



شبكة المعلومات الجامعية
التوثيق الإلكتروني والميكروفيلم

بسم الله الرحمن الرحيم



MONA MAGHRABY



شبكة المعلومات الجامعية
التوثيق الإلكتروني والميكرو فيلم



شبكة المعلومات الجامعية التوثيق الإلكتروني والميكرو فيلم



MONA MAGHRABY



شبكة المعلومات الجامعية
التوثيق الإلكتروني والميكروفيلم

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات



يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار



MONA MAGHRABY



**Assessment of the therapeutic potential of gold
nanoparticles against Collagen-Induced Arthritis in rats**

A Thesis Submitted for the degree of Ph.D in Biochemistry

Submitted by

Samar Mohamed Amin Abou El-Fotouh Shahan

(M.Sc. Molecular Immunology)

Genetic Engineering and biotechnology Institute
University of Sadat City

Under the supervision of

Prof. Dr. Mohamed Ragaa Mohamed

Professor of Biochemistry and Molecular Biology

Faculty of Science
Ain Shams University

Prof. Dr. Roba Mohamed Talaat

Professor of Molecular Immunology
Genetic Engineering and Biotechnology Institute
University of Sadat City

Dr. Germine Mohsen Hamdy

Assistant Professor of Biochemistry
Faculty of Science
Ain Shams University

2021

Approval sheet

Name of candidate/ **Samar Mohamed Amin Abou El-Fotouh Shahen.**

Title of the thesis / **Assessment of the therapeutic potential of gold nanoparticles against Collagen-Induced Arthritis in rats.**

This thesis has been approved for submission by:

Supervisors:

Prof. Dr. Mohamed Ragaa Mohamed

Professor of Biochemistry and Molecular Biology, Faculty of Science,
Ain shams University.

Prof. Dr. Roba Mohamed Talaat

Professor of Molecular Immunology, Genetic Engineering and
Biotechnology Institute, University of Sadat City.

Dr. Germine Mohsen Hamdy

Assistant Professor of Biochemistry, Faculty of Science, Ain Shams
University

Examiners committee:

Prof. Dr. Tarak Mohamed Motawaa

Professor of Biochemistry – Faculty of Pharmacy- Cairo University.

Prof. Dr. Sameh Hamdy suroor

Emeritus Research Professor, Department of Biochemistry - National
Research Center

Prof. Dr. Mohamed Ragaa Mohamed

Professor of Biochemistry and Molecular Biology, Faculty of Science,
Ain shams University.

Prof. Dr. Roba Mohamed Talaat

Professor of Molecular Immunology, Genetic Engineering and
Biotechnology Institute, University of Sadat City.

Declaration

I declare that this thesis has been composed by myself and that the work of which is a record that has been done by myself. This thesis has not been submitted previously for a degree at this or any other university.

Samar M. Shahan

Biography

Name: Samar Mohamed Amin Abou El-fotouh Shahen.

Degree Awarded: Master in Molecular Immunology.

Faculty: Genetic Engineering and Biotechnology Institute.

University: University of Sadat City

Date of Registration: 2016

Date of Appointment: 2021

ACKNOWLEDGEMENT

Thanks are due first and last to **Almighty GOD** as I deeply owe **HIM** mercy, support and guidance in my whole life.

I would like to express my sincere gratitude to **Prof. Dr. Mohamed Ragaa Mohamed** (Professor of Biochemistry and Molecular Biology, Faculty of Science, Ain Shams University) for his continuous support, guidance, motivation and valuable advice and criticism. It is a great honor to me to work under his supervision.

My sincere thanks also go to **Prof. Dr. Roba Mohammed Talaat** (Professor of Molecular Immunology, Genetic Engineering and Biotechnology Research Institute, University of Sadat City) who provided me an opportunity to join her team and gave me an access to the laboratory and research facilities. Without her precious support it would not be possible to conduct this research. Also, I would like to thank all members of the Molecular Biology Lab for their continuous help and support specially **Nehal Abd-Alhakem**.

I cannot find words to express the deepest appreciation to **Dr. Germin Mohsen Hamdy** (Ass. professor of Biochemistry, Faculty of Science, Ain Shams University) for her insightful comments, encouragement and her guidance. It was a pleasure and honor to work with her.

Special thanks to **Dr. Mustafa R Ali** (Biological Engineering Department, Massachusetts Institute of Technology, Cambridge University) for synthesis of Gold nanoparticles, his cooperativity and

continuous help and to **Dr. Rehab M. Samaka** (Pathology Department, Faculty of Medicine, Menoufia University) for explaining and writing comments on histopathology pictures.

This thesis is dedicated to my Dad who always believed in me and wanted the best for me. Thanks for your endless love, encouragement and for teaching me that success always comes with persistence. Secondly, I would like to thank my family for continuous help.

Samar Mohamed Shaken

List of contents

Contents	Page
<i>List of abbreviations</i>	I
<i>List of tables</i>	V
<i>List of figures</i>	VI
<i>Abstract</i>	1
<i>Introduction</i>	2
<i>Aim of the Work</i>	4
<i>Chapter (I). Review of literature</i>	5
1.1. Rheumatoid arthritis (RA).	5
1.1.1. Epidemiology of RA.	7
1.1.2. Diagnosis of RA.	7
1.1.3. Symptoms and complications of RA.	10
1.1.4. Risk factors of RA.	11
1.1.5. Prevention of RA.	13
1.2. Immunopathogenesis of RA.	13
1.2.1. Role of B Cells in RA pathogenesis.	15
1.2.2. Role of T Cells in RA pathogenesis.	15
1.2.2. Role of macrophages in the pathogenesis of RA	16
1.2.4. The role of synovial Fibroblast (SF) in RA pathogenesis.	16
1.2.5. The role of osteoclasts in RA pathogenesis.	17
1.2.6. Role of chondrocytes in the pathogenesis of RA.	17
1.3. Role of Cytokines in RA	20
1.4. Angiogenesis in RA.	23
1.5. Treatment of RA.	29
1.5.1. Disease modifying anti-rheumatic drugs (DMARDs).	29
1.5.2. Glucocorticoids treatment.	30
1.5.3. Non-steroidal anti-inflammatory drugs.	30
1.5.4. Biological agents in RA treatment.	30
1.6. Superiorities of Nanotherapeutics.	34
1.7. Gold nanoparticles (AuNPs).	35
1.7.1. AuNPs in treatment of RA.	36
1.8. Animal Models of RA	37
1.8.1. Collagen-induced arthritis (CIA)	38
<i>Chapter (II). Materials and methods</i>	41

2.1. Materials	41
2.1.1. Chemicals and reagents	41
2.1.2. Kits.	42
2.1.3. Animals	43
2.2. Methods	43
2.2.1. Reagents preparation	43
2.2.1.2. Preparation of collagen-IFA emulsion	43
2.2.1.3. Aqua regia	44
2.2.2. Synthesis, Conjugation and Characterization of AuNPs	45
2.2.3. Experimental design	48
2.2.3.1. Induction of CIA model	49
2.2.4. Imaging of the joints	50
2.2.5. Histopathological analysis	50
2.2.6. Bio-distribution of AuNPs in internal organs	52
2.2.7. Biochemical analysis	54
2.2.8. Cytokines measurement	54
2.2.8.1. Pro-inflammatory cytokines assessment	54
2.2.8.2. Anti-inflammatory cytokines evaluation	54
2.2.8.3. Antigenic mediator's evaluation.	54
Principle of ELISA assay	55
□ Determination of cytokine concentration	56
2.2.9. Statistical analysis of data	58
Chapter III. Results	59
3.1. Radiographic analysis of anti-arthritis effects of AuNPs	59
3.2. Anti- arthritis effects of AuNPs on collagen-induced histopathological changes	61
3.3. Biodistribution of AuNPs in internal organs	69
3.4. Biochemical analysis	72
3.5. Effect of AuNPs on cytokines	73
3.5.1. Effects of AuNPs on pro-inflammatory cytokines.	73
3.5.2. Effects of AuNPs on angiogenic mediators	77
3.5.3. Effects of AuNPs on anti-inflammatory cytokines	79
3.5.4. Correlation between cytokines in AuNSs@NLS group	81
Chapter IV. Discussion	83
Summary	94
Reference	97
Arabic summary	ب
Arabic abstract	أ

List of abbreviations

ACR	American College of Rheumatology
ADAMTS	A disintegrin and metalloproteinase with thrombospondin motifs
ADCC	Antibody-dependent cellular cytotoxicity
ALT	Alanine transaminase
Ang1	Angiopoietin1
ANOVA	One-way analysis of variance
Anti-CCP	Antibodies to cyclic citrullinated peptides
APC	Antigen-presenting cells
AST	Aspartate transaminase
AuNPs	Gold nanoparticles
AuNRs	Gold nano-rods
AuNSs	Gold nano-spheres
BMI	Body mass index
CAIA	Collagen antibody-induced arthritis
CCL2	C-C Motif Chemokine Ligand 2
CFA	Complete Freund's adjuvant
CIA	Collagen-induced arthritis
CII	Collagen type II
COX	Cyclooxygenase
CRP	C-reactive protein
CTBA	Cetyltrimethylammonium bromide
CXCL	Chemokine (C-X-C motif) ligand
DMARDs	Disease modifying anti-rheumatic drugs

ELISA	Enzyme-linked immunosorbent assay
ESR	Erythrocyte sedimentation rate
EULAR	European League against Rheumatism
FGF	Fibroblast growth factor
FLS	Fibroblast-like synoviocytes
GAL	Galectin
GCs	Glucocorticoids
GM-CSF	Granulocyte-macrophage colony-stimulating factor
HB-EGF	Heparin-binding endothelial growth factor
HGF	Hepatocyte growth factor
HIF-1α	Hypoxia inducible factor-1 α
HLA	Human leukocyte antigen
HRP	Horseradish peroxidase
ICP-MS	Inductively coupled plasma mass spectrometry
IFA	Incomplete Freund's Adjuvant
IFN-γ	Interferon gamma
IL	Interleukin
KGF	Keratinocyte growth factor
MCP	Metacarpophalangeal
MCP-1	Monocyte chemoattractant protein-1
MIC	Macrophage inhibitory cytokine
MIF	Macrophage migration inhibitory factor
MMP	Matrix metalloproteinase
mPEG-SH	Methoxy polyethylene glycol thiol
MRI	Magnetic Resonance Imaging
MTP	Metatarsophalangeal

MTX	Methotrexate
NLS	Nuclear localization sequence peptide
NPs	Nanoparticles
NSAIDs	Non-steroidal anti-inflammatory drugs
OPG	Osteoprotegerin
PBS	Phosphate-buffered saline
PDGF	Platelet derived growth factor
PDGFR	Platelet derived growth factor receptor
PEG	Polyethylene glycol
PGE2	Prostaglandin E2
PGs	Prostaglandins
PIP	Proximal interphalangeal
RA	Rheumatoid arthritis
RANK	Receptor activator of nuclear factor- κ B
RANKL	Receptor activator of nuclear factor kappa B ligand
RF	Rheumatoid factor
RGD	Arginine-glycine aspartic acid
ROS	Reactive oxygen species
SD	Standard deviation
SF	Synovial fibroblast
SLE	Systemic lupus erythematosus
SPR	Surface plasmon resonance
SPSS	Statistical Package for the Social Sciences
TCZ	Tocilizumab
TGF	Transforming growth factor
Th	T-helper cell
