: Body weights of mice in the five experimental groups over the mechanistic study period
Figure 20: Histopathological findings in the five experimental groups after the mechanistic study
Figure 21: The effect of treatment with galangin, sulfasalazine, and a combination of both on serum LDH activities
Figure 22: The effect of treatment with galangin, sulfasalazine, and a combination of both on the mRNA expression levels of TLR4
Figure 23: The effect of treatment with galangin, sulfasalazine, and a combination of both on nuclear concentrations of NF-κB p65
Figure 24: The effect of treatment with galangin, sulfasalazine, and a combination of both on tissue IL-6 concentrations
Figure 25: The effect of treatment with galangin, sulfasalazine, and a combination of both on tissue TNF-α concentrations
Figure 26: The effect of treatment with galangin, sulfasalazine, and a combination of both on the mRNA expression levels of HMGB1
Figure 27: The effect of treatment with galangin, sulfasalazine, and a combination of both on tissue GSH concentrations

Figure	28:	The	effect	of	treatment	with	galangin,	sulfasalazine	, and a
combin	ation	l	of	both	n on	tiss	ue MI	OA concer	ntrations
									124