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Anti-Inflammatory, Oxidative, and Antioxidant Status in Women Patients with Rheumatoid Arthritis affected by Niosomal Curcumin

A Thesis

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By

Nasreen Ghalib Obeaid

B.Sc. in Chemistry- College of Science, University of Kerbala, (2021-2022)

Supervised by

Prof. Dr. Fadhil Jawad Al-Tu'ma — **Department of Chemistry and Biochemistry**

Dr. Ali Mohammed Kadhim — **Department of Internal Medicine**

College of Medicine – University of Kerbala

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

﴿يُؤْتِي الْحِكْمَةَ مَن يَشَاءُ ۚ وَمَن يُؤْتَ الْحِكْمَةَ فَقَدْ أُوتِيَ خَيْرًا كَثِيرًا ۗ وَمَا يَذَّكَّرُ إِلَّا أُولُو الْأَلْبَابِ﴾

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Supervisor Certification

We certify that this thesis entitled **"Anti-Inflammatory, Oxidative, and Antioxidant Status in Women Patients with Rheumatoid Arthritis affected by Niosomal Curcumin "** was carried under our supervision at the College of Medicine, University of Kerbala, as partial fulfillment for the requirement of the degree of Master of Science in **"Clinical Biochemistry"**.

Supervisors

Prof. Dr. Fadhil J. Al-Tu'ma

Department of Chemistry and Biochemistry

College of Medicine

University of Kerbala

Dr. Ali M. Kadhim Al-Tu'ma

Department of Internal Medicine

College of Medicine

University of Kerbala

In view of the available recommendations, I am forwarding this M.Sc. thesis for debate by the examining committee.

Assist. Prof.

Dr. Atheer H. AL-Ghanimi

Head of Chemistry and Biohemistry Department

College of Medicine

University of Kerbala

Examination Committee Certification

We the examining committee, certify that we have read this M.Sc. thesis entitled:

Anti-Inflammatory, Oxidative, and Antioxidant Status in Women Patients with Rheumatoid Arthritis affected by Niosomal Curcumin

We have examined the student **"Nasreen Ghalib Obeaid"** in its content and our opinion; it meets the standard of thesis for the Master's degree in **"Clinical Chemistry"** with an appreciated **"Excellent"**.

Signature
Assist. Prof. Dr. Atheer H. Al-Ghanimi
College of Medicine
University of Kerbala
Date: / / 2024
(Member)

Signature
Assist. Prof. Dr. Hameed H. Al-jameel
College of Medicine
University of Kerbala
Date: / / 2024
(Member)

Signature
Prof. Dr. Fadhil J. Al-Tu'ma
College of Medicine
University of Kerbala
Date: 1 / 10 / 2024
(Member / Supervisor)

Signature
Dr. Ali M. Kadhim Al-Tu'ma
College of Medicine
University of Kerbala
Date: / / 2024
(Member / Supervisor)

Signature
Prof. Dr. Hanaa A. Ali AL-Sultani
Faculty of Science / University of Kufa
Date: / / 2024
(Chairman)

Approved by the College of Medicine - University of Kerbala

Signature:
Date: / / 2024
Prof. Dr. Riyadh Dayhood Al-Zubaidi
Dean of the College of Medicine

Dedication

Praise be to Allah who facilitated the beginnings, perfected the endings, and enabled us to reach our goals. Praise be to Allah for His abundant gifts and abundant grace, grace, and generosity upon us.

To my master, my support and my asset in this world and the hereafter

Imam Mahdi

(May Allah hasten his honorable return)

To the pure soul of

My Father

To the soul of my dear

My Mother

To my beloved **Brothers and Sisters**

To my friends **Ghufran and Jullanar**

To everyone who helped me

To all these people, I dedicate this work

I ask God to make him a beacon for every student.

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Summary

Rheumatoid arthritis (RA) is a chronic autoimmune disease with a global prevalence of over 1%. It is middle-aged and elderly patients, with higher rates in females. Obesity is linked to an increased risk of autoimmune disorders due to pro-inflammatory environments. Nanomedicine, using nanoparticles, is a promising approach for treating and targeting RA. Curcuminoids, active components of turmeric powder, have numerous biological activities, including anti-inflammatory, anticancer, antiviral, antibacterial, antifungal, and immunomodulatory properties. Genes are genomic sequences encoding functional products, while Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases in the metzincin protease superfamily. Tissue inhibitors of metalloproteinase (TIMPs) a crucial regulator of MMPs, play a significant role in biological processes and diseases.

The objectives aim to study oxidant-antioxidant imbalance in RA, identify inflammatory and anti-inflammatory biomarkers, synthesize curcumin-loaded hyaluronic acid-decorated niosomal nanoparticles using thin layer hydration technique, and examine the application of Hyalo-Nio-cur NPs as antioxidant and anti-inflammatory in RA patients. In addition, shows the effect of Hyalo-Nio-cur NPs on MMP-2 and TIMP-2 in patients with RA.

Methods of study on RA patients' blood samples from 130 healthy controls and outpatients used SMART-120 chemical analyzer to quantify lipid profile levels. Enzyme-linked immunosorbent Assay (ELISA) was used to evaluate Malondialdehyde (MDA), reduced glutathione (GSH), catalase, Interleukin-6 (IL-6), and interleukin-10 (IL-10) levels. Hyalo-Nio-cur NPs were synthesized to examine antioxidant and anti-inflammatory properties in rheumatoid arthritis (RA) patients. Real-time polymerase chain reaction (RT- PCR) was used to evaluate MMP-2 and TIMP-2 genes in the NPs.

The results indicated that the obese RA had significantly higher mean ± SD levels of Malondialdehyde than non-obese RA as compared with control groups (5.06 ± 1.05, 4.07 ± 1.22, and 1.04 ± 0.28 nmol/mg respectively. The catalase activity levels were significantly different between obese and non-obese RA as compared with control groups (4.40 ± 2.28, 6.62 ± 2.52, and 15.77 ± 1.16 U/L respectively, Glutathione levels were lower in obese RA (51.26 ± 2.52), non-obese RA (54.44 ± 7.76), and control group (63.22 ± 10.14 μM/ml). The mean ± SD of IL-6 in obese RA was significantly higher than in non-obese RA and control groups, with values of (71.02 ± 11.39, 59.8 ± 14.50, and 5.87 ± 1.9) pg/ml, respectively. Non-obese RA patients had significantly lower IL-10 levels compared to obese RA patients, with mean levels of (20.62 ± 6.80, 25.31 ± 5.25, and 7.37 ± 1.33) pg/ml, respectively. The study found that Nio-cur NPs and Hyalo-cur-Nio NPs significantly reduced Malondialdehyde levels in PBMCs, and increased catalase, and glutathione levels. Treatment also decreased IL-6 and increased IL-10, increased TIMP2 expression, and reduced MMP-2 in healthy and RA.

Conclusion Obesity affects rheumatoid arthritis patients, leading to increased inflammation and increased oxidant status. Obese patients have higher levels of MDA and antioxidants, while pro-inflammatory cytokines like IL-6 are higher in obese RA. Nanomedicine, specifically curcumin-loaded nanoparticles coated with HA, has shown promise in managing RA by reducing inflammation, increasing anti-inflammatory indicators, and enhancing antioxidant enzyme activity. This research suggests a promising approach for RA treatment.

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Abbreviations	Complete Name
ACPA	Anti-citrullinated peptide antibody
ACR	The American College of Rheumatology
AFM	Atomic Force microscopy
Anti-CD20	Anti-CD20 antibody immunotherapy
APC	Antigen-presenting cell
B-cell	B lymphocyte
BMI	Body mass index
C5a	Complement component 5
CAT	Catalase
CD20	Cluster of differentiated 20
CD4+	Clusters of differentiation 4
CD44	Clusters of differentiation 44
cDNA	Complementary deoxyribonucleic acid
CETP	Cholesteryl ester transfer protein
CRP	C-reactive protein
CT	Computed tomography
CVD	Cardiovascular diseases
DETBA	1,3-Diethyl-2-thiobarbituric acid
DLS	Dynamic Light Scattering
DMARDs	Disease-modifying anti-rheumatic drugs
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
DTNB	Dithiobis (2 - nitro benzoic acid)
ELISA	Enzyme-linked immune sorbent assay
ESR	Erythrocyte sedimentation rate
EULAR	The European League Against Rheumatism
FDRs	First-degree relatives
FE-SEM	Field emission scanning electron microscopy
FLS	Fibroblast-like synovial cells
FT-IR	Fourier Transform Infrared Spectroscopy
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GPXs	Glutathione peroxidases
GSH	Glutathione
HA	Hyaluronic acid
HDL-C	High-density lipoprotein cholesterol
HLA	Human leukocyte antigen
HLA-DR	Human leukocyte antigen-DR iso-type

HLA-DRB1	Major Histocompatibility Complex, Class II, DR Beta 1
HRP	Horseradish peroxidase
HRQoL	Health-related quality of life
Hyalo-Nio NPs	Hyaluronic acid-coated niosomal nanoparticles
Hyalo-Nio-cur NPs	Curcumin-loaded hyaluronic acid-coated niosomal nanoparticles
IL1	Interlaken-1
IL-10	Interlaken-10
IL-12	Interlaken-12
IL-17	Interleukin-17
IL-19	Interlaken-19
IL1-RA	Interlaken-1 receptor antagonists
IL-1β	Interleukin-1β
IL-22	Interlaken-22
IL-23	Interlukins-23
IL-24	Interlaken-24
IL-26	Interlaken-26
IL-28A	Interlaken-28A
IL-28B	Interlaken-28B
IL-29	Interlaken-29
IL-4	Interlaken- 4
IL-6	Interleukin-6
IL-6R	Interleukin-6 receptor
IL-8	Interleukin-8
LDL-C	Low-density lipoprotein cholesterol
MDA	Malondialdehyde
MDA-TBA	Malondialdehyde and thiobarbituric acid
MD-HAQ	Multi-Dimensional Health Assessment Questionnaire
MMP-2	Matrix metalloproteinases 2
MMPs	Matrix metalloproteinases
MRI	Magnetic resonance imaging
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide
MTX	Methotrexate
Nio-cur NPs	curcumin-loaded niosomal nanoparticles
NK	Natural killer cells
Nm	Nanometer
NPs	Nanoparticles
Nrf2	nuclear factor erythroid 2–related factor 2
NSAIDs	Non-steroidal anti-inflammatory drugs

ORs	Odds ratios
PBMCs	Peripheral blood mononuclear cells
PBS	Phosphate buffer solution
PDI	Polydispersity index
PET	Positron emission tomography
RA	Rheumatoid arthritis
Real-time PCR	Real-time polymerase chain reaction
RF	Rheumatoid factor
RNA	Ribonucleic acid
ROC	The receiver operating characteristic
ROS	Reactive oxygen species
RPM	Revolutions per minute'
SO	Sarcopenic obesity
SOD	Superoxide dismutase
SPSS	Statistical Package for the Social Sciences
TAC	Total antioxidant capacity
TBARS	Thiobarbituric acid reactive substances
TC	Total cholesterol
T-cell	T –Lymphocyte
TDD	Targeted drug delivery
TG	Triglycerides
Th-1	T- helper 1
Th-17	T- helper 17
Th-2	T- helper 2
TIMP	Tissue inhibiter Matrix metalloproteinases
TIMP-2	Tissue inhibiter Matrix metalloproteinases-2
TNF	Tumor necrosis factor
TNF-α	Tumor necrosis factor-alpha
Trx	Thioredoxin
ULN	Upper limit of normal
25(OH)D3	25-hydroxyvitamin D3
WHO	World Health Organization

Chapter One

Introduction
and
Literature Review

1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease that affects the human body's joints. The global prevalence of RA is between 0.5 and 1.3% **(Meade, *et al*., 2024).**

RA is a disease of unknown origin, which is characterized by inflammatory changes of the synovial tissue of joints, cartilage, and bone, and, less frequently, of extra-articular sites **(Scherer, *et al*., 2020)**. Knee and finger joint swelling, destruction, and pain are the common symptoms of RA **(Zhang, *et al*., 2023)**.

Phagocytic cells like neutrophils and macrophages are crucial in the pathogenesis of RA, as they are linked to RA onset and development through mechanisms like reactive oxygen species degranulation or neutrophil extracellular traps release (**Fresneda, *et al.,* 2021)**. Monocytes /macrophages are important in RA pathogenesis due to the release of pro-inflammatory cytokines **(Pascual, *et al*., 2024)**. The former is pro-inflammatory and produces cytokines like interleukin (IL)-6 and tumor necrosis factor (TNF)-α, while the latter has an anti-inflammatory function by secreting IL-10 and repairing damaged tissue **(Wang, *et al.,* 2020)**.

Obesity represents a severe and multifactorial condition influenced by environmental phenomena and genetic aspects **(de Carvalho Braga, *et al.,* 2024)**. The association between body mass index (BMI) and the clinical course of RA remains debated, with evidence suggesting a relationship between inflammation and obesity. Excess production of adipocytokines, especially in obese individuals, perpetuates chronic inflammation **(Imane El Mezouar, 2024)**.

Disease-modifying anti-rheumatic drugs, DMARDs are standard therapy for manifest RA, starting with methotrexate and adding TNF-inhibitors or other biological DMARDs as needed to achieve disease control in RA **(Fraenkel, *et al*., 2021)**.

The overproduction of pro-inflammatory cytokines stimulates neutrophils and leads to the secretion of reactive oxygen species (ROS) by macrophages in the synovial fluid, which causes tissue injury **(Mueller, *et al*., 2021**). MDA is one of the recognized markers of oxidative damage in RA **(Kondo, *et al*., 2023)**. It has been reported that MDA–acetaldehyde adducts localize with citrullinated proteins in patients with RA **(León Fernández, *et al*., 2024)**. The increased ROS content in arthritic rat livers appears to be the product of both a stimulated pro-oxidant mechanism and defective antioxidant protection, such as reduced glutathione (GSH) and catalase (CAT) **(Khawaja and El-Orfali, 2024).**

Curcumin, the primary active component of turmeric, has been extensively studied for its anti-inflammatory, antioxidant, immunomodulatory, and anticancer properties **(Deng, *et al*., 2024)**. Curcumin has demonstrated significant therapeutic potential, particularly in autoimmune diseases like RA, by effectively inhibiting inflammatory reactions and reducing pain and swelling symptoms **(Chamani, *et al*., 2022; Abdollahi, *et al*., 2023)**.

1.1. Rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic autoimmune disease with aggressive, symmetrical polyarthritis as the major clinical manifestation **(Luo, *et al*., 2024)**.

The origin of term "rheumatoid arthritis" comes from the Greek phrase that describes inflammation and fluid build-up in the joints **(Taher and Nassir, 2024)**. RA was first described in 1800 by Dr. Landré-Beauvais and was initially regarded as a form of gout **(Sokolova, *et al*., 2022)**. Later, British rheumatologist Dr. Alfred Baring Garrod، in 1859, separated the two diseases, naming one as rheumatoid arthritis **(Rathore, *et al*., 2023)**.

The prevalence rate of RA is more than 1% worldwide, Most of the patients are middle-aged and elderly, and the rate in females is higher than that

in males **(Finckh, *et al.*, 2022).** The prevalence of the disease varies greatly, depending upon geographical, environmental as well as genetic factors **(Mushtaq, *et al*., 2024)**.

Rheumatoid arthritis is characterized by persistent synovial inflammation, and erosion of bones and cartilage, leading to joint destruction **(Jahid, *et al*., 2023)**. Clinical manifestations are morning stiffness, pain in the shoulder, neck, and pelvic girdle, loss of mobility with fever, fatigue, malaise, loss of body weight, and development of rheumatoid nodules (**Santos, *et al*., 2019).** The main pro-inflammatory cytokines that promote osteoarthritis damage and inflammatory response are interleukin-6 (IL-6), interleukin-1(IL-1), interleukin-18 (IL-18), and tumor necrosis factor (TNF) as shown in Fig. (1.1) **(Alippe, *et al*., 2019)**.

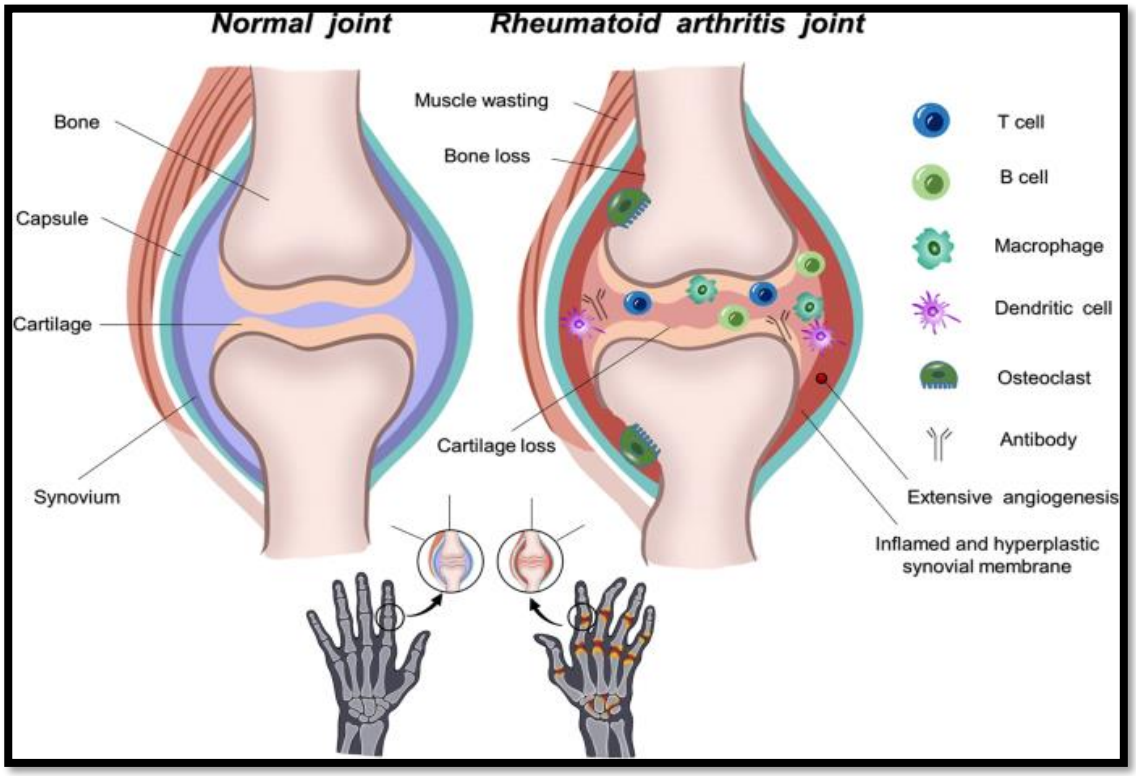


Fig. (1.1): Normal and rheumatoid arthritis joints (Ding, Hu et al. 2023).

1.1.1. Stages of rheumatoid arthritis

1. First stage

Early-stage RA is characterized by synovitis, or an inflammation of the synovial membrane, causing swelling of involved joints and pain upon motion. During this stage, there is a high cell count in synovial fluid as immune cells migrate to the site of inflammation. However, there is generally no x-ray evidence of joint destruction, except for swelling of soft tissues and possibly evidence of some bone erosion.

2. Second stage

In moderate RA, there is a spread of inflammation in synovial tissue, affecting joint cavity space across joint cartilage. This inflammation will gradually destroy cartilage, accompanied by a narrowing of the joint.

3. Third stage

Severe RA is marked by the formation of pannus in the synovium. Loss of joint cartilage exposes bone beneath the cartilage. These changes will become evident on X-rays, along with erosions around the margins of the joint. Joint deformities may also become evident.

4. Fourth stage

Stage IV is called terminal or end-stage RA. The inflammatory process has subsided and the formation of fibrous tissue and/or fusing of bone results in ceased joint function. This stage may be associated with the formation of subcutaneous nodules **(Al-Rubaye, *et al.*, 2017)**.

1.1.2. Causes and risk factors of rheumatoid arthritis

Rheumatoid arthritis is an inflammatory disease with unclear etiology that affects the synovial tissue of joints, bone, cartilage, and, less commonly, extra-

articular areas **(Scherer, *et al.*, 2020)**. Risk factors for the development of RA were reported to include smoking, obesity, sex hormones, drugs, changes in the *microbiome* of the gut, and infections **(Littlejohn, *et al*., 2018)**.

1. Genetic factors

There is an increased prevalence of RA in those families with and ~40–50% risk for seropositive RA, being strongest in first-degree relatives (FDRs) **(Frisell, *et al*., 2016)**. Previous studies have evaluated the risk of RA development in FDRs **(Bemis, *et al*., 2021)**. The strongest genetic association with susceptibility to RA is located at the HLA-DRB1 gene locus - an HLA class II gene **(Xu, *et al*., 2023)**. The HLA-DR gene has a role in RA pathogenesis by influencing the development of autoantibodies that are involved in the progression and prognosis of RA patients **(Karami, *et al.*, 2019)**.

2. Environmental factors

Environmental risk factors include smoking and gut *microbiome* **(Kondo, *et al.*, 2023)**. The association between smoking and RA-risk genes increases the chance of developing ACPA-positive RA **(Tang, *et al.*, 2023)**. A 12% increase in the prevalence of RA was observed in those exposed to passive smoking in adulthood and 34% higher in individuals exposed to passive smoking during childhood than in unexposed individuals **(Zhang, *et al.*, 2023)**. Smoking leads to decreased health-related quality of life (HRQoL) and increased disease activity in RA patients **(Alfredsson, *et al.*, 2023)**. The *Microbiota* may be considered a risk environmental factor for RA **(Alghamdi, *et al.*, 2022)**. The gut microbiota can influence the immune system and inflammation **(Miyauchi, *et al*., 2023)**. The connection between the gut microbiome and RA in recent years, the depletion of *Bifidobacteria,* the *Bacteroides-Porphyromonas-Prevotella* group, and the *Bacteroides fragilis* group in the microbiome of RA patients

compared with that in patients with non-inflammatory fibromyalgia (**Vaahtovuo, *et al*., 2008)**.

3. Sex and hormones

The prevalence of RA is 0.5-1%, with a woman-to-men ratio of 3:1 **(Venetsanopoulou, *et al.*, 2023)**. Sex hormones have immunomodulatory and pleiotropic effects on the immune system, lowering estrogen levels throughout menopause and postpartum is consistently related to an increased risk and severity of RA **(Raine and Giles, 2022)**. Men have prevalence increases after the age of 40 years **(Nilsson, *et a*l., 2021)**. Androgens have direct and indirect anti-inflammatory effects on the immune system with a significantly lower risk of developing autoimmune rheumatic diseases in men than in women **(Cutolo and Gotelli, 2023).**

4. Obesity

Obesity is linked to an increased risk of some autoimmune disorders due to a pro-inflammatory environment induced by adipocytokines, and an increased risk of developing RA in women **(Moroni, *et al*., 2020)**. High levels of adipokines, including IL-6, IL-12, and TNF, can increase inflammation and disease activity in obese patients with RA, affecting both the innate and adaptive immune systems **(Guimarães, *et al*., 2019)**. Obese patients with RA have indeed been shown to report greater pain, patients global scores, poorer quality of life, and greater Multi-Dimensional Health Assessment Questionnaire (MD-HAQ) scores compared to non-obese patients with RA **(Poudel, *et al.*, 2020)**.

5. Age

Accelerated biological aging could reduce the life duration of individuals with RA and raise the risk of the condition, especially for those with a high hereditary genetic makeup **(Chen, *et al*., 2024)**.

6. Vitamin D3 deficiency

Vitamin D is a steroid hormone precursor that undergoes chemical conversion in the liver and kidney **(Lin, *et al*., 2016)**. Vitamin D is traditionally known for its impact on bone homeostasis, it also regulates immune function in multiple domains including autoimmune diseases such as rheumatoid arthritis **(Malakooti, *et al*., 2024)**. Over the past two decades, research has shown a connection between the immune system and vitamin D, with low levels and deficiency being common in RA patients, prompting the exploration of vitamin D as a potential RA protective factor **(Romão and Fonseca, 2021).** Research indicates that methotrexate, a medication used to treat RA, may affect the level of 25(OH) D3 in RA patients by affecting its metabolic pathways and reducing its bioavailability **(Cieślewicz, *et al*., 2024)**.

1.1.3. Signs and symptoms of rheumatoid arthritis

Common symptoms of RA start with mild or moderate inflammation affecting just a few joints, RA can cause other medical problems, such as

- Joint pain, edema, and fever are present, as is joint discomfort during action.
- Joint stiffness for more than half an hour, usually following a long time of repose or after waking up in the morning.
- Joint swelling can make it difficult to do routine duties
- Fatigue
- Irregular low-grade fever
- Appetite loss **(Kadhim, *et al.*, 2023)**.

1.1.4. Pathophysiology of rheumatoid arthritis

The pathogenesis of RA is multifactorial and complex, including environmental factors, genetic variables, and immune dysregulation **(Zhao, *et***

***al*., 2022)**. The pathological of RA include, the immune system becoming active, leading to the infiltration of various inflammatory cells such as mast cells, macrophages, monocytes, fibroblasts, and chondrocyte-like cells into the synovial tissues of the affected joints. As the infiltration rises, RA gets progressively more severe and the synovial tissue is severely infiltrated by leukocytes, which finally leads to the proliferation of the endothelium layer **(Meng, *et al*., 2023; Zhu, *et al*., 2023)**. Many pathogenic mechanisms, including antigen-presenting cells (APC), autoreactive T cell formation, leading to B-cell activation and the production of autoantibodies against its cellular structure (such as rheumatoid factor (RF) and anti-citrullinated protein antibody (ACPA) **(Malmström, *et al*., 2017)**. Autoantibodies are mainly secreted and produced by autoreactive B cells after they differentiate into plasma cells **(Wu, *et al*., 2021)**. Immune complexes containing RF or ACPA activate the complement pathway, leading to the production of C5a and membrane attack complex, both of which can cause damage to the joints **(Laurent, *et al*., 2015)**.

B-cells also facilitate T-cell activation, T cells, and other immune cells are recruited to the synovial tissue, where they produce large amounts of pro-inflammatory cytokines and interact with synovial fibroblasts and macrophages **(Mellado, *et al*., 2015).** Cytokines and chemokines play a crucial role in driving inflammation through their ability to activate endothelial cells, stimulate fibroblasts, and facilitate the accumulation of activated T and B cells, as shown in Fig. (1.2) **(Wang, *et al*., 2024).** pro-inflammatory cytokines, including tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-6, IL-17 and the mediators produced through downstream pathways in the arthritic joints, constitute the milieu driving cartilage and bone destruction **(Alghasham, Rasheed, 2014)**.

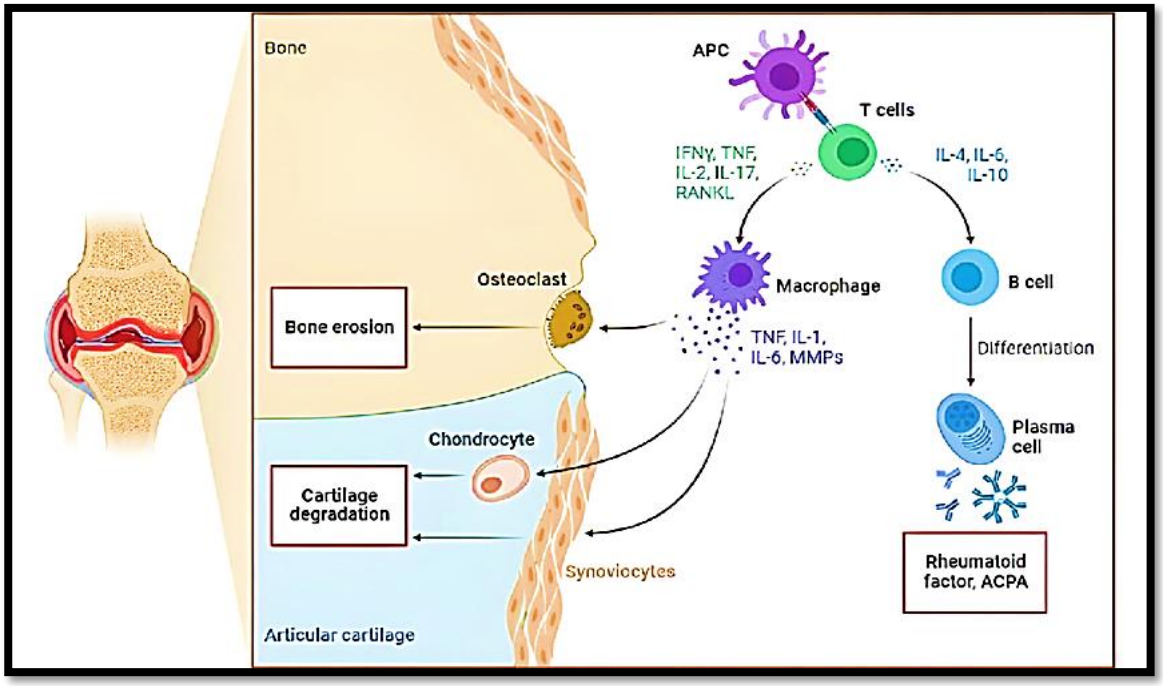


Fig. (1.2): The pathogenesis of rheumatoid arthritis

1.1.5. Epidemiology of rheumatoid arthritis

The epidemiology of RA influences approximately 0.5-1% of the adult population and presents two- to three-fold more frequently in women than in men **(Venetsanopoulou, *et al.*, 2023)**. RA incidence varies globally, with industrialized countries experiencing higher rates due to genetics and environmental risk factors **(Finckh, *et al.*, 2022)**. The prevalence of RA is expected to vary between Africa and the Middle East due to differences in socioeconomic status, environment, and ethnicity **(Almoallim, *et al.*, 2021)**.

Risk factors include age and sex; as people age, their risk of developing RA disease increases **(Al-Nasrallah, *et al.*, 2019)**. The incidence of RA in Iraq was 1.1 in 2014 and 2.2 in 2019, whereas the incidence of RA in 2001 and 2011 was 1.6 and 2.1, respectively. This variation, although it is not significantly large, may be due to the disturbance in the healthcare system **(Al_Badran, *et al.*, 2022).**

1.1.6. Clinical diagnosis of rheumatoid arthritis

Rheumatoid arthritis is commonly diagnosed through an extensive diagnosis that includes patient symptoms, a physical examination by a doctor, an assessment of risk factors and family history, joint assessment using ultrasound sonography, and laboratory tests to measure elevated levels of CRP and ESR in the blood serum, as well as the detection of RA-specific autoantibodies **(Lin, *et al*., 2020)**. The diagnosis dividing a corroding to:

1.1.6.1. Criteria of rheumatoid arthritis

The ACR/EULAR-2010 categorization criteria The European League Against Rheumatism (EULAR) and the American College of Rheumatology (ACR) are utilized in the diagnosis of RA when these criteria have applied as shown in Table (1.1) **(Altaha, *et al*., 2010).** Application of these the maximum score of the criteria is 10, with a score ≥ 6 allowing the classification as RA **(Steiner, *et al*., 2024).**

Table (1.1): The 2010 ACR/EULAR classification criteria for RA.

Classification Criteria for RA(Total Score ≥ 6 is Considered Satisfactory for the Diagnosis of RA)		Score
Joint involvement (swollen or tender joint)	1 large joint (shoulders, elbows, hips, knees, and ankles)	0
	2–10 large joints	1
	1–3 small joints (with or without involvement of large joints) *	2
	4–10 small joints (with or without involvement of large joints)	3
	>10 joints (at least 1 small joint) **	5
Serology	Negative RF and negative ACPA (≤upper limit of normal (ULN))	0
	Low-positive RF or low-positive ACPA (≤ULN and ≤3 times)	2
	High-positive RF or high-positive ACPA (≤3 times)	3
Acute-phase reactants	Normal CRP and normal ESR	0
	Abnormal CRP or abnormal ESR	1
Duration of symptoms	<6 weeks	0
	≥6 weeks	1

1.1.6.2. Imaging diagnosis

The ACR-EULAR 2010 classification includes ultrasonography, computed tomography (CT), and magnetic resonance imaging (MRI) as imaging tools for early diagnosis of RA, due to their much higher accuracy than in the case of conventional radiographs **(Zabotti, *et al*., 2020)**.

Ultrasonography is a favored imaging technique because of its low cost, lack of exposure to hazardous radiation, and speed with which images can be acquired, recent advancements in ultrasound image technology have enabled the development of sonographic equipment for image inflammatory joints in patients with RA **(Prado, *et al*., 2018; Kaeley, *et al*., 2020)**. Technique MRI is the ability to provide highly sensitive multiplanar information on both the bone and soft tissue structures in and around the joints without using ionizing radiation **(Harnden, *et al*., 2022).** However, MRI has certain limitations such as high cost, and prolonged examination time **(Nicoara, *et al*., 2023)**. CT is a widely used radiographic technique for detecting bone damage, including erosion, new bone formation, calcifications, and sclerosis due to its inherent contrast between bone and soft tissue **(Østergaard and Boesen, 2019).**

1.1.7. Inflammatory and anti-inflammatory marker in rheumatoid arthritis

The pathogenesis of RA involves a complex network of various cytokines and cells that trigger synovial cell proliferation and cause damage to both cartilage and bone **(Kondo, *et al*., 2021).** The network of cytokines can be divided into two groups, the pro-inflammatory and anti-inflammatory cytokines **(Wautier and Wautier, 2023)**, and both affect the regulation of responses to self (auto) antigen **(Markovics, *et al*., 2021)**.

Pro-inflammatory mediator factors of RA, IL-1, IL-6, and TNF-α, are produced because of CD4+ T-cell activation, they cause bone erosion, which leads to the production of autoantibodies **(Singh, *et al*., 2020).** Inflammatory

cytokines are responsible for stimulating destructive mechanisms in the joint **(Zhang, 2021).** Anti-inflammatory cytokines, such as IL-10, IL-4, and IL-1 receptor antagonists (IL1-RA) **(Oliveira, *et al*., 2023)**. In RA, anti-inflammatory cytokines balance the innate and adaptive immune cells' prolonged activation **(Chen, *et al*., 2019)**. Such an increase in IL-10 is a response to inflammatory decreases in the effects of inflammatory cytokines and can prevent cartilage degradation (**Brennan and Beech, 2007)**. Two types of interleukin were studied.

1. Interleukin-6

Interleukin-6 is a cytokine with numerous biological functions that is produced by monocytes macrophages, endothelial cells, and lymphoid cells **(Liu, *et al*., 2022)**. The biological activities only bind to its specific IL-6 receptor (IL-6R) and these cytokine-receptor complexes associate with the gp130 IL-6R β-subunit leading to intracellular signaling **(Rose-John, *et al*., 2017)**. IL-6 secretion is stimulated during inflammatory response secondary to tissue injury or infection **(Grebenciucova and VanHaerents, 2023)**.

Interleukin-6, a cytokine with 212 amino acid residues, enhances the activity of helper and cytotoxic T cells and the differentiation of Th17 involved in inflammation. In addition, this cytokine induces antibody production by B-lymphocytes and causes osteoporosis and joint edema by affecting articular fibroblasts **(Li, *et al*., 2017)**. The human IL-6 structure consists of four long α-helices, A-D, and three connecting loops. Two disulfide bonds are contained in the loop (A and B). Site I on the C-terminal end of helix D interacts with the IL-6 receptor (IL-6R); site II, located on helices A and C, interacts with one gp130; and site III on the N-terminal end of helix D interacts with another gp130 as shown in Fig. (1.3) **(Tanaka, *et al*., 2013).**

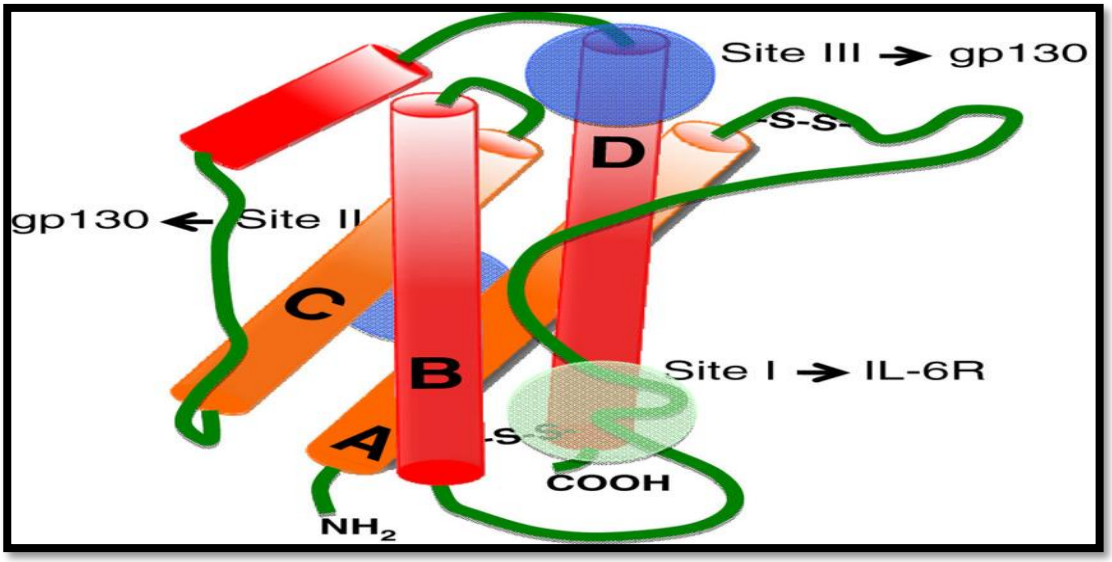


Fig. (1.3): Explain the domain structure of human IL-6.

The roles of IL-6 in RA pathogenesis are *via* the activation and maturation of B and T cells, as well as the production of autoantibodies **(Zhang, 2021)**. In RA, increased serum concentration of IL-6 is associated with joint damage, which may be because IL-6 is involved in the promotion of osteoclast formation **(Wu, *et al*., 2021)**. Elevated levels of IL-6 in serum, synovial fluid, and various tissues have correlated with increased RA disease activity **(Burska, *et al*., 2014).**

2. Interleukin-10

Interleukin-10 is a pleiotropic cytokine known for its potent anti-inflammatory and immunosuppressive effects. Originally identified as a product of T helper2 cells, it is produced by various myeloid- and lymphoid-derived immune cells participating in both innate and adaptive immunity **(Kuncara, *et al*., 2022).** IL-10 has been shown to exist in solution predominantly as a homodimer, composed of two polypeptide chains of 160 amino acids each **(Abbasa, *et al*., 2023)**. IL-10 family members are helical cytokines formed by six or seven helices and connecting loops. A four-helix bundle, generally formed by helices A, C, D, and F, comprises the protein core, which is a characteristic

feature of all these helical cytokines as shown in Fig. (1.4) **(Trivella, *et al*., 2010)**.

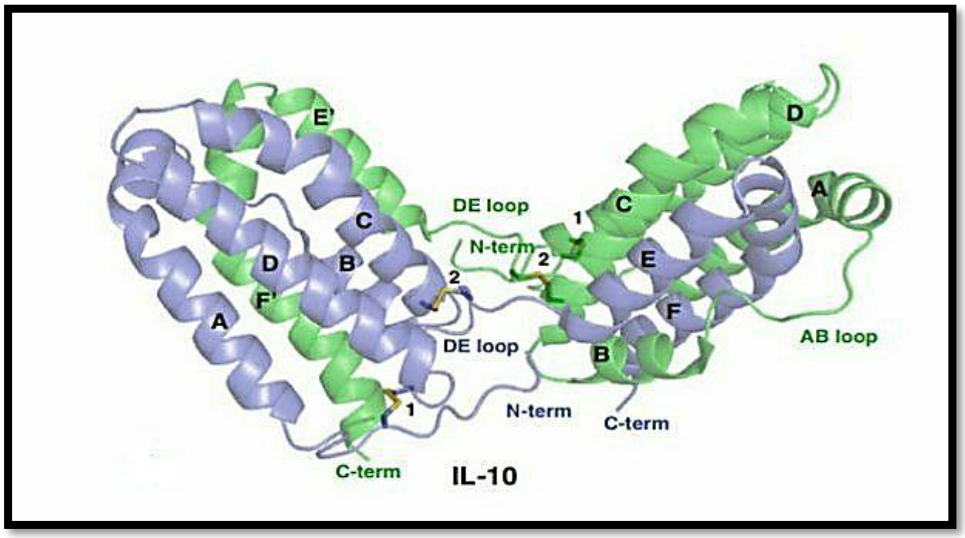


Fig. (1.4): Explain the domain structure of human IL-10.

The IL-10 family cytokines consist of IL-10, IL-19, IL-20, IL-22, IL-24, IL-26, IL-28A, IL-28B, and IL-29, they have diverse biological functions, including maintaining the integrity of epithelial layers, promoting innate immune responses against pathogenic infections and facilitating the tissue-healing **(Ni, *et al.*, 2020)**. IL-10 can inhibit the synthesis and secretion of related pro-inflammatory cytokines, such as IL-6, thus regulating inflammatory immunity **(Liu, *et al*., 2022)**.

Medical uses have been limited because IL-10 is not found enough in lymphoid organs **(Yuba, *et al*., 2021)**. IL-10 has been shown to suppress pro-inflammatory cytokine in rheumatoid joints and to induce joint swelling and deformity reduction, while also cartilage necrotic decreases **(Atallah, *et al*., 2023)**. IL-10, as an immune-regulatory cytokine that is elevated in patients with RA appears to be a response to increased inflammation and destructive cytokines due to increased production of pro-inflammatory cytokines and reduced production of anti-inflammatory cytokines may influence the Th1/Th2 **(Krishna Priya, *et al*., 2020)**.

1.1.8. Oxidants and antioxidants in rheumatoid arthritis

Oxidative stress is defined as an excess of reactive oxygen species (ROS) compared to antioxidants **(Hayes, *et al*., 2020)**. ROS is strongly associated with the development of cancer, diabetes, neurodegeneration, cardiovascular disease, rheumatoid arthritis, renal disease, and eye disease **(Pisoschi, *et al*., 2021)**. Several reactive oxygen and nitrogen species are produced during normal metabolism in the human body **(Demirci-Cekic, *et al*., 2022)**. ROS production in RA tissues is influenced by factors like phagocytic cell burst and ischemia-reperfusion damage, which increases cellular oxidative phosphorylation, activates osteoclasts, breaks down cartilage, and reduces hyaluronic acid **(Dixit, *et al*., 2020)**. RA pathogenesis is linked to increased oxidative stress, lower antioxidant levels, and reduced antioxidant defenses **(Ferreira, *et al*., 2021).** ROS are elevated in the serum of patients with RA, it is produced by the synovial tissues **(Wang, *et al.,* 2022)**. MDA assessment of oxidative stress in RA patients does not function as a biomarker for the degree or activity of the disease **(Merino de Paz*, et al*., 2023).**

Antioxidants are agents and molecules that stabilize or deactivate free radicals **(Deshmukh and Gaikwad, 2024).** The antioxidant system, consisting of low molecular antioxidants and endogenous enzymatic antioxidants like GPXs, CAT, SODs, and Trx, helps regulate oxidative stress levels **(Jomova, *et al.*, 2023)**. Endogenous non-enzymatic antioxidants, such as glutathione, coenzyme Q, bilirubin, uric acid, and lipoic acid, are small molecules found in lipid or aqueous environments **(Malekmohammad, *et al*., 2019).** Exogenous antioxidants are a group of organic and synthetic substances found in fruits and vegetables, including vitamin C, carotenoids, polyphenols, sulforaphane, and anthocyanins **(Korczowska-Łącka, *et al*., 2023)**. The antioxidant CAT activity difference between patients and controls is similarly substantial, and greater catalase activity was identified in the control group than in the patients' group

with RA **(Abdulrahman, *et al*., 2023)**. GSH, a crucial intracellular ROS quencher, is upregulated in RA patients, indicating an adaptive defense against oxidative stress **(Vu, *et al*., 2024)**. RA patients require a balanced oxidative environment, as thiol groups of proteins and low molecular weight compounds like glutathione are oxidized by oxidant molecules, forming reversible disulfide bonds **(Alisik, *et al*., 2021)**.

1. Malondialdehyde

Malondialdehyde (MDA) is the end product of lipid peroxidation of polyunsaturated fatty acids. It has been utilized as a biomarker to evaluate oxidative stress in diverse biological samples in individuals who suffer from a wide range of disorders **(Cordiano, *et al*., 2023)**.

When a free radical attacks a carbon-carbon double bond in polyunsaturated fatty acids, unsaturated lipid radicals with H_2O release are formed. Lipid hydroperoxides and peroxyl radicals will develop when more O_2 is absorbed. On the one hand, because of their homoallylic cis-double bond with the peroxyl group, peroxyl radicals can undergo cyclization. After cyclization, intermediate free radicals are created. These radicals can cyclize once more to create bicycle endoperoxides, which are structurally linked to prostaglandins, and then they can cleave to make MDA. As shown in Fig. (1.5) **(Mas-Bargues, *et al*., 2021)**.

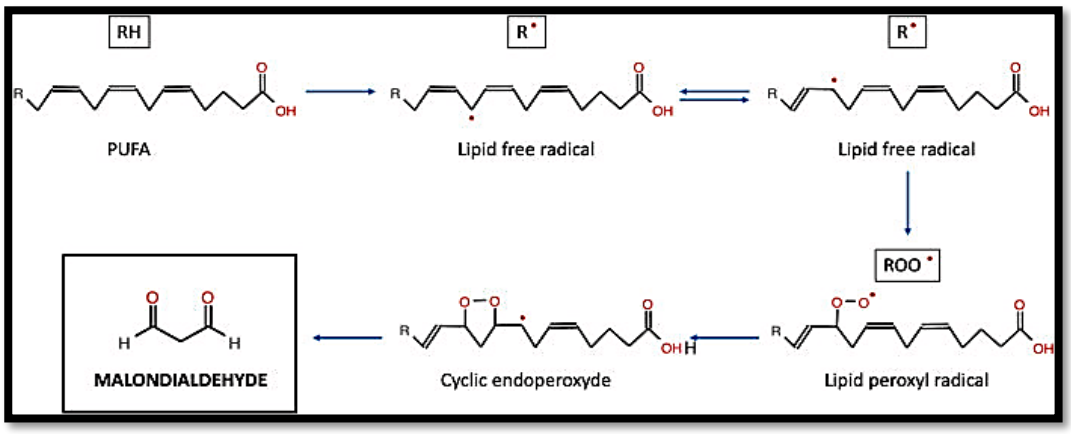


Fig. (1.5): MDA formation through lipid peroxidation

2. Catalase

Catalase is one of the most significant antioxidant enzymes, it is found in nearly all aerobic organisms. Catalase converts two molecules of hydrogen peroxide into one molecule of oxygen and two molecules of water in a two-step process **(Nandi, *et al*., 2019).**

$$\text{Catalase} + H_2O_2 \text{ ----------►Compound I}+H_2O$$

$$\text{Compound I} + H_2O_2 \text{ ----------► Catalase} + H_2O + O_2$$

Humans have a common nonfunctional heme-containing catalase with a replacement group of ferric protoporphyrin IX that works with hydrogen peroxide **(Gebicka and Krych-Madej, 2019).** Catalase is expressed in all major bodily organs (especially in the liver and kidneys) and in erythrocytes, where it plays an essential role in cell defense against oxidative stress. The importance of the enzyme can be gauged from the fact of its direct and indirect involvement in many diseases and infections. An attempt has been made to correlate the role of catalase with the pathogenesis and progression of oxidative stress-related diseases **(Rakotoarisoa *et al*., 2019).**

3. Reduced Glutathione

Glutathione is a low molecular weight thiol compound that contains a sulfhydryl group in its structure; it has an essential role as an antioxidant that functions by various mechanisms **(Kim, *et al*., 2023)**.

An important function of GSH is cell detoxification via the formation of S-adducts with harmful electrophilic compounds by Glutathione-S-transferase, as well as the elimination of cytotoxic α-oxoaldehydes by glyoxalases I and II **(Ishkaeva, *et al.*, 2021).** GSH synthesis occurs in the cytosol using glutamate-cysteine ligase and glutathione synthase enzymes. The process is controlled by negative feedback from the end product, GSH. If feedback inhibition is lost, cysteine availability as a precursor can become a limiting factor. This process is

a rate-limiting enzyme in all organisms as shown in Fig. (1.6), **(Wrotek, *et al*., 2020)**.

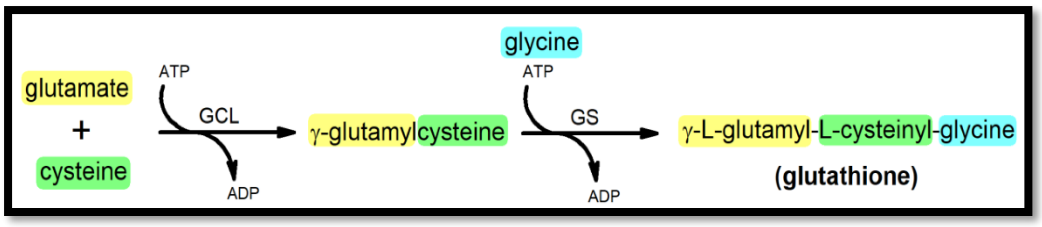


Fig. (1-6): Glutathione synthesis

Synthesis and degradation of GSH, together with its export, translocation to cell organelles, utilization for multiple essential functions, and regeneration from glutathione disulfide by glutathione reductase, are relevant to GSH homeostasis and metabolism **(Kennedy, *et al*., 2020).**

1.1.9. Obesity

1.1.9.1. Obesity according to the world health organization

Obesity is a chronic and complex disease, associated with an increased risk of developing serious health conditions **(Juul-Hindsgaul, *et al*., 2024).** The World Health Organization (WHO) defines obesity as "abnormal or excessive fat accumulation that presents a health risk **(Nutter, *et al.*, 2024)**. The WHO guidelines classify the participants according to their weight profiles, and the prevalence of overweight and obesity **(Nasaif, *et al*., 2024)**. The WHO classification is beneficial in distinguishing individuals who may be at increased risk of morbidity and mortality due to obesity as shown in **Table (1.2) (Safaei, *et al.*, 2021)**.

Table (1.2): BMI classification of adult weights based on WHO schema

Classification	BMI (kg/m^2)	Risk of co-morbidities
Underweight	<18.5	Low
Normal weight	18.5–24.9	Average
Overweight	25.0–29.9	Mildly increased
Obese	≥30	
Obese I	30.0–34.9	Moderate
Obese II	35.0–39.9	Severe
Obese III	≥40	Very severe

1.1.9.2. The Relationship between obesity and rheumatoid arthritis

Adipose tissue is a growing concern for the development and progression of RA, especially in weight-bearing joints like feet, legs, and knees, as it causes mechanical stress, increases joint pain, and accelerates the disease's progression **(Bilski, *et al*., 2023).** Adipokines play a cardinal role in the regulation of adipogenesis, insulin secretion, and sensitivity. They influence other biological processes, including inflammation/immune responses and myocardial contractility **(Soliman, *et al*., 2022)**.

Adipose tissue increase is linked to pro-inflammatory molecule production, leading to RA. 23.1% of RA patients have average weight, 27.9% are overweight, and 49% are obese, with significant female predominance **(Abdellatif, *et al*., 2024)**. Patients with RA may be at risk for sarcopenic obesity (SO), a disease in which their body fat content is more than that of healthy people and their skeletal muscle mass is reduced **(Letarouilly, *et al*., 2021)**.

Despite having lower lipoprotein levels than the general population, RA patients have a significantly greater risk of death from CVD, an observation known as the "lipid paradox." RA patients have elevated levels of TC, LDL-C, and the LDL-C/HDL-C ratio and decreased levels of HDL-C **(Chavan, *et al*., 2015; Dessie, *et al*., 2022).** The increase in LDL is because small dense LDL

particles more easily penetrate endothelial cells than their larger counterparts and are more susceptible to oxidation **(Jin, *et al*., 2022)**. Chronic inflammation in patients with RA leads to decreased HDL-C levels, leading to an increase in atherogenic factors, HDL-C undergoes conformational changes, transforming it into a pro-inflammatory and proatherogenic molecule, eliminating its antioxidant and antiatherogenic properties, which leads to oxidative changes, altering HDL-C structure **(González-Gay, *et al*., 2024)**.

Treatment of RA can influence obesity in complex ways; treatment may lead to weight gain due to medication side effects, such as corticosteroids, which can increase appetite and fat accumulation. Studies indicate that obese patients often experience poorer responses to therapy and higher disease activity, potentially due to inflammatory adipokines affecting immune response (**Poudel, *et al*., 2020; George and Baker, 2016)**.

1.1.10. Treatment of rheumatoid arthritis

Rheumatoid arthritis is a challenging condition with no complete and effective treatment available **(Zhao, *et al*., 2023)**.

The first line of treatment is non-steroidal anti-inflammatory drugs (NSAIDs), which inhibit cyclo-oxygenase to prevent joint pain and prevent the production of prostaglandins, prostacyclin, and thromboxanes **(Bullock, *et al*., 2019)**. Corticosteroids are potent anti-inflammatory medications but have side effects and are recommended for short periods at low doses **(Combe, *et al*., 2017).**

The second line is disease-modifying anti-rheumatic drugs (DMARDs), such as methotrexate (MTX), which reduces inflammation, controls symptoms, prevents joint damage, and potentially increases survival **(Tanaka, 2023)**. TNF inhibitors are often used as the first line bDMARD in methotrexate non-responders due to their long-term familiarity with their use, efficacy, and safety

profile **(Findeisen, *et al*., 2021)**. Kinase inhibitors and cytokine antagonists are developed to treat RA by inhibiting cytokines, preventing joint damage, and showing great results due to their specificity and selectivity **(Shen and Du, 2023)**. Tocilizumab and sarilumab, which are IL-6 receptor antagonists, have been shown to have greater efficacy than TNF inhibitors when used alone without concurrent DMARDs **(Yip and Yim, 2021)**. Rituximab, an anti-CD20 antibody, effectively treats RA by depleting CD20+ B cells **(Ercoli, *et al*., 2021).**

Nanomedicine has emerged as a novel therapeutic strategy to effectively localize anti-rheumatoid arthritis medications in inflamed joints **(Jeong and Park, 2020)**.

The text highlights ongoing research on biomarkers for therapy, understanding treatment mechanisms, and optimizing patient outcomes. Future perspectives include precision medicine for individual patients and novel therapeutics targeting specific pathways to optimize RA care using natural materials to reduce side effects.

1.2. Nanotechnology and nanomedicine

Nanomedicine is a multidisciplinary field that integrates various medical applications, including disease treatment, prevention, pain relief, health improvement, and nanoscale technology against traumatic injuries **(Vaishampayan, *et al*., 2023)**. Nanomedicine provides a versatile platform for delivering active cargo, allowing for tuning nanomaterials' properties like material, size, shape, surface chemistry, and targeting molecules **(Hamilton and Kingston. 2024).** Nanomedicine is developing nanoscale materials like gold and silver nanoparticles, dendrimers, nanorods, carbon buckyballs, nanoshells, and nanocubes, each with specific properties for targeted tissue and organ use **(Husain, *et al*., 2023; Dirisala, *et al*., 2022)**.

1.2.1. Nanoparticle structures

Nanoparticles (NPs) are small particles with dimensions ranging from 1 to 100 nm, with properties influenced by size and surface functionalities, used in cosmetics, electronics, and diagnostic and therapeutic medical applications (**Najahi-Missaoui, *et al.*, 2020)**. Nanoparticles below 100 nm have sparked immense interest for their unique physical and chemical properties **(Khan, *et al.*, 2022)**. Nanoparticles are commonly employed in delivery systems to enhance the solubility and absorption of lipophilic nutrients **(Chen, *et al.*, 2024)**. Shape and size play powerful roles in determining the properties of a material **(Pearce, *et al.*, 2021)**.

The classification of nanoparticles into crystalline and non-crystalline shapes is based on their composition, with crystalline nanoparticles being fragments of bulk crystal, while noncrystalline nanoparticles are not **(Nelli, *et al.*, 2023)**. The NP classification is based on their composition into three types as shown in Fig. (1.7) **(Sandhiya and Ubaidulla, 2020)**.

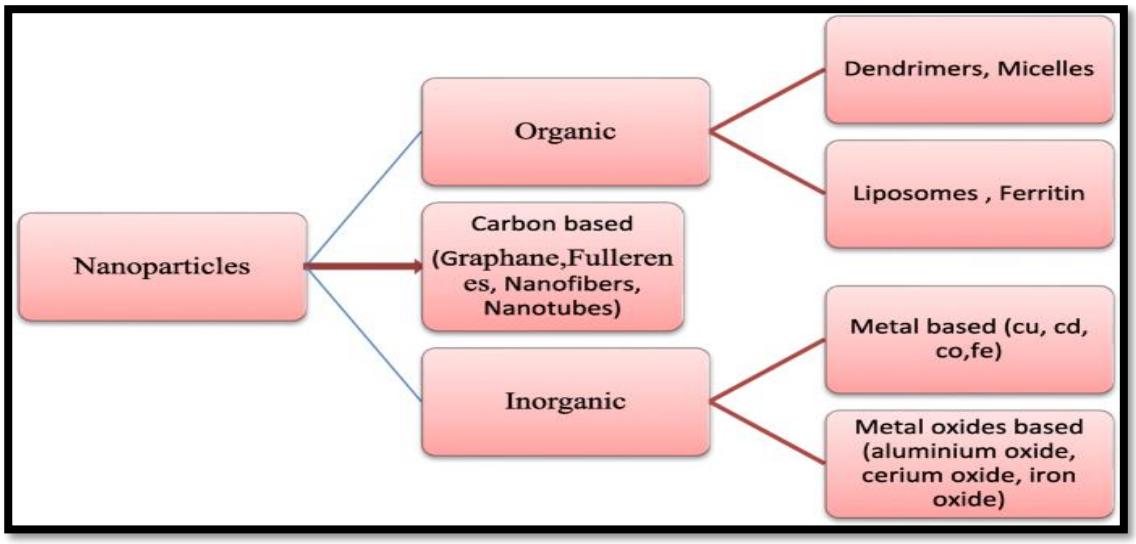


Fig. (1.7): Classification of nanoparticles.

1.2.2. Application of nanoparticles in the medical field

Diagnostic sciences are now using nanodevices for early and rapid disease identification for further medical procedural recommendations **(Malik, *et al*., 2023)**. diagnostic imaging, in which nanoparticles can be attached to specific biomarkers to enhance imaging modalities such as MRI, CT scans, and positron emission tomography (PET) scans, making them more sensitive, accurate, and specific **(Singh and Amiji, 2022)**. Nanotechnology is utilized in medicine for drug delivery, medical imaging, and other fields due to its unique physicochemical properties and photothermal effects **(Zhao, *et al*., 2023)**. The medical field focuses on disease diagnosis, targeted delivery, and minimally invasive surgical procedures due to their small size, lightweight, flexibility, and non-destructive penetration into biological tissues **(Fadeel, *et al*., 2020)**.

1.2.3. Drug delivery

Nanotechnology has revolutionized drug delivery for oral, topical, and parenteral medications, enhancing drug retention and allowing tunable release kinetics at the disease site **(Krishnan and Mitragotri, 2020)**. The drug delivery system improves therapeutic response by releasing the active pharmaceutical ingredient, unlike conventional systems that struggle with poor bioavailability and fluctuating plasma drug levels **(Adepu and Ramakrishna, 2021)**.

Nanoparticles are essential in targeted drug delivery TDD systems due to their unique physicochemical properties, including surface-to-volume ratio, surface energy, size, structure, and electrical, and magnetic properties **(Kumar, *et al*., 2024)**.

1.2.4. Curcumin

Curcumin, a yellow compound, is the primary active component of turmeric powder, collectively known as curcuminoids **(Sable, *et al*., 2024)**. It has a low molecular weight of lipophilic molecules that can pass through the

cellular membrane easily **(Hosseini, *et al*., 2018)**. Because of its intense yellow color, it is used as a natural food coloring agent **(Raduly, *et al*., 2021)**. Curcumin's interaction with various proteins allows for selective modulation of multiple cellular signaling pathways linked to various chronic diseases **(Hassan, *et al*., 2019).**

Curcumin's chemical structure, including phenyl rings, 1,3-diketone system, and carbon-carbon double bonds, enhances its potent antioxidant activity, regulating enzymes like reductase, catalase, glutathione peroxidase, and superoxide dismutase **(Abd El-Hack, *et al*., 2021)** as shown in Fig. (1.8) **(Sharifi-Rad, *et al*., 2020).**

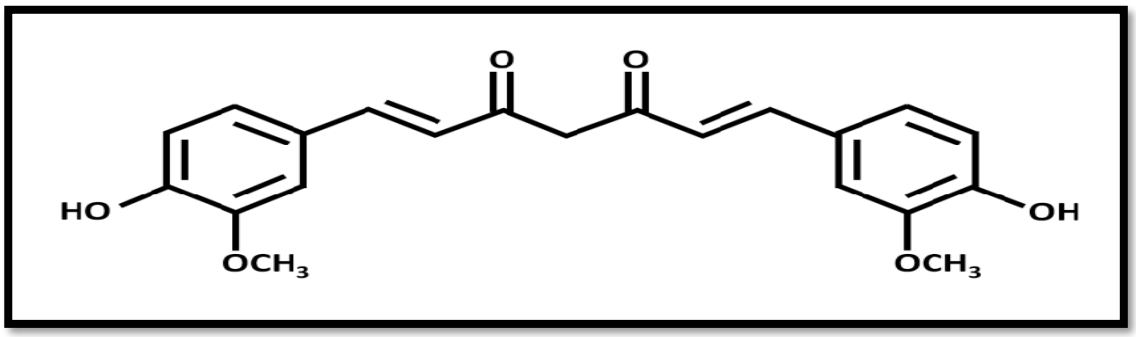


Fig. (1.8): Chemical structure of curcumin.

1.2.4.1. Applications of curcumin

Curcumin exhibits many biological activities, including anti-inflammatory, anticancer, antiviral, antibacterial, antifungal, and immunomodulatory activities **(Azad, *et a*l., 2024)**.

1. Role as antioxidant

Curcumin, according to research, can effectively serve as an antioxidant by reducing the effects of oxidants and oxidative stress through various molecular mechanisms **(Jakubczyk, *et al.*, 2020)**.

2. Anti-inflammatory

Curcumin regulates immune cell responses by affecting dendritic, T helper 17, and T regulatory cells, which produce pro-inflammatory cytokines and inhibit the inflammatory response **(Ghoushi, *et al*., 2024)**.

3. Anticancer property

Curcumin has been shown to have antitumor properties in various cancer cell lines, including breast, lung, head, and neck squamous cell carcinoma, prostate, and brain tumors **(Tomeh, *et al*., 2019).**

4. Neuroprotective

Curcumin's positive impact on cognition is due to its ability to enhance neurogenesis, promoting neurite outgrowth and proliferation of neural stem cells in vitro and in vivo **(Namgyal, *et al*., 2020)**.

5. Antimicrobial

Modern studies confirm curcumin's strong antimicrobial potential despite its poor solubility in water, low bioavailability, and pharmacokinetic profile **(Namgyal, *et al*., 2020)**. Antibiofilm properties by inhibiting bacterial quorum sensing systems and removing pre-existing biofilms **(Shukla, *et al.,* 2020).**

1.2.5. Niosomes

Niosomes are vesicles formed by the self-assembly of non-ionic amphiphilic surfactants. Cholesterol, and occasionally, charged molecules, are added to provide rigidity to the bilayers and to enhance the stability of the system. Niosomes share structure similarities with liposomes, and they were introduced as an alternative to overcome limitations associated with stability, sterilization, and large-scale production of liposomes **(Thabet, *et al*., 2022)**. The structure of niosomes as shown in Fig (1.10), **(Mishra, *et al*., 2020)**.

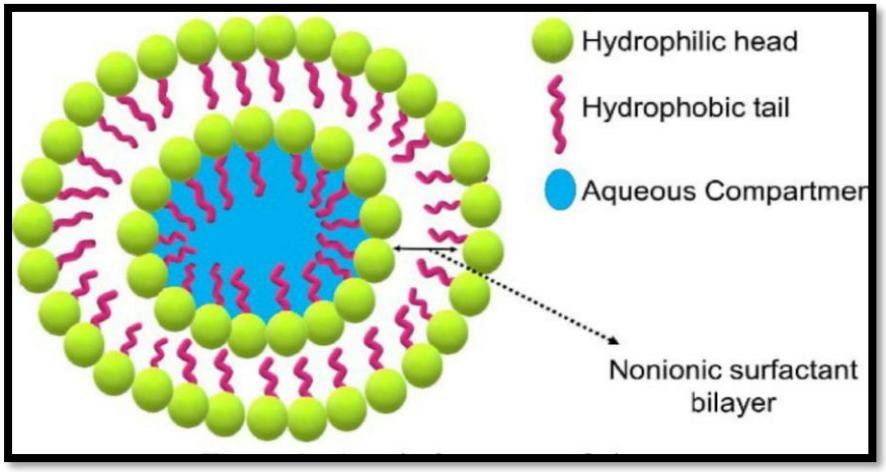


Fig (1.9): Structure of niosomes.

1.2.6. Hyaluronic acid

Hyaluronic acid is a polysaccharide with characteristics that play a pivotal role in various fields such as medicine, cosmetics, and materials science for biomedical and cosmetic uses. With its water retention, capacity HA is essential for skin hydration and wound healing **(Papakonstantinou, *et al*., 2012; Li, *et al*., 2022)**. Hyaluronic acid has been found to improve the precision of drug carriers like liposomes in targeting cancer cells, potentially improving treatment outcomes **(Huang and Huang, 2018)**. In the medical field, HA has been employed in treating osteoarthritis of shoulder and knee joints due to its attributes **(Uemura, *et al*., 2019)**. Hyaluronic acid has been utilized in various drug delivery systems, including nanoparticle, gel, and microsphere drug delivery systems, owing to its ability to serve as a drug carrier **(Huang and Huang, 2018)**. Given its biocompatibility as a polysaccharide HA functions by binding to CD44 receptors on specific cell surfaces; hence making it an attractive choice for targeted therapy **(Puluhulawa, *et al*., 2022)**.

1.2.7. Peripheral blood mononuclear cells

Peripheral blood mononuclear cells (PBMCs) include T and B-lymphocytes, NK cells, as well as monocytes and dendritic cells. PBMCs may

enhance endometrial receptivity and improve the implantation rates **(Melo, *et al*., 2022).** PBMCs include lymphocytes (T cells, B cells, and NK cells), monocytes, and dendritic cells. In humans, the frequencies of these populations vary across individuals, but typically, lymphocytes are in the range of 70–90 %, monocytes from 10 to 20 %, and dendritic cells are rare, accounting for only 1–2 %. The frequencies of cell types within the lymphocyte population include 70–85 % CD3 + T cells, 5–10 % B cells, and 5–20 % NK cells, and the isolation of PBMCs from blood as in Fig. (1.9) **(Kleiveland, 2015)**.

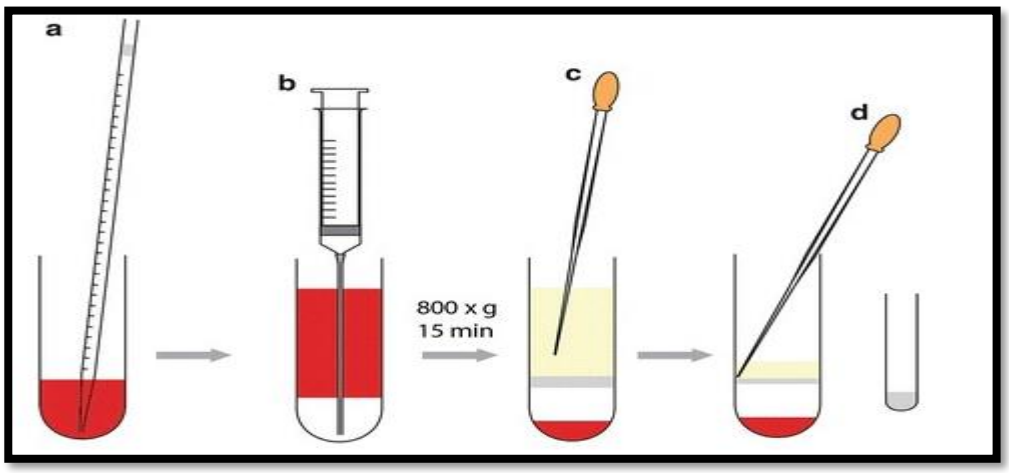


Fig. (1.10): Schematic illustration of how to prepare the density gradient for isolation of PBMCs from blood (a, b) and where in the gradient the PBMCs (*grey layer*) are found after centrifugation (c, d).

In this study, nanoparticles were synthesized to treat in-vitro rheumatic joint pain. The curcumin was coated with noisome particles to improve the efficiency of curcumin, and this compound was coated with hydronic for the compound to stimulate accession to the specific location and targets of rheumatoid cells.

1.3. Molecular genetic

1.3.1. Gene and gene expression

The genetic component of an organism is referred to as its genome. The fundamental microbiological components of DNA and RNA are therefore included in the genome, which is made up of a collection of "genes" a union of

genomic sequences encoding a coherent set of potentially overlapping functional products **(Gerstein, *et al*., 20 07)**. The word "gene" was not coined until early in the 20th century, by the Danish botanist Johannsen (1909), but it rapidly became fundamental to the then-new science of genetics, and eventually to all of biology **(Portin and Wilkins, 2017)**.

Gene functions are the activities performed by a gene product at the molecular level, and the locations, relative to cellular structures, a biological program comprising molecular activities acting in concert to achieve a particular outcome; this program can be at the cellular level or the organism level of multicellular organisms **(Aleksander, *et a*l., 2023)**.

Gene expression is a complex process involving numerous steps. The initial step in gene expression is the transcription of the DNA molecule into an exact RNA copy. In translation, polypeptides are synthesized sequentially stepwise via three distinct steps initiation, elongation, and termination. The generated protein may undergo several post-translational modifications before it is used in its dedicated role **(Gibney and Nolan, 2010)**.

1.3.2. Analysis of matrix metallopeptidase-2 and tissue inhibitor metalloproteinases-2 genes in rheumatoid arthritis

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases belonging to the superfamily of metzincin proteases **(Bian, *et al*., 2023)**. MMP-2 (gelatinase A, type IV collagenase) is one of the two discovered human gelatinases that are named for their ability to degrade gelatin proteolytically **(Nikolov and Popovski, 2021)**. MMP2 is a protein-coding gene MMP-2's gene locus occupies the position on chromosome 16q **(Gajewska, *et al*., 2022).**

A recent study showed that the MMP-2 enzyme is excessively secreted in the joints of patients with rheumatoid arthritis and plays an important role in inflammation and immunity **(Yu, *et al*., 2022)**. MMP-2 is a key factor in the

development of RA by degrading the extracellular matrix, leading to cartilage and bone destruction, and activating pro-inflammatory cytokines like IL-1β, IL-6, and TNF-α, thereby enhancing inflammation and immune responses in the synovium **(Itoh, *et al*., 2002; Bian, *et al*., 2023).**

Tissue inhibitor of metalloproteinase 2 (TIMP2) is a crucial regulator of matrix metalloproteinases (MMPs) and plays significant roles in various biological processes and diseases **(Costanzo, *et al*., 2022**). TIMP2, a 194 amino acid mature protein, is encoded by the TIMP2 gene on chromosome 17q involved in tissue remodeling and associated with conditions like cancer, rheumatoid arthritis, asthma, and neurodegenerative diseases **(Wang, *et al*., 2022).** Tissue inhibitor MMP (TIMP) prevents uncontrolled degradation of ECM components, the increased proteolytic activity of MMPs is due to an imbalance between the proteolytic and inhibitory activities of MMPs and TIMP **(Cabral-Pacheco, *et al*., 2020)**. TIMP-2 acts as a natural inhibitor of MMPs, which are involved in the degradation of extracellular matrix and joint destruction in RA, gene transfer of TIMP-2 and other TIMPs has been shown to inhibit cartilage degradation and invasion by rheumatoid synovial fibroblasts **(Mohammed, *et al*., 2003)**.

Curcumin has been shown to significantly affect the levels of (MMP-2) and (TIMP-2). In a study involving liver fibrosis in rats, curcumin treatment led to a notable decrease in serum levels of both MMP-2 and TIMP-2 after two weeks of administration, suggesting its potential role in modulating extracellular matrix homeostasis **(Supriono, *et al*., 2020)**. Additionally, curcumin's inhibitory effects on MMP-2 have been observed in various cancer models, indicating its broader therapeutic implications **(Wroński, *et al*., 2021; Davoodvandi, *et al*., 2021)**.

Aim of Study:

The aim of the study measure oxidant-antioxidant, inflammatory, and anti-inflammatory status and gene expression of rheumatoid arthritis patients, which could achieved by the following points:

1. Investigation the oxidant-antioxidant status in sera of (obese and non-obese) RA of Iraqi women and compared with the control group by investigations of reduced glutathione, catalase, and Malondialdehyde.
2. Study the inflammatory and anti-inflammatory status by investigation of interleukin-6 and interleukin-10 in sera of (obese and non-obese) RA and compared with that in apparently healthy women.
3. Synthesize the curcumin-loaded hyaluronic acid-decorated niosomal nanoparticles by thin layer hydration technique.
4. Characterization of curcumin-loaded hyaluronic acid-decorated niosomal nanoparticles via DLS, FT-IR, and FE-SEM
5. Investigation of their effect on each of oxidant-antioxidant and inflammatory-anti-inflammatory status.
6. Investigation of curcumin-loaded hyaluronic acid-decorated niosomal nanoparticles on gene expression of RA patients by real-time PCR.

Chapter Two

Materials and Methods

2. Subjects, Materials, and Method

2.1. Study design and ethical approval

A case-control research approach was used to collect samples from 130 female subjects in October. 2023 – May 2024. They are classified as shown in Fig. (2.1). The study's ethical approval was confirmed by the Kerbala College of Medicine, Kerbala University (23-41 on 21/12/2023), and Kerbala Health Directorate. After describing the nature of the study and objectives to each patient, the management of the consultant rheumatology clinic and the female patients and control themselves gave their consent. The questionnaire included name, age, BMI, type of treatment, and history of diseases.

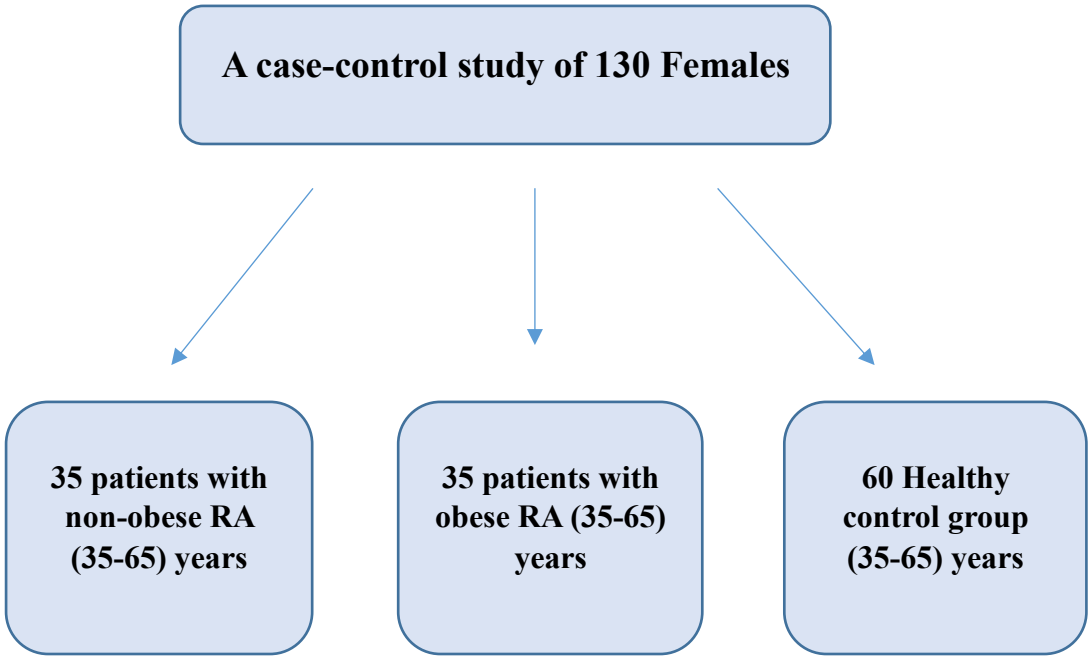


Fig. (2.1): Study design scheme

2.2. Subjects

2.2.1. Patients

The study was conducted on 70 female patients aged between 35-65 years old as shown in Fig. (2.1). The patients were divided into two subgroups: 35 non-obese RA and 35 obese RA according to ACR/EULAR 2010 by a healthy control. They have been observed and diagnosed by specialist physicians. Patients were collected from the consultant rheumatology clinic, Al-Hassan Teaching Hospital, Kerbala Health Directorates / Kerbala – Iraq. A particular questionnaire form including descriptive information was designed and filled with each patient.

Patients inclusion criteria

Every patient had a complete physical examination, necessary laboratory testing, and a review of their clinical history. Patients with rheumatoid arthritis achieved each requirement for the 2010 ACR/EULAR (American College of Rheumatology/European League against Rheumatism) RA classification. Increased RF, ESR, and CRP were discovered, which aligns with this classification.

Patients exclusion criteria

Patients with the presence of rheumatologically disorders or other autoimmune disorders and Infectious diseases. As well, as exclude patients with liver disease, kidney disease, pregnancy, diabetic disorder, and smoking.

2.2.2. Healthy control

The control group includes another 60 female subjects obtained from apparently healthy women. Through a self-report method, demographic data of the participants were also collected.

2.2.3. Blood sample collection and storage

The study involved obtaining 5 ml of whole blood from each sample and dividing it into two parts.

The first part venipuncture was used to obtain three-milliliter blood samples, which were then put in gel tubes at room temperature for ten minutes. Approximately 3000 x g centrifugation was used to separate the serum for 10 minutes. Serum samples were divided into three samples in Eppendorf, and kept at -20 °C, to be used later for additional testing.

Separate peripheral blood mononuclear cells

The second part was used to separate peripheral blood mononuclear cells (PBMCs) from the blood sample. These PBMCs were used for nanotechnology procedures and were synthesized into curcumin-loaded hyaluronic acid-decorated niosomal nanoparticles.

2.3. Chemicals and Kits

A list of the kits utilized in this investigation is provided in

Table (2.1): Kits and Chemicals used in this study and their suppliers

No.	Kits and Chemicals	Company and Country
1	ELISA kits for measurement of IL-6	Karmania pars gene/ Iran
2	ELISA kits for measurement of IL-10	Karmania pars gene/ Iran
3	MDA assay kit	Karmania pars gene/ Iran
4	Catalase activity assay kit	Karmania pars gene/ Iran
5	Reduced glutathione kit	Karmania pars gene/ Iran
6	Cholesterol reagent kit	AFLO/ Germany
7	HDL-cholesterol reagent kit	AFLO/ Germany
8	LDL-cholesterol reagent kit	AFLO/ Germany
9	Triglycerides reagent kit	AFLO/ Germany

2.4. Instrument and lab equipment

Table (2.2): List of the equipment used with their companies and origin.

No.	Equipment	Company	Origin
1	SMART-120 chemistry analyzer	AFLO	Germany
2	Centrifuge	Hettich	Germany
3	Deep freezing	Kirtsh	Germany
4	Light cycler	Roche Molecular	China
5	Real-time PCR	Roche Molecular	China
6	Incubator	Memmert	Germany
7	Water path	Memmert	Germany
8	ELISA instrument system	Bio Tek	USA
9	Scanning electron microscopy	TESCAN	Czech
10	Atomic force microscopy	Nanowizard II	Germany
11	Dynamic light scattering system	Malvern	UK
12	FT-IR spectrophotometer	Shimadzu 8400 S	Japan
13	Ultraviolet spectrophotometry	Fremont	USA

2.5. Methods

Part I

2.5.1. Measurement of body mass index

Body mass index (BMI) is a population-based index that is used to approximate body size by providing a ratio between body height and weight. BMI was calculated from the following equation **(Giersch, Taylor, *et al*., 2023)** :

$$\textbf{BMI (Kg/m}^2\textbf{) = Weight (kg) / Height (m}^2\textbf{).}$$

WHO classification was used for BMI evaluation. Normal BMI levels range between (20-24.9) kg/m^2 while overweight range between (25- 29.9) kg/m^2 and when BMI ≥ 30 kg/m^2, the woman is considered obese **(Namjou, Stanaway, *et al*., 2021)**.

2.5.2. Measurement of serum lipid profile

2.5.2.1. Measurement of serum total cholesterol concentration

Principle:

Esterified cholesterol is hydrolyzed into free cholesterol and fatty acid by cholesterol esterase (CHE). 4-Cholesten-3-one and hydrogen peroxide (H_2O_2) are then formed from the released free cholesterol by the action of cholesterol oxidase. In the presence of peroxidase (POD), hydrogen peroxide reacts with a derivative of phenol and 4-amino antipyrine (4-AAP) to produce a colored complex whose color intensity is directly proportional to the total cholesterol concentration in the sample. A schematic representation of the reaction is shown in the following equations:

$$\text{Cholesterol ester} \xrightarrow{\textbf{CHE}} \text{Cholesterol + fatty acids}$$

$$\text{Cholesterol} \xrightarrow{\textbf{CHOD}} \text{4-Cholesten-3-one + } H_2O_2$$

$$H_2O_2 + \text{Phenol + 4-AAP} \xrightarrow{\textbf{POD}} \text{colored complex + } H_2O$$

Reagents

Table (2.3): Reagents used for total cholesterol assay.

	Buffer	**mmol/L**
Reagent A (100 mL)	4-AAP	1mmol/L
	CHE	300 U/l
	CHOD	300 U/l
	POD	1500 U/l
	Derivative of phenol	1mmol/L
Standard (5 mL)	Cholesterol	200 mg/dL

4-AAP, 4-aminoantipyrine; CHE, cholesterol esterase; CHOD, cholesterol oxidase; POD, peroxidase.

Procedure

Concentrations of total cholesterol were measured by using a SMART-120 chemistry analyzer and the procedure for blank, standard, and sample measurement is demonstrated. The mixture was prepared and incubated at 37°C for 5 minutes. The sample-to-reagent ratio was 1:100, and the absorbance of the sample (Ax) and the standard (As) were read against a blank reagent at 510 nm.

Table (2.4): The procedure of total cholesterol assessment.

Pipette	Blank (µL)	Sample (µL)	Standard (µL)
Reagent (A)	1000 µL	1000 µL	1000 µL
Water	10	-	-
Sample	-	10	-
Standard	-	-	10

Cholesterol (mg/dL) = Ax/As × 200 (standard value)

2.5.2.2. Measurement of serum triglyceride concentration

Triglycerides, esters of fatty acids and glycerol, do not circulate freely in plasma but are bound to proteins and transported as macromolecular complexes called lipoproteins. Methods for triglyceride determination generally involve enzymatic hydrolysis of triglycerides to glycerol and free fatty acids followed by enzymatic measurement of the glycerol released. The enzyme lipase hydrolyzes triglycerides to produce glycerol and free fatty acids. The glycerol participates in a series of coupled enzymatic reactions, in which glycerol kinase (GK) and glycerol phosphate oxidase (GPO) are involved, and hydrogen peroxide (H_2O_2) is generated. Produced H_2O_2 reacts with TOOS and 4-AAP to form a colored complex, whose absorbance is directly proportional to the concentration of triglycerides in the sample.

A schematic representation of the reaction is shown in the following equations:

$$\text{Triglyceride} \xrightarrow{\text{LPL}} \text{Glycerol} + \text{Fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{Glycerol-1-phosphate} + \text{ADP}$$

$$\text{Glycerol-1-phosphate} + O_2 \xrightarrow{\text{GPO}} \text{Dihydroxyacetone phosphate} + H_2O_2$$

$$H_2O_2 + \text{4-AAP} + \text{TOOS} \xrightarrow{\text{POD}} \text{colored complex} + H_2O$$

Reagents

Table (2.5): Reagents used for triglycerides assay.

Reagent A (100 mL)	**buffer**	**100 mmol/L**
	Magnesium chloride	15 mmol/L
	ATP	4 mmol/L
	4-AAP	1 mmol/L
	TOOL	0.1 mmol/L
	PLP	2500 U/I
	POD	1800 U/I
	Gk	1000 U/I
	GPO	5500 U/I
Standard (10 mL)	Glycerol	200 mg/dL

ATP, adenosine triphosphate; 4-AAP, 4-amino antipyrine; LPL, lipoprotein lipase; POD, peroxidase; GK, glycerol kinase; GPO, glycerol phosphate oxidase.

Table (2.6): The procedure of triglycerides assessment.

Pipette	Blank (µL)	Sample (µL)	Standard (µL)
Reagent (A)	1000 µL	1000 µL	1000 mL
Water	10	-	-
Sample	-	10	-
Standard	-	-	10

Triglycerides (mg/dL) = Ax/As × 200 (standard value)

Procedure

Concentrations of triglycerides were measured by using a SMART120 chemistry analyzer and the procedure for blank, standard, and sample measurement. The mixture was prepared and incubated at 37°C for 5 minutes.

The sample-to-reagent ratio was 1:100, and the absorbance of the sample (Ax) and the standard (As) were read against a blank reagent at 510 nm.

2.5.2.3. Measurement of serum high-density lipoprotein cholesterol

High-density lipoprotein (HDL), low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL) are the lipoproteins responsible for the vast majority of cholesterol transport in the blood. Of the lipoprotein classes, HDL has the highest proportion of protein relative to lipids, containing slightly more than 50% protein, as well as having the densest and generally smallest size among the lipoproteins. Thus, high LDL levels and low HDL levels are strongly associated with an increased risk of adverse cardiovascular events. Specific polyanions in the first phase block the interfering lipoproteins LDL-C, VLDL-C, and chylomicrons (CM), and a specific surface-active agent inhibits the coloration of VLDL-C, LDL-C, and chylomicrons in the second phase. The intensity of the color is directly proportional to the HDL-C in the sample.

A schematic representation of the reaction is shown in the following equations:

HDL-C + LDL-C + VLDL-C + Chylomicron (CM) → HDL-C + (LDL-C +VLDL-C +CM)

$$\text{HDL-C} \xrightarrow{\text{CHE, CHOD}} \text{Fatty acids} + H_2O_2$$

$$H_2O_2 + \text{4-AAP} + \text{HDAOS} \rightarrow \text{colored complex} + H_2O$$

Reagents

Table (2.7): Reagents used for high-density lipoprotein cholesterol assay.

Reagent A (90 mL)	**Buffer**	**100 mmol/L**
	Polianions	1 mmol/L
	4-AAP	4 mmol/L
Reagent B (30 mL)	CHE	800 U/l
	CHOD	500 U/l
	Peroxidase	1500 U/l
	HDAOS	1 mmol/L
	Detergent	4 mmol/L

4-AAP, 4-aminoantipyrine; CHE, cholesterol esterase; CHOD, cholesterol oxidase.

Procedure

The mixture was prepared and incubated at 37 °C for 5 minutes, and the absorbance of the blank sample (Abx) was read against a blank reagent at 600 nm. In the next step, reagent B was added, mixed, and incubated at 37°C for 5 minutes and the absorbance of the sample (Ax) and the standard (As) were read against a blank reagent.

Table (2.8): The procedure of high-density lipoprotein cholesterol assessment.

Pipette	Blank (µL)	Sample (µL)	Standard (µL)
Reagent (A)	300 mL	300 µL	300 µL
Water	4	-	-
Sample	-	4	-
Standard	-	-	4
Reagent (B)	100	100	100

HDL (mg/dL) = (Ax – Abx)/ (As – Abs) × (Standard Value)

2.5.2.4. Measurement of serum low-density lipoprotein cholesterol

LDL-C (mg/dL) = Total Cholesterol – HDL-C – (Triglycerides/5)

2.5.3. Measurement of oxidative stress biomarker

2.5.3.1. Measurement of serum lipid peroxidation

Principle:

Thiobarbituric acid reactive substances (TBARS) evaluated lipid peroxidation in sera. A simple, dependable, and standardized method for calculating serum lipid peroxidation is the TBARS test. The MDA-TBA adduct is created by the reaction between MDA and 1,3-Diethyl-2-thiobarbituric acid (DETBA) in an acidic environment at high temperatures (90–100 °C). It can be detected calorimetrically at 530–540 nm or fluorometrically at 515 nm for

excitation and 555 nm for emission. Fluorometrically measurements reveal a significantly higher sensitivity for this reaction.

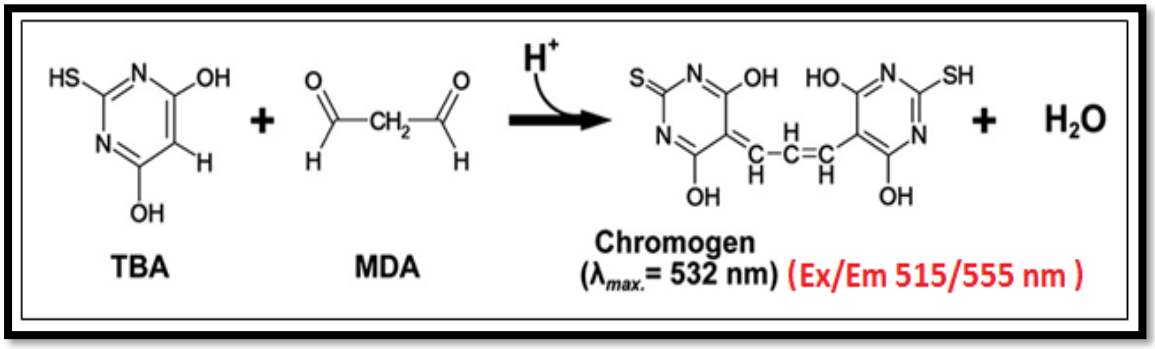


Fig. (2.2): The MDA-(TBA) $_2$ compound design.

Table (2.9): Contents of the kit of MDA

Type of solution	The components
Solution A	(CN, KPG - MDA)
Solution B	(CN, KPG - MDAB)
Solution C	(CN, KPG - MDAC)
Standard	(CN, KPG - MDAs)

Procedure:

1. The solutions were prepared by adding 10 mL of distilled water to each solution A and solution B.
2. The working solution, A/B/C solution was prepared in a ratio of 2/1/1.
3. The standard solution was prepared, and 20.5 microliters of the standard solution prepared in the kit were diluted with 1 ml of 96% ethanol and then added to 49 ml of distilled water. The current solution contains 500 µmol of standard. The standard solution to a ratio of 1/10 diluted, for the obtained main standard. For this purpose, 200 microliters of the standard was dissolved with 800 microliters of distilled water. Subsequent standards at dilutions of 10. 5. 2.5. 1.25, 0.62. 0.31 and 0.15 µmol were prepared.

4. 200 microliters of the working solution were added to all microtubes related to standards, blanks, and samples.
5. 100 microliters of standards and 100 microliters of distilled water were added to the negative control and 100 microliters of the sample to the corresponding vial.
6. The microtubes were incubated for 60 minutes at 90 ° C.
7. The samples were incubated for 10 minutes at room temperature until the temperature of the samples reached room temperature.
8. The micro tubes were Centrifuged at 1900 RPM for 10 minutes.
9. 150 µl of standards, blanks, and samples were added to ELISA wells immediately.

Calculation

In Fig. (2.3), concentrations of standards, the corresponding OD values, and the linear regression equation of the standard curve were calculated. Additionally, according to the OD value of the samples, the concentration of the corresponding sample was calculated as the sample value using the standard curve and the formula y = ax + b. To calculate the MDA concentration as µM / mg or nmol/mg.

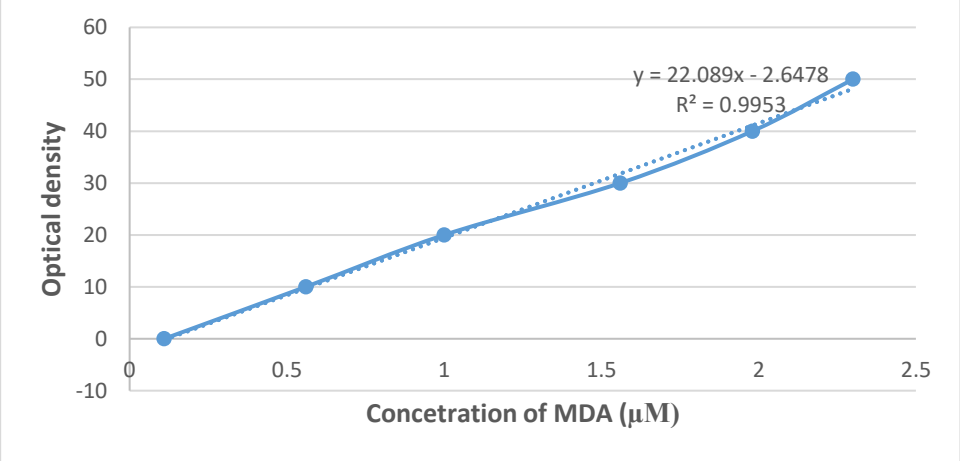


Fig. (2.3): standard diagram of the MDA activity

2.5.3.2. Measurement catalase activity

Principle

The method is based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2 with the formation of perchloric acid as an unstable intermediate. The acetate produced is measured calorimetrically at 610 nm. This was assayed by the method described by Sinha **(Ogbuagu, *et al*., 2022).**

Table (2.10): Contents of the kit of catalase

Type of solution	The components
Solution A	(CN. KPG - CATa)
Solution B	(CN. KPG - CATb)
Solution C	(CN. KPG - CATc)

Procedure

1. To prepare the solution A, 20 ml of distilled water was added.
2. The prepared solution A was mixed in a ratio of 1 to 3 with acetic acid and in the required amount, solutions B and C were mixed before testing. Accordingly, they were mixed in proportions of 1/200.
3. The kit components were kept at room temperature for 20 minutes before starting the test.
4. The ingredients were added according to the table below in 1.5 ml microtubes.

Table (2.11): The procedure of catalase activity assessment

Tubes / Solutions	Test	Negative control	Standard	Blank
Sample	50 µl	50 µl	-	-
Distilled water	-	250 µl	50 µl	250 µl
Mix B and C solution	250 µl	-	250	-

5. All tubes were incubated for 3 minutes at 37 ° C and then 500µL of mixed solution A and acetic acid was added.
6. All tubes were incubated at 100 ° C for 10 minutes and then centrifuged at 2500 rpm for 5 minutes.
7. 200 µL of the supernatant was transferred to the wells of the ELISA plate and assess its OD at 570 nm.

The following formula is used to calculate catalase activity:

Catalase activity (U / L) =2.303/3x [S'/S - M] x VT / Vs

S'= Absorbance of standard, S= Absorbance of Test, M =Absorbance of negative control, VT = Total Volume, and VS= Volume of the sample.

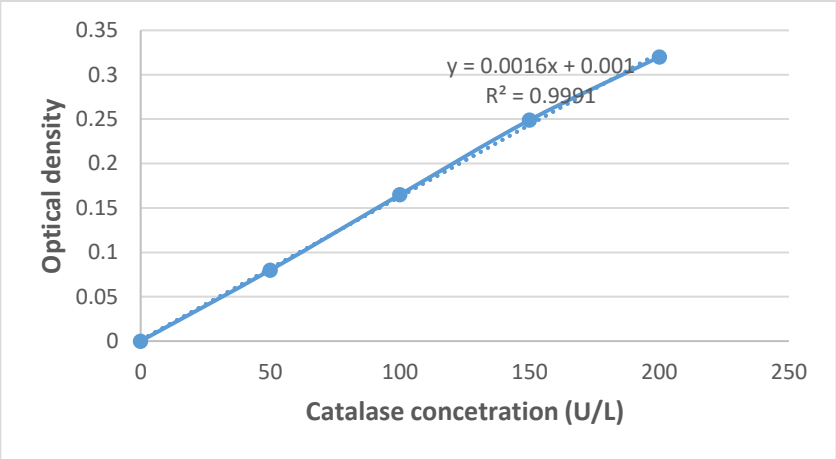


Fig. (2.4): standard curve of catalase activity

2.5.3.3. Measurement of reduced glutathione

Principle:

The sulfhydryl group of GSH easily reduces the adichromogen dithiobis (2-nitrobenzoic acid) (DTNB) to form a brightly yellow compound. The maximal absorbance of reduced chromogen is 412 nm, and it is directly correlated with the content of GSH, Fig. (2.5).

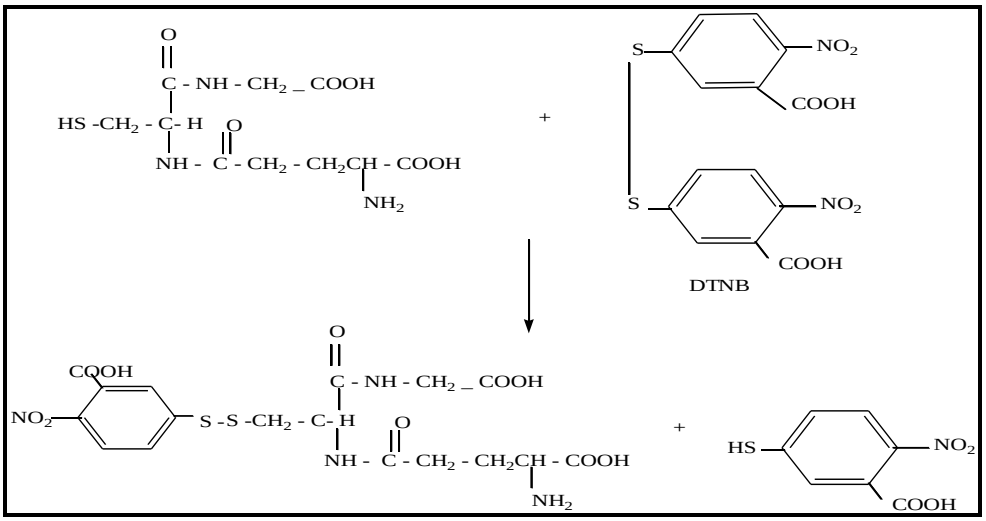


Fig. (2-5): Interaction between DTNB & GSH

Procedure

Sample GSH was determined by using a modified procedure utilizing Ellman's reagent (DTNB), which is summarized as the following. Duplicates of each standard and sample test tube are prepared and then pipette into test tubes.

Table (2.12): The procedure of reduced glutathione activity assessment

Tubes / Reagents	Sample µl	Reagent	Standard
Sample	100 µl	-	-
Standard	-	-	100 µl
DDW	800 µl	900 µl	100 µl
TCA	100 µl	100 µl	100 µl

Calculation

In Fig. (2.6), concentrations of standards, the corresponding OD values, and the linear regression equation of the standard curve were calculated. Additionally, according to the OD value of the samples, the concentration of the corresponding sample was calculated, and the best-fit line can be determined by regression analysis.

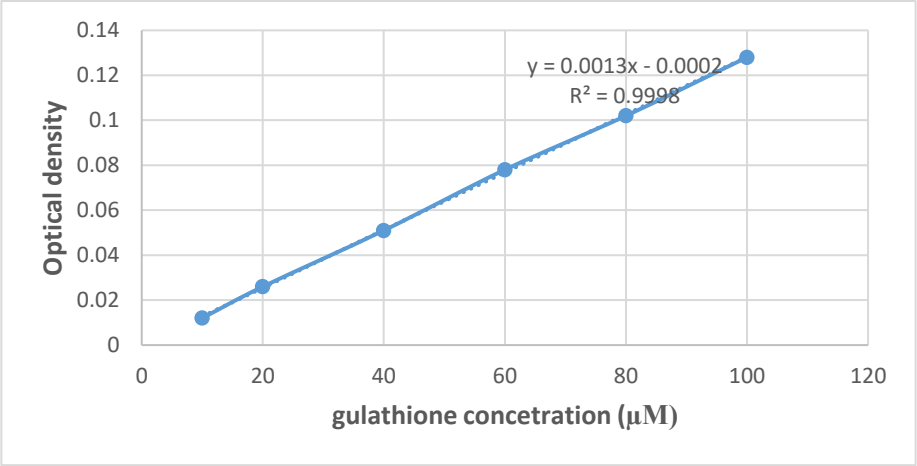


Fig. (2.6): The standard curve of glutathione concentration

2.5.4. Measurement of interleukins

2.5.4.1. Measurement of serum interleukin-6 levels

Principle

The Human IL-6 solid-phase sandwich ELISA (enzyme-linked immunosorbent assay) is designed to measure the amount of the target bound between a matched antibody pair. A target-specific antibody has been pre-coated in the wells of the supplied microplate. Samples, standards, or controls are then added into these wells and bind to the immobilized (capture) antibody. The sandwich is formed by the addition of the second (detector) antibody, and a substrate solution is added that reacts with the enzyme-antibody-target complex to produce a measurable signal. The intensity of this signal is directly proportional to the concentration of the target present in the original specimen.

Table (2.13): Kit component of IL-6

Item	Catalog number	volume
Human anti-IL-6 pre-coated plate	KPG-IL6 P	96 vials
Standards	KPG-IL6SN1-4	200µL
HRP-Avidin buffer	KPG-HA	5.5mL
HRP	KPG-HAA	540µL
Substrate	KPG-SU	5.5mL
Stopping	KPG-ST	3.5mL
10X washing buffer	KPG-WB	40mL

Standard

The available standards in the ready-to-use kit are as described in the following **table (2.14)**:

Table (2.14): Standard kit component OF IL-6

OD	Pg/ml	CN	Standards
2-1.8	**200 pg/ mL**	**KPG-IL6 S4**	**Standard 4**
1.4-0.9	**100 pg/ mL**	**KPG-IL6 S3**	**Standard 3**
0.6-0.3	**50 pg/ mL**	**KPG-IL6 S2**	**Standard 2**
0.08-0.1	**0 pg/ mL**	**KPG-IL6 S1**	**Standard 1**

Procedure

1. To prepare the washing solution, this solution with distilled water 10 times was diluted
2. The plate was removed from the desired package and brought to room temperature in a dry environment, 50 pl was added to standards #4 to #1 first to fourth wells. to the
3. 50 microliters of the desired sample were added to the rest of the wells and incubated for 60 minutes on a 200-RPM shaker at room temperature.
4. After proper incubation, the plates were washed 3 times using the washing solution (after adding the washing solution, the plates were incubated for approximately 1 minute at room temperature and then drained).

5. 50 microliters of conjugated antibody (Detection ab) was added to all wells and incubated for 60 minutes on a 200-RPM shaker at room temperature.
6. After proper incubation, the plates were washed 3 times using washing solution.
7. 50 microliters of HRP-Avidin solution were added to all wells and incubated for 30 minutes on a shaker (at least at RPM 200).
8. After proper incubation, the plates were washed 5 times using a washing solution.
9. 50 microliters of substrate were added to all wells and incubated for 15 minutes.
10. 25 microliters of the stopping solution were added to all the wells and the absorption of the samples in an ELISA at a wavelength of 450 nm.

Calculation

In Fig. (2.7), concentrations of standards, the corresponding OD values, and the linear regression equation of the standard curve were calculated. Additionally, according to the OD value of the samples, the concentration of the corresponding sample was calculated, and the best-fit line can be determined by regression analysis.

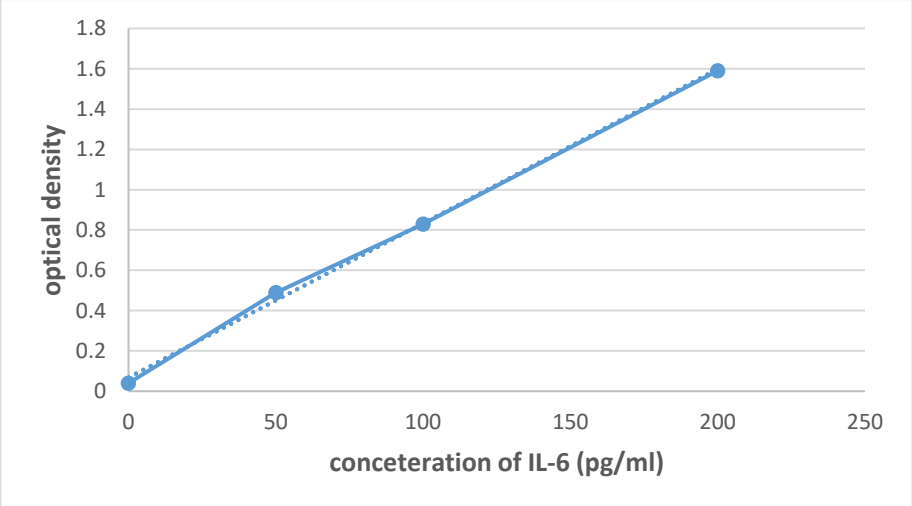


Fig. (2.7): The standard curve of human serum IL-6 levels

2.5.4.2. Measurement of serum interleukin-10 levels

The principle, procedure, and Calculation of Results are mentioned in Human IL-6 ELISA Test section 2.5.4.1

Calculation As mentioned in 2.5.4.1.

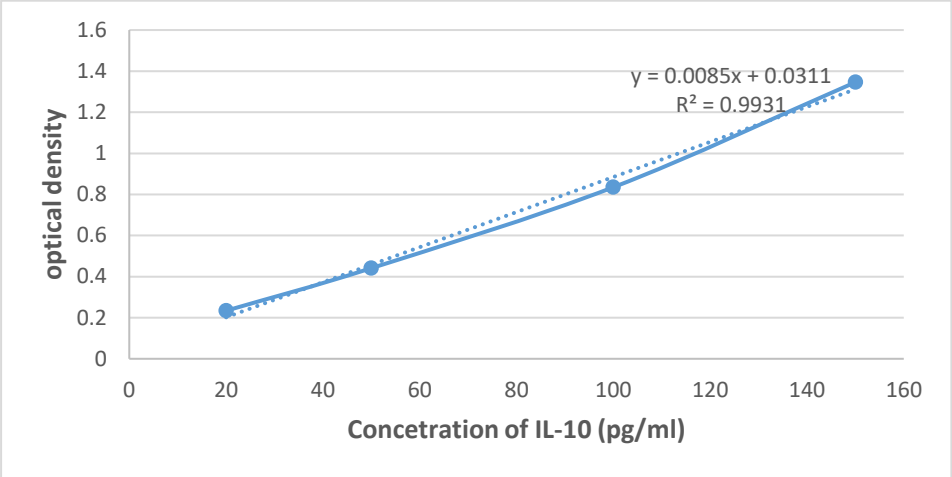


Fig. (2.8): The standard curve of human serum IL-10 levels

Part II

2.5.5. Synthesis of nanoparticles

2.5.5.1. Peripheral blood mononuclear cell isolation and culture

Centrifugation was used to separate peripheral blood mononuclear cells (PBMC) over Histopaque 1077 density gradients. The isolated cells were then washed three times with PBS (pH 7.4) and resuspended at a concentration of 1 × 10^6 cells/ml in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 10 U/ml of penicillin, and 10 µg/ml of streptomycin. The cells were incubated at 37 °C with 5% CO_2 to reach 90% confluence to get used in other experiments.

2.5.5.2. Synthesis of niosome nanoparticles

The thin-film hydration method was used for the synthesis of niosome nanoparticles (NPs). 10 ml of blank niosomal NPs were prepared by dissolving cholesterol (6 mg) and Span 60 (36 mg) in Methanol (6 ml) and Chloroform (3 ml). A rotary evaporator was utilized for 1 hour at 55-60 ◦C and 0.46 atm to mix

substances and remove solvents. The mixture was hydrated with 10 ml of phosphate buffer solution (PBS) by the rotary evaporator at 55-60 ˚C and 0.9 atm for another hour. The final product was ultra-sonicated for 30 minutes at 24 ˚C to reduce the size of synthesized niosomal NPs.

2.5.5.3. Synthesis of curcumin loaded niosomal nanoparticles

To synthesize curcumin-loaded niosomal NPs (Nio-curcumin NPs), the same methods and amount of substances were used, with the addition of 3.6 mg of curcumin in the first step. To synthesize hyaluronic acid-coated niosomal NPs (Hyalo-Nio NPs), and curcumin-loaded hyaluronic acid-coated niosomal NPs (Hyalo-Nio-curcumin NPs), 10 ml of normal saline containing 0.1% (w/v), hyaluronic acid solution was added dropwise to blank niosomal NPs and Nio-curcumin NPs, while the mixtures were stirring at ambient temperature for 1 h to reform the NPs and coating the hyaluronic acid onto the NPs surface.

2.5.5.4. Characterization of synthesized NPs

An FT-IR spectrophotometer was used to perform spectral analysis of the compounds before and after nanoparticle preparation. The spectral analysis was conducted in the region of 4000-400 cm^{-1} with a spectra resolution of 4 cm^{-1}. The zeta sizer dynamic light scattering system was utilized to analyze the size, polydispersity index (PDI), and zeta potential of the synthesized niosomal nanoparticles. Scanning electron microscopy and atomic force microscopy (AFM) were employed to record the surface morphological properties of the synthesized niosomal nanoparticles**.**

2.5.5.5. Curcumin release from niosomal nanoparticles

To evaluate the *in-vitro* drug release properties of curcumin from niosomal nanoparticles, the dialysis method was employed in the following manner: 5 mL of Hyalo-Nio-cur nanoparticles were transferred to a dialysis membrane tube (12 kDa) and magnetically stirred at 120 rpm in PBS (pH = 7.4) while being

incubated at 37 ◦C. At specified intervals, 2 mL of immersing solution was replaced with an equal volume of fresh PBS solution, and the absorbance of the released curcumin was measured at 425 nm (λ_{max} of curcumin) using ultraviolet spectrophotometry.

2.5.5.6. *In-vitro* cytotoxicity and cell uptake assay

The viability of PBMCs was assessed by conducting an MTT reduction assay to determine the impact of different doses of pure cur (2.5, 5, 10, 20, 30 μM), Nio-cur NPs (2.5, 5, 10, 20, 30 μM), and Hyalo-Nio-cur NPs (2.5, 5, 10, 20, 30 μM). Initially, 1×10^5 cells were placed in each well of 96-well plates and incubated at 37 °C with 5% CO_2 for 24 hours. Subsequently, the cells were exposed to pure curcumin, Nio-cur NPs, and Hyalo-Nio-cur NPs at 37 °C with 5% CO_2. After 48 hours, the treatment substances were replaced with 200 μL of MTT solution and incubated in the dark at 37 °C for 4 hours. The MTT solution was then removed, and 100 μL of DMSO was added to each well. The plate was shaken for 20 minutes on a plate shaker. Finally, the optical density of the wells was measured at 570 nm using the EL × 800 Microplate Absorbance Reader, and the highest proliferation values for curcumin, Nio-cur NPs, and Hyalo-Nio-cur NPs were calculated using Graph Pad 8.4 (Prism) software.

2.5.5.7. Malondialdehyde level

The levels of Malondialdehyde in the serum of rheumatoid patients were assessed using a spectrophotometric method that relies on measuring the color produced during the reaction between thiobarbituric acid and Malondialdehyde. PBMCs were seeded in a 6-well plate (1×10^5 cells per well) and incubated at 37 °C with 5% CO_2 for 24 hours to attach to the plates. Curcumin, Nio-cur NPs, and Hyalo-Nio-cur NPs were used for cell treatment for 48 hours at 37 °C with 5% CO_2. The concentration of thiobarbituric acid in treated and untreated

PBMCs was determined using the absorbance coefficient of the Malondialdehyde-thiobarbituric acid complex and reported in nmol/mg.

2.5.5.8. Measurement of glutathione and catalase

The Goth method was employed to assess catalase activities in the plasma of treated and untreated PBMCs. The yellow complex formed by molybdate, and hydrogen peroxide was quantified at 405 nm against a blank utilizing a spectrophotometer. The evaluation of glutathione levels in treated and untreated PBMCs was conducted using the techniques outlined by Flohe and Gunzle **(Günzler and Flohé, 2018)**

2.5.5.9. Inflammatory cytokine levels

In a 6-well plate, 1×10^5 PBMCs were seeded and allowed to attach for 24 hours at 37 °C with 5% CO_2. Subsequently, the PBMCs were treated with pure curcumin, Nio-cur NPs, and Hyalo-Nio-cur NPs, for 48 hours at 37 °C with 5% CO_2. A control group of cells remained untreated. Finally, the levels of IL-10 and IL-6 in both treated and untreated PBMCs were quantified using an enzyme immunoassay and the human ELISA Kit.

Part III

2.5.6. Molecular studied

2.5.6.1. Real-time polymerase chain reaction

The principle techniques underlying both RT-PCR and real-time PCR are total RNA isolation, reverse transcription (RT), and PCR. Reverse transcription involves the synthesis of DNA from RNA by using an RNA-dependent DNA polymerase. Quantitative polymerase chain reaction (PCR) analysis was conducted utilizing a Light Cycler instrument. The amplification protocol involved an initial denaturation step at 95.8 °C for 10 minutes,

Table (2.15) lists the primer sequences for quantitative PCR. Fluorescence emitted by SYBR Green I was detected after each amplification cycle to assess the accumulation of PCR products throughout the cycling process. Following each run, melting curve profiles were generated to validate the specificity of transcript amplification. The monitoring and quantification of fluorescence emission readings from cycle to cycle were conducted utilizing the second derivative maximum method through Light-cycler software.

Standard curves for GAPDH and other primers were established by serially diluting total cDNA. All determined concentrations are expressed relative to the concentration of the respective standards.

Table (2.15): Primer sequences utilized for quantitative PCR

Genes	Forward	Reverse
MMP2	AATGCCATCCCCGATAACCTG	CTCAGCAGCCTAGCCAGTCG
TIMP2	CCCCTCCAACCCATATAACACC	CACCCGGCTCTTCTTAACCTG
GAPDH	CTTCCAGGAGCGAGATCCCT	CCTGTTGCTGTAGCCAAATTCGT

2.5.6.2. RNA extraction

RNase-free microtubes, tubes, and falcons were used for RNA extraction. First, the flask cells were separated by trypsin and counted. About 1 x 10^6 cells along with 2 ml of medium containing 10% FBS were transferred to each well of a 6-well plate and the plate was incubated for 24 hours at 37 °C and 5% carbon dioxide for the cells to adhere to the wells. After 24 hours, the medium of the wells was emptied and each well was washed twice with PBS. Then the plates were incubated for 48 hours in an incubator at 37 °C and 5% carbon dioxide. After 48 hours, the contents of the wells were emptied and 500 microliters of Trizol was added to each well. After 10 minutes, the triazole was collected from the wells and transferred to microtubes, and 200 µl of chloroform was added to each microtube. Next, the microtubes were transferred to a refrigerated

centrifuge and centrifuged for 10 minutes at 12,000 RPM and 4°C. After centrifugation, 3 phases were formed inside the microtubes: the upper phase (transparent color) containing RNA, the middle phase (dark and white color) containing DNA, and the lower phase (pink color) containing protein. The supernatant phase of each microtube was collected and transferred to other microtubes. 200 microliters of isopropanol were added to each microtube and the microtubes were incubated for 10 minutes at 4°C and centrifuged for 10 minutes at 12000 RPM with a refrigerated centrifuge at 4°C.

cDNA synthesis

The synthesis of DNA from the RNA template is called cDNA, which is done by the reverse transcription enzyme. Reverse transcription enzymes require primers for cDNA synthesis. Since this day, two types of random hexamers and oligo primers have been used to make cDNA. The difference between these two types of primers is that oligo is attached to the polyadenine tail of mRNA, while random hexamers are randomly attached to different parts of mRNA. After connecting the primers, the reverse transcription enzyme synthesizes cDNA from template RNA.

Procedure

cDNA synthesis was done according to the method mentioned by the kit manufacturer. In this way, 5 microliters of extracted RNA were mixed with 1 microliter of random primer and 14 microliters of cDNA master mix, and the samples were incubated for 30 minutes at 50°C. To inactivate the reverse transcriptase enzyme, the samples were incubated at 90°C for 5 minutes. After cDNA synthesis, the samples were transferred to a -20-degree refrigerator until use.

PCR analysis was carried out using a Light Cycler instrument from Roche Diagnostics. The reaction mix included 20 ml of Light Cycler DNA-Master,

SYBR Green I, 25 pmol of oligonucleotide primers, 2 ml of cDNA solution, and 2.4 ml of 25 mmol/l $MgCl_2$. The amplification protocol consisted of an initial denaturation step at 95.8 °C for 10 minutes, followed by 40 cycles for the detection of MMP2 and TIMP2 with the following conditions: 95.8 °C for 5 seconds, primer annealing at 58.8 °C for 10 seconds, and primer extension at 72.8 °C for 20 seconds. GAPDH expression was used as a reference to normalize mRNA expression levels. Fluorescence emitted by SYBR Green I was detected after each amplification cycle to assess PCR product accumulation. Melting curve profiles were generated after each run to confirm transcript amplification specificity. Lightcycler Software was employed for monitoring and quantifying fluorescence emission readings using the second derivative maximum method. Standard curves for GAPDH and other primers were established by serially diluting total cDNA, and all determined concentrations are expressed relative to the concentration of the respective standards.

2.6. Statistical analysis:

Data collected through a questionnaire from all participants was recorded in a data sheet and allocated a unique identification number. Multiple entries were employed to mitigate mistakes. The data analysis for this study was conducted using two software programs; the Statistical Package for the Social Sciences (SPSS) version 26.0 by IBM in Chicago, Illinois, USA, and the Real Statistics Resource Pack software for Mac (Release 7.2) for Excel 2016. The content is protected by copyright from 2013 to 2020. Analyzed were the participants' data from each group using descriptive statistics. The categorical values were represented as percentages (n %). The normality of the data distribution was assessed using the Shapiro-Wilk test, which is a numerical method.

The relationship between the examined components was assessed using odds ratios (ORs) and a 95% confidence interval range determined by non-conditional logistic regression.

Analytical and statistical testing indicated substantial disparities in categorical variables among the parameters. Statistical significance was attributed to all hypothesis tests with p-values less than 0.05 (two-sided). The receiver operating characteristic (ROC) study identified the ideal threshold that maximizes specificity and sensitivity for crucial instances. All the values of P were determined to be two-sided, and a P value less than 0.05 was regarded as statistically significant.

Chapter Three

Results

3. Results

Part I

3.1. Demographic data characteristic

The demographic characteristics of patients and healthy control groups are summarized in Table (3.1). The age range of participants was between 35 and 65 years old, with 22.85% of patients with non-obese RA and 45.71% of patients with obese RA, in the 35-44 age group, 37.17% in patients with non-obese RA and 34.28% in obese RA in the 45-54 age group. Moreover, 40% of patients with non-obese RA and 20% of obese RA in the 55-64 age group. Overall, the findings show that both non-obese and obese RA made up the majority of the patient samples. The medical history of the patients was collected through a self-report questionnaire, revealing that approximately 62.85 % have non-obese RA and 65.71% have obese RA have a family history of the disease.

Table (3.1): Demographic Data according to different factors in RA patients and healthy control groups.

Variable \ Type		Non-obese RA N = 35		Obese RA N = 35		Healthy Control N = 60	
		No.	(%)	No.	(%)	No.	(%)
Age group	(35-44)	8	22.85%	16	45.71%	31	51.66%
	(45-54)	13	37.17%	12	34.28%	22	36.66%
	(55-64)	14	40%	7	20%	17	28.33%
BMI	Non-obese	35	100%	0	0	60	100%
	Obese	0	0	35	100%	0	0
Family History	Yes	22	62.85%	23	65.71%	0	0
	No	13	37.14%	12	34.28%	60	100%

BMI: body mass index, RA: Rheumatoid arthritis

3.2. Examination of the data in the studied groups.

3.2.1. Anthropometric characteristics

Table (3.2) and Fig. (3.1) show the age of the control group shows no significant differences compared to the other study groups (non-obese RA and obese RA) but non-obese RA and obese was a significant difference compared to the RA group. BMI of patients and control show significant differences between groups, and each patient group's non-obese and obese RA is significant to each other.

Table (3.2): Comparison between study groups in Age and BMI

Type / Variable	Mean ± SD			*P-value*
	Non-obese RA N=35	**Obese RA N=35**	**Healthy Control N=60**	
Age (year)	48.62 ± 6.62 a	45.0 ± 6.88 b	46.07 ± 7.99 ab	0.102
BMI (kg/m^2)	22.14 ± 1.96 b	32.35 ± 2.20 a	21.0 ± 1.57 c	0.0001
Using the Post Hoc test, the mean's horizontal differences in letters differ significantly, while the mean having (ab) did not significantly differ with other groups vertically.				

One-way ANOVA was significant at 0.05; SD: Standard deviation, S: Significant; BMI: Body mass index; RA: Rheumatoid Arthritis.

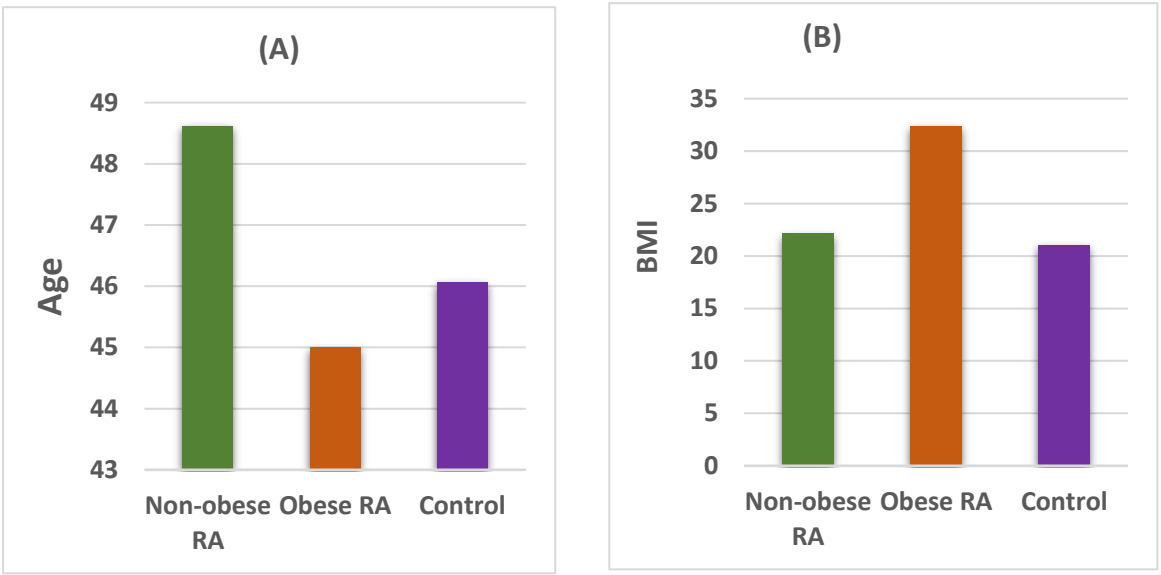


Fig. (3.1): Comparison between patients and control groups in (A) Age and (B) BMI.

3.2.2. Comparison of lipid profile between patients and healthy control

Table (3.3) and Fig. (3.2) explain the distribution of serum levels of the lipid profile in the RA patients group and the healthy control.

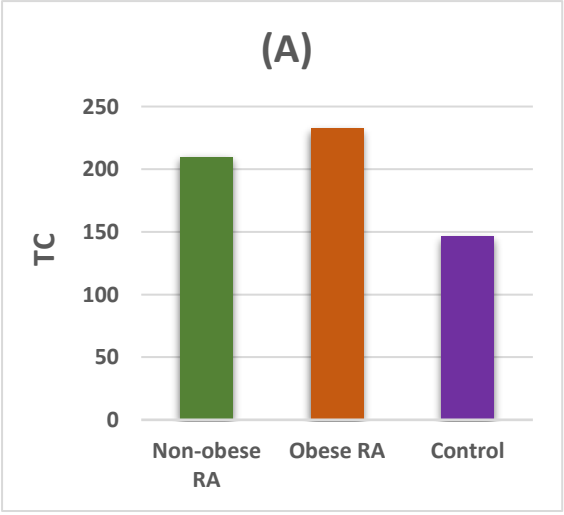
The mean ± SD levels of total cholesterol are significantly higher in obese RA (232.31 ± 16.85 mg/dl) than in non-obese RA (209.85 ± 13.64mg/dl) (*P* <0.0001) and each of them is significantly higher as compared with the healthy control group (146.18 ± 25.61 mg/dl), (*P* <0.0001). The data of serum TG levels indicated that the mean ± SD levels of TG are significantly higher in obese RA (166.08 ± 22.14 mg/dl) than non-obese RA (147.28 ± 17.33 mg/dl) (*P* <0.0001) and each of them is significantly higher than that obtained in the healthy control group (94.97 ± 19.1 mg/dl), (*P* <0.0001). The mean ± SD of HDL-C levels in sera of RA obtained was decreased and reached (35.46 ± 4.71 mg/dl) in non-obese RA which is non-significantly higher than that in obese RA (34.44 ± 2.94 mg/dl), and both of them were significantly lower than that found in apparently healthy women (58.35 ± 8.02 mg/dl) respectively, (*P* <0.0001).

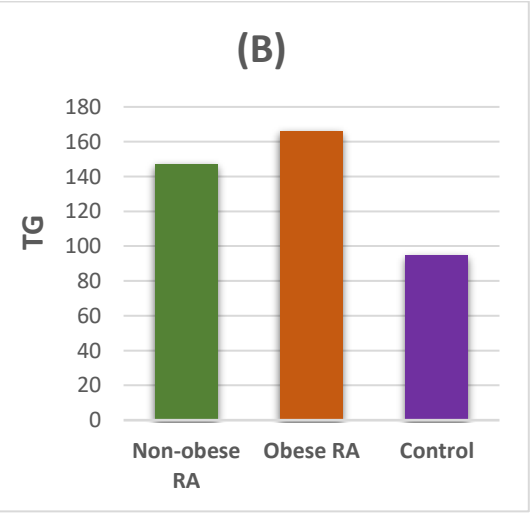
In addition to that, LDL-C levels were increased in RA patient groups in which its level in non-obese RA (166.47 ± 25.75 mg/dl) was non-significantly higher than that in sera of obese RA women (160.57 ± 29.59 mg/dl). While its level in sera of non-obese and obese RA patients was significantly higher than that found in apparently healthy women (99.0 ± 15.54 mg/dl) respectively, (*P* <0.0001).

Table (3.3): Comparison between study groups regarding lipid profile

Type / Variable	Mean ± SD			
	Non-obese RA N=35	**Obese RA N=35**	**Healthy Control, N=60**	***P-value***
TC, (mg/dl)	209.85 ± 13.64 b	232.31 ± 16.85 a	146.18 ± 25.61 c	0.0001
TG, (mg/dl)	147.28 ± 17.33 b	166.08 ± 22.14 a	94.97 ± 19.10 c	0.0001
HDL-C, (mg/dl)	35.46 ± 4.71 b	34.44 ± 2.94 a	58.35 ± 8.02 a	0.0001
LDL-C, (mg/dl)	166.47 ± 25.75 a	160.57 ± 29.59 a	99.0 ± 15.54 b	0.0001
Using the Post Hoc test, the mean's Horizontal differences in letters differ significantly.				

One-way ANOVA was significant at 0.05; SD: standard deviation, S: significant; RA: Rheumatoid Arthritis, TC: Total Cholesterol, TG: Triglycerides, HDL-C: High-Density Lipoprotein, LDL-C: Low-Density Lipoprotein.





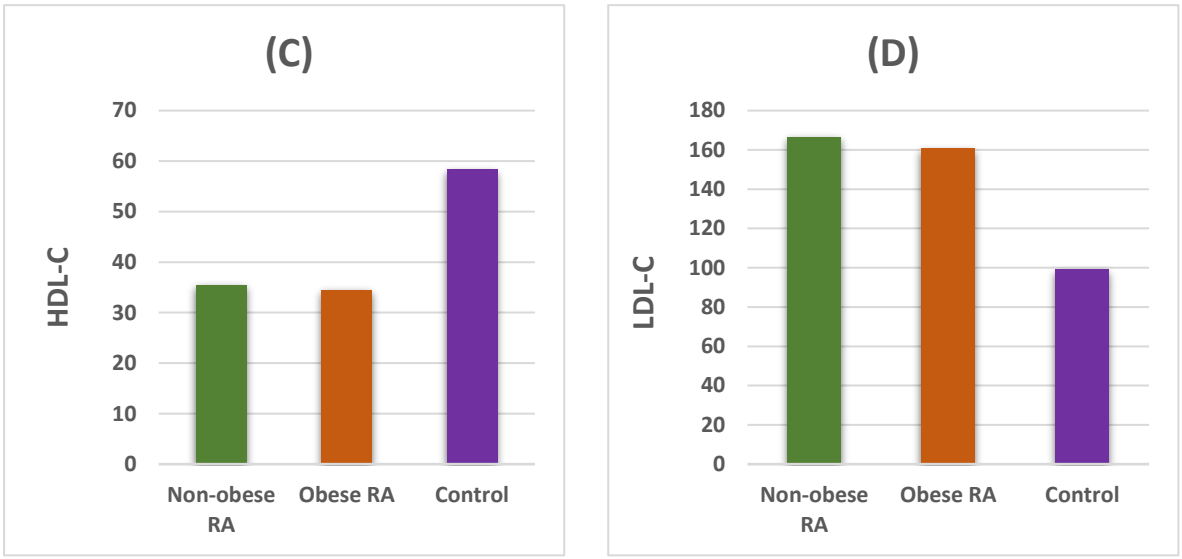


Fig. (3.2): Comparison between patient and control groups in (A) TC, (B) TG, (C) HDL, and (D) LDL

3.2.3. Oxidative and antioxidant status in RA patients and healthy control

Table (3.4) and Fig. (3.3) indicated the observed results as mean ± SD levels of each of MDA, CAT activity, and GSH in sera of non-obese and obese rheumatoid arthritis women as compared with the healthy control group.

A significantly higher mean ± SD levels of lipid peroxidation end-product Malondialdehyde in the obese RA patient group (5.06 ± 1.05 nmol/mg) were obtained which is more than in the non-obese RA group (4.07 ± 1.22 nmol/mg). These levels in RA patient groups were significantly higher than the levels investigated in sera of apparently healthy women control group (1.04 ± 0.28 nmol/mg) respectively, (P <0.0001). The mean ± SD activity levels of the endogenous antioxidant enzyme catalase were significantly lower (4.40 ± 2.28 U/L) in the sera of the obese RA patient group than that determined in the non-obese RA patient group (6.62 ± 2.52 U/L). These levels of catalase activity were significantly decreased as compared with that found in sera of the health control group (15.77 ± 2.16 U/L), (P <0.0001).

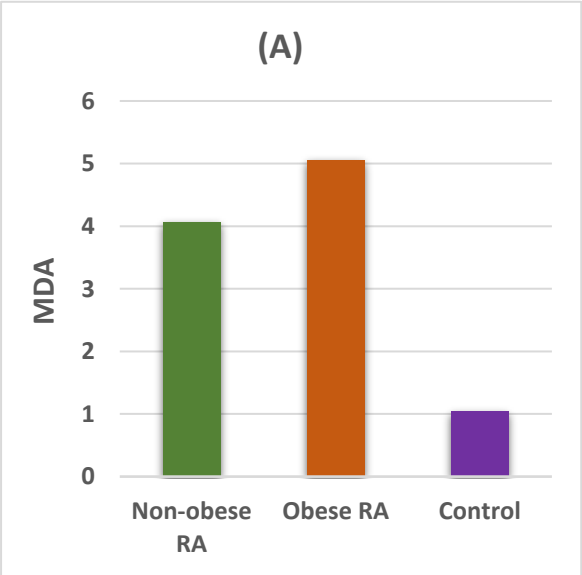
In addition to that, a significantly different mean ± SD level was observed regarding endogenous non-enzymatic antioxidant reduced glutathione (GSH) in

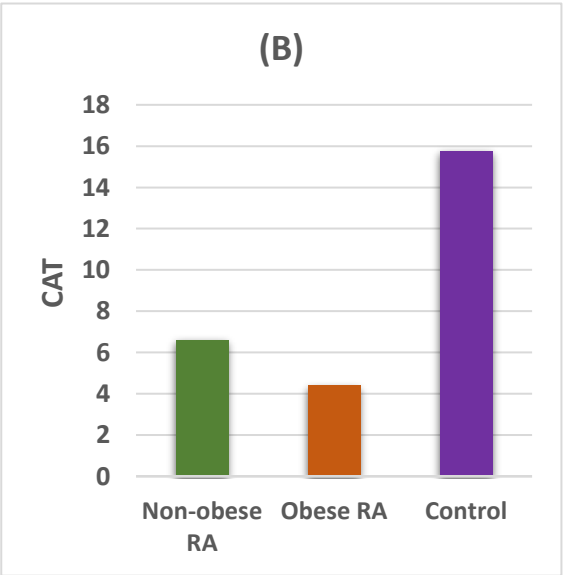
sera of RA patient groups in which its level in obese RA was significantly the lowest and reached (51.26 ± 6.99 µM). While sera of the non-obese RA group reached (54.44 ± 7.76 µM) as compared with that in the healthy control group which was significantly higher than both of the RA patient groups (63.22 ± 10.14 µM), (*P*<0.0001).

Table (3.4): Comparison between oxidative status parameters in studying groups regarding MDA, CAT, and GSH.

Variable \ Type	Mean ± SD			*P-value*
	Non-obese, RA, N = 35	**Obese RA N = 35**	**Healthy Control, N=60**	
MDA,(nmol/mg)	4.07 ± 1.22 b	5.06 ± 1.05 a	1.04 ± 0.28 c	0.0001
CAT, (U/L)	6.62 ± 2.52 b	4.40 ± 2.28 c	15.77 ± 2.16 a	0.0001
GSH, (µM)	54.44 ± 7.76 a	51.26 ± 6.99 b	63.22 ± 10.14 a	0.0001
Using the Post Hoc test, the mean's horizontal differences in letters differ significantly.				

One-way ANOVA was significant at 0.05; SD: standard deviation, S: significant; RA: Rheumatoid Arthritis, CAT: catalase; GSH: reduced glutathione; MDA: Malondialdehyde.





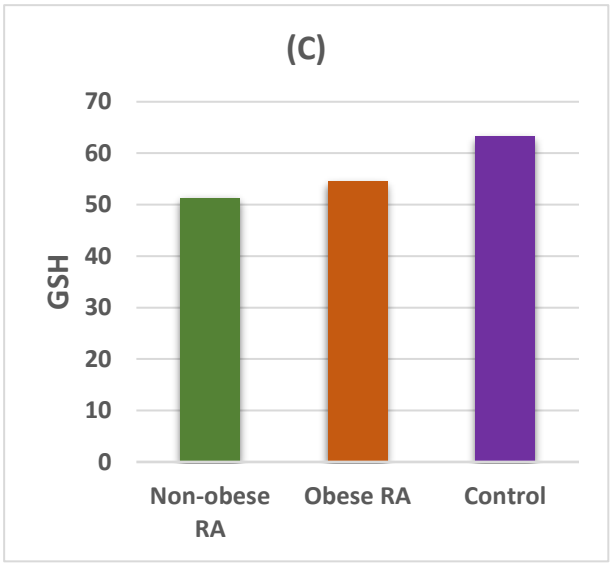


Fig. (3.3): Comparison between study groups regarding (A) MDA, (B) CAT, and (C) GSH in RA patients and healthy control groups.

3.2.4. Inflammatory and anti-Inflammatory status in RA patients and healthy control

Table (3.5) and Fig. (3.4) indicated the observed results as mean ± SD levels of each of IL-6, and IL-10 in sera of non-obese (N = 35) and obese rheumatoid arthritis (N = 35) women as compared with the healthy control group (N = 60).

The data observed indicated that a significantly higher mean ± SD level of each of IL-6 and IL-10 in the sera of RA patient groups was determined and reached (71.02 ± 11.39 ng/ml) and (25.31 ± 5.25 ng/ml) in the sera of the obese RA group. Which were significantly higher than that found in sera of non-obese RA patient group (59.8 ± 14.50 ng/ml) and (20.62 ± 6.8 ng/ml), and both of these levels in patient groups were significantly higher than that investigated in sera of apparently healthy control group respectively (5.87 ± 1.91 ng/ml) and (7.37 ± 1.33 ng/ml), (P<0.0001).

Table (3.5): Comparison between study groups regarding IL-6, and IL-10

Variable \ Type	Mean ± SD			*P-value*
	Non-obese RA, N=35	**Obese RA N=3**	**Healthy Control, N=60**	
IL-6, (pg/ml)	59.8 ± 14.50 b	71.02 11.39 a	5.87 ± 1.91 c	0.0001
IL-10, (pg/ml)	20.62 ± 6.80 b	25.31 ± 5.25 a	7.37 ± 1.33 c	0.0001
Using the Post Hoc test, the mean's horizontal differences in letters differ significantly.				

One-way ANOVA was significant at 0.05; SD: standard deviation, S: significant; RA: Rheumatoid Arthritis; IL-10: interlukine-10; IL-6: interlukine-6

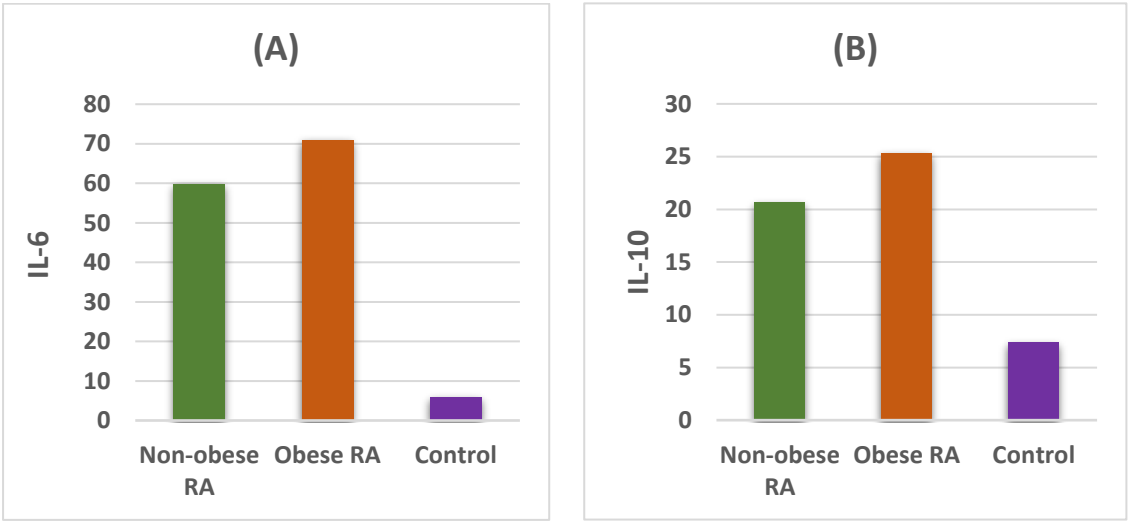


Fig. (3.4): Comparison between study groups regarding (A) IL-6 and (B) IL-10 in RA patients and healthy control groups.

3.3. Correlation between biomarker and studied parameters in patient groups

3.3.1. Correlation between MDA levels and other parameters

Table (3.6), indicates the correlation between serum levels of MDA and biochemical parameters and anthropometric characteristics in RA patient groups. The results found that in the non-obese RA group. A significant positive correlation between MDA and TC (*P*=0.0001) was observed, and a non-significant positive correlation between MDA with each age, BMI, TG, LDL-C, IL-10, and IL-6 was found. However, a non-significant negative correlation

with each of HDL-C, CAT activity, and GSH levels was observed. In the obese RA group, a significant positive correlation between MDA and BMI (*P*=0.008), TC (*P*=0.01), TG (*P*=0.004), and LDL-C (*P*=0.02) was observed. In addition, a significant negative correlation between MDA and HDL-C (*P*=0.004), and a non-significant negative correlation between MDA and GSH were investigated, and a non-significant positive correlation between MDA with each of age, IL-10, and IL-6 was determined.

Table (3.6): Correlation coefficient of MDA with parameters regarding patient groups.

Parameters	MDA (nmol/mg)			
	Non-obese RA		Obese RA	
	r	*P-value*	*r*	*P-value*
Age, (year)	0.13	0.27	0.12	0.30
BMI, (kg/m^2)	0.21	0.08	0.32**	0.008
TC, (mg/dl)	0.59**	0.0001	0.29*	0.01
TG, (mg/dl)	0.16	0.18	0.34**	0. 004
HDL-C, (mg/dl)	-0.13	0.25	-0.33**	0.004
LDL-C, (mg/dl)	0.08	0.50	0.27*	0.02
CAT, (U/l)	-0.01	0.90	-0.11	0.35
GSH (µM)	-0.07	0.54	-0.23	0.052
IL-6, (pg/ml)	0.06	0.57	0.03	0.77
IL-10, (pg/ml)	0.03	0.77	0.11	0.35

r: Pearson correlation coefficients; P: P value; S: significant; BMI: Body mass index; RA: Rheumatoid Arthritis, CAT: catalase; GSH: reduced glutathione; MDA: Malondialdehyde; IL-10: interleukin-10; IL-6: interleukin-6; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein-cholesterol; LDL-C: low-density lipoprotein cholesterol; **Correlation is significant at the 0.01 level, *Correlation is significant at the 0.05 level, - = negative.

3.3.2. Correlation between CAT and other biomarkers

The correlation between serum activity levels of CAT and biochemical parameters and anthropometric characteristics in patient groups is in Table (3.7).

In the non-obese RA group, there is a significant positive correlation between CAT and HDL-C (*P*=0.0001), while a significant negative correlation between CAT and each of age (*P*=0.009), and IL-6 (*P*=0.01) was found. BMI,

TC, TG, LDL-C, MDA, and IL-10 had a negative non-significant correlation with CAT, while GSH non-significant positive correlation with CAT.

In the obese RA group, a significant negative correlation was found with IL-10 (*P*=0.03), IL-6 (*P*=0.001), and a non-significant positive correlation with GSH and HDL-C, while the non-significant negative correlation with age, BMI, TC, TG, LDL-C, and MDA.

Table (3.7): Correlation coefficient of CAT with parameters regarding patient groups.

Parameters	CAT, (U/l)			
	Non-obese RA		Obese RA	
	r	*P-value*	*r*	*P-value*
Age, (year)	-0.311**	0.009	-0.08	0.50
BMI, (kg/m^2)	-0.82	0.49	-0.08	0.50
TC, (mg/dl)	-0.06	0.58	-0.11	0.34
TG, (mg/dl)	-0.04	0.72	-0.02	0.81
HDL-C, (mg/dl)	0.48**	0.0001	0.05	0.66
LDL-C, (mg/dl)	-0.20	0.09	-0.05	0.64
MDA, (nmol/mg)	-0.14	0.90	-0.11	0.35
GSH, (µM)	0.10	0.37	0.01	0.91
IL-6, (pg/ml)	-0.28*	0.01	-0.41**	0.0001
IL-10, (pg/ml)	-0.05	0.67	-0.35**	0.003

****Correlation is significant at the 0.01 level, *Correlation is significant at the 0.05 level, - = negative; r: Pearson correlation coefficients; P : P value; S: significant; BMI: Body mass index; RA: Rheumatoid Arthritis, CAT: catalase; GSH: reduced glutathione; MDA: Malondialdehyde; IL-10: interleukin-10; IL-6: interleukin-6; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein-cholesterol; LDL-C: low-density lipoprotein cholesterol.**

3.3.3. Correlation between GSH and other studied parameters

The correlation between serum levels of GSH and biochemical parameters and anthropometric characteristics in patient groups is in Table (3.8). In non-obese RA, the results significant positive correlation between GSH and HDL-C (*P*=0.03), a significant negative correlation between GSH and age (*P*=0.03), and a non-significant positive correlation between GSH and CAT.

Furthermore, a non-significant negative between GSH and BMI, TC, TG, LDL-C, MDA, IL-10, and IL-6.

While in obese RA, the result is a significant positive correlation between GSH and HDL-C (*P*=0.02). A non-significant positive correlation between GSH and CAT, in addition to a non-significant negative correlation between GSH and age, BMI, TC, TG, LDL-C, IL-10, and IL-6.

Table (3.8): Correlation coefficient between GSH with other parameters

Parameters	GSH, (µM)			
	Non-obese RA		Obese RA	
	r	*P-value*	r	*P-value*
Age, (year)	-0.25*	0.03	-0.07	0.53
BMI, (kg/m^2)	-0.02	0.83	-0.06	0.61
TC, (mg/dl)	-0.11	0.33	-0.16	0.16
TG, (mg/dl)	-0.06	0.61	-0.07	0.52
HDL-C, (mg/dl)	0.25*	0.03	0.27*	0.02
LDL-C, (mg/dl)	-0.11	0.34	-0.21	0.07
MDA, (nmol/mg)	-0.07	0.54	-0.23	0.052
CAT, (U/l)	0.10	0.37	0.01	0.91
IL-6, (pg/ml)	-0.03	0.80	-0.141	0.24
IL-10, (pg/ml)	-0.21	0.07	-0.04	0.84

****Correlation is significant at the 0.01 level, *Correlation is significant at the 0.05 level, - = negative; r: Pearson correlation coefficients; P: P value; S: significant; BMI: Body mass index; RA: Rheumatoid Arthritis, CAT: catalase; GSH: reduced glutathione; MDA: Malondialdehyde; IL-10: interleukin-10; IL-6: interleukin-6; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein-cholesterol; LDL-C: low-density lipoprotein cholesterol.**

3.3.4. Correlation between IL-6 and other studied parameters

The correlation between serum levels of IL-6 and biochemical parameters and anthropometric characteristics in patient groups is in Table (3.9). The results found in the non-obese RA group indicated a non-significant positive correlation between IL-6 with each of age, BMI, TC, TG, LDL-C, and MDA, while a non-significant negative correlation between IL-6 with HDL-C, GSH, and IL-10,

and a significant negative correlation between IL-6 and CAT (*P*=0.01)**.** In the obese RA group, a significant positive correlation between IL-6 with each of TC (*P*=0.008), TG (*P*=0.09), and LDL-C (*P*=0.0001). Moreover, a significant negative correlation between IL-6 and HDL-C (*P*=0.003), and CAT (*P*=0.001). In addition, a non-significant positive correlation between IL-6 and age, BMI, and MDA, furthermore a non-significant negative correlation between IL-6 with IL-10, and GSH.

Table (3.9): Correlation coefficient of IL-6 with various parameters regarding patient groups.

Parameters	IL-6, (pg/ml)			
	Non-obese RA		Obese RA	
	r	*P-value*	*r*	*P-value*
Age, (year)	0.18	0. 12	0.01	0.89
BMI, (kg/m^2)	0.15	0.18	0.10	0.38
TC, (mg/dl)	0.18	0.29	0.44**	0.008
TG, (mg/dl)	0.17	0.30	0.43**	0.01
HDL-C, (mg/dl)	-0.20	0.09	-0.48**	0.003
LDL-C, (mg/dl)	0.21	0.07	0.68**	0.0001
MDA (nmol/mg)	0.06	0.57	0.03	0.77
CAT, (U/l)	-0.28*	0.01	-0.41**	0.0001
GSH (µM)	-0.03	0.80	-0.14	0.24
IL-10, (pg/ml)	-0.006	0.96	-0.11	0.34

P: P value; BMI: Body mass index; RA: Rheumatoid Arthritis, CAT: catalase; GSH: reduced glutathione; MDA: Malondialdehyde; IL-10: interleukin-10; IL-6: interleukin-6; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein-cholesterol; LDL-C: low-density lipoprotein cholesterol.

3.3.5. Correlation between IL-10 and other studied parameters

The correlation between serum levels of IL-10 and biochemical parameters and anthropometric characteristics in patient groups is in Table (3.10). The results found in the non-obese RA group indicated a significant positive correlation between IL-10 and age (*P*=0.002). Moreover, a non-significant positive correlation between IL-10 and TC, TG, LDL-C, MDA, and

BMI, while a non-significant negative correlation between IL-10 and HDL-C, CAT, and IL-6. While a significant negative correlation between IL-10 and CAT (*P*=0.04), a non-significant positive correlation between IL-10 and BMI, TC, TG, LDL-C, and MDA, furthermore a non-significant negative correlation between HDL-C, IL-6, and GSH.

Table (3.10): Correlation coefficient of IL-10 with parameters regarding patient groups.

Parameters	IL-10 (pg/ml)			
	Non-obese RA		Obese RA	
	r	*P-value*	*r*	*P-value*
Age, (year)	0.36**	0. 002	0.35**	0.003
BMI, (kg/m^2)	0.10	0.39	0.15	0.19
TC, (mg/dl)	0.08	0.49	0.10	0. 38
TG, (mg/dl)	0.12	0.30	0.18	0.20
HDL-C, (mg/dl)	-0.21	0.08	-0.01	0.93
LDL-C, (mg/dl)	0.16	0.16	0.20	0.09
MDA (nmol/mg)	0.03	0.77	0.11	0.35
CAT, (U/l)	-0.05	0.67	-0.35**	0.04
GSH (µM)	-0.21	0.07	-0.02	0.14
IL-6, (pg/ml)	-0.006	0.96	-0.11	0.34

****Correlation is significant at the 0.01 level, *Correlation is significant at the 0.05 level, - = negative; r: Pearson correlation coefficients; P: P value; S: significant; BMI: Body mass index; RA: Rheumatoid Arthritis, CAT: catalase; GSH: reduced glutathione; MDA: Malondialdehyde; IL-10: interleukin-10; IL-6: interleukin-6; TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein-cholesterol; LDL-C: low-density lipoprotein cholesterol.**

3.4. Receiver operating characteristic

The receiver operating characteristic (ROC) curve and AUC analysis for the MDA, CAT, GSH, IL-6, and IL-10 for the RA group were performed and the results obtained indicated an important role as a diagnostic parameter in which IL-10 has a good performance, data are presented in Table (3.11). In the non-obese RA group, IL-10 had the highest AUC with a value of 0.819 [95%

CI (confidence interval) =0.722 – 0.914, Sensitivity = 83%, Specificity= 77%, Cut-off point = 14.92 pg/ml]. IL-6 had an AUC of 0.785 [95%CI (confidence interval) = 0.684-0.887, sensitivity= 69%, Specificity = 73%, Cut-off point =51.52ng/ml. MDA had the AUC with a value of 0.724 [95% CI (confidence interval) =0.630 – 0.854, Sensitivity = 63%, Specificity = 70%, Cut-off point = 3.27nmol/mg].

Table (3.11): Optimal threshold, AUC, sensitivity, and specificity of MDA, IL-10, and IL-6 obtained by ROC curve in patients with non-obese RA.

Parameters	Cut-off	Sensitivity	Specificity	AUC	*P*-value	95% Cl	
MDA (nmol/mg)	≥3.27	%63	%70	0.724	0.0001	0.630	0.854
IL-6 (pg/ml)	≥51.52	%69	%73	0.785	0.0001	0.684	0.887
IL-10 (pg/ml)	≥14.92	%83	%77	0.819	0.0001	0.722	0.914

MDA: Malondialdehyde; IL-10: interlukine-10; IL-6: interlukine-6

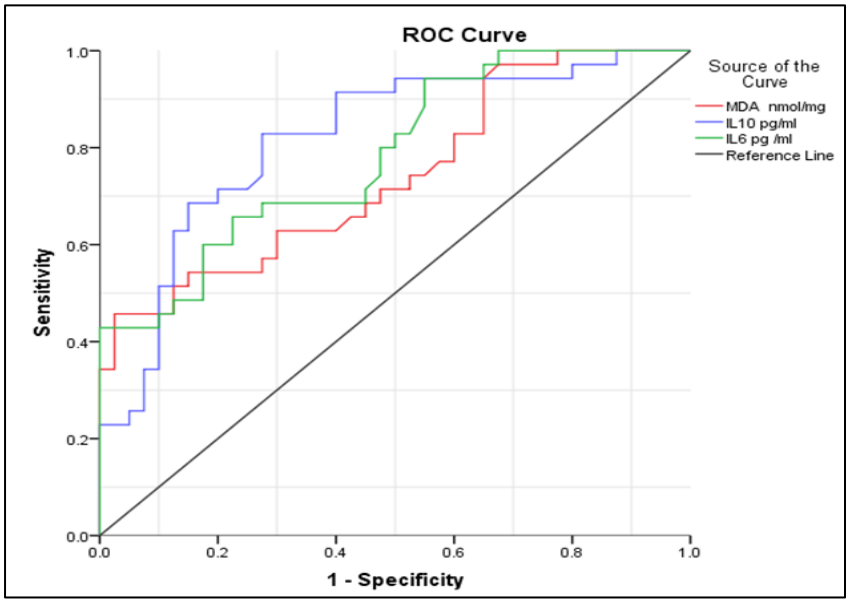


Fig. (3.5): Receiver Operating Characteristic (ROC) curve of serum MDA, IL-6, and IL-10 levels as discriminators of patients with non-obese RA.

In the non-obese RA group, CAT had the highest AUC of 0.871 [95%CI (confidence interval) = 0.788-0.954, sensitivity= 80%, Specificity = 80%, Cut-

off point =8.91U/l. GSH had an AUC of 0.825 [95%CI (confidence interval) = 0.732-0.918, sensitivity= 74%, Specificity = 70%, Cut-off point = 56.4µM

Table (3.12): Optimal threshold, AUC, sensitivity, and specificity of CAT and GSH obtained by ROC curve regarding patients with non-obese RA.

Parameters	Cut-off	Sensitivity	Specificity	AUC	*P*-value	95% CI	
CAT, (U/l)	≤8.91	%80	%80	0.871	0.0001	0.788	0.954
GSH, (µM)	≤56.4	%74	%70	0.825	0.0001	0.732	0.918

CAT: catalase; GSH: reduced glutathione

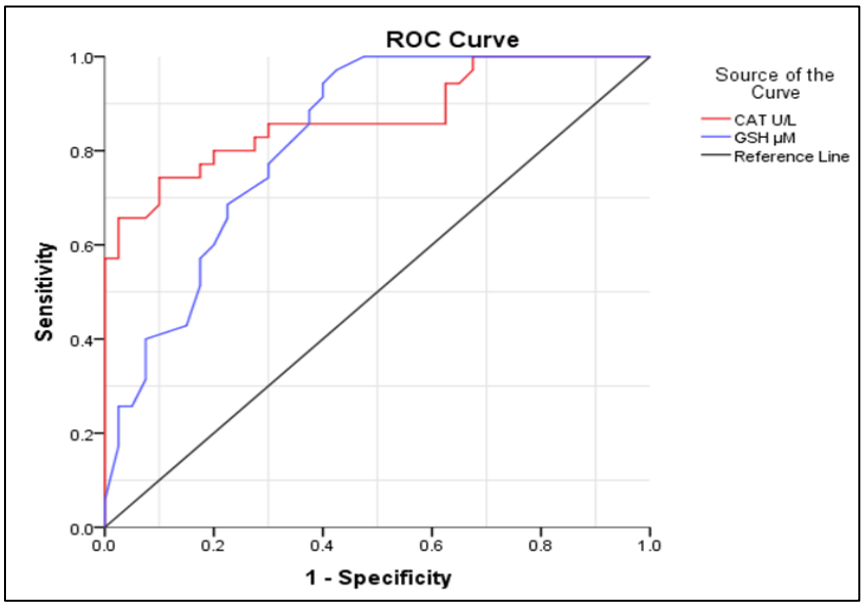


Fig. (3.6): Receiver Operating Characteristic (ROC) curve of serum CAT and GSH levels as discriminators of patients with non-obese RA.

In the obese RA group, IL-6 had the highest AUC with a value of 0.945 [95% CI (confidence interval) =0.899– 0.991, Sensitivity = 91%, Specificity = 83%, Cut-off point = 54.35 pg /ml]. MDA had an AUC of 0.933 [95%CI (confidence interval) = 0.881-0.955, sensitivity= 87%, Specificity = 80%, Cut-off point =3.65 nmol/mg]. IL-10 had the AUC with a value of 0.885 [95% CI (confidence interval) =0.817 – 0.999, Sensitivity = 89%, Specificity = 90%, Cut-off point = 18.8 pg/ml].

Table (3.13): Optimal threshold, AUC, sensitivity, and specificity of MDA, IL-6, and IL-10 obtained by ROC curve regarding patients with obese RA.

Parameters	Cut-off	Sensitivity	Specificity	AUC	*P-value*	95% Cl	
MDA, (nmol/mg)	≥3.65	%87	%80	0.933	0.0001	0.881	0.955
IL-6, (pg/ml)	≥54.35	%91	%83	0.945	0.0001	0.899	0.991
IL-10, (pg/ml)	≥18.8	%89	%90	0.885	0.0001	0.917	0.999

MDA: Malondialdehyde; IL-10: interlukine-10; IL-6: interlukine-6

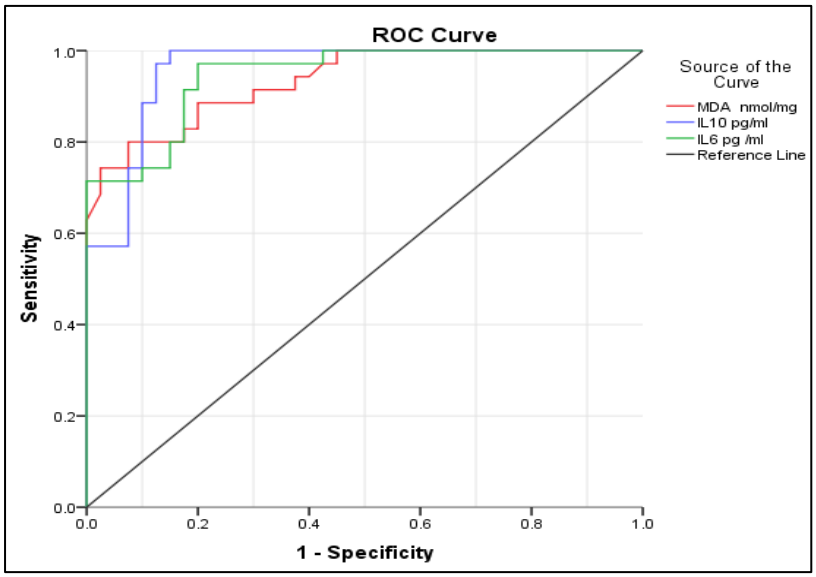


Fig. (3.7): Receiver Operating Characteristic (ROC) curve of serum MDA, IL-10, and IL-6 levels as discriminators of patients with obese RA.

In the obese RA group, CAT had the highest AUC of 0.983 [95%CI (confidence interval) = 0.962-1.000, sensitivity= 97%, Specificity = 90%, Cut-off point =8.39U/l. GSH had an AUC of 0.753[95%CI (confidence interval) = 0.642-0.863, sensitivity= 80%, Specificity = 60%, Cut-off point =61.5µM

Table (3.14): Optimal threshold, AUC, sensitivity, and specificity of CAT and GSH obtained by ROC curve regarding patients with obese RA.

Parameters	Cut-off	Sensitivity	Specificity	AUC	P-value	95% CI	
CAT, (U/l)	≤8.39	%97	%90	0.983	0.0001	0.962	1.000
GSH, (µM)	≤61.5	%80	%60	0.753	0.0001	0.642	0.863

CAT: catalase; GSH: reduced glutathione

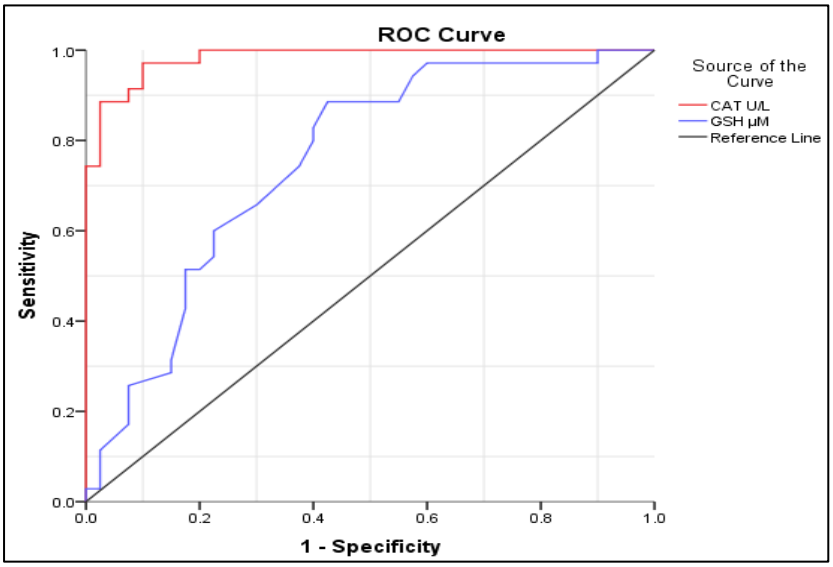


Fig. (3.8): Receiver Operating Characteristic (ROC) curve of serum CAT, and GSH levels as discriminators of patients with RA with obesity.

Part II

3.5. The properties of the structure Hyalo-Nio-cur NPs

The results of DLS analysis for synthesized niosomal NPs are presented in Table (3.15). According to these results, the Hyalo-Nio-cur NPs have the largest size compared to other NPs, the size of synthesized niosomal NPs varies from 115 nm to 179 nm (noisome NPs = 115 ± 9.4 nm, Nio-cur NPs = 158 ± 17.3 nm, and Hyalo-Nio-cur NPs = 179 ± 21.6 nm). A low PDI value indicates a narrow size distribution so these results show the distribution and homogeneity of NPs (noisome NPs =0.372, Nio-cur NPs = 0.628, and Hyalo-Nio-cur NPs = 0.596). The zeta potential value is critical in preventing the aggregation of particles the result shows no aggregation of these particles.

Table (3.15): The zeta potential, PDI, and size of synthesized niosomal NPs

Nanoparticle	Size	PDI	Zeta potential
niosomal NPs	115 ± 9.4	0.372	−9.7 ± 3.4
Nio-cur NPs	158 ± 17.3	0.628	−24.9 ± 2.5
Hyalo-Nio-cur NPs	179 ± 21.6	0.596	−28.1 ± 6.3

The surface morphology and structure of synthesized niosomal NPs were studied using an SEM system Fig. (3.10) at different magnification ranges, the results obtained images show spherical morphology for all NPs.

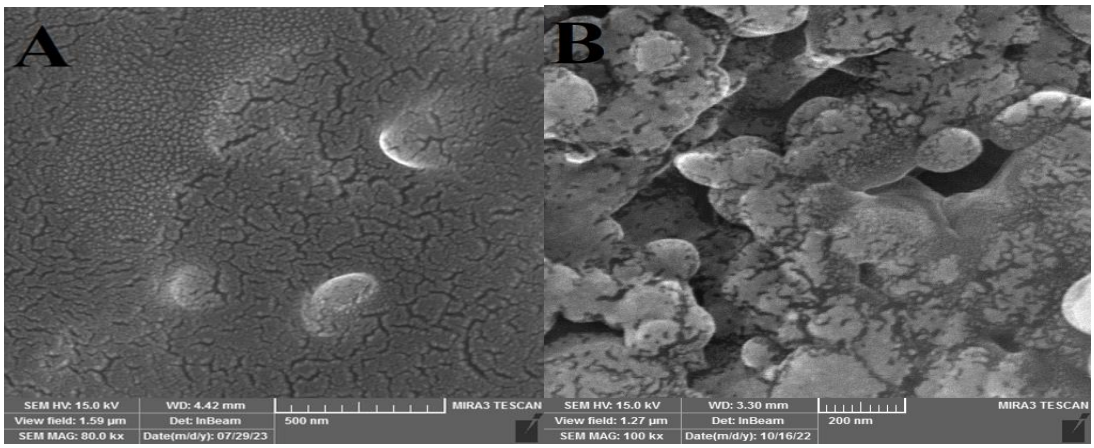


Fig. (3-9): The SEM images of (A) Nio-cur NPs, (B) Hyalo-Nio-cur NPs

The AFM results revealed particles with a maximum size of Hyalo-Nio-cur NPs 149 nm, demonstrating uniform dispersion of nanoparticles without any aggregation, as depicted in Fig. (3.10).

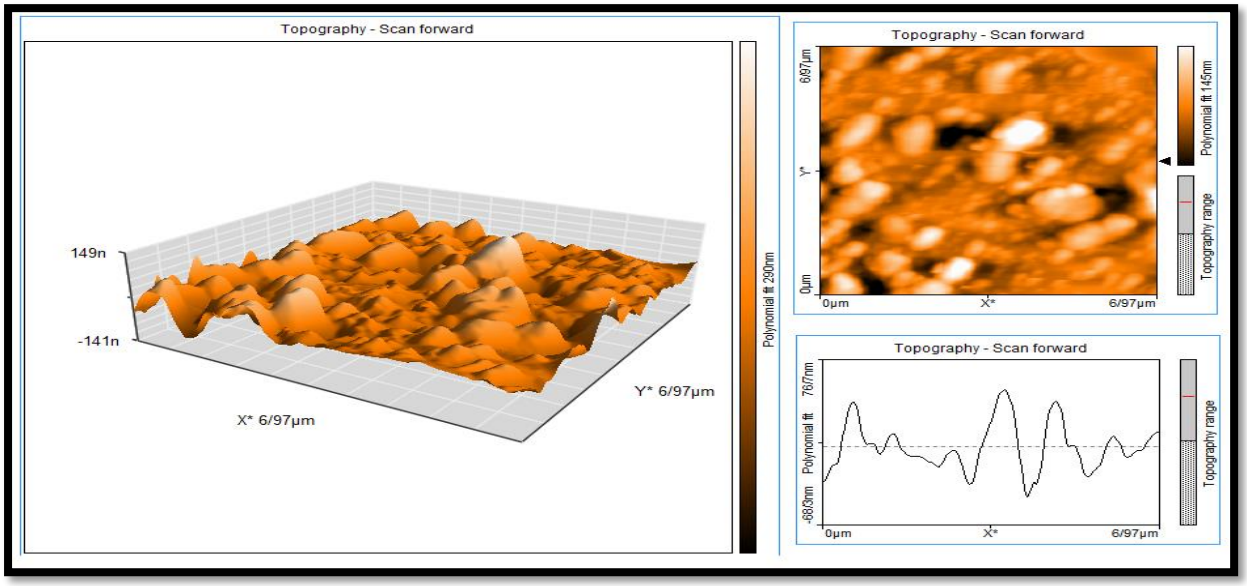


Fig. (3.10): AFM image of Hyalo-Nio-cur NPs

The TEM image Fig. (3.11) clearly illustrates the spherical morphology of the Hyalo-Nio-cur NPs, with an average size consistent with the results obtained from DLS.

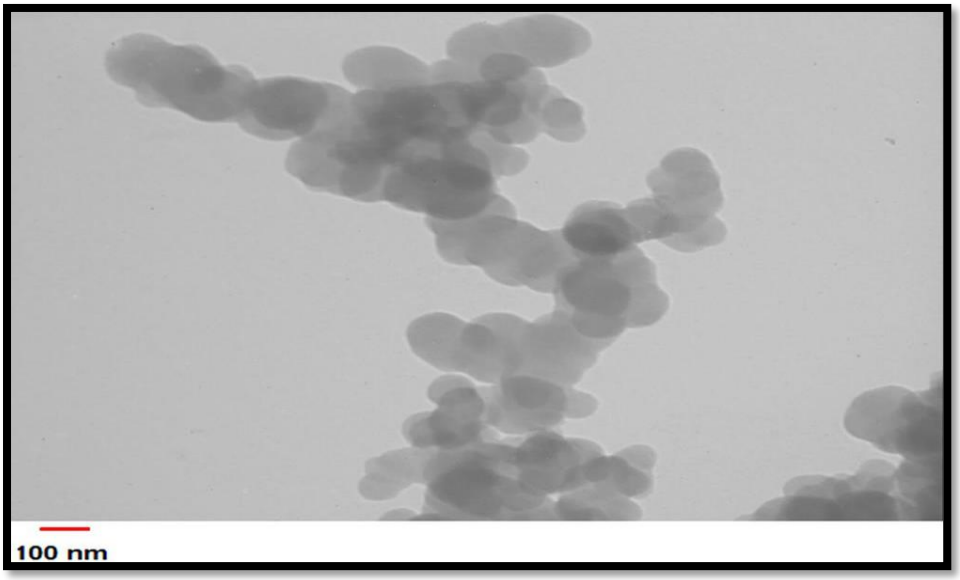


Fig. (3.11) TEM image of the synthesized Hyalo-Nio-cur NPs

3.5.1. FTIR Analysis of synthesized Nio NPs, Hyaluronic Acid

The molecular interactions of the Cholesterol, Span 60, HA, and curcumin were obtained using FT-IR Fig. (3.12). The FTIR spectra analysis

revealed characteristic peaks indicative of primary and secondary amine functional groups at 3400 cm^{-1} in both cholesterol and Span 60. Notably, the blank niosomal NPs displayed distinct peaks associated with various functional groups, including C = O stretching (1746 cm^{-1}), C–O stretching (1172 cm^{-1}), aliphatic C–N stretching (1000–1250 cm^{-1}), C–H stretching (2800–3000 cm^{-1}), C–H symmetric stretching (1469 cm^{-1}), and O–H stretching (3452 cm^{-1}). Span 60 exhibited characteristic peaks at approximately 3395 cm^{-1} (O–H stretching), 2956 cm^{-1} (–CH stretching), and 1736 cm^{-1} (C = O ester bond). Upon the integration of hyaluronic acid into the drug-loaded niosome, a discernible peak at 1600 cm^{-1} corresponding to the amide group emerged.

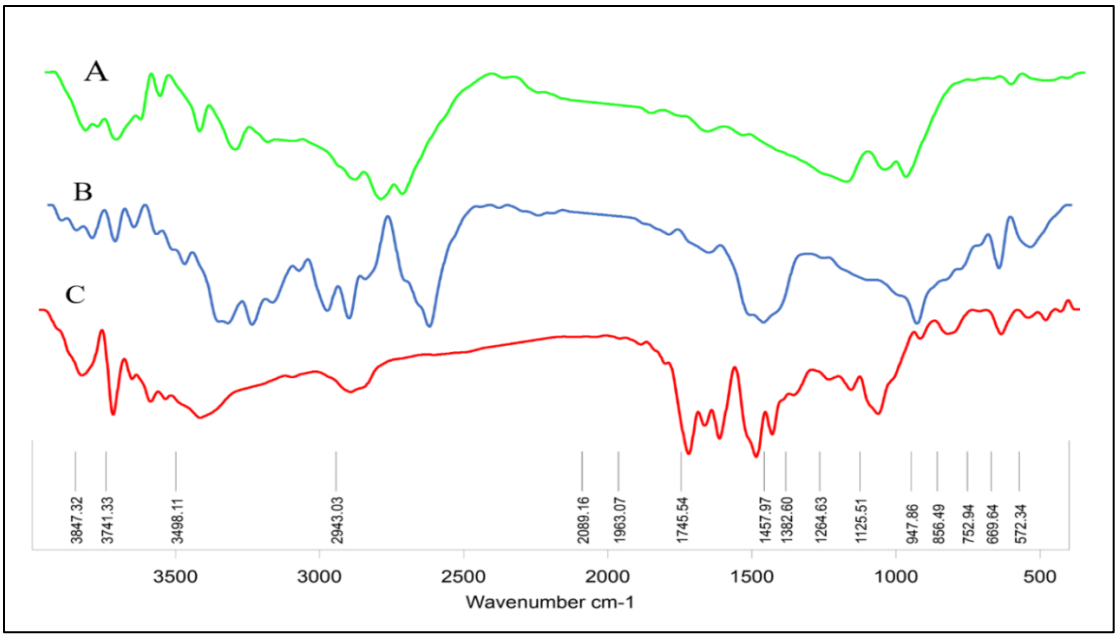


Fig. (3.12): The FTIR spectra of HA and curcumin confirm their presence in niosomal NP structures. A) Blank niosomal NPs, B) HA, and C) Hyalo-Nio-cur NPs

Fig. (3.13) demonstrates the *in-vitro* release of curcumin from the Nio-cur NPs and Hyalo-Nio-cur NPs. The release pattern of the curcumin from the Nio-cur NPs and Hyalo-Nio-cur NPs was biphasic, with a maximum release rate of 62% and 76% within 120 h of the experiment and decreased afterward, subsequently. The steep slope of the initial release is due to the presence of

curcumin on the surface of the nanoparticles, which is attached to the surface of niosomal NPs with weak bonds instead of loading inside them.

The effect of curcumin and curcumin-loaded NPs on the proliferation of PBMCs was investigated by MTT assay Fig. (3.14). The maximum enhanced survival of PBMCs was achieved with a 10 µM incubated group. The inclusion of hyaluronic acid on the surface of curcumin facilitates result in localizing the curcumin approximately PBMCs, thereby enhancing the cellular before and after therapeutic impact in 48 hours.

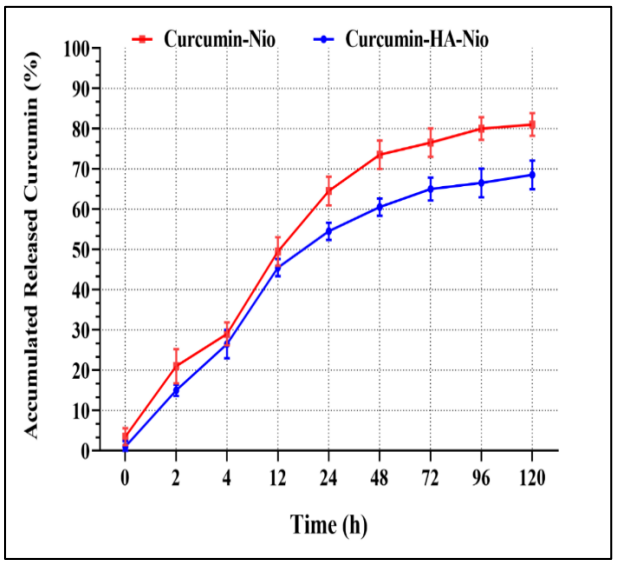


Fig. (3.13): The 120 hours release pattern of the curcumin from the Hyalo-Nio-cur NPs.

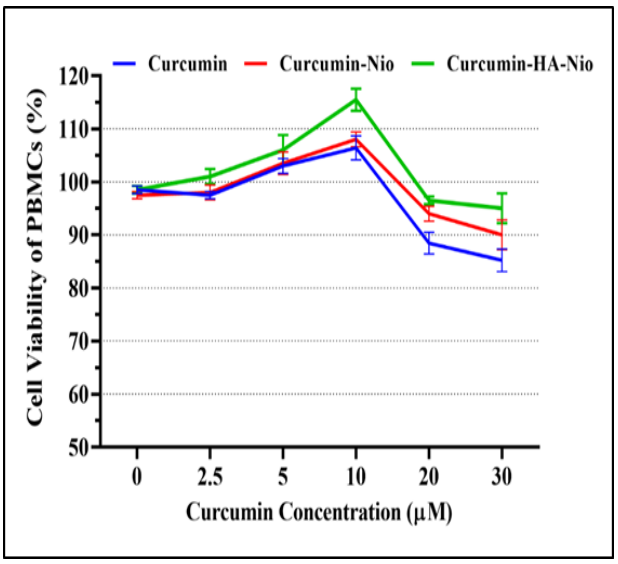


Fig. (3.14): The proliferation effect of substances on PBMCs in 48 hours of treatment

2.5.2. Oxidative status

Fig. (3.15 A) The MDA levels in PBMCs are depicted before and after being treated with curcumin, Nio-cur NPs, and Hyalo-cur NPs, Interestingly incubated using curcumin and Nio-cur NPs did not lead to a reduction in MDA levels compared to PBMCs. Reduction in Malondialdehyde level achieved with Hyalo-Nio-cur NPs (3.73 to 2.28). The GSH levels in treated and untreated PBMCs are shown in Fig. (3.15 B). Demonstrated the catalase level in PBMCs.

Generally, the treated PBMCs demonstrated higher levels of catalase compared to the untreated group. The highest increase in catalase level belongs to the Hyalo-Nio-cur NPs treated group (6.61 to 12.81). Fig. (3.15 C) Accordingly, treatment with curcumin, Nio-cur NPs, and Hyalo-Nio-cur NPs. Successfully increased the GSH levels in PBMCs (38.11 to 57.27).

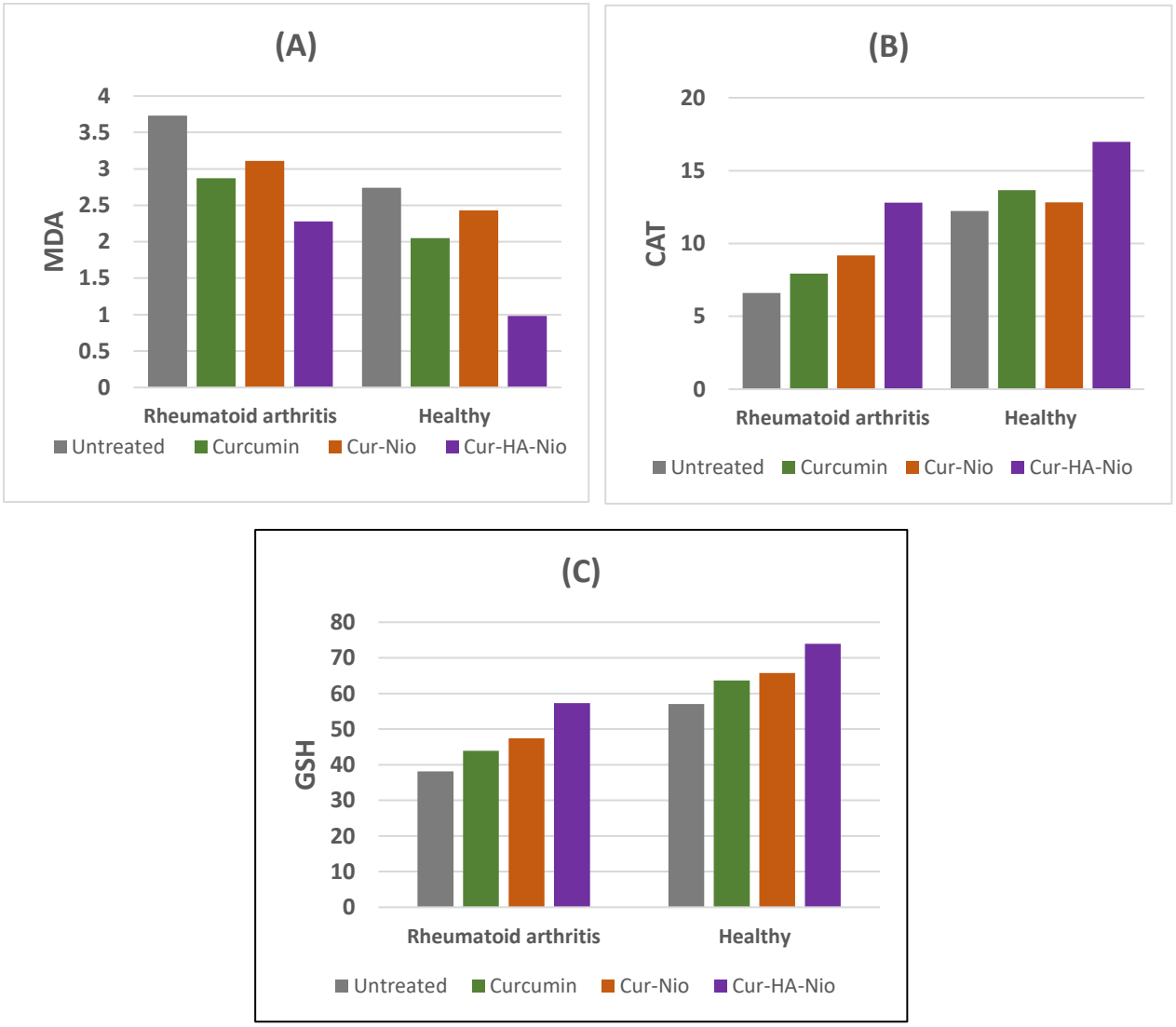


Fig. (3.15): The Malondialdehyde, glutathione, and catalase level changes in PBMCs. (A) Malondialdehyde, (B) Catalase, (C) Glutathione (* = p < 0.05, ** = p < 0.01, and *** = p < 0.01)

Fig. (3.16) demonstrates these cytokines changes in rheumatoid and healthy cells. Treatment of healthy and RA cells with curcumin, Nio-cur NPs, and Hyalo-Nio-cur NPs resulted in a decrease of IL-6 (43.28 to 18.61), whereas

the IL-10 level was increased. A significant change was observed in IL-10 levels between untreated healthy and untreated RA patients (38.11 to 61.98). In all RA treatment groups, the Hyalo-Nio-cur NPs could acquire significant results compared to untreated RA.

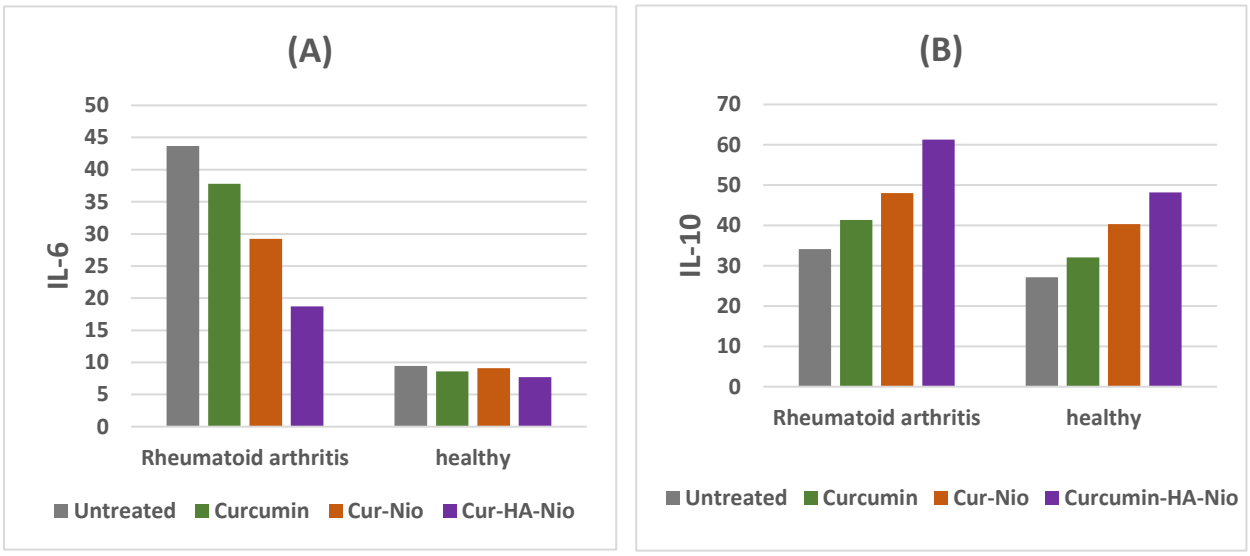


Fig. (3.16): Variation of (A) IL-6 and (B) IL-10 levels in healthy and RA patients. (* = $p < 0.05$, ** = $p < 0.01$, and *** = $p < 0.01$).

Part III

3.6. Gene expression

Fig (3.17) shows the gene expression level of MMP2 and TIMP2 in the treated and untreated rheumatoid arthritis patient's cells. A real-time PCR technique was applied and the result obtained after treating RA patients with each of curcumin, Nio-cur NPs, and Hyalo-Nio-cur NPs indicated that there was a change in gene expression. This leads to an increase in the expression level of TIMP2 as compared with non-treated subjects, this increase (1.02 to 1.78) was due to a significant correlation with the presence of Hyalo-Nio-cur NPs, ($p < 0.01$).

On the other hand, results of real-time PCR demonstrate the expression level of MMP-2 expression was decreased when using curcumin, indicating

curcumin-specific inhibition of MMP-2 as compared to untreated RA patients, and this reduction (1 to 0.37) has a significant correlation with the presence of Hyalo-Nio-cur NPs, ($p < 0.01$).

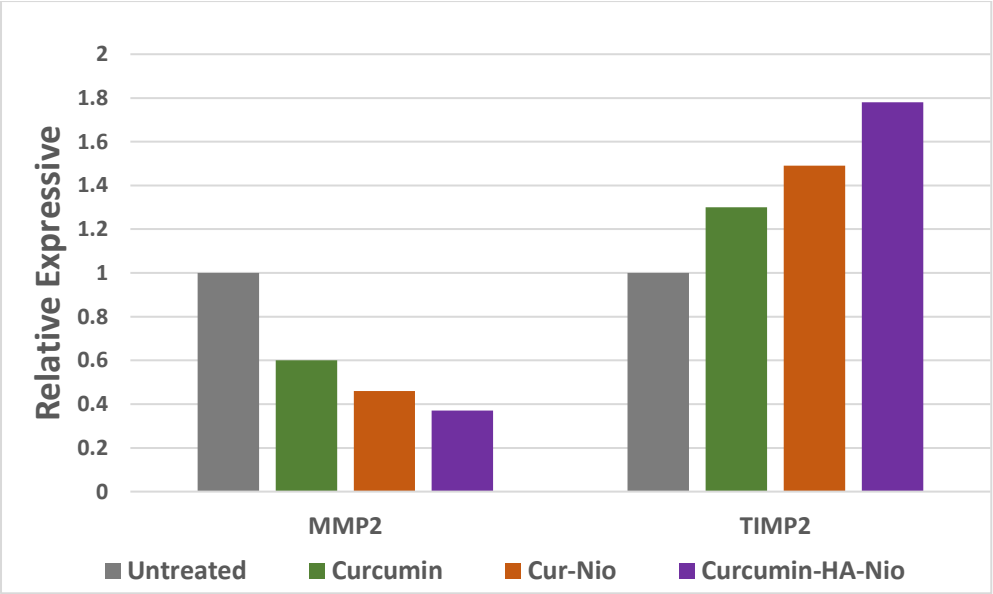


Fig. (3.17): The gene expression level of MMP2 and TIMP2 in the treated and untreated RA patients cells (* = $p < 0.05$, ** = $p < 0.01$, and *** = $p < 0.01$).

Chapter Four

Discussion

4. Discussion

Part I

4.1. Demographic and anthropometric characteristics

According to this study, Table (3.1), it was noticed that most patients were in the age groups 35-44 (45.71) and 55–64 (40%). Due to aging being associated with an increased incidence of autoimmune diseases such as RA, immunosenescence may have special importance for the development of all immune-mediated conditions **(Barbé-Tuana, *et al*., 2020)** .

Several features of premature immunosenescence have been observed in RA, these alterations include decreased thermic functionality, expansion of late-differentiated effector T cells, increased telomeric attrition, and increased production of pro-inflammatory cytokines **(Bauer, 2020)**. In the last few years, it has been seen that immunosenescence occurs despite two different mechanisms, patients with RA have the same clinical features as immune-compromised patients, and RA exhibits irreversible bone and cartilage destruction and results in a short life span due to increased chances of CVS diseases. Immune ageing generally affects people after the age of 50 years, but it is faster in RA patients **(Serhal, *et al*., 2020)**. Aging is characterized by an accumulation of genetic alterations that may be accelerated by obesity **(Qiang, *et al*., 2020)**.

In the current study, it was noticed that a percentage of patients were obese RA (100%) compared to non-obese RA. A previous meta-analysis by Qin et al. reported a positive association between overweight/obesity and RA compared to the association in normal-weight individuals **(Qin, *et al*., 2015)**. In addition, the Percentage ratio of RA Patients according to BMI was found Patients with that had BMI equal to 25 -33 kg/m² comprised 70% higher than those patients who had a BMI ranging between 19-23 kg/m² (30%) **(Yahya, *et al*., 2022)**.

Previous studies have shown that more than 60% of RA patients fall into the obese or overweight category (BMI≥25 kg/m2). Furthermore, obesity is a significant and increasingly common comorbidity, even in the earliest stages of RA presentation **(Feng, *et al*., 2019).** The association between BMI and RA occurs in the overweight stage is the beginning of the accumulation and intensification of chronic inflammation and the beginning of excess energy. Excess energy may initially induce chronic inflammation, prompt the hypertrophy of adipocytes, leading to the relative hypoxia of intracellular organelles, increase the oxidative stress of the endoplasmic reticulum, and cause the release of proinflammatory factors into the blood. Increased proinflammatory cause RA **(Bedeković, *et al*., 2023; Hotamisligil, 2010)**.

The family history of RA patients in the current study is positive as shown in Table (3.1), Family history significantly influences the risk of developing RA. Studies indicate that individuals with a first-degree relative with RA have a twofold to fourfold increased risk of the disease, with heritability estimates ranging from 40% to 60% (**Frisell, *et al*., 2016).** Moreover, a family history of other autoimmune diseases, such as lupus and thyroid disease, and decreased immunity also correlates with a higher risk of RA. This suggests shared genetic or environmental factors that may predispose individuals to RA **(Kronzer, *et al*., 2021; De Roos, et al., 2008)**. Despite advances in understanding RA's genetic basis, family history remains a crucial predictor for clinical assessment and potential interventions **(Murata, *et al*., 2020)**.

Serum lipid profile axis

In this study, the results indicated that the levels of lipid profiles were elevated in patient groups (non-obese and obese) RA compared to the healthy control group, as shown in Table (3.3) and Fig. (3.2).

A previous study indicated that patients with RA have higher cholesterol, LDL-C, and triglyceride levels and lower HDL-C levels than the control group **(Anoze and Nassir, 2008**). RA is associated with significant changes in lipid profiles, often resulting in dyslipidemia. Studies show that approximately 65% of RA patients exhibit dyslipidemia, characterized by altered levels of TG, TG, LDL, and HDL **(Toosi, *et al*., 2018)**. Dyslipidemia in RA may be considered a secondary impact of the chronic inflammatory state seen in RA patients **(Erum, *et al*., 2017)**.

High cholesterol levels are among the risk factors for the development of rheumatoid arthritis in women. Early menopause and a high body mass index (BMI) all contribute to an increase in cholesterol **(Turesson, *et al*., 2015).** Another study reported that RA is associated with an abnormal lipoprotein phenotype. Increased cholesterol levels are associated with increased TG and lead to greater progression of disease activity **(Dessie, *et al*., 2020)**. Some studies show that reduction in inflammation is associated with improvements in HDL cholesterol efflux capacity, consistent with the limited human studies to date **(Liao, *et al*., 2015)**. HDL cholesterol efflux capacity is impaired in RA subjects with high disease activity compared with those with low disease activity **(Charles-Schoeman, *et al*., 2012)**. Moreover, some treatments for rheumatoid arthritis like prednisolone and methotrexate can exacerbate lipid abnormalities **(Yan, *et al*., 2023)**.

In this study, it was found that there was a higher significance of TC, TG, LDL-C, and a lower significance of HDL-C in obese RA patients. In a previous study it explains that body weight was independently associated with changes in TC, LDL-C, HDL-C, and TG **(Kiriyama, *et al*., 2021)**. High TG, TC, and LDL levels in obese RA patients are primarily driven by systemic inflammation and metabolic syndrome. Chronic inflammation from RA alters lipid metabolism, often leading to increased TG and LDL levels. Obesity exacerbates this

dyslipidemia due to additional inflammatory mediators and insulin resistance, which further disrupt lipid profiles **(Choi, *et al*., 2023)**. The increase of triglyceride-rich lipoproteins is a causal factor for low HDL-C levels in obesity (**Stadler and Marsche, 2020)**. The increase in the release of free fatty acids from the adipocytes was caused by obesity increases their uptake by the liver, resulting in liver accumulation and enhanced production of VLDL and its release into the bloodstream **(Feng, *et al*., 2017)**. This increase of acceptor lipoproteins further stimulates the transfer of triglycerides on HDL in exchange for cholesteryl-esters mediated by CETP **(Castillo-Núñez, *et al*., 2022)**.

In some studies, in obese individuals, B-cells have been observed to produce various cytokines, infiltrate adipose tissue, and produce IgG autoreactive antibodies that trigger local inflammation as well as support Th17 function, which promotes a pro-inflammatory T-cell ratio **(Srikakulapu, *et al*., 2020)**. In other studies, tissue-associated immune cells, in particular T-cells have been discovered to play a contributing role in obesity-associated inflammation **(Liu and Nikolajczyk, 2019)**.

Oxidants and antioxidants axis

In this study, the results indicated that the levels of MDA were elevated in patient groups (obese and non-obese) RA compared to healthy controls as shown in Table (3.4) and Fig. (3.3).

Consistent with previous research, investigations were conducted by others **(Aljoboury, *et al*., 2020; Das, *et al*., 2021; Merino de Paz, *et al*., 2024)** which reported higher significance than MDA levels in RA patients. The increase of serum MDA in RA patients indicates an increase in lipid peroxidation (Oxidative stress). Rising in lipid peroxidation may be due to an increase in ROS formation, which occurs during chronic inflammation and hence causes damage to the joints **(Mateen, *et al*., 2016)**.

Previous studies suggest that elevated lipid peroxidation is the source of increased oxidative stress. Lipid peroxidation produces MDA, which can worsen inflammation by damaging cells and releasing immunogenic chemicals. Polyunsaturated fatty acids are particularly sensitive to lipid peroxidation. And one of the most significant effects of exposing the synovial fluid to superoxide and hydrogen peroxide **(Duryee, *et al*., 2021; Tudorachi, *et al*., 2021; Zhang, *et al*., 2023).** Studies indicate that obesity negatively influences disease activity and therapeutic response in RA, with higher MDA levels correlating with increased inflammation and disease severity. Suggesting that adipokines and inflammatory cytokines play a crucial role in this relationship **(Abuhelwa, *et al*., 2020; El-barbary, *et al*., 2011)**.

A previous study found that obesity is linked to higher overall oxidant status and MDA levels (a measure of lipid oxidation) than normal weight, additionally, a positive correlation between MDA and body mass index (BMI) was observed by other researchers that agree with the current study **(Bhale, *et al*., 2014; Cota-Magaña, *et al*., 2024)**.

Studies indicate a positive correlation between Malondialdehyde levels and lipid profiles in obese patients. Elevated MDA levels are associated with increased BMI and higher total cholesterol and triglycerides, suggesting that oxidative stress may contribute to dyslipidemia in obesity **(Klisic, *et al*., 2023; Lima, *et al*., 2004)**. In particular, the research found that obese individuals exhibited significantly higher MDA levels compared to their non-obese counterparts, reinforcing the link between oxidative stress and lipid abnormalities **(Huang, *et al*., 2023)**.

This study found a positive correlation between MDA levels and TC and TG in obese patients As shown in Table (3.6). Hypertriglyceridemia and hypercholesterolemia were associated with oxidative modification of LDL, protein glycation, and glucose-autoxidation, thus leading to excess production

of lipid peroxidation products which may cause elevation of oxidative stress these increased levels could be attributed to increased ROS production and/or deficiency of antioxidant defense system **(Yang, *et al*., 2008)**. JHA, *et al*. found that positive correlation between MDA and LDL cases, however, LDL-C is prone to be oxidized and forms oxidized LDL, thus leading to the high level of MDA as an oxidative stress marker **(JHA, *et al*., 2019)**.

Previous research indicates that as MDA levels increase, HDL-C levels tend to decrease, with a correlation coefficient of ($r = -0.301$, $p < 0.05$), these suggest that elevated oxidative stress, indicated by higher MDA, may contribute to lower HDL-C levels, which is a risk factor for cardiovascular diseases in obesity **(Yilmaz, *et al*., 2021)**. Another study shows no linear relationship was observed between MDA and HDL cholesterol **(Rao, Kiran, 2011)**.

In this study, the obtained results demonstrated that patients with RA had significantly lower activity of the antioxidant enzyme catalase (CAT) compared to the control group. This study has similar reports of reduced catalase activity in rheumatoid arthritis (**Kumar, *et al*., 2016; Zamudio-Cuevas, *et al*., 2022)**.

Catalase is an endogenous antioxidant, catalase levels are reduced due to oxidative stress, and it is used to reduce the formation of free radicals **(Handajani and Sukmana, 2024)**. Catalase protects the cells from the buildup of hydrogen peroxide by dismutating it to generate water and oxygen or by consuming it as an oxidant in which it serves as a peroxidase **(Raducan, *et al*., 2012).** Low levels of CAT in RA patients have been reported because of ROS attacks **(El-barbary, *et al*., 2011)**. Studies indicate a significant negative correlation between catalase activity and age in patients with RA that is similar to this study as shown in Table (3.7), due to age increases, catalase levels tend to decrease, contributing to oxidative stress in RA patients markers **(Mahdi, *et al*., 2018)**.

This study observed catalase levels lower in obese RA as compared with other groups. Obesity decreases the activities of the body's protective antioxidants and can enhance systemic oxidative stress **(Amirkhizi, *et al*., 2010)**. A previous study found that catalase activity was significantly reduced in adipose tissue from obese individuals compared to control individuals **(Salman, 2023)**. A relationship that maintains catalase levels could be beneficial for managing lipid profiles and reducing cardiovascular risks in RA patients, as HDL-C is often linked to better cardiovascular health outcomes in the context of inflammation **(Ioannidou, *et al*., 2024)**. Some studies suggest elevated IL-6 is associated with increased systemic inflammation and disease severity, while catalase, an antioxidant enzyme, may counteract oxidative stress linked to inflammation **(Yang, *et al*., 2023)**. A study found that elevated oxidative stress in RA might lead to reduced catalase activity, which is inversely related to IL-10, an anti-inflammatory cytokine, lower catalase activity correlates with higher IL-10 levels, suggesting a complex interplay between oxidative stress and inflammation in obesity-related **(Anwar, *et al.*, 2024; Kim, *et al*., 2021)**.

In our investigation, a lower significance of GSH in (obese and non-obese) RA patients compared with the control. Many previous studies showed the same results, the lower significance of GSH in rheumatoid arthritis **(Arsalan, *et al*., 2019; Al-Jawadi and Jankeer, 2021)**. GSH is a low molecular-weight tripeptide that provides antioxidant protection against oxidative, GSH can be used to scavenge those excessive intracellular ROS and be converted into GSSG, resulting in a higher concentration of intracellular GSSG and a lower concentration of intracellular GSH **(Zhang, *et al*., 2023)**. reduced levels of GSH in RA due to increased oxidative stress and inflammation and reduced activities of enzymes like glutathione reductase (GR) and glutathione peroxidase (GPx) contribute to lower GSH levels **(Kondo, *et al*., 2023).** Negative correlation between age and GSH, due to antioxidant levels' tendency to decrease with age,

which would render older people more susceptible to free radical stress and DNA damage during the biological process of aging **(Reddy Thavanati, *et al*., 2008)**. Many studies reported decreases in GSH content in vivo to elevated energy metabolism and prevention of obesity in humans and animal models **(Picklo, *et al*., 2015)**. In current study has shown a positive correlation between GSH and HDL-C, which supports studies showing that lower GSH levels correlate with reduced HDL-C, suggesting that oxidative stress may contribute to dyslipidemia in obesity. However, some research indicates a positive correlation between GSH and HDL-C at certain obesity levels **(Goutzourelas, *et al*., 2018; Čolak, *et al*., 2020)**.

Inflammatory and anti-inflammatory Axis

Interleukin-6 was found to be higher in RA patients compared to control as shown in Table (3.5). It agreement with the study for *Ahmed* that showed higher levels of IL-6 were present in serum, synovial tissue, and synovial fluid from patients with RA compared to those with non-inflammatory arthritis **(Ahmed, 2022**). Another study similar to this study discovered by researchers that IL-6 has a critical regulatory role in the development and course of a variety of inflammatory diseases **(Mohammed, et al., 2023)**. Through Trans signaling, IL-6 can activate primary afferent sensory neurons in the periphery, which is the first step toward experiencing pain **(Sebba, 2021)**.

Chronic joint inflammation in RA leads to the production of IL-6 and its receptor (IL-6R), which is expressed in effector cells that cause and prolong inflammation **(Woś and Tabarkiewicz, 2021)**. A previous study suggests that IL-6 is an important mediator of inflammation and plays a pivotal role in the development and progression of RA, due to the biological actions of IL-6 appear relevant to inflammatory diseases, where chronic immune activation and recurrent acute phase responses are characteristic **(Shrivastava, *et al*., 2015)**.

In current study, has shown significant positive correlations between IL-6 levels and lipid profiles in obese patients, particularly in those with RA. Due to increased adipose tissue in obesity leads to higher expression of inflammatory cytokines like IL-6, contributing to a more inflammatory state, and chronic high inflammatory activity in RA is associated with lower muscle mass, fat mass, and increased visceral adipose tissue, which is linked to higher cardiovascular risk **(Favalli, 2020)**. Adipose tissue is an important source of circulating IL-6 and the expanding adipose tissue in obesity may contribute to high levels of IL-6 in the circulation **(Sindhu, *et al*., 2015)**. Elevated IL-6 levels are associated with increased triglycerides and decreased HDL-C due to its inhibitory effect on lipoprotein lipase, leading to impaired lipid metabolism. This cytokine also contributes to systemic inflammation, which exacerbates insulin resistance and cardiovascular risks **(Patsalos, *et al*., 2020)**.

Interleukin-10 levels investigated were found to be higher in rheumatoid arthritis patients compared to control as shown in Table (3.5), which agreed with the previous study **(Dissanayake, *et al*., 2021)**.

A previous study indicates that IL-10 also plays a role in facilitating immunostimulatory properties that aid in the clearance of both infectious and noninfectious particles while minimizing excessive inflammation, IL-10, an anti-inflammatory mediator of inflammation, is secreted in active RA **(Al-Jumaily, *et al*., 2023)**. Moreover, another study indicates that IL-10 was higher in individuals with a longer duration of morning stiffness and lower in subjects **(Patschan, *et al*., 2020)**. IL-10 acts as an anti-inflammatory cytokine, inhibiting the production of pro-inflammatory cytokines like TNF-α and IL-6, and deactivating macrophages **(Hernández-Bello, *et al*., 2017)**.

In addition, it has been shown is higher IL-10 in obese RA patients. Obesity is associated with a chronic increase in circulating inflammatory mediators **(da-Cruz Nascimento, *et al*., 2022)**. Previous studies showed that IL-10, which can

be targeted to the site of inflammation and has better in vivo pharmacokinetics, did alleviate disease severity in preclinical RA models **(Trachsel, *et al*., 2007)**. In this, studies have shown a positive correlation between IL-10 levels and age. As individuals age, the immune response often shifts, leading to increased production of IL-10, which may serve as a compensatory mechanism to counteract chronic inflammation associated with RA **(Atallah, *et al*., 2023)**.

Part II

4.2. Nanoparticles study

According to these results in Table (3.15), the Hyalo-Nio-curcumin NPs have the largest size compared to other NPs this could be a result of the encapsulation of curcumin inside the NPs and the surface decoration of the NPs with hyaluronic acid. The same result found that, indicates curcumin encapsulation can be higher for other carriers, above 50% may be considered good for a lipophilic drug and varies strongly depending on different systems and processes **(Zamarioli, *et al*., 2015)**. The size range of nanoparticles, typically 1-100 nm, makes them suitable for various biomedical applications, including drug delivery systems **(Odeniyi, *et al*., 2018)**. A low PDI value indicates a narrow size distribution. PDI is a crucial parameter in the characterization of nanoparticles, as it provides information about the size distribution and homogeneity of the particle, which is essential for the stability and performance of nanoparticles in various applications **(Kunasekaran and Krishnamoorthy, 2015; Danaei, *et al*., 2018)**. The zeta potential value is a small and negative charge so no aggregation of particles, as it indicates the electrostatic repulsion between particles of similar electric charge, thereby stabilizing them **(Chourasiya, *et al*., 2021)**. The value of zeta potential is useful for understanding and predicting interactions between particles in suspension, and it has been used to study cell adhesion, which is related to surface charge properties **(Metwally, *et al*., 2019)**. A high zeta potential value, whether positive

or negative, indicates a high electrostatic force, which prevents phase separation and contributes to the stability of nanoparticles **(Saryanti, *et al*., 2020)**.

Fig. (3.9) shows the spherical morphology of nanoparticles during preparation by using an SEM system means preparing the nanoparticles correctly due to reducing the surface area to volume ratio, leading to lower energy configurations that agree with this study **(Sau and Rogach, 2010)**.

Fig. (3.10) used AFM imaging is typically conducted in semi-contact mode, allowing for detailed visualization of the NPs' morphology and size distribution. This technique is valuable for assessing the structural characteristics and stability of the nanoparticles, which are crucial for their application in drug delivery systems **(Bharmoria, *et al*., 2021)**.

Fig. (3.11), the TEM image clearly illustrates the spherical morphology of the Hyalo-Nio-cur NPs. The high-resolution TEM image enables the detailed observation of the nanoparticle surface and internal structure **(Chen, *et al*., 2013)**. Confirming the effective encapsulation of curcumin within the niosomes.

Fig. (1.12) FTIR spectra analysis revealed characteristic peaks indicative of primary and secondary amine functional groups in both cholesterol and Span 60. Moreover, peaks corresponding to alkanes and aromatic ring stretch functional groups were observed at 1457 cm^{-1}, 1458 $cm^{-1,}$ and 1459 cm^{-1} in cholesterol and Span 60 **(Rehman, *et al*., 2018)**. The blank niosomal NPs displayed distinct peaks associated with various functional groups, the disappearance of peaks related to C–C stretching in the aromatic ring (at 1506 cm^{-1}) and C = C stretching (at 1674 cm^{-1}). The FTIR spectra of the niosome NPs indicated the incorporation of cholesterol molecules into the lipid bilayer membrane, confirming the formation of niosomes **(Mansoori-Kermani, *et al*., 2022)**. Span 60 exhibited characteristic peaks consistent with previous findings. Cholesterol displayed peaks at 3432 cm^{-1} (O–H stretching), 2930 cm^{-1} (C–H

stretching), 1454 cm^{-1} (C = C stretching), and 1054 cm^{-1} (C–O bending vibrations). Additionally, the FTIR spectra of HA exhibited a sharp carbonyl stretching peak at 1600 cm^{-1} **(Arshad, *et al*., 2021)**.

Fig. (3.13) explains the release pattern of the curcumin from the Nio-cur NPs and Hyalo-Nio-cur NPs within 120 h of the experiment and decreased afterward. Various studies have investigated the various drug release profiles from noisome NPs. Niosomes are known for their ability to provide sustained and controlled release of drugs due to their unique characteristics **(Jadid, *et al*., 2023)**. The sustained release of drugs from niosomes is attributed to several factors. Firstly, niosomes can form a depot, allowing for the controlled and sustained release of drugs **(Rajera, *et al*., 2011)**. Additionally, the entrapment of drugs within niosomes can be enhanced through proper formulation and adjustment of process parameters, leading to sustained release **(Ruckmani and Sankar, 2010)**. Niosome formulations also have been observed to exhibit higher entrapment efficiency compared to other NPs, leading to extended drug release over time **(Durak, *et al*., 2020)**.

The effect of curcumin and curcumin-loaded NPs on the proliferation of PBMCs was investigated by MTT assay which is shown in Fig. (3.14). The MTT assay method is a sensitive technique for fast analysis of cell metabolic activity upon cell exposure to various biological molecules **(Ganjooei, *et al*., 2021).** The MTT assay is widely used to assess cell proliferation and viability due to its simplicity, safety, and cost-effectiveness **(Molaae, *et al.,* 2017).**

Fig. (3.15) that showed the highest reduction in Malondialdehyde level was achieved with Hyalo-Nio-cur NPs. It is a product formed during oxidative stress, specifically lipid peroxidation **(Jové, *et al*., 2020)**. Studies indicate that curcumin-loaded nanoparticles can cross the blood-brain barrier and significantly decrease MDA levels in treated groups, suggesting their potential to mitigate oxidative damage and inflammation in various conditions, including

neurodegenerative diseases **(Yavarpour-Bali, *et al*., 2019).** This reduction in MDA levels underscores the antioxidant properties of curcumin when delivered through nanoparticle formulations.

Hydro-Nio-Cur NPs have been shown to increase catalase levels in treated groups. This enhancement indicates an upregulation of antioxidant enzymes, contributing to improved cellular defense against oxidative stress **(Lin, *et al*., 2019)**. The increase in catalase activity suggests that these NPs may effectively mitigate oxidative damage, potentially through the activation of signaling pathways like Nrf2, which regulates the expression of various antioxidant proteins, including catalase **(Memarzia, *et al*., 2021)**.

In our investigation, found an increase in GSH level belonging to the Hyalo-Nio-cur NPs treated group. This enhancement in GSH, a critical antioxidant, indicates the potential of these nanoparticles to improve cellular antioxidant defenses and reduce oxidative stress. The mechanism likely involves the modulation of various signaling pathways, including the activation of Nrf2, which regulates antioxidant responses, thereby promoting GSH synthesis and overall cellular health **(Colaço, *et al*., 2023; Alizadeh and Kheirouri, 2019)**.

Treatment with Hydro-Nio-Cur has been shown to significantly decrease IL-6 levels. Studies indicate that curcumin can inhibit the expression of IL-6, particularly in inflammatory conditions **(Devi, *et al*., 2015)**. Curcumin treatment reduced IL-6 levels in cultured monocytes and diabetic rat models, demonstrating its potential to lower pro-inflammatory cytokines and mitigate vascular inflammation **(Jain, *et al*., 2009)**. Highlighting curcumin's role as an anti-inflammatory agent.

Treatment with Hydro-Nio-Cur has been shown to increase IL-10 levels. Curcumin, a natural anti-inflammatory compound, enhances IL-10 production, which plays a crucial role in mediating anti-inflammatory responses

(Mollazadeh, *et al*., 2019). Research indicates that curcumin can induce IL-10 expression in various cell types, contributing to its beneficial effects in inflammatory diseases and conditions such as preeclampsia and neurodegenerative disorders **(Fadinie, *et al*., 2019)**. This increase in IL-10 levels suggests that Hydro-Nio-Cur may effectively modulate immune responses and promote anti-inflammatory effects.

Part III

4.3. Molecular studied

The results obtained after treating RA patients with the curcumin, Nio-cur NPs, and Hyalo-Nio-cur NPs led to an increase in the expression level of TIMP2 and a decrease in MMP-2 as compared with non-treated patients.

Curcumin reduces the activity of key transcription factors such as NF-kB and AP-1, which are crucial for MMP-2 gene expression. This leads to decreased MMP-2 mRNA levels in various cell types, including vascular smooth muscle cells, and by exerting anti-inflammatory properties, curcumin may indirectly reduce MMP-2 levels, as inflammation is a known stimulus for MMP expression **(Mogharrabi, *et al*., 2020)**. Moreover, Curcumin promotes the upregulation of tissue inhibitors of metalloproteinases (TIMPs), which counteract MMP activity. Increased TIMP levels can further inhibit MMP-2 expression and activity, enhancing extracellular matrix stability **(Supriono, *et al*., 2020)**. Curcumin inhibits MMP-2 in rheumatoid arthritis by reducing its expression and activity in fibroblast-like synoviocytes (FLSs). It achieves this by downregulating inflammatory cytokines such as TNF-α, IL-6, and IL-17, which are involved in the activation of MMP-2 **(Xu, *et al*., 2022; He, *et al*., 2015)**. These mechanisms collectively contribute to curcumin's potential therapeutic effects in conditions characterized by elevated MMP-2 activity, such as rheumatoid arthritis.

Treatment with Hydro-Nio-Cur has been shown to increase the expression of TIMP-2 in rheumatoid arthritis. Curcumin's anti-inflammatory properties may enhance TIMP-2 levels, which inhibit matrix metalloproteinases (MMPs) like MMP-2, thus promoting extracellular matrix stability **(Chauhan, *et al*., 2017)**. Studies indicate that curcumin modulates pathways involved in inflammation and synovial hyperplasia, leading to increased TIMP-2 expression and reduced MMP activity, which is beneficial in managing rheumatoid arthritis symptoms and progression **(Xiao, *et al*., 2022)**.

Conclusions and Recommendations

5. Conclusions and recommendations

5.1. Conclusions

1. The results showed the effect of obesity on the patients with rheumatoid arthritis on biomarker was found elevated MDA in obese RA patients compared with non-obese RA due to obesity-increased inflammation that leads to increased accidents in rheumatoid arthritis, Antioxidants decreased in obese RA patients compared with non-obese RA.
2. The results presented here contribute to inflammatory and anti-inflammatory biomarkers in obese and non-obese RA, IL-6 considered pro-inflammatory cytokines increased in obese RA more than in non-obese RA, considered as maker for the inflammation in RA disease.
3. Nanomedicine offers a path for RA therapy by using specially designed nanoparticles for precise drug delivery and improved treatment results. This research delves into the use of curcumin-loaded nanoparticles coated with HA to target rheumatoid cells specifically. Our results show the development of Hyalo-Nio-cur NPs with positive attributes such as controlled drug release and acceptable size. Treatment using these NPs led to reductions in Malondialdehyde and interleukin-6 levels with alterations in MMP-2 genes associated with inflammation. Furthermore, we observed an increase in inflammatory indicators and antioxidant enzyme activity displaying the therapeutic promise of Hyalo-Nio-cur NPs in managing RA.

5.2. Recommendations & future work

1. More patients are required to further investigate to assessment of MDA, CAT, and GSH levels in patients with rheumatoid arthritis.
2. In essence, our study adds to the growing body of research supporting the benefits of Nanomedicine in addressing the complexities of RA. More research is needed to study the effect of Nano-cur on the gene expression of genes associated with rheumatoid arthritis, both in blood serum and through a broader range of analyses.
3. The *in vitro* inhibitory effect of curcumin on the expression of MMP-2 genes, which are very important in rheumatoid arthritis and its establishment, encourages us to continue this project in the form of in vivo research.
4. More research is needed to know the relationship between lipid profile levels and rheumatoid arthritis disease and the correlation between them is necessary.

Chapter Five

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Appendices

University of Kerbala – College of Medicine

Department of Chemistry and Biochemistry

Student Name: **Nasreen Ghalib Obeaid**

Supervisors:

Prof. Dr. Fadhil Jawad Al-Tu'ma-College of Medicine – University of Kerbala

Dr. Ali Mohammed Kadhim -College of Medicine – University of Karbala

Experimental Data

Anti-Inflammatory and Oxidative Biomarkers Levels Affected by Curcumin-Loaded Hyaluronic Acid Decorated Niosomal Nanoparticles on the Peripheral Blood Mononuclear Cells of Rheumatoid Arthritis

Sample NO:
Type of study: a case-control/cross-sectional
Inclusion Criteria:
Exclusion Criteria:
Rheumatoid arthritis description : severe(chronic) , moderate , slightly
Age:
Family History:
Duration of RA : ( ) years
Type of Treatment:
Clinical Biochemical Markers
C-reactive protein CRP
ESR
RF

Malonaldyhade (MDA)	
Catalase	
Reduced Glutathione (GSH)	
Interlukin-6 (IL-6)	
Interleukin 10 (IL-10)	
<u>Lipid Profile</u>	
Total Cholesterol:	
HDL-Cholesterol:	
Triglycerides:	
LDL-Cholesterol:	
Height:	**Weight:**
BMI:	
Nanoparticles studies and Gene	
Synthesize and Characterization of Curcumin- Loaded Hyaluronic Acid – Decorated Niosomal Nanoparticles and detection gene in rheumatoid arthritis	

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جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة كربلاء
كلية الصيدلة
مجلة كربلاء للعلوم الصيدلانية

Number: 502
Date: 22/7/2024

Acceptance Letter

Dear Sir/Madam,

I am pleased to inform you that your manuscript titled **Association between Serum Interleukin-6 Level in obese and non obese Rheumatoid Arthritis** " authored by " **Nasreen Ghalib Obeai ; Fadhil Jawad Al-Tu'ma and Ali Mohammed Kadhim**" has been accepted for publication in the Kerbala Journal of Pharmaceutical Sciences. After careful consideration and peer review, we believe that your work makes a significant contribution to the field and will be of great interest to our readers.

Your manuscript will be an excellent addition to our upcoming issue.

Additionally, please ensure that you have completed and returned all necessary copyright forms and any other documentation required for publication.

Once again, congratulations on your acceptance, and thank you for submitting your outstanding work to Kerbala Journal of Pharmaceutical Sciences. We look forward to publishing your article and hope to receive more of your valuable research in the future.

Thanks,

Prof. Dr. Ahmed Salih Sahib

Editor in Chief

Email:kerbala.jps@uokerbala.edu.iq

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ORIGINAL PAPER

Enhancement of Anti-Inflammatory Activity of Curcumin Through Hyaluronic Acid Decorated Niosomal Nanoparticles for Effective Treatment of Rheumatoid Arthritis Patients

Nasreen Ghalib Obeaid[1] · Fadhil Jawad Al-Tu'ma[1] · Ali Mohammed Kadhim Majeed[2]

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Abstract
Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by joint pain, swelling, and erosion, affecting approximately 0.5–1.0% of the global population. Nanomedicine offers a promising approach for targeted rheumatoid arthritis therapy by utilizing modified nanoparticles for treatment, diagnosis, and targeted delivery in RA management. In this study, we aimed to enhance the bioavailability of curcumin, an anti-rheumatoid agent, using niosomal nanoparticles (NPs) coated with hyaluronic acid for targeted delivery to rheumatoid cells. Peripheral blood mononuclear cells were isolated from blood samples of rheumatoid arthritis patients and healthy individuals. The Hyalo-Nio-curcumin NPs were synthesized using the thin-film method. The drug release pattern of curcumin was studied, and these NPs were examined using dynamic light scattering (DLS), scanning electron microscopy (SEM), atomic force microscopy (AFM), and fourier transform infrared spectroscopy (FT-IR) techniques. Various factors such as superoxide dismutase) SOD), interleukin-1 beta) IL-1β, (interleukin-6) IL-6, interleukin-10 (IL-10), malondialdehyde (MDA), catalase (CAT), glutathione peroxidase (GPx), tissue inhibitor of metalloproteinases 2 (TIMP2), matrix metalloproteinase 2 (MMP2), and receptor activator of nuclear factor kappa-B ligand (RANKL) were assessed in peripheral blood mononuclear cells. The synthesized Hyalo-Nio-curcumin NPs exhibited a spherical morphology with sizes of 179 ± 21.6 nm, a polydispersity index of 0.596, and a zeta potential of − 28.1 ± 6.3. FT-IR analysis confirmed the effective encapsulation of substances within the Hyalo-Nio-curcumin NPs. Treatment with Hyalo-Nio-curcumin NPs led to a significant reduction in MDA, as well as decreased activity of pro-inflammatory cytokines (IL-1β, IL-6) and downregulation of inflammation-related genes (MMP2, RANKL). Conversely, there was an increase in the activity of IL-10, CAT, GPx, SOD, and gene expression of TIMP2. This study demonstrates the significant potential of hyaluronic acid-coated niosomal curcumin nanoparticles in enhancing the bioavailability and therapeutic efficacy of curcumin for RA treatment. The novel use of these targeted NPs resulted in substantial anti-inflammatory and antioxidant effects, suggesting a promising approach for improving RA management through nanomedicine.

✉ Fadhil Jawad Al-Tu'ma
fadhil.jawad.altuma@gmail.com; jawad@uokerbala.edu.iq

[1] Department of Chemistry and Biochemistry, College of Medicine, University of Kerbala, Kerbala, Iraq
[2] Department of Internal Medicine, College of Medicine, University of Kerbala, Kerbala, Iraq

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Acceptance of article for publication in Medical Journal of Babylon

Dear Dr.

Date: 08-07-2024

Nasreen Ghalib Obeaid*, Fadhil Jawad Al-Tu'ma and Ali Mohammed Kadhim

I am pleased to inform you that your manuscript (**MJBL_540_24**) titled as:

Comparison between Oxidant and Antioxidant Marker levels in Obese and non-obese Rheumatoid Arthritis Patients

has been accepted for publication in Medical Journal of Babylon.

We have received the payment for publication. So, you will receive the galley proof within 4-5 weeks. You must have to solve the query if we point out any in the galley proof.

After correction of galley proof, your article will be published online at https://www.medjbabylon.org

Best Regards

Prof. Mufeed J. Ewadh
Editor-in-Chief-MJBL

Corresponding author: Nasreen Obeaid. Department of Chemistry and Biochemistry, College of Medicine, University of Kerbala / Kerbala – Iraq.
E. mail: e05173410@s.uokerbala.edu.iq

Certificate

3rd International Conference

For Pathological Analyses

26-27 February 2024
Basrah-Iraq

This Certificate is Awarded to
Nasreen Ghalib Obaied ; Fadhil Jawad Al-Tu'ma and

Ali M. Kadhim Al-Tu'ma

In the recognition of his/her participation in the Third International Scientific Conference for Pathological Analyses as a (Participant)

Prof. Dr. Mohanad Jawad Kadhim
Chancellor of Basra University

Asst. Prof. Dr. Majid Noori Humoud
Dean of Science College

The 12th International Scientific
Conference of Al-Nahrain University
College of Medicine

Certificate of Participation

This is to certify that **Dr. Nasreen Ghalib Obaied** has Participated as a speaker in the 12th International Scientific Conference of Al-Nahrain University College of Medicine, held on **15-16th April 2024, Baghdad, Iraq.**

Prof. Dr. Anees K. Nile
Dean of College of Medicine/ AL-Nahrain University
President of Conference

الخلاصـــــة

التهاب المفاصل الروماتويدي (RA) هو مرض مناعي ذاتي مزمن مع انتشار عالمي يزيد عن 1٪. إنه المرضى في منتصف العمر وكبار السن، مع معدلات أعلى في الإناث. ترتبط السمنة بزيادة خطر الإصابة باضطرابات المناعة الذاتية بسبب البيئات المؤيدة للالتهابات. الطب النانو، باستخدام الجسيمات النانوية، هو نهج واعد لعلاج واستهداف التهاب المفاصل الروماتويدي. الكركمينويدات، المكونات النشطة لمسحوق الكركم، لها العديد من الأنشطة البيولوجية، بما في ذلك الخصائص المضادة للالتهابات، والمضادة للسرطان، والمضادة للفيروسات، والمضادة للبكتيريا، والفطريات، والمناعة. الجينات عبارة عن تسلسلات جينومية تشفر منتجات وظيفية، في حين أن Matrix metalloproteinases (MMPs) عبارة عن ببتيدات endopeptidasesتعتمد على الزنك في عائلةthe metzincin protease و (TIMPs) منظم حاسم ل MMPs، تلعب دورا هاما في العمليات البيولوجية والأمراض.

تهدف إلى دراسة اختلال التوازن المؤكسد ومضادات الأكسدة في التهاب المفاصل الروماتويدي، وتحديد المؤشرات الحيوية الالتهابية والمضادة للالتهابات، وتوليف الجسيمات النانوية النيوزوسوم المزينة بحمض الهيالورونيك المحمل بالكركمين باستخدام تقنية ترطيب الطبقة الرقيقة، وفحص تطبيق Hyalo-Nio-cur NPs كمضاد للأكسدة ومضاد للالتهابات في مرضى التهاب المفاصل الروماتويدي. بالإضافة إلى ذلك، يظهر تأثير Hyalo-Nio-cur NPs على MMP-2 وTIMP-2 للمرضى الداخليين المصابين بالتهاب المفاصل الروماتويدي.

استخدمت طرقا لعمل الدراسة عينات دم مرضى التهاب المفاصل الروماتويدي من 130 ضابطا صحيا ومرضى SMART-120 chemistry analyzerلتحديد مستويات الدهون. تم استخدام مقايسة الممتز المناعي المرتبط بالإنزيم (ELISA) لتقييم مستويات Malondialdehyde (MDA)، وانخفاض الجلوتاثيون (GSH)، والكاتلاز، وإنترلوكين -6 (IL-6)، وإنترلوكين -10 (IL-10). تم تصنيع Hyalo-Nio-cur NPs لفحص الخصائص المضادة للأكسدة والمضادة للالتهابات في مرضى التهاب المفاصل الروماتويدي (RA). تم استخدام تفاعل البوليميراز المتسلسل في الوقت الفعلي (RT- PCR) لتقييم جينات MMP-2 وTIMP-2 في NPs.

أشارت النتائج إلى أن التهاب المفاصل الروماتويدي البدين كان لديه ±متوسط مستويات SD من Malondialdehyde أعلى بكثير من RA غير البدين مقارنة بالمجموعات الضابطة (5.06 ± 1.05 و4.07

± 1.22 و1.04 ± 0.28 نانومول / مجم على التوالي. كانت مستويات نشاط الكاتلاز مختلفة بشكل كبير بين التهاب المفاصل الروماتويدي البدين وغير البدناء مقارنة بالمجموعات الضابطة (4.40 ± 2.28، 6.62 ± 2.52، و15.77 ± 1.16 وحدة / لتر على التوالي، وكانت مستويات الجلوتاثيون أقل في التهاب المفاصل الروماتويدي البدين (51.26 ± 2.52)، والتهاب المفاصل الروماتويدي غير البدين (54.44 ± 7.76)، ومجموعة التحكم (63.22 ± 10.14 مايكرومتر / مل). كان متوسط ± SD ل IL-6 في التهاب المفاصل الروماتويدي البدين أعلى بكثير منه في RA غير البدناء والمجموعات الضابطة، بقيم (71.02 ± 11.39، 59.8 ± 14.50، و5.87 ± 1.9) بيكوغرام / مل، على التوالي. كان لدى مرضى التهاب المفاصل الروماتويدي غير البدناء مستويات IL-10 أقل بكثير مقارنة بمرضى التهاب المفاصل الروماتويدي الذين يعانون من السمنة المفرطة، بمتوسط مستويات (20.62 ± 6.80، 25.31 ± 5.25، و7.37 ± 1.33) بيكوغرام / مل، على التوالي. وجدت الدراسة أن Nio-cur NPs وHyalo-cur-Nio NPs قللت بشكل كبير من مستويات Malondialdehyde في PBMCs، وزادت مستويات الكاتلاز الجلوتاثيون. كما قلل العلاج من IL-6 وزاد IL-10، وزاد من تعبير TIMP2، وخفض MMP-2 في الأصحاء والتهاب المفاصل الروماتويدي.

الاستنتاجات تؤثر السمنة على مرضى التهاب المفاصل الروماتويدي، مما يؤدي إلى زيادة الالتهاب وزيادة حالة الأكسدة. المرضى الذين يعانون من السمنة المفرطة لديهم مستويات أعلى من MDA ومضادات الأكسدة، في حين أن السيتوكينات المؤيدة للالتهابات مثل IL-6 أعلى في التهاب المفاصل الروماتويدي البدين. أظهر الطب النانوية، وتحديدا الجسيمات النانوية المحملة بالكركمين المطلية ب HA، واعدة في إدارة التهاب المفاصل الروماتويدي عن طريق تقليل الالتهاب، وزيادة المؤشرات المضادة للالتهابات، وتعزيز نشاط الإنزيم المضاد للأكسدة. يقترح هذا البحث نهجا واعدا لعلاج التهاب المفاصل الروماتويدي.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

كلية الطب – جامعة كربلاء

فرع الكيمياء والكيمياء الحياتية

حالة مضادة للالتهابات والأكسدة ومضادات الأكسدة لدى النساء المصابات بالتهاب المفاصل الروماتويدي المصاب بالكركمين النيوسومال

رسالة ماجستير

مقدمة إلى مجلس كلية الطب ـ جامعة كربلاء كجزء من متطلبات نيل درجة الماجستير في

[الكيمياء السريرية]

من قبل

نسرين غالب عبيد

بكالوريوس علوم (قسم الكيمياء) – جامعة كربلاء (2021 – 2022)

بأشراف

الاستاذ الدكتور فاضل جواد آل طعمة المدرس الدكتور علي محمد كاظم مجيد آل طعمة

كلية الطـــب – جامعة كـــربلاء

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