Iraq-Kurdistan Region
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ADJUVANT USE OF RESVERATROL IN PATIENTS WITH KNEE OSTEOARTHRITIS: A COMPARATIVE STUDY WITH MELOXICAM

A THESIS SUBMITTED TO THE DEPARTMENT OF PHARMACOLOGY AND TO THE GRADUATE STUDIES DEPARTMENT AT THE COLLEGE OF MEDICINE-UNIVERSITY OF SULAIMANI IN A PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN PHARMACOLOGY AND TOXICOLOGY

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August 2018 **2018گەلاوێژ** **2018آب**

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وَيَسْأَلونَكَ عَنِ الرُّوحِ قُلِ الرُّوحُ مِنْ أَمْرِ رَبِّي وَمَا أُوتِيتُمْ مِنَ الْعِلْمِ إِلاَّ قَلِيلاً

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I the undersigned, PhD candidate declare that this thesis is my original work and has never been presented in any other University and that all resources of materials have been duly acknowledged

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SUPERVISOR CERTIFICATION

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This thesis is dedicated to

My parents ....
My beloved husband .....The entire work would not have been possible without your Love and Support
My most precious gifts;
My daughter.....Roya
My son.....Rawsht
My sisters and brothers for their endless support and encouragement.

ACKNOWLEDGMENTS

First of all, I would like to thank Almighty God for giving me the opportunity to conduct this work. I would most like to thank my supervisor *Professor Saad A. Hussain* for his advanced supervision and guidance throughout my PhD study. I am very grateful for his hard work and extraordinary knowledge that he has put into the project. I acknowledge his endless help and advices. Many thanks to the dean of college of medicine Assistant Professor Kosar Mohamed Ali for his kind support. I am grateful to the Out Patient Department (OPD)-Orthopedic Section of Shar Teaching Hospital, Rheumatology and Rehabilitation Center, General Teaching Hospital for hosting my project. I would like to thank all the patients participating in the studies voluntarily. It was a pleasure to have had the opportunity to work with the orthopedicians Dr. Ziyad S. Ali, Runj S. Ahmmad at the Department of Orthopedics, at Shar Teaching Hospital in Sulaimani city. Many thanks for their cooperation and support. I appreciate all kind cooperation of the head of Department of Pharmacology Professor Mohammed Omer Mohammed for his kind support and encouragement. I would also like to acknowledge the facilitation provided by the rheumatologists, Dr. Rauf Rahem Mirza and Dr. Hawar Ali Ehsan in the Rheumatology and Rehabilitation Center in Sulaimani. I would especially like to thank the senior radiologist Dr. Bakhtyar K.Talabani at the Radiology Department in Shar Teaching Hospital for confirmation of the grading process of the knee Osteoarthritis which was a critical part in the enrollment of the candidates in our clinical trial. I am also deeply thankful to the Biochemistry Clinic of Dr. Saman Hussein and all his staff members for their help in biochemical analysis of my samples. Their hard work has helped me complete the practical laboratory part of the project. Finally, I am thankful to all who shared their words of advice and encouragement to finish this study.

Bushra

ABSTRACT

Background and Objective: Knee osteoarthritis (OA) is a chronic degenerative and disabling disease of the articulating knee joints. Inflammation and pain play an important role in the pathogenesis of knee OA and could cause tissue damage and morbidity. There is a growing interest in developing therapeutic strategies to decrease progressive degeneration of joint tissues with fewer side effects, and many efforts have recently focused on the potential effects of natural compounds to halt OA progression and reduce the associated symptoms. Resveratrol is a non-flavonoid small polyphenol compound that accumulates in plants in response to exogenous stress, it is a powerful antioxidant with anti-inflammatory properties and may be considered as a rational candidate with a potential therapeutic interest in joint disorders. The present study was designed to evaluate the clinical benefits, the biochemical changes associated with the use of resveratrol and safety of resveratrol as an adjuvant treatment with meloxicam in patients with mild to moderate knee OA.

Methods: The study was a double-blind, randomized placebo-controlled clinical trial involving 110 patients with knee OA conducted at Shar Teaching Hospital, Sulaimani General Hospital and the Specialized Rheumatology Center, Sulaimani City from December 2016 to September 2017. The participants consumed 15 mg/day meloxicam with either 500 mg/day of resveratrol or placebo for 90 days. The effect of treatment was evaluated by measuring the changes from baseline in the clinical parameters; Knee Injury and Osteoarthritis Outcome Score (KOOS), Western Ontario and McMaster Universities (WOMAC) index, and the Visual Analog Scale (VAS) score and after 30, 60 and 90 days of treatment. Blood was collected to determine serum interleukins 1β and 6,

tumor necrosis factor-α, C-reactive protein, and the complement proteins C3 and C4. Safety and tolerability of resveratrol on hematological, liver function, kidney function parameter and lipid profile in knee OA patients was also assessed.

Results: The adjuvant use of resveratrol with meloxicam was well tolerated and significantly improves total Knee Injury and Osteoarthritis Outcome Score (KOOS) after 30 days, compared with both baseline value and that of Mlx+placebo group within the same period ($P<0.05$). Although the effect of resveratrol continues to improve Knee Injury and Osteoarthritis Outcome Score (KOOS) after 60 and 90 days, these values are not significantly different ($P>0.05$). There was also a significant reduction in Western Ontario and McMaster Universities (WOMAC) score over the treatment period. The post intervention Western Ontario and McMaster Universities (WOMAC) score in Mlx+Res was significantly improved in all time ranges, compared with both baseline value and those of Mlx+placebo group ($P<0.05$); Meanwhile the post-intervention Western Ontario and McMaster Universities (WOMAC) score was not significantly changed in Mlx+placebo group among different times of follow-up, i.e., between baseline, day 30, day 60 and day 90. Post-intervention Visual Analog Scale score was also significantly improved in both groups at the end of the treatment period compared to baseline value; however, the degree of changes in the Mlx+Res group were significantly higher compared to that reported in the Mlx+placebo group ($P<0.05$). Serum levels of the pro-inflammatory cytokine TNF-α, IL-1β and IL-6 were also significantly reduced ($P<0.05$) compared with both the baseline level and that of the group treated with meloxicam and placebo. Inflammatory biomarkers associated with knee OA hs-CRP and the complement proteins C3 and C4, were reported to be

significantly lower in the Mlx+Res group compared to the Mlx+placebo group at day 90 ($P<0.05$)

Conclusion: These findings suggest that resveratrol was effective adjuvant therapy with meloxicam in the treatment of patients with mild to moderate knee OA.

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List of Abbreviations

Abbreviation	Stands for
AAOS	American Academy of Orthopaedic Surgeon
ACR	American College of Rheumatologists
ADAMTS	A Disintegration and Metalloproteinase with Thrombospondin-like Motifs
ADL	Function, daily living ;
AhR	Aryl Hydrocarbon Receptors
ALP	Alkaline phosphatase
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AMPK	Adenosine Monophosphate- Activated Protein Kinase
AP-1	Activator protein 1
AST	Aspartate Aminotransferase
ATPases	Adenosine triphosphatase
BCL-2	B cell leukemia/lymphoma-2
BMI	Body Mass Index
C3	Complement proteins C3
C4	Complement proteins C4
COPCORD	Community Oriented Program for Control of Rheumatic Disorders
COX-2	Cyclooxygenase-2
CRP	C-Reactive Protein
CTX-I	C-Telopeptide of Type I Collagen
DNA	Deoxyribonucleic acid
ECM	Extracellular Matrix
ELISA	Enzyme-Linked Immunosorbent Assay
ER	Estrogen receptor

ERK1/2	Extracellular signal-regulated protein kinases
ESR	Erythrocyte Sedimentation Rate
EULAR	European League against Rheumatism
FSTL1	Follistatin-like protein 1
GOT	Glutamate Oxaloacetate Transaminase
GPT	Glutamate Pyruvate Transaminase
HA	Hyaluronic Acid
HDL-c	High Density Lipoprotein cholesterol
HK2	Hexokinase II
HPLC	High-Performance Liquid Chromatography
IL-1β	Interleukins IL-1β
IL-6	Interleukins IL-6
IL-8	Interleukins IL-8
iNOS	Inducible Nitric Oxide synthase
IκB-α	Inhibitor of Kabba B
KOOS	Knee Injury and Osteoarthritis Outcome Score
LDL	Low-Density Lipoprotein
LPS	Lipopolysaccharide
MAC	Membrane Attack Complex
MAP	Mitogen-activated protein
MMP	Matrix Metalloproteinase
MRI	Magnetic Resonance Imaging
NADH	Nicotinamide Adenine Dinucleotide Hydrogen
NF-κB	Nuclear Factor κB
NO	Nitric Oxide
NOAEL	No-Observed-Adverse-Effect-Level
NRf2	Nuclear factor (erythroid-derived 2)-like 2

NTX-I	N-Telopeptide of Type I Collagen
OA	Osteoarthritis
OARSI	OA Research Society International
PGE_2	Prostaglandin E_2
PIICP	Procollagen Type II C-Propeptide
PIINP	Procollagen Type II N-Propeptide
QoL	Quality of Life
RF	Rheumatoid Factor
RNA	Ribonucleic acid
SF	Synovial Fluid
SF-36	Short Form-36
Sirt1	Sirtuin 1
SNRIs	Serotonin–norepinephrine reuptake inhibitors
SOD	Superoxide Dismutase
Sport/Rec	Sports and Recreational activities
STAT-3	Signal Transducers and Activators of Transcription-3
TNF-α	Tumor Necrosis Factor
VAS	Visual Analog Scale
WBC	White blood cell count
WHO	World Health Organization
WOMAC	Western Ontario and McMaster Universities

1. Introduction

Knee osteoarthritis (OA) is a slow progressive chronic degenerative and disabling disease of the articulating knee joints, which mostly arises after hyaline cartilage damage [1]. It is described as a disease of the entire joint unit with pathological changes in all tissues, including articular cartilage degradation, osteophyte formation, subchondral bone thickening, degeneration of ligaments, degeneration of the menisci in the knee, hypertrophy of the joint capsule and presence of inflammation in synovial membrane [2,3]; the disease is also described by progressive retrogression and erosion of articular cartilage with concomitant structural and functional changes in the whole joint [4,5]. In spite of advances in the elaboration of cellular and molecular mechanisms of OA pathogenesis and the identification of more sensitive and reliable biomarkers as diagnostic tools [6] there is no optimal therapy that slows cartilage breakdown or reverses the progression of cartilage degradation and other tissue damage. In addition to non-pharmacological approaches to treatment, many drugs are used in the pharmacological treatment of OA; non-steroidal anti-inflammatory agents (NSAIDs) are the most widely used for their analgesic and anti-inflammatory activities, which effectively reduce the symptoms of OA [7]. However, the long-term use of these drugs was correlated with many important adverse events, including an increased risk of gastrointestinal bleeding, cardiovascular disorders and an increased bleeding tendency due to platelet activation [8]. Currently, there is a growing interest in developing therapeutic strategies to decrease the progressive degeneration of joint tissues with fewer side effects. Resveratrol is a natural polyphenol extracted from *Polygonum cuspidatum*, which shows pleiotropic properties that suggest it may be applied for many therapeutic uses, including the treatment of

cancer, cardiovascular diseases, and metabolic disorders [9]. The current research study was designed to evaluate the effect of resveratrol, as an adjuvant with meloxicam (Mlx) in patients with mild to moderate knee osteoarthritis on the pain and functional activity during a 90-day period, to investigate the biochemical changes associated with the use of resveratrol as an adjuvant with meloxicam and monitor the adverse effects on hematological markers, kidney, liver functions and lipid profile. Therefore, based on the prospective outcomes, the study design includes three distinct parts; the clinical effect of resveratrol, anti-inflammatory effects of resveratrol as an adjuvant with meloxicam on biomarkers of inflammation in patients with knee OA and assessment of the tolerability of resveratrol based on a three-month follow-up. The literature review provides an overview of knee OA, including epidemiology and prevalence of knee OA, etiology and risk factors, its pathology, as well as methods for measuring OA and current management strategies.

CHAPTER ONE

LITERATURE REVIEW

CHAPTER ONE

LITERATURE REVIEW

1.1. Literature Review

1.1.1. Knee Osteoarthritis; Epidemiology and Prevalence

Knee Osteoarthritis is the most prevalent joint disease characterized by pain and degenerative lesions of the cartilage, subchondral bone, and other joint tissues. [10]. It has traditionally known as a non-inflammatory disorder of articular cartilage; however, most recent evidence suggests that OA is generally accepted to be an inflammatory and biomechanical disease [6]. Recently, it has been considered as the most common type of inflammatory joint diseases, with chronic and painful inflammatory changes that associated with physical impairment and chronic disability in millions of patients around the world [11].

Knee osteoarthritis is a highly prevalent age-related degenerative disease of synovial joint [12]. It is a leading cause of chronic pain and disability in many countries including United States (US) [13] and other developed nations [14]. The prevalence and incidence of OA continue to accelerate regularly throughout the world not only in the aging population, but also in active young and middle-aged individuals [15]. It has been forecasted that 25% of the adult population, or more than 50 million people in the US, will be affected by this disease by the year 2020 and that OA will be a major cause of morbidity and physical limitation among individuals over the age of 40 [16]. However, this is found as a geographical variation in OA epidemiology. Data on clinically-diagnosed knee OA in

the Community Oriented Program for Control of Rheumatic Disorders (COPCORD) studies in Asian region showed that the prevalence within this area was ranged from 1.4% in urban Filipinos to 19.3% of rural populations in Iran [17]. Meanwhile, studies from China, which utilized similar methods and definitions to a population-based study such as the Framingham Study, found that the prevalence of bilateral knee OA and lateral compartment disease were two to three times higher in Chinese cohorts, compared with estimates from the the Framingham Knee Osteoarthritis Study [18]. Prevalence of knee OA increases with age and is considered as a major cause of pain, functional disability worldwide and an economic burden on healthcare systems [19,20]. The impact of obesity and metabolic alteration on OA prevalence is parallel to what the ageing process displays [21,22]. Increases in life expectancy and ageing populations are expected to make OA the fourth leading cause of disability by the year 2020 [23,24].

In the USA, approximately 13% of women and 10% of men aged 60 years and older have symptomatic knee OA. These proportions are likely to increase due to the aging of the population and the growing rate of obesity in the general population [21]. There is a growing recognition that OA affects people at younger ages. Recent US data demonstrated that half of people with symptomatic knee OA are diagnosed by age 55 [25]. Men before the age of 50 have a higher prevalence and incidence of OA than women, this is probably related to the secondary changes following trauma, while after the age of 50, women have both a higher prevalence and incidence [26]. Age is considered as a significant contributor to the sex differences in prevalence of OA, females tend to have more severe knee and hand OA than men, particularly after menopausal age [27]. Based on radiographic data, meta-analysis reported that females tend to

have more severe knee OA than males, and that the gender differences increase with age > 55 years.

1.1.2. Etiology and Risk Factors

Osteoarthritis is traditionally categorized as a primary or idiopathic disease when the original cause of OA is not completely addressed and secondary when it follows a clearly defined pathology such as post-traumatic, congenital or metabolic alteration [28]. Currently, OA is recognized as a disease of complex etiology that occurs as a result of both mechanical and biological events. Although the causes of OA remain unknown, various approaches recognize OA as a multifactorial disease that set different associated factors for its incidents [27]. Moreover, OA has a considerable hereditary origin and is considered to be a polygenic disease [29]. Modern imaging techniques recognize OA as a whole joint disease, which may involve multiple tissues of different phenotypes; subchondral bone is fundamentally involved in the pathogenesis and progression of OA. In particular, the area of subchondral bone at the tibiofemoral articulation is larger in OA knees than healthy controls and correlates with knee joint space narrowing and osteophytes formation [30]. The initiation of OA is based on a number of risk factors including prior joint injury [19], overweight and obesity [31], joint shape and dysplasia [32], synovial membrane inflammation [33], complement proteins [34], release of inflammatory cytokines [35], age related inflammation [36], innate immunity, the low-grade inflammation, metabolic diseases [37] and diabetes mellitus [38]. Risk factors associated with OA have been broadly divided into person-linked factors and joint-linked factors. Person-linked factors include age, gender, obesity, genetics, race/ethnicity and diet, while joint-linked factors refer to biomechanical factors that are targeting a particular joint such as, prior

knee injury, heavy work load, and muscle strength [39]. Factors associated with OA can further be classified either to OA initiation or to disease progression. Age, gender, occupation, body mass index (BMI) and recreational activity can play a role in the development of OA, and weight status and dietary factors may play a role in its progression [40].

1.1.3. Pathogenesis of OA

The pathogenesis of OA is a complicated concern and its management represents a challenge for the rheumatologist and orthopedician. Pathogenically, knee OA is characterized by structural changes in knee joint tissues. The predominant structural alterations are the loss of cartilage, formation of osteophytes, joint tissues inflammation [3], and subchondral bone remodeling in early stages of cartilage degeneration [41]. These changes are easily demonstrated radiographically, and the objective criteria of disease severity are based on the level of joint space loss that would reflect the loss of cartilage and the presence of osteophytes [42]. In recent years, it has been revealed that genetic, mechanical, and environmental factors are associated with the development of OA. At the cellular and molecular level, OA is characterized by the alteration of the healthy homeostatic state toward a catabolic state [43]. In addition to these structural alterations of the hard tissues, a number of changes in articular and periarticular tissues also occur with knee OA. These include synovial hyperplasia and joint effusions. Although the pathophysiology of joint degeneration in OA is extensively studied, there is still a lack of understanding how it leads to the clinical syndrome of OA and as a result, efforts of prevention of the disease or slowing its progression are impeded. While knee OA has typically been defined as a prototypical non-inflammatory arthropathy, there is convincing evidence to suggest that in addition to being a disease

of biomechanics, it has inflammatory and metabolic inherent [44]. A common sign of knee OA is the synovial inflammation. Clinically, breakdown of the extracellular matrix (ECM) results in the gradual dysfunction of the articular cartilage, often accompanied by pain and physical disability. As a result of the cartilage alteration, an obvious abnormal remodeling in the subchondral bone occurs frequently in the form of sclerosis and osteophyte formation (Figure 1-1) [45].

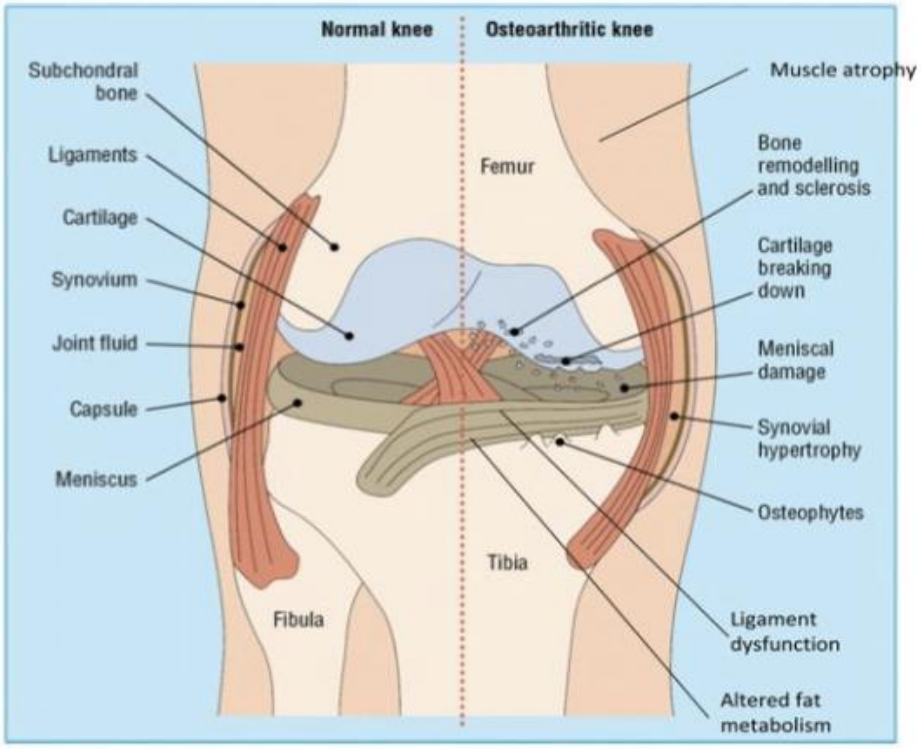


Figure 1-1: Cross-sectional picture of healthy knee joint on the left and characteristic changes to those structures in OA on the right [45].

1.1.3.1. Joint compartments included in knee OA

In knee OA, dysregulation of the biochemical and biomechanical environment is attributed to the degeneration and alteration of the joint compartments that involved in the development of OA. These compartments include articular cartilage, subchondral bone, synovial membrane, menisci, and ligaments and molecules mediating pain generation [45].

1.1.3.1.1. Articular Cartilage

Chondrocytes play an important role in the maintenance of articular cartilage homeostasis as they secrete matrix constituents and matrix-degrading enzymes as well as having surface receptors for various cytokines and growth factors [3]. In OA, the dynamic equilibrium between synthesis and degradation of ECM which is maintained by chondrocytes is interrupted, with degradation being dominated. Chondrocytes produce and release Matrix Metalloproteinase (MMP) and A Disintegrin and Metalloproteinase with Thrombospondin-like Motifs (ADAMTS) aggrecanase enzymes. The aggrecanase ADAMTS – ADAMTS5 and MMP3, which degrade aggrecan followed by MMP13 which degrades type II collagen, may be responsible for matrix breakdown in early OA [3].

1.1.3.1.2. Subchondral Bone

In early OA, the alteration rate of subchondral bone and bone remodeling increase and new bony structure arise, which leads to reduced thickness of the subchondral plate. In the advance stage of degeneration, changes in remodeling balance occur in four main processes: reduced bone turnover, subchondral sclerosis, thickening of calcified cartilage and thinning of trabeculae [46]. The defining hallmark of the subchondral bone in late-stage OA is bone volume enlargement and the increased apparent density. There will be an active bone formation at the joint margins which may lead to osteophyte formation in primary and secondary OA or bone erosion in inflammatory arthritis [47]. A Perturbed bone could boost abnormal cartilage metabolism through reduced structural support, impede nutrient supply, and provision of catabolic factors. On the other hand, bone is highly innervated and may be a potential source of pain in OA [48].

1.1.3.1.3. Synovial Membrane

The histological pattern of synovium in knee OA patients is characterized by synovial lining hyperplasia, sub-lining fibrosis and stromal vascularization and macrophage infiltration in the synovium is common in OA. The synovium membrane has a crucial role in cartilage degeneration [49]. Phagocytosis, lubrication and cartilage nutrition are also favored by the synovium due to its vascular and neural nature. While OA traditionally is considered primarily as a disease of hyaline cartilage and the related bones due to repetitive use and over burden of the movable joint, large number of evidence suggests that synovitis and the originated pro-inflammatory mediators are important in the pathogenesis of OA with effects on articular cartilage. The synovium may show significant changes, even before detectable cartilage degeneration has occurred, with infiltration of mononuclear cells, thickening of the synovial lining layer and production of inflammatory cytokines [50]. Products of cartilage degradation may initiate a state of inflammation and oxidative stress because they diffuse into the synovial membrane and phagocytized by macrophages thereafter reactive oxygen species (ROS), pro-inflammatory cytokines, and degradative enzymes are highly initiated [51] (Figure 1-2)

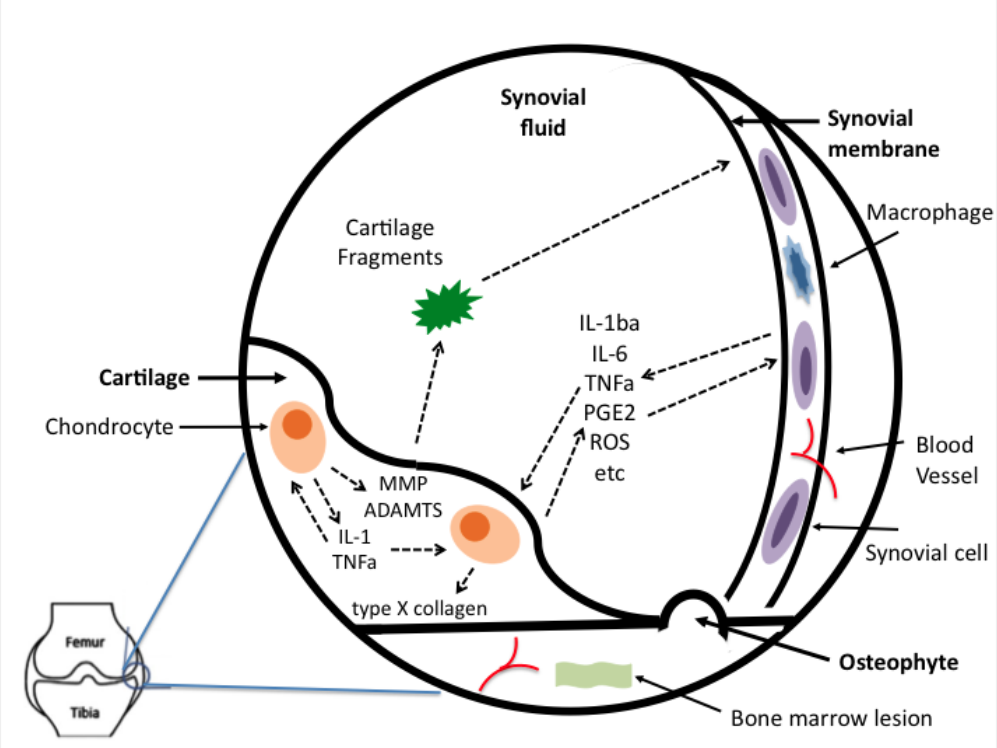


Figure 1-2: Complex cyclic processes in cartilage, bone, and synovial membrane in knee OA contribute to the disease pathogenesis. Chondrocytes produce matrix degrading enzymes (MMP and ADAMTS) and inflammatory mediators that can act on the chondrocytes or enter the synovial membrane to cause inflammation. Chondrocytes produce type X collagen in a repair attempt but it is not suitable for adult ECM because it can not maintain the same mechanical features as healthy adult cartilage. In the bone, osteophytes and bone marrow lesions lead to an adverse biomechanical environment and are potentially painful [51].

1.1.3.1.4. Menisci and Ligaments

Pathological changes in the menisci and ligaments are common in older adults with knee OA, even in those without previous injury. These pathological changes include chondrocalcinosis, calcification of the articular cartilage and meniscus that usually accompanied by the presence of crystals in the joint, matrix disruption, fibrillation, cell clusters, and cell death which can alter knee kinematics and strain areas of the cartilage that are not accustomed to those loads, promoting cartilage degradation [3]. Additionally, increases in vascular penetration and innervation have been reported in OA menisci, suggesting a potentially important source of pain in OA [52].

1.1.3.2. Molecules Mediating Pain in OA

Pain is a multifactorial symptom in OA. The process of joint degeneration causes pain; the same inflammatory mediating molecules involved in joint destruction play a role in the pain processing of OA [51]. Although the origins of pain in OA are undetermined, the pain fibers that innervate the synovial membrane, ligaments, menisci, and subchondral bone are involved in pain generation. Pain is believed to arise from nociceptive and neuropathic mechanisms [53]. Subchondral bone, periostium, synovium, ligaments, and joint capsule are all highly innervated and contain nerve endings that may be the source of pain in OA patients. The severity of the synovitis as detected by Magnetic Resonance Imaging (MRI) has been reported to be associated with joint pain in the knee. Subchondral bone has been proposed as a source of joint pain in knee OA. During inflammatory conditions, the number of cytokine receptors on neurons is increased. Meanwhile, the cytokines released in the osteoarthritic joint may act on the innervating joint nociceptors that carry pain signals from peripheral tissues to the central nervous system and generate pain [54]. The neuropeptides, substance P, calcitonin-gene-

related-peptide, and neurokinin-1, are believed to play a role in OA pain [55].

1.1.3.3. Inflammatory Aspect of OA

OA is now viewed as an inflammatory disease with multiple phenotypes [56]. Many recent studies have shown the presence of synovitis in patients with knee OA and demonstrated a direct association between joint inflammation and disease progression [57]. Accumulating number of studies has presumed the role of systemic inflammatory cytokines in the origination and progression of osteoarthritis.

The basic mechanisms of OA pathogenesis are the involvement of pro-inflammatory cytokines such as interleukins IL-1β, IL-6, IL-8 and tumor necrosis factor-α (TNF-α) and pro-catabolic mediators through their signaling pathways of nuclear factor kappa B (NF-κB) and mitogen-activated protein (MAP) kinase signaling responses [51]. Excessive mechanical loading causes an alteration in the metabolism of joint cells, leading to the release of enzymes and inflammatory cytokines such as IL-1β. IL-1β, then stimulates the synthesis of nitric oxide (NO), prostaglandin E_2 (PGE_2) and matrix metalloproteinases (MMPs) [58]. Among these proteases, MMP-13 is overexpressed in OA and catalyzes the destruction of type II collagen and aggrecan which are the essential constituents of the cartilaginous extracellular matrix (ECM). Molecules of aggrecan and collagen are released from the cartilage and activate the synoviocytes and macrophages, resulting in paracrine secretion of cytokines and MMPs into the synovial fluid [59]; those catabolic molecules can further mediate the inflammation process in the joint.

1.1.4. Biomarkers and Inflammatory Mediators Involved in OA

In OA, biomarkers can be categorized as a dry biomarkers or wet soluble biomarkers. Imaging variables such as radiographs, Magnetic Resonance Imaging (MRI), and ultrasound and questionnaires with data from visual analog scales are consider as dry biomarkers, while genetic (RNA, DNA) and biochemical (carbohydrates, proteins, protein fragments, peptides, metabolites) molecules are belong to soluble biomarkers [60]. Biochemical markers in OA can be measured in blood, serum, urine and synovial fluid. Currently, there are no reliable, quantifiable and easily measured biomarkers that provide an earlier diagnosis of OA. Novel biomarkers should have some characteristics in order to be used for prognostic and diagnostic purposes at early stage [61]. In the past decades, biochemical biomarkers have raised as anticipating tools in OA diagnosis, with more sensitivity and reliability than plain radiography to detect joint changes that occur in OA. Such biomarkers could facilitate early diagnosis of joint destruction, disease prognosis and progression monitoring, which could be detectable with an early biochemical test [62]. Over the years, a series of markers have been proposed that may reflect the synthesis or degradation of the joint tissues. However, despite the active research in this field, currently no single marker is sufficiently validated for its use in OA diagnosis [61]. In OA, the cytokines profile is altered due to the inflammatory process; some may induce damage of the extracellular matrix of the joint tissue, while others may act as biochemical markers of disease severity and pain. Currently, many researchers have focused on two groups of biomarkers including non-inflammatory and inflammatory biomarkers [63].

1.1.4.1. Non-Inflammatory Biomarkers

Non-inflammatory biomarkers include biomarkers related to the metabolism of collagen such as Procollagen Type II C-Propeptide (PIICP) and Procollagen Type II N-Propeptide (PIINP), Cross linked N-Telopeptide of Type I Collagen (NTX-I) and cross linked C-Telopeptide of Type I Collagen (CTX-I), and biomarkers in metabolism of non-collagen, such as aggrecan and non-aggrecan biomarkers. Follistatin-like protein 1 (FSTL1) is also considered as a novel OA biomarker in serum [64] and hyaluronic acid (HA) is a marker for early stage of OA [65]. Matrix metalloproteinases (MMPs), expressed in synovial cells and chondrocytes, are also potential biomarkers for OA.

1.1.4.2. Inflammatory Biomarkers

Inflammatory cytokines are the most important biomarkers involved in the pathogenesis of OA. Inflammatory biomarkers include two groups which are pro-inflammatory and anti-inflammatory [66]. The majority of these well-recognized mediators involved in the pathogenesis of OA are demonstrated in Table 1-1.

Table 1-1: Recommended inflammatory biomarkers that reflect different tissues and mechanisms involved in OA [64, 67,68,34].

Biomarkers	Mechanisms
Pro-inflammatory Biomarkers	
IL-1β	Mediates catabolic activity independently and with other mediators by inducing the expression and release of proteolytic enzymes, such as MMPs and aggrecanases, induces production of IL-6 and IL-8, and suppresses the expression of ECM components, including type II collagen and aggrecan
IL-6	Inhibit the production of type II collagen, inhibit proteoglycan synthesis, reduce chondrocyte proliferation , increase MMP-2 activity, increase aggrecanase-mediated proteoglycan catabolism, strongly activate the immune system and enhances inflammatory response.
IL-15	Recommended as a progressive marker for OA diagnosis in the serum, it correlates with both the

	sensation of pain and radiological severity, it can stimulate the secretion of certain types of metalloproteinases from the MMPs group
IL-18	Recommend as a biomarker for OA assess the disease severity, stimulates the COX-2 and TNF-α expressions in primary synovial cells, and inducing high PGE_2 level production.
TNF-α	Associated with cartilage erosion, induce IL-6, 1L-8 production, MMP-1,-3, and -13 , PGs, and inhibit proteoglycans and type II collagen synthesis it promotes the production of nitric oxide (NO), a potent catabolic and pro-apoptotic mediator, in the synovial tissue.
Complements	Results in the formation of membrane attack complex (MAC) on chondrocytes, which either kills the cells or causes them to produce matrix-degrading enzymes and inflammatory mediators.

CRP (a marker of low-grade systemic inflammation)	It is associated with decreased knee cartilage volume and increased knee pain.
Obesity-related inflammatory biomarkers (Adipokines; leptin, adiponectin, visfatin and resistin**)**	Can increase production of MMPs-1,-3, and -13, induce cartilage degradation.
Anti-Inflammatory Biomarkers	
IL-13, IL-10, IL-7 and IL-4	Involve in anabolic or catabolic mechanism of chondrocytes , induces bone loss through increased osteoclastogenesis.

IL: Interleukin; TNF-α: Tumor necrosis factor alpha; CRP: C-Reactive Protein

1.1.5. Diagnosis of OA

Diagnosis of OA can be performed with the aid of self-reported osteoarthritis process obtained from a questionnaire and radiographic interpretation of OA. The European League against Rheumatism (EULAR) [69] and the American College of Rheumatologists (ACR) [70] adopted classification strategy for knee OA with the aim of differentiating OA from other types of arthritis (Table 1-2).

Table 1-2: ACR (1986) Criteria for classification of idiopathic osteoarthritis of the knee.

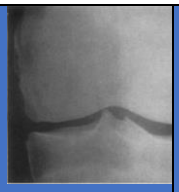
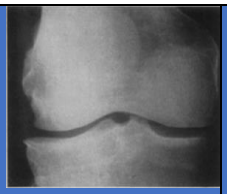
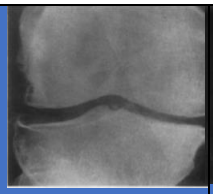
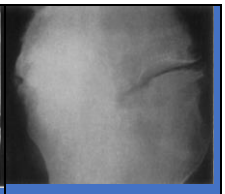
Clinical	**Clinical and Laboratory**	**Clinical and Radiographic**
Knee pain + At least 3 of 6: Age >50 year Stiffness <30 minutes Crepitus Bony tenderness Bony enlargement No palpable warmth	Knee pain + At least 5 of 9: Age >50 year Stiffness <30 minutes Crepitus Bony tenderness Bony enlargement No palpable warmth ESR <40 mm/h RF <1:40 SF signs of OA: clear viscous, or WBC <2000/mm^3	Knee pain & Osteophytes +At least 1 of 3: Age >50 year Stiffness <30 minutes Crepitus
95% sensitive 69% specific	92% sensitive 75% specific	91% sensitive 86% specific

ESR, erythrocyte sedimentation rate; OA, osteoarthritis; RF, rheumatoid factor; SF, synovial fluid; WBC, white blood cell count [70].

Based on this strategy, the clinical classification for knee OA depends on knee pain plus three of the six criteria included in the system, such as age > 50 years, morning stiffness < 30 minutes, crepitus, bony tenderness, bony enlargement and the absence of palpable warmth [70]. The European League against Rheumatism (EULAR) in 2009 recommended a similar criterion for diagnosis of knee OA. In their recommendations, detection of three symptoms (sustain knee pain, short lived morning stiffness and physical function limitation) and three signs (crepitation, movement restriction, and bony enlargement) was adequate to precisely (99%) diagnose knee OA [69]. Additionally, the radiographic evidence

associated with OA includes presence of osteophytes, narrowing intra-articular space, subchondral bone sclerosis and subchondral bone cysts. These radiographic features are based on Kellgren-Lawrence grading system for knee OA classification, which grades the extent of radiographic osteoarthritis from 0 to 4 [71]. Kellgren and Lawrence developed the first measuring method for a stable grading system of knee OA by using X-rays in 1957, which was accepted by World Health Organization [WHO (1961)] as the standard method for scoring knee OA [71] and it is considered as the most confirmed imaging modalities for the assessment of knee OA till now. Radiographic grading of knee OA according to the Kellgren-Lawrence score was shown in table 1-3:

Table 1-3 Kellgren and Lawrence radiographic criteria for assessment of OA*:

					
Radiographic grade	0	I	II	III	IV
Classification	Normal	Early OA	OA	OA	Advanced OA
Description	Healthy	Doubtful narrowing of joint space and possible osteophyte lipping	Definite osteophyte and possible narrowing of joint space	Moderate multiple osteophytes, definite narrowing of joint space, some sclerosis and possible deformity of bone contour	Large osteophytes, marked narrowing of joint space, severe sclerosis and definite deformity of bone contour
*Radiology does not reliably correlate with symptoms [71]					

There are different descriptions for Kellgren-Lawrence criteria since its development; almost five different versions of these criteria documented by many authors, which affect the classification and distribution of severity of knee OA on radiographs. However, the association between clinical knee complaints and the different descriptions has not yet well-recognized [72]. Therefore, a well-recognized discordance exists between the pathological evidence of OA and severity of clinical symptoms and signs for the less severe grades of OA. In general, pain is more frequent in the more severe grades of OA. However, there are patients with moderate to severe knee OA (Kellgren-Lawrence grade 3 or 4) without any pain in the knee. The reason for such discrepancy is not well verified so far [72].

1.1.6. Clinical Symptoms of Knee OA

Knee OA is associated with various clinical complaints such as joint pain, functional impairment, and joint morning stiffness, which last no more than half an hour, together with swelling, tenderness and occasionally locking, which may cause disability and extremely affect the life quality of patients. Knee OA can affect the life of a person in four dimensions: symptoms of pain, loss of function, limited physical activities and decrease in quality of life [73].

1.1.6.1. Pain

Pain is the most problematic symptom for most of the OA patients. Knee joint pain observed in the early stages of OA when patient starts to move and usually increases as the day progresses. Weight load can worsen the joint pain, and in the later stage pain can also be felt in rest time and during the night. Pain origin of knee OA is most often come from patellofemoral joints. As well as knee pain is emerged either from lateral tibiofemoral compartment or medial tibiofemoral compartment [74].

1.1.6.2. Physical Function Limitation

Functional limitations are considered as important clinical manifestations of symptomatic knee OA. Since knee OA is a chronic and slow progressive disease, clinically worsening of the physical function over time can be seen in OA patients, and a rising number of people end up with a total knee replacement [75]. Recently, the adjusted analyses of a new study also demonstrated either stable or worsening physical function in OA patients [76]. Additionally, other study revealed that physical function impairment can be observed in OA when there is neuromuscular dysfunction of the knee [77].

1.1.6.3. Poor Quality of Life in Knee OA

In patients with knee OA, physical impairment, joint pain and disability tend to increase with disease progression. Knee OA burden can be significant for knee OA patients, where pain and functional impairment are the main domains of that burden. Additionally, depression and anxiety, and reduced participation in activities are common in adults with knee OA [78], and taken together, they often exert a significant decline in the quality of life. Thus, in these individual the disability displays a negative influence on their activities of daily living, which leads to losses in pleasure, social life activity, and sleeping quality that lead also to decrease in their quality of life [79].

1.1.7. Measurement of Clinical Symptoms

Many strategies are available for measurement of clinical symptoms of OA and various approaches are used in the literature. In addition, quality of life, sleep quality, mood, pain coping strategies, participation in valued experiences, and others are recognized as contributors to the full OA experience and are being measured in research settings [1].

1.1.7.1. Measurement of Pain

Pain is a subjective feeling which is affected by physiological, psychological and demographic factors (age, sex and comorbidity). Therefore, the patient-reported pain provides great variation in individual pain perception. Measurement of pain includes the severity, intensity, frequency, inactivity, and immobilization, emotional state, sleep, and quality of life [73]. There are a number of multidimensional questionnaires for measuring pain, which are suggested to provide a more extensive estimation of pain and overall response to a treatment in OA [80]. Each questionnaire is chosen based on the clinical or research objective. There are different methods of assessing pain in individuals with knee OA including:

One-dimensional Pain Assessments: Such as Visual Analog Scale 100mm (VAS-100) or numerical rating scale [81].

Multidimensional Pain Questionnaires: Such as Short Form-36 **(**SF-36) -Bodily Pain Subscale [82], McGill Pain Questionnaire, Lequesne Algofunctional Index and Western Ontario and McMaster Universities (WOMAC) pain subscale [83].

VAS is a one-dimensional measure of pain severity and composed of a horizontal or vertical line, usually 10 centimeters (100 mm) in length, hold 2 verbal descriptors, one for each symptom extreme. It is typically utilized in epidemiologic and clinical studies to measure the intensity or frequency of pain ranges from none to an extreme amount of pain. It has been extensively used in distinct adult populations, incorporating those with OA [84].

Meanwhile, Western Ontario and McMaster Universities (WOMAC) osteoarthritis index is a disease-specific tool developed for the elderly to assess osteoarthritis-induced pain, stiffness, and functional limitation. It is defined by a validated, disease-specific questionnaire (24 questions)

addressing severity of joint pain (five questions), stiffness (two questions), and restriction of physical function (17 questions), and attributing to the 48 h before assessment. A higher WOMAC score represents worse symptom severity [85].

Additionally, Knee Injury and Osteoarthritis Outcome Score (KOOS) is a questionnaire-based assessment of knee associated complains. This questionnaire was developed in the 1990s as an extension of the WOMAC for use in a younger and more active patient group with knee injuries or knee OA [86]. Psychometric features of KOOS and VAS (validity and reliability) have been investigated in different languages and age group [87]. This self-explanatory tool has five patient-relevant subscales, which embraces 42 items: Symptoms including stiffness (seven items); Pain (nine items); activity of daily living (ADL) Function (17 items); Sport and Recreation Function (five items); Quality of Life (QoL) (four items); the five patient-relevant subscales of KOOS can be scored separately. Recently automated administration provides scoring software which is available online and can be freely downloaded elsewhere. The KOOS and VAS has been used in numerous studies for determining OA severity, such as comparing different methods of reconstruction of the anterior cruciate ligament, investigating the effectiveness of total knee replacement, and reporting functional outcome scores in the total knee arthroplasty for knee osteoarthritis [88]. Moreover, it is utilized to evaluate the efficiency and safety of pharmacological interventions in total knee and hip arthroplasties [89] and other advanced clinical intervention [90].

1.1.7.2. Measurement of Physical Function

Performance-based assessment and self-reported measures are used for assessment of physical function. The process of self reporting is simple and uncomplicated, required less time and does not have an influence of the observer bias, although other factors such as culture, language, and education level may affect on its reliability in a way that it may not reflect the actual ability of the participants [91].

1.1.8. Management of Knee OA

Currently, OA is not a curable disease and there is no optimal therapy for OA that regenerate damaged joints. Therefore, treatment goals, as stated in many of the guidelines, aimed to alleviate pain and improve physical function and mobility and slow or halt progression of OA [92]. The therapeutic scale includes:

1. **Non-pharmacological treatment modalities:** physiotherapy, orthopedic aids and orthosis, and general measures. [92]
2. **Pharmacological treatment modalities (Pharmacotherapy)**
3. **Surgery:** The most radical treatment to knee OA is partial or total knee replacement. Although this invasive technique became less critical with smaller incisions and less time consuming in the hospital, but still it is only recommended for patients with end-stage osteoarthritic and those who have attempted all available conservative interventions. However, it has been associated with feared and challenging complications such as peri-prosthetic joint infection and deep vein thrombosis [93].

1.1.8.1. Non-pharmacological treatment modalities

1.1.8.1.1. Education and Self-care

The approach of patient-education describes a method for achieving self-management through the capability of an individual to manage OA-

related pain, physical, and psychological impact of a chronic disease state. Although education is widely recognized as an important management protocol, evidences from the literature are not dominantly positive. It is unlikely that self-management and education play a great role in alleviation of pain in adults with OA. In 2013, a meta-analysis comparing self-management programs to attention control, usual care, information alone, or another intervention concluded that low to moderate quality evidence exists for beneficial effects of self-management programs in persons with OA [94].

1.1.8.1.2. Weight Reduction and Exercise

Reduction of weight is defined as uncomplicated method for reducing knee OA complaints. Evidences reveal that obese people have higher rates of knee OA than non-obese control subjects [31]. Although weight loss is included in all the guidelines, and considered as a logical step to improve clinical symptoms in the affected joints, weight loss as a treatment for knee OA has not been extensively investigated in the literature. Investigation in meta-analysis includes weight-loss in knee OA patients found insignificant improvements of pain while significant improvements in physical function. Weight reduction is frequently an outcome of exercise interventions trials; however, there are also benefits of exercise without weight reduction. Many systematic reviews and meta-analyses investigated aerobic and strength exercises for the treatment of knee OA [95]. They concluded that exercise has a mild to moderate positive effect on pain and physical function in adults with knee OA. On the other hand, the ACR OARSI guidelines for management of knee OA completely recommend weight loss and regular exercise to relieve OA pain [92,96].

1.1.8.1.3. Biomechanical Measures and Others

Biomechanical approaches are the most widely used remedy for knee OA. These include physical therapy, rest, ice bracing, knee sleeves, taping, foot orthoses and supports include canes, crutches and walkers. There are different types of braces for knee OA, from simple sleeve to a complicated brace. The main objective of using this technique is to distribute the weight bearing load on the knee compartment. Additional biomechanical measures are physical therapy and rehabilitation-centered approach, and home centered programs [97]. Despite the common use of these biomechanical interventions, it is generally accepted that none of them are sufficient as an exclusive therapy for OA [98].

1.1.8.2. Pharmacological Treatment Modalities (Pharmacotherapy)

1.1.8.2.1. Traditional Therapy for Osteoarthritis

Currently, there are five kinds of commonly used medications in clinical treatment of OA, and based on the recommendations of the American Academy of Orthopaedic Surgeon (AAOS), ACR and OA Research Society International (OARSI) they are summarized as [96,99]:

a. Acetaminophen; according to ACR it is considered as a first-line that also recommended by AAOS for symptomatic knee OA.
b. Non-selective NSAIDs and Selective COX-2 inhibitors recommended for symptomatic knee OA by both AAOS and ACR.
c. Opioid analgesics (tramadol); recommended for symptomatic knee OA and refractory pain in patients with hip or knee OA by AAOS and ACR.
d. Serotonin–norepinephrine reuptake inhibitors (SNRIs); duloxetine Conditionally recommended for patients $\geqslant 75$
e. Intra-articular corticosteroids recommended for symptomatic knee OA by AAOS, ACR and OARSI recommend it for patients with

moderate-to-severe pain who are not respond to oral analgesic and anti-inflammatory agents

f. Intra-articular hyaluronic acid: no longer recommended by AAOS, ACR. It was inconclusively recommended in the 2008 edition.

1.1.8.2.2. New Osteoarthritis Therapy

The insufficient efficacy and intolerable adverse effects of traditional OA therapy necessitate an advance search for more optimal therapy. Various types of new OA drugs have demonstrated a positive result to be a potential therapy to reverse the progression of the articular cartilage degradation and other tissue damages. Although small number of them have received approval for the clinical trials, and depend on their molecular target and mechanism of action, they are categorized as: chondrogenesis inducers, osteogenesis inhibitors, matrix degradation inhibitors, apoptosis inhibitors, and anti-inflammatory cytokines [100,101].

1.1.8.3. Nutraceuticals as Alternative Medicine in Treatment of OA

Nutraceuticals are food-derived bioactive products that have beneficial role in improvement of health by prevention and treatment of a disease. The term nutraceutical is a combination of "Nutrition" and "Pharmaceutical". The advantages of nutraceuticals are their wide availability, safety and possibility of self-medication [102]. Long-term therapy will likely be required to halt or slow progression of articular knee joint. Therefore, there is a serious need for OA disease-modifying therapies that ameliorate OA-associated symptoms, with none or less adverse effect over long periods of time. Nutraceuticals are dietary compounds that play a valuable role in keeping the structural integrity of articular cartilage by preserving the balance between anabolic and catabolic processes, which dysregulates chondrocytes and synovial fluid

of the OA-patient joints [103,104]. Nutraceuticals have displayed anti-inflammatory, anti-catabolic and anti-oxidative stress activities which are important features of drugs targeting OA [103]. The most commonly used nutraceuticals for OA are: *Boswellia serrata,* Bromelain (pineapple extract), *Caesalpinia Sappan* extract, Capsaicin, Cat's claw, Chicory root, Diallyl-sulphide (garlic extract), Willow bark, *Aloe vera*, Avocado/soybean unsaponifiables, Genistein, Green-Lipped Mussel extract, *Lactobacillus casei*, Methylsulfonylmethane, Polyunsaturated fatty acids, Green tea as a source of polyphenol epigallocatechin 3-gallate (EGCG), Pomegranate, Ginger, Tumeric, Rosehip powder and resveratrol [105,103].

1.1.8.3.1. Molecular Targets for Nutraceuticals in OA

Molecular targets in OA can be classified as inflammatory, oxidative stress, or catabolic (Figure 1-3). Nutraceuticals that have the ability to immediately target these aspects of OA may be definable for molecular targeting of OA. Many nutraceuticals demonstrated obvious anti-inflammatory action that effectively inhibit activated inflammation and suppress catabolic activity, and oxidative stress-induced deleterious responses. Furthermore, an optimal nutraceutical therapy may require a combination of many compounds to produce greater effects on OA targets, such as inflammatory processes and catabolism, oxidative stress and relieve chronic pain. Additionally, they may produce additive, complementary, and/or synergistic anti-arthritic effects when used concomitantly with other compounds in the same formulation. The findings of clinical and preclinical studies recommended the idea of nutraceuticals combination to act on the multiple molecular targets during treatment of OA [103].

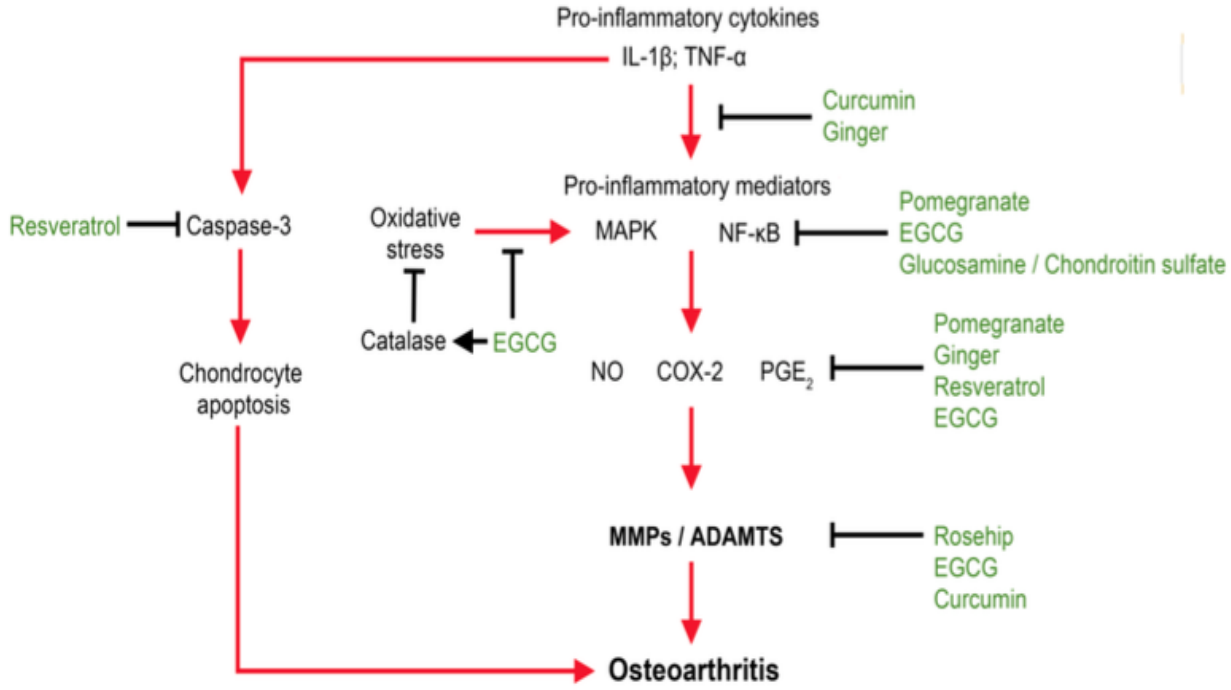
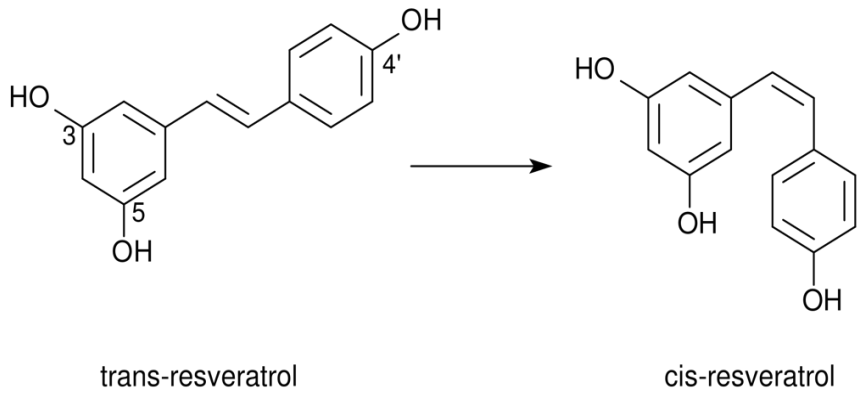


Figure 1-3: Molecular OA targeting of select nutraceuticals, EGCG; epigallocatechin 3-gallate [103].

1.1.8.3.2. Resveratrol

1.1.8.3.2.1. Sources of Resveratrol

Resveratrol (Res) (3,5,4′-trihydroxystilbene) is a non-flavonoid small polyphenol compound that accumulates in plants in response to exogenous stress factors such as injury, fungal infections or UV irradiation [106]. It is the parent compound of a family of hydroxyl-stilbenes existing in cis- and trans-configurations in a variety of spermatophyte plants such as grapevine, peanuts, pine or Chinese knotweed (Figure 1-4). Trans-Res is the predominant form found in dietary sources and supplements [107].



trans-resveratrol cis-resveratrol

Figure 1-4 Chemical structure of trans-resveratrol and cis-resveratrol [107].

It is found in grape skins, various berries such as blueberries, cranberries, mulberries, peanuts and pistachios [108,109,110] and other plants sources, including traditional Asian medicines [111]. Resveratrol was first isolated by a Japanese scientist Michio Takaoka in 1939 from the roots of *Veratrum grandiflorum* [112] and later in the dried roots of *Polygonum cuspidatum*; it is used in traditional Chinese and Japanese medicine to treat suppurative dermatitis, gonorrhea favus, athlete's foot and hyperlipidemia [113,114].

1.1.8.3.2.2. Bioavailability and Pharmacokinetics of Resveratrol

In human, resveratrol is absorbed through the small intestine by passive diffusion or forming complexes with membrane transporters, such as integrins at a relatively high rate following oral administration [115]. Although 75% of administered resveratrol is absorbed, its oral bioavailability is around 1%. Poor bioavailability of resveratrol is related to rapid and extensive metabolism in the intestine and liver [116,117]. The major metabolites detected in the plasma and urine by pharmacokinetic studies includes resveratrol glucuronides and sulphates [118,119]. The primary sites of resveratrol metabolism are the intestine

and liver, although colonic bacterial metabolism may be a significant metabolic pathway [120]. At the intestinal level and after resveratrol absorption, it can be detected in plasma essentially in three different forms: free form, glucuronide and sulfate conjugates. The free form can bound to albumin and lipoproteins such as low-density lipoprotein (LDL); these complexes, in turn, can be dissociated at cellular membranes that have receptors for albumin and LDL, leaving the resveratrol free and penetrates cells. Resveratrol's affinity for albumin suggests that it could be a natural reservoir for resveratrol and play an important role in its distribution and bioavailability [121]. Resveratrol is rapidly and extensively biotransformed by first pass metabolism [122]. Short half-life, non retention ability, rapid elimination, and undesirable degradation/biotransformation lead to the low bioavailability of the parent molecule of resveratrol at the site of action [123,124]. To control and improve the matter of poor bioavailability and pharmacokinetics of resveratrol, different strategies have been practiced [125] such as: (i) co-administration of resveratrol with metabolism inhibitors; (ii) use of naturally or chemically-synthesized resveratrol analogs; and (iii) develop a new and advanced drug delivery systems like adjuvants, nanoparticles, liposomes, micelles, phospholipid complexes, dendrimers, nano-emulsions, nano-gels, and nano-gold [126,127]. Some studies have suggested that metabolites of resveratrol may be converted back into resveratrol at the tissue level, although this has not been thoroughly demonstrated [123,128]. Although the bioavailability of the parent resveratrol molecule is relatively low, many preclinical in vivo studies demonstrated its remarkable biological activities. Pharmacokinetic studies displayed high levels of stilbene metabolites, therefore some debated that these metabolites may be considered as reservoirs for the stilbenoids either by the direct action of the metabolites [129] or via the

enterohepatic recycling pathway [130,131]. Stilbenoids are mainly eliminated as the metabolites via renal excretion in the urine and via non-renal excretion in the faeces. Non-renal routes appear to predominate over the renal route of elimination for resveratrol [132] suggesting an important role for the enterohepatic cycling. Thus, biological activities may still be obtained with low circulating levels of the parent stilbenoid compounds [133].

1.1.8.3.2.3. Chronopharmacology of Resveratrol

The pharmacokinetics study of resveratrol demonstrated its circadian-dependent features; therefore, the plasma level of resveratrol as shown by Area Under the Curve (AUC) diagram is larger after morning oral administration than afternoon. Consequently, resveratrol bioavailability would be higher if administered in the morning. A pharmacokinetic study of repeated doses of resveratrol for a couple of days (48 hours) concludes that tolerance is good, concentrations in plasma do not increase over time and even decrease, and the bioavailability is higher when administered in the morning [134].

1.1.8.3.2.4. Pharmacodynamic Profile of Resveratrol

Resveratrol is a highly pleiotropic molecule with an excellent safety profile. Strong molecular evidence has been published to support its potency for targeting multiple inflammatory diseases. The pleiotropic effect of resveratrol is responsible for its substantial use [135]. This compound can provide protective effects against carcinogenesis [136,137] cardiovascular [138] and neurodegenerative diseases [139]. Additionally, it exerts metabolic effects on glucose homeostasis in type 2 diabetes mellitus and other metabolic disorders [140]. Recently, due to the anti-apoptotic, anti-inflammatory and antioxidant properties of

resveratrol, it exerts apparent anti-osteoarthritic effects [141]. It provides numerous health benefits including antiaging, antidiabetic, antimicrobial, anticancer, and neuroprotection activities [142]. Resveratrol has multiple mechanisms of action that may be correlated with its health benefits. Similar to most polyphenols, resveratrol has intrinsic anti-oxidant capacity, but it also induces the expression of a number of anti-oxidant enzymes [143]. Resveratrol also interacts with many receptors, kinases, and other enzymes that probably account for its major contributions to various biological effects. According to many *in vitro* and *in vivo* studies, the administration of resveratrol stimulates the activities of sirtuin 1 (Sirt1) and adenosine monophosphate- activated protein kinase (AMPK), both of which influence the regulation of metabolism in multiple tissues [144,145,146]. Furthermore, resveratrol inhibits cyclooxygenases [147,148] and could therefore act through some of the same mechanisms as aspirin. The involvement of resveratrol in the detoxification processes may be attributed to its ability to antagonize the aryl hydrocarbon and dioxin receptors (AhR). Resveratrol is a strong AhR competitive antagonist; therefore, it inhibits many effects related to AhR activation like endocrine disruption, oxidative stress, inflammation, apoptosis and immunosuppression. Resveratrol is a modulator of membrane ATPases [149] which may be correlated to its analgesic effects. Resveratrol is also a weak agonist of the Estrogen Receptor (ER)-α and an agonist of ER-β [150] a feature relevant to its bone protecting effects (Figure 1-5).

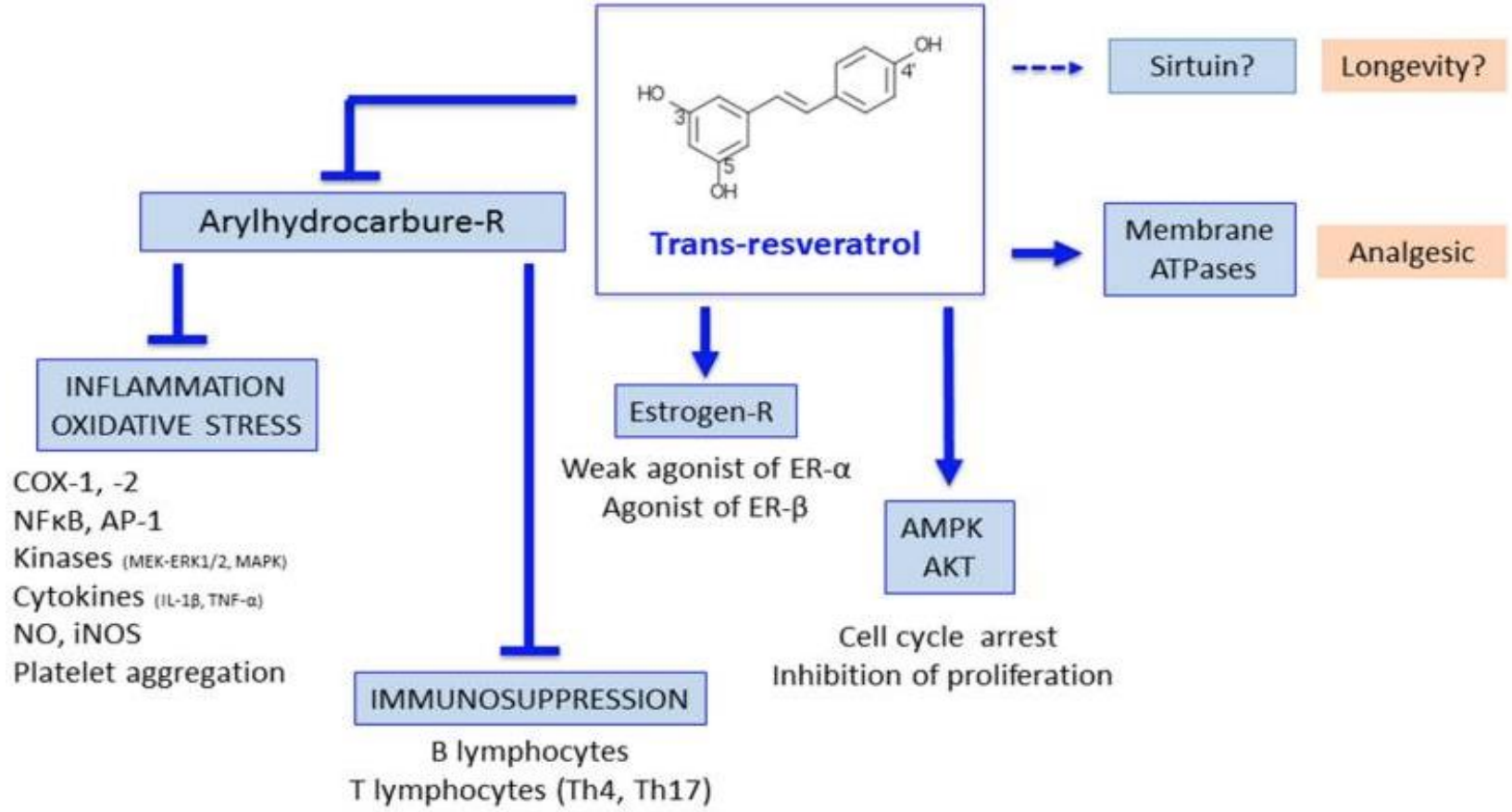


Figure 1-5: The pleiotropic effects of Resveratrol. AKT: Protein kinase B; AMPK: 5' adenosine monophosphate-activated protein kinase; AP-1: Activator protein 1; COX: cyclooxygenase; ER: estrogen receptor; ERK1/2: Extracellular signal-regulated protein kinases **[**148].

1.1.8.3.2.5. Pleiotropic Effects of Resveratrol

1.1.8.3.2.5.a. Anticancer Activity

Large number of recent works focused on the anticancer effects of resveratrol, with its mechanisms of action [151]. Resveratrol was evaluated in many pre-clinical studies as a chemo-sensitizing agent to enhance the cytotoxicity of anticancer drugs including adriamycin. Based on the obtained results of these studies, it has been concluded that resveratrol can effectively reverse drug resistance through a molecular mechanism that may involve the alteration of multidrug resistance protein 1, lung resistance protein, glutathione S-transferase, B cell leukemia/lymphoma-2 (BCL-2) and topoisomerase-II. Correspondingly, resveratrol could be a potential candidate for reversing drug resistance in bladder cancer chemotherapy [152]. Additionally, the chemo-sensitizing effects of the co-administered resveratrol with cisplatin on malignant mesothelioma cells have been newly observed [153]. Similarly, resveratrol may produce anticancer effect through other mechanisms such as inhibition of proliferation, migration and invasion, and promotes apoptosis in a time-dependent manner. These effects also can be achieved through the inhibition of mRNA and protein expression of STAT-3, suggesting that the anticancer effects of resveratrol are mediated by STAT-3 signaling [154]. In addition, resveratrol substantially decreases hexokinase II (HK2)-mediated glycolysis, lowers the epidermal growth factor receptor and downstream kinases Akt and Extracellular signal-regulated protein kinases ERK1/2 activation, and impairs glucose metabolism by suppressing the expression of HK2 mediated by the Akt signaling pathway [155].

1.1.8.3.2.5.b. Antidiabetic Effects

Administration of resveratrol alleviates diabetic symptoms, including reduction of body weight, polyphagia and polydipsia, hinder insulin resistance, and enhance glucose uptake by hepatic cells, adipocytes, and skeletal muscle, and hepatic glycogen synthesis in diabetic rodents [156]. Treatment with resveratrol has ameliorative effect on the diabetic complications including cardiomyopathy, nephropathy, neuropathy, ketoacidosis, retinal vasculopathy, hypertension, stroke, and hyperosmolar hyperglycemic non-ketotic syndrome in diabetic animal models, suggesting potential anti-diabetic activity. Resveratrol treatment has also been reported to improve insulin sensitivity and glycemic control in type 2 diabetic patients [157,158]. Furthermore, resveratrol demonstrates a considerable suppression of advanced glycation end products (AGE) formation. Accordingly, this natural polyphenol offers the potential to reduce glycation and to impede carbohydrate-hydrolyzing enzyme activities [159].

1.1.8.3.2.5.c. Neuroprotection and Improvement of Cognitive Function

Pre-clinical and epidemiological studies have demonstrated that resveratrol displays protective effect against neurodegeneration and preserve cognitive functions. An improvement in memory performance and hippocampal functional connectivity along with higher brain glucose metabolism have been reported after co-administration of resveratrol with quercetin [160]. Animal studies on long-term therapy of resveratrol in diabetic rats reduced neuronal damage and improved cognitive performance by reducing oxidative stress and inflammation, and by impeding synapse loss through inhibition of hippocampal apoptosis [161, 162].

1.1.8.3.2.5.d. Anti-oxidant Effect

The antioxidant action of resveratrol is related to its intrinsic structural features. It has a powerful free radical scavenging activity toward reactive oxygen and nitrogen species. Meanwhile, resveratrol can stimulate the expression of the enzymes responsible for homeostasis of the cellular redox reactions, such as: superoxide dismutase (SOD), catalase, heme oxygenase, and glutathione peroxidase. Moreover, it is an excellent metal ions chelator that has ability to counteract creation of free radicals through Fenton's reaction [163]. Resveratrol inhibits lipid peroxidation mainly by removing lipid peroxides produced in the plasma membrane, and demonstrates cardioprotective effect against lipid peroxidation induced by doxorubicin [164].

1.1.8.3.2.5.e. Anti-Obesity Effects

Resveratrol effectively enhances thermogenesis in brown and white adipose tissues along with the appearance of multi-molecular brown adipocytes and elevated thermogenic gene expression. It also enhances energy expenditure and insulin sensitivity [165]. Resveratrol also dampens the accumulation of fat via decreasing adipogenesis and/or de novo lipogenesis in white adipose tissue. Additionally, lipid oxidation in the liver and skeletal muscle was increased by resveratrol, and also increases the capacity for adaptive thermogenesis. Therefore, in rodents, there is a general consensus concerning the effect of resveratrol on reducing body fat accumulation. By contrast, in humans, the studies are scarce, and no clear anti-obesity action has been revealed so far [166].

1.1.8.3.2.5.f. Cardiovascular Effects of Resveratrol

In vitro and *in vivo* studies demonstrated the inhibitory action of resveratrol on platelet aggregation through binding to calcium channels and inhibition of thrombin, which shows a valuable role for the cardiovascular system [167]. The molecular mechanism of the antiplatelet effect of resveratrol revealed that it can suppress the production of collagen-induced superoxide anion in platelets, collagen-induced phosphorylation of p47, a major regulatory subunit of NADPH oxidase [168]. Other cardiovascular effects of resveratrol may be attributed to the decrease in triglycerides levels via induction of lipolysis, activating lipase for adipose triglyceride, and by enhancing lipid mobilization [169]. Resveratrol also stimulates relaxation in superior mesenteric arterial rings in a concentration-dependent manner through the endothelium-dependent pathway and release of nitric oxide, and through endothelium-independent pathway by opening voltage-dependent K+ channels and ATP-sensitive K+ channels and blockade of extracellular Ca2+ influx [170].

1.1.8.3.2.5.g. Anti-inflammatory Effects

At the molecular and cellular levels, resveratrol can inhibit several signaling pathways involved in inflammation and in biological processes leading to the alleviation of the clinical symptoms associated with inflammatory processes including pain [171,172]. It also regulates neuro-inflammation through the activation of Sirtuin 1 (Sirt1) [173]. Different mechanisms are involved in the anti-inflammatory action of resveratrol such as phosphorylation of mitogen-activated protein kinases (MAPKs), down-regulating the expression of inflammatory biomarkers such as TNF-α and IL-6, and suppressing signal transducer and activator of transcription (STAT)1/STAT3 in lipopolysaccharide (LPS)-stimulated

murine macrophages. It also suppresses the production of NO and prostaglandin E2 (PGE2), slightly lowers PGE2 levels and the expression of COX-2, induces nuclear factor like 2 (NRf2) nuclear translocation [174,175]. Resveratrol in another experimental study in cytokine-challenged cells also suppress the production of free radicals, increases hemoxygenase-1 and glutamate cysteine ligase mRNA expression, induces NRf2 activation, and enhances reduced-to-oxidized ratio of glutathione [176]. Resveratrol also protects against hepatic damage in high-fat diet fed mice. It considerably impeded excessive activation of the NF-κB pathway and enhanced hepatic steatosis. It also improved sirtuin1 (Sirt1) protein and AMP-activated protein kinase-α (AMPKα) phosphorylation levels. Taken together, resveratrol supplementation attenuates the inflammatory cascades and improves hepatic steatosis through activation of the AMPKα–Sirt1 pathway [177]. Resveratrol also reduced the levels of IL-1β and TNF-α and immunoglobulin G extravasation in brain. [178].

1.1.8.3.2.6. Resveratrol as a Candidate Treatment in OA

Resveratrol may be considered as a rational nutraceutical candidate with a potential therapeutic interest in joint disorders. There is growing evidence of an effect of resveratrol on pathological chondrocytes and synoviocytes [179]. Because of its anti-apoptotic, anti-inflammatory and antioxidant properties, it exerts potential anti-osteoarthritic effects in many experimental models of OA [141,148]. Pre-clinical studies also provided accumulating evidence on the efficacy of resveratrol in ameliorating the degenerative articular damage [180,181,146]. Resveratrol inhibits the progression of OA through different mechanisms, including reducing degradation of type II collagen, suppressing chondrocyte apoptosis and body weight reduction; thereby, therapeutic worthiness and the potential

preventive effect of resveratrol is further intensified for obesity-associated OA [181]. More recently, a new approach, through using resveratrol in an experimental model of induced OA as chondroprotective agent through chondrocyte autophagy, was initiated for the aim of attenuating disease progression [182]. Additionally, in an *in vitro* study, resveratrol prevents the degradation of cartilage matrix by protecting the major cartilage matrix proteins, proteoglycans, collagen type II and aggrecan, from the matrix degrading enzyme or inflammatory stimuli (i.e., iNOS, COX2) [183,184,185]. Furthermore, in animal models of osteoarthritis, resveratrol demonstrates joint protective effects, which may be attributed to decreased production of pro-inflammatory and pro-degradative mediators, and modulation of cellular and humoral responses to the inflammatory insults [148]. In addition to the inhibition of COX-2 and PGE2 production by resveratrol, intra-articular injections of resveratrol in rabbit inflammatory arthritis model had a chondroprotective effect on the cartilage [186, 187].

Several *in vitro* studies have shown that IL-1β-induced suppression of chondrocyte proliferation and morphological alterations are inhibited by resveratrol. Furthermore, treatment of IL-1β-stimulated cells with resveratrol ceases the activation of caspase-3, apoptosis and accumulation of tumor suppressor gene protein p53 and induces ubiquitin-independent degradation of p53. Resveratrol arrests IL-1β-induced, NF-κB-dependent pro-inflammatory and matrix degrading gene products including MMPs, caspase-3, and COX-2. Resveratrol also suppressed IL-1β-induced NF-κB dependent expression of apoptosis-related gene products by the accumulation of phosphorylated IκB-α, ubiquitinated IκB-α and inhibition of proteasome activity [188,189].

Resveratrol controls the progression of experimental OA model by lowering of the apoptosis rate of chondrocyte and reducing the production

of NO, therefore it decreases destruction of cartilage tissue and provide a protection mechanism against OA progression [190]. Many preclinical studies showed that resveratrol may act on the sirtuin system [191]. Sirt1 has been confirmed to enhance cell survival and inhibit apoptosis through regulating several transcription factors, including p53, the transcriptional coactivator p300 and NF-κB [192-196]. The ability of sirtuin to promote chondrocyte survival and affect chondrocyte differentiation and proliferation has been documented by previous investigations, indicating that Sirt1 can protect cartilage degeneration via inhibiting apoptosis and elevating cartilage-specific gene expression [197,198,199]. Previous findings detected down-expression of Sirt1 in articular cartilage of patients with knee OA, and the expression levels are negatively associated with the disease severity; therefore, Sirt1 is highly expressed in the less damaged and normal human articular cartilage, while it is decreased in severely degenerated cartilage [200,201]. The effects of resveratrol-activated Sirt1 were observed in the articular chondrocytes [202]. Therefore, Sirt1 activation induced by resveratrol appears to improve the survival and metabolism of OA chondrocytes [203,201]. Moreover, resveratrol attenuates the formation of osteoclasts, production of inflammation-related proteins and of circulating ROS in rat model of periodontitis [204].

1.1.8.3.2.7. Safety Profile of Resveratrol

Resveratrol is unlikely to be toxic or cause remarkable adverse events in humans. The tolerability and safety of resveratrol was reported by many clinical studies, including cancer patients where high doses of resveratrol have been used [205]. The safety of resveratrol has been assessed in different clinical and animal studies, utilizing different doses (8, 150, 300, 1000, 2000, 5000mg/day) for various term-therapy periods including, 1,

3, 9 and 12 months. The findings of these studies suggest that adverse reactions to resveratrol in doses of less than 1,000 mg/day are scarce or mild, and short-term resveratrol supplementation is well tolerated [206,129]. In a clinical trial assessing the safety of oral resveratrol in 10 subjects, it has been reported that a single dose of 5,000 mg caused no serious adverse reactions [207]. Moreover, in a 29 consecutive days follow-up study, only mild-to-moderate gastrointestinal side effects, including nausea, abdominal pain, flatulence, and diarrhea, were reported in healthy participants who administered more than 1,000 mg/day of resveratrol [208]. Mild diarrhea was also reported in six out of eight participants who consumed 2,000 mg of resveratrol twice daily for two periods of eight days in an open-label and within subject-control study [209]. No hematological, coagulation, general biochemical, or electrocardiographic changes were observed in the obese participants who used 150 mg of pure resveratrol for 30 days [210]. Additionally, toxicity and adverse events of resveratrol in different animal models has already been investigated, including its acute, subchronic and chronic toxicity. In rats, daily oral intake of resveratrol at doses up to 700 mg/kg of body weight for 90 days caused no obvious adverse effects [211]. On the other hand, toxicological studies performed in animal models concluded that the no-observed-adverse-effect-level (NOAEL) for resveratrol was 200 mg/kg/days and 600 mg/kg/day in rats and beagle dogs, respectively [212].

1.2. Aim of the Study

The present study was designed to:

- Evaluate the clinical benefits of resveratrol, as an adjuvant therapy with meloxicam, in patients with mild to moderate knee osteoarthritis.
- Investigate the biochemical changes associated with the use of resveratrol, as an adjuvant with meloxicam, in patients with mild to moderate knee osteoarthritis.
- Assess the tolerability of Resveratrol.

CHAPTER TWO

MATERIAL AND METHODS

CHAPTER TWO

MATERIAL AND METHODS

2.1. Materials

All the chemicals; the diagnostic kits for biochemical analysis, medications and reagents used in this study were purchased from one of the following suppliers (Table 2-1):

Table 2-1: The chemicals and reagents with their suppliers.

No.	Chemicals	Suppliers
1	Trans-Resveratrol natural pure powder ≥98% (HPLC on anhydrous basis); Botanical source: *Polygonum Caspidutum*	Apollo Healthcare Resources- Singapore
2	Pre-gelatinized starch powder	Pioneer Company for Pharmaceutical Industries, Iraq
3	Meloxicam (Mobic) 15mg tab	Boehringer Ingelheim, Germany
4	Empty Gelatin Capsule size 0 white opaque	Pioneer Company for Pharmaceutical Industries, Iraq
5	IL-1β: Enzyme-Linked Immunosorbent Assay (ELISA) Kit	KORAIN BIOTECH CO., LTD, Shanghai, China
6	IL-6: Enzyme-Linked Immunosorbent Assay (ELISA) Kit	KORAIN BIOTECH CO., LTD, Shanghai, China
7	TNF-α: Enzyme-Linked Immunosorbent Assay (ELISA) Kit	KORAIN BIOTECH CO., LTD, Shanghai, China
8	high-sensitivity CRP (hs-CRP) Kit	Roche Diagnostics GmbH, Mannheim, Germany
9	Complement proteins C3, C4 Kit	Roche Diagnostics GmbH, Mannheim, Germany
10	Glutamate Pyruvate Transaminase (GPT) Kit	Roche Diagnostics GmbH, Mannheim, Germany

11	Glutamate Oxaloacetate Transaminase (GOT) Kit	Roche Diagnostics GmbH, Mannheim, Germany
12	Alkaline phosphatase (ALP) Kit	Roche Diagnostics GmbH, Mannheim, Germany
13	Cholesterol Kit	Roche Diagnostics GmbH, Mannheim, Germany
14	Triglyceride Kit	Roche Diagnostics GmbH, Mannheim, Germany
15	High Density Lipoprotein cholesterol (HDL-c) Kit	Roche Diagnostics GmbH, Mannheim, Germany
16	Low Density Lipoprotein cholesterol (LDL-c) Kit	Roche Diagnostics GmbH, Mannheim, Germany
17	Vitamin D_3 Kit	Roche Diagnostics GmbH, Mannheim, Germany
18	Creatinine Kit	Roche Diagnostics GmbH, Mannheim, Germany
19	Urea Kit	Roche Diagnostics GmbH, Mannheim, Germany
20	Swelab Alfa Diluent + Swelab AlfaLyse for Hematological parameters.	BOULE MEDICAL AB Sweden

2.2. Instruments and the tools:

The the instruments, disposables, tools used in the present study and their suppliers are listed in table 2-2:

Table 2-2: The Instruments, tools and disposables with their suppliers.

No	Instruments, tools and disposables	Suppliers
1	SWELAB hematology analyzer	BOULE MEDICAL AB Sweden
2	MICROPLATE READER; AWARENESS TECHNOLOGY CHROMATE, INC. FOR RUNNING ELISA	Awareness Technology inc., Palm City, Fl U.S.A.
3	Cobas e 411 Roche-HITACHI Analyzer	Roche Diagnostics GmbH, Mannheim, Germany
4	Cobas c 311 Roche-HITACHI Analyzer	Roche Diagnostics GmbH, Mannheim, Germany
5	Refrigerated centrifuge, Maximum speed;15000 rpm	Sigma-Laboratories centrifuges, Germany
6	Phoenix Capsule Filling Machine Device	UK-warehouses
7	Visual Analogue Scale (WONG-BAKER FACE PAIN RATING SCALE)	PRESTIGE MEDICAL-Printed in TAIWAN
8	Assay Tip for cobas e 411 analyzer	Roche Diagnostics GmbH-Made in Germany
9	Sample Cup	Roche Diagnostics GmbH-Made in Austria
10	Pipette Tip vol 1000µL blue tips	CITOTEST LABWARE MUNFACTURING CO.LTD-CHINA
11	Pipette Tip vol 200µL yellow tips	CITOTEST LABWARE MUNFACTURING CO.LTD-CHINA
12	Flat-Bottom Blood collection tube with screw cap 12ml	CITOTEST LABWARE MUNFACTURING CO.LTD-CHINA

2.3. Study Design and Ethical Consideration

The study was a prospective double-blind placebo-controlled randomized trial. Based on the prospective outcomes, the study design includes three distinct parts:

1. Clinical effect of resveratrol as an adjuvant with meloxicam in knee OA patients.
2. Anti-inflammatory effects of resveratrol as an adjuvant with meloxicam on biomarkers of inflammation in patients with knee OA.
3. Assess the tolerability of resveratrol based on a three-month follow-up of co-administration of resveratrol and meloxicam on hematological, liver function, kidney function parameter and lipid profile in knee OA patients.

Diagrams of the study design are shown in (Figure 2-1, Figure 2-2).

The present study was conducted over a period of 12 months. The screening and enrollment process was started in December 2016 and the follow up of all the patients with all data analysis completed in December 2017. The research was conducted at Shar Teaching Hospital, Sulaimani General Hospital and the Rheumatology and Physical Rehabilitation Center, Sulaimani City. The research protocol was approved by the Research Ethics Committee of the College of Medicine, University of Sulaimani (Registration No: 42 in 21/11/2016) in accordance with the Declaration of Helsinki and its amendments, and the Guidelines for Good Clinical Practices issued by the Committee of Propriety Medicinal Products of the European Union.

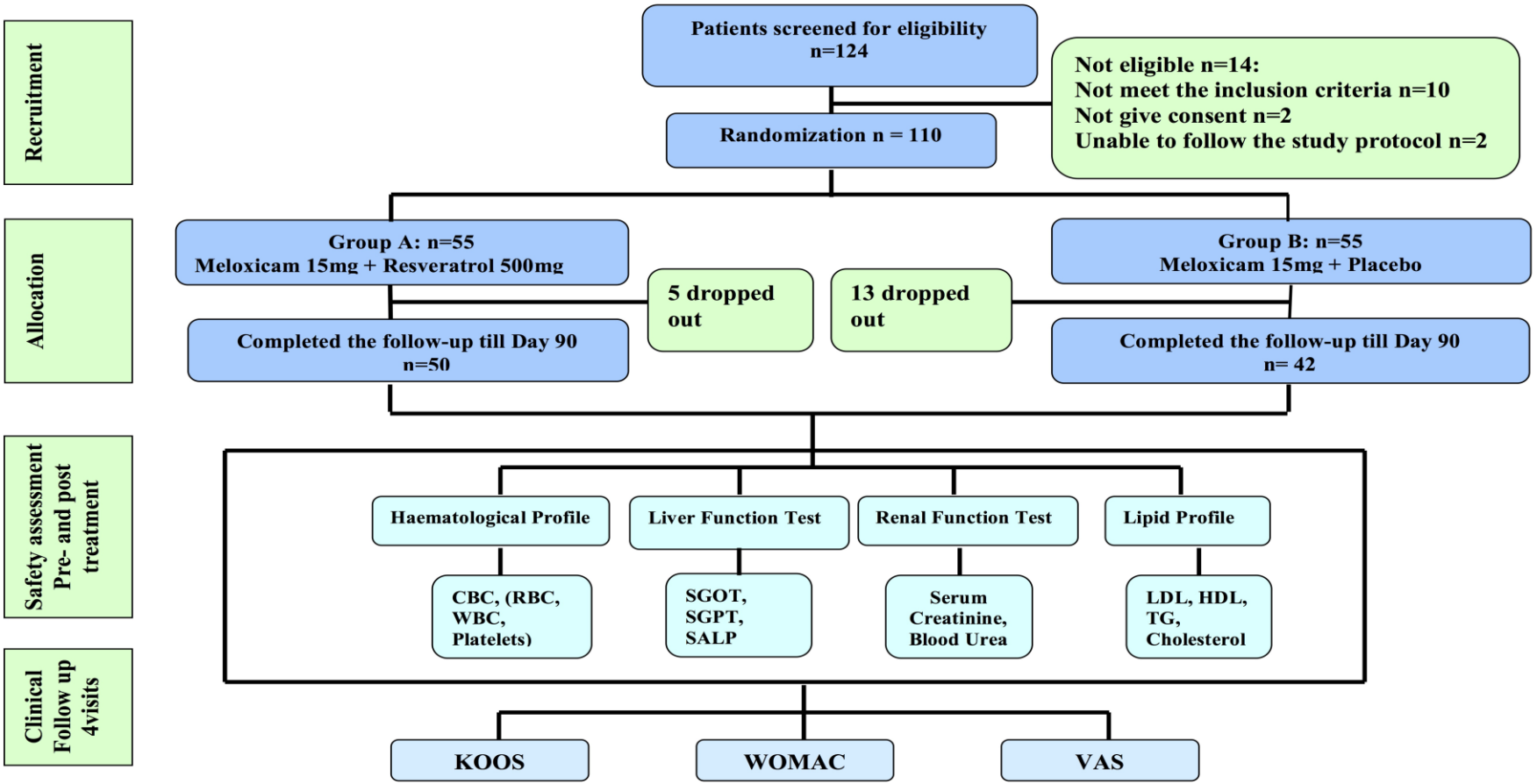


Figure 2-1 Flow-chart of the study that demonstrates participant's recruitment, randomization, intervention, dropout rate and completion of 90-day trial. *n*: number of patients; OA: osteoarthritis; WOMAC: Western Ontario and McMaster Universities Arthritis Index; VAS: Visual Analogue Score; KOOS: Knee injury and Osteoarthritis Outcome Score.

2.4. Patients Inclusion and Exclusion Criteria

The patients with painful knee OA who were attended the Out Patient Department (OPD) of Shar Teaching Hospital, General Teaching Hospital and Rheumatology and Physical rehabilitation center were screened for eligibility. Based on the American College of Rheumatology (ACR) criteria of OA diagnosis [70], and the methods found in previously published literatures (Table 2-3), the eligible patients of both sexes were enrolled in the study.

2.5. Enrollment and Consent

A total of one hundred and twenty-four patients with painful knee OA who were attended the Out Patient Department (OPD) of Shar Teaching Hospital, General Teaching Hospital and Rheumatology and Physical Rehabilitation Center were screened for eligibility. One hundred and ten patients who met the inclusion criteria were enrolled in the current study. All candidates have gone through a standardized interview process and all demographic data including gender, age, body weight, height, OA grade and any other diseases were recorded in a form specially designed for the study and they received more information about the study protocol and the treatment intervention. All participants were asked to sign a written informed consent (Appendix A-English version, Appendix B-Kurdish version) voluntarily before participation in the study. The purpose, procedures, and potential side effects and benefits of the study were also explained thoroughly to the participants.

Table 2-3 Patients inclusion and exclusion criteria:

Inclusion Criteria : Patients meeting the following criteria were included:	
1	Male or female of age range 45–75 years.
2	Patients with symptomatic tibiofemoral knee osteoarthritis according to the ACR criteria [70].
3	Symptoms lasting for more than 6 months.
4	Patients with radiographic diagnosis of mild to moderate knee OA (grade I–III) according to the Kellgren–Lawrence classification [71], defined by the presence of typical knee symptoms (pain, stiffness, disability).
5	Pain evaluation of the past 24 hours > 40 mm on the 100 mm Visual Analogue Scale (VAS) at baseline without NSAID or other analgesic for more than 48 hours.
6	Agreed to expel from NSAIDs use and other analgesics for the duration of the study.
6	Accept to sign informed consent form, willing to participate in and comply with the study.
8	No known drug allergies.
Exclusion Criteria : Patients with one or more of the following criteria were excluded:	
1	Secondary OA due to metabolic arthropathy: chondrocalcinosis.
2	Pain associated with acute joint trauma.
3	Patients with grade IV OA on the Kellgren-Lawrence grading system.
4	Patient with pre-existing joint disorders such as RA, gouty arthritis, infectious arthritis, etc.
5	Patients with severe cardiovascular, lung, liver (viral hepatitis), kidney (renal insufficiency) and hematopoietic system disease and cancer.
6	Using NSAIDs and receiving corticosteroid infiltration within 2 and 4 weeks, respectively.
7	Infiltration of hyaluronic acid in one of the knees within 6 months before recruitment.
8	Predominant symptomatic Patellofemoral joint OA.
9	Any pathological condition that could interfere with the assessment of the OA symptoms.
10	Joint replacement (any site).
11	Previous knee surgeries.
12	Taking coumarin anticoagulants and heparins and anti-platelets such as aspirin or

	clopidogrel.
13	Pregnant or lactating women.
14	Premenopausal women not using a contraceptive method.
15	Unable to discontinue supplements, vitamins and anti-OA drugs over the last 4 months.
16	Patient has contraindication to NSAIDs use.

2.6. Randomization and Intervention

The participants were randomized by independent outpatient unit person. A simple randomization technique was followed, in which predetermined sample size for treatment group (n=55) and for placebo (n=55) as an opaque envelope containing an identifier for treatment group and an identifier for placebo, has been shuffled. The order of the shuffled envelopes determines the allocation of participants to one of the treatments. The process is relatively simple to organize, preserves the predetermined design parameters, and can be readily extended to situations of long recruitment duration. The eligible patients were randomly allocated into one of two groups; Group A, those who received 15 mg meloxicam with 500 mg resveratrol (Mlx+Res) orally once daily for 90 days; the dose of resveratrol was selected based on the previous published articles [117, 206]. Group B; those who initiated treatment with 15 mg meloxicam and placebo formula (Mlx+placebo) which is specially prepared for the study, orally once daily for 90 days. Based on the literature review, most of the investigations on bioactive polyphenols needs not less than 90-day treatment period to achieve satisfactory response as analgesic and/or anti-inflammatory effects. The placebo was formulated as hard gelatin capsules composed of pre-gelatinized starch that have identical color, form and size as the resveratrol capsules. All those involved in the trial (investigators, participants and staff collecting data) were blinded from the randomization. The patients using anti-inflammatory drugs before inclusion were kept in 2-week washout period.

During this period, oral or local administration was gradually withdrawn within a week after enrollment and the patients were not receiving any drug for the next week and then he/she was assigned to one treatment group of the trial. Recruited patients were asked to carry on their regular daily activities that they had been doing before inclusion and also advised to continue the same till the end of the trial period. Additionally, all the patients were asked to return the empty containers of the trial drugs on every follow-up visit in order to check their compliance with the treatment protocol. Adherence to the medication protocol was also monitored through behavioral and telephone-based interventions on regular monthly follow-up basis. The patients were free to terminate participation in the study at any time without prior permission of the investigator or any reason. Furthermore, the investigator may discontinue any patient who develop any adverse effect or show non-compliance with the treatment protocol.

2.7. Outcome Measures

2.7.1. Primary Outcome

The primary assessment parameters in the present study include Knee injury and Osteoarthritis Outcome Score (KOOS), Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and Visual Analogue Score (VAS) for pain at day 0, 30, 60 and 90 for evaluation of the clinical efficacy of resveratrol in KOA patients.

2.7.2. Secondary Outcome

The secondary assessment parameters include evaluation of the levels of many biomarkers, including the pro-inflammatory cytokines TNF-α, IL-1β, IL-6, and high-sensitivity CRP (hs-CRP) inflammatory markers, and C3, C4 complement proteins twice at baseline (Day 0) and at day 90 (post treatment).

2.8. Clinical Efficacy and Follow-up Assessment

The included patients were attained the assigned out-patient clinic for clinical assessment on day 0 (First visit), day 30 (Second visit), day 60 (Third visit), and day 90 (Last visit). During each follow-up visit, patient's general and systemic physical examinations were performed. Evaluation of the knee OA symptoms was performed as a primary outcome utilizing the following evaluation systems (Figure 2-1):

1. Total KOOS score (Appendix C) [213].
2. Total WOMAC index (Appendix D) [214].
3. Visual Analogue Score for pain (VAS-100) (Appendix E) [17].

The disease severity score was assessed by both the investigators and the subjects on every follow-up visit to determine the efficacy of the treatment. As a primary outcome, the efficacy of treatment was evaluated by measuring the change from baseline in the KOOS score, WOMAC index, and the VAS-100 score; they were measured at baseline, and at 30, 60 and 90 day of the out-patient visits. The other outcomes measure in clinical assessment were measurement of the changes in KOOS subscale scores [symptom and stiffness; pain; Function and daily living (ADL); sports and recreational activities (Sport/Rec) and quality of life (QoL)] of knee OA severity, the changes in WOMAC subscale scores (stiffness, pain and daily functions), adverse drug reactions, and compliance with treatment protocol reporting.

2.8.1 Calculation of KOOS Score

The Knee injury and Osteoarthritis Outcome Score (KOOS) is a 42-item self-report questionnaire that has 5 reported subscales. KOOS scoring system applies a 5-point Likert scale, and all items have five possible answer options scored from 0 (No Complains) to 4 (Extreme Complains). Each of the five scores is calculated as the sum of the items included. Obtained scores are transformed to a 0–100 scale by a specific formula with zero representing extreme knee complains and 100 representing no knee complains as common in orthopedic assessment scales and generic measures. Scores between 0 and 100 represent the percentage of total possible score achieved. Although an aggregate score can be calculated in KOOS but analysis and interpretation of the five dimensions separately is more preferable [215]. In the present study, an automated administration that provides scoring software online has been used for calculation of the total KOOS score and its subscales. The five patient-relevant subscales of KOOS should be considered as secondary outcomes and are scored separately in the automated system: KOOS Symptoms and stiffness (7 items); KOOS Pain (9 items); KOOS ADL (17 items); KOOS Sport/Rec (5 items); KOOS QoL (4 items).

2.8.2. Calculation of WOMAC Index Score

Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) is a self-administered health status measure that assesses the dimensions of pain, stiffness and function (either separately or as an overall index). The five pain questions in WOMAC reflect pain experienced on five different activities. The patient's response to each question produces a score that is then summed to derive an aggregated score for each dimension. It produces three subscale scores (pain, stiffness and physical function) and a total score (WOMAC index) that reflects overall disability. The present study used the Likert version 3.0 [214] of WOMAC index (Appendix D) which is consist of 24 items with five response levels for each item, representing different degrees of intensity (none, mild, moderate, severe, and extreme) that were scored from 0 to 4. The total score for the WOMAC was determined by adding the aggregate scores for pain (score range 0–20), stiffness (score range 0–8), and physical function (score range 0–68). Scores range from 0 to 96 for the total WOMAC where 0 represents the best health status and 96 the worst possible status. The higher the score, the poorer the function or greater pain; i.e., the score is directly proportional to the severity of disease. Therefore, an improvement is achieved by the reducing of the overall score [216]. At the baseline both values (maximal points for WOMAC and minimal score for KOOS) represent relatively extreme knee complains.

2.8.3. Calculation of VAS

Visual Analogue Scale (VAS) composes of a horizontal or vertical line, usually 10 centimeters (100 mm) in length [217]. Perception of pain severity is recorded on a VAS scale, and the severity is reflected by the scores of 0–10. In the present study, pain was assessed by the patients themselves by marking ‘‘no pain, mild pain, moderate pain, and severe

pain'' on the pain chart on each visit [218].

2.9. Biochemical Measurements of the Anti-inflammatory Effect:

Blood sample (9.0 ml) was obtained from each participant twice, at the baseline time (Day 0) and at the end of the intervention (Day 90). Six milliliter of the collected blood was put in a standard sampling tubes i.e tubes containing separating gel. The blood was left to clot and the resulted serum was isolated by centrifugation at 4000 rpm for 10 min and stored at −70 °C until the time of analysis. The remaining three milliliters of the collected blood was kept in EDTA tube for hematological analysis.

In this part of the study, serum levels of the pro-inflammatory cytokines TNF-α, IL-1β, IL-6, and the high-sensitivity CRP (hs-CRP), and C3, C4 complement proteins were measured. Analysis was done for TNF-α, IL-1β and IL-6 using enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer instructions. Meanwhile, the analysis of high-sensitivity CRP (hs-CRP) and the complement proteins C3, C4 were performed by spectrophotometric method utilizing ready-made kits according to the manufacturer's recommendations. (Figure 2-2)

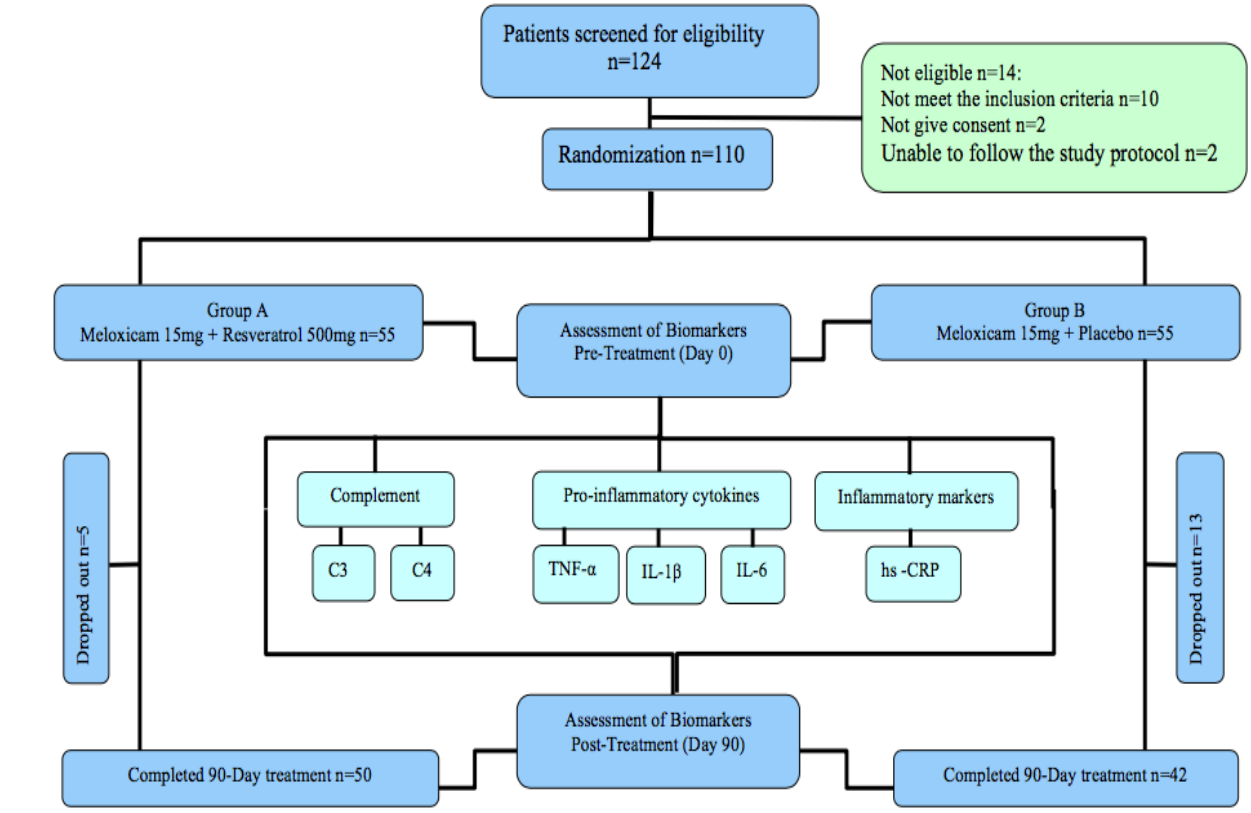


Figure 2-2 Flow chart of the followed design to assess the biomarkers of inflammation in patients with symptomatic knee OA. IL: Interleukin; TNF-α: Tumor necrosis factor alpha; CRP: C-Reactive Protein; C: complement protein.

2.10. Procedures for Determination of Anti-inflammatory Biomarkers:

2.10.1. Determination of serum level of Tumor Necrosis Factor-α (TNF-α)

Measurement of Tumor Necrosis Factor α (TNF-α) was based on the Enzyme-Linked Immunosoebent Assay (ELISA). Ready-made kit was utilized based on the manufacturer's instruction. TNF-α was added to the wells pre-coated with TNF-α monoclonal antibody. After incubation a biotin-conjugated anti-human TNF-α antibody was added and binds to human TNF-α. Streptavidin- Horse Radish Peroxidase (HRP) was added and binds to the biotin-conjugated anti-human TNF-α antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution was then added and color develops in proportion to the amount of human TNF-α. The reaction was terminated by addition of acidic stop solution and absorbance is measured at 450 nm, the results expressed as ng/L.

2.10.2. Determination of serum level of Interleukin-1β (IL-1β)

Interleukin-1β (IL-1β) was estimated based on the Enzyme-Linked Immunosoebent Assay (ELISA). Ready-made kit was utilized based on the manufacturer's instruction. IL-1β was added to the wells pre-coated with IL-1β monoclonal antibody. After incubation a biotin-conjugated anti-human IL-1β antibody was added and binds to human IL-1β. Streptavidin-HRP was added and binds to the biotin-conjugated anti-human IL-1β antibody. After incubation unbound Streptavidin-HRP was washed away during a washing step. Substrate solution was then added and color develops in proportion to the amount of human IL-1β. The reaction was terminated by addition of acidic stop solution and absorbance is measured at 450 nm. The results express as pg/L.

2.10.3. Determination of serum level of Interleukin-6 (IL-6)

Interleukin-6 (IL-6) is estimated based on the Enzyme-Linked Immunosoebent Assay (ELISA). Ready-made kit was utilized based on the manufacturer's instruction. IL-6 was added to the wells pre-coated with IL-6 monoclonal antibody. After incubation a biotin-conjugated anti-human IL-6 antibody was added and binds to human IL-6. Streptavidin-HRP was added and binds to the biotin-conjugated anti-human IL-6 antibody. After incubation unbound Streptavidin-HRP was washed away during a washing step. Substrate solution was then added and color develops in proportion to the amount of human IL-6. The reaction was terminated by addition of acidic stop solution and absorbance is measured at 450 nm. The results expressed as ng/L.

2.10.4. Determination of high- sensitivity CRP (hs-CRP)

The quantitative determination of C-reactive protein (CRP) in serum was based on immunoturbidimetric assay utilizing Roche/Hitachi cobas c systems. [219]. Human CRP agglutinates with latex particles coated with monoclonal anti-CRP antibodies. The precipitate was determined turbidimetrically. The results expressed as mg/L.

2.10.5. Determination of the complement protein C3

The quantitative determination of Complement C3c in human serum was performed based on Immunoturbidimetric assay using Roche/Hitachi cobas c systems. Human C3c forms a precipitate with a specific antiserum which was determined turbidimetrically [220]. The results expressed as mg/dl.

2.10.6. Determination of the complement protein C4

For quantitative determination of serum C4, immunoturbidimetric assay was used on Roche/Hitachi cobas c systems [220]. Human C4 forms a

precipitate with a specific antiserum which was determined turbidimetrically. The results expressed as mg/dl.

2.11. Tolerability of Resveratrol

This part of the study involves the assessment of hematological parameters, liver function, renal function, and lipid profile and a comprehensive short-term follow-up of clinical and physical examination, vital signs, body weight alteration and occurrence of drug adverse events. Furthermore, serum concentration of vitamin D was measured for each patient twice, in a pre and post interventional schedule to investigate the expected effect of resveratrol on Vitamin D level [221].

The collected blood in the EDTA tube was used for determination of hematological parameters; hemoglobin concentration (Hb), haematocrit (Hct%) value, erythrocyte count (RBCs), white blood cell count (WBC) and platelets count. The collected serum was used for biochemical analysis. Both hematological and biochemical analysis were done at the baseline time and at the end of the treatment. For liver function markers serum alanine aminotransferase (ALT)/Glutamate Pyruvate Transaminase (GPT), aspartate aminotransferase (AST)/Glutamate Oxaloacetate Transaminase (GOT) and alkaline phosphatase (ALP) activities were measured. Meanwhile for the renal function markers, serum creatinine and urea were measured. Measurement of total serum cholesterol, triglyceride, high density lipoprotein cholesterol (HDL-c) and low density lipoprotein cholesterol (LDL-c) levels were also carried out spectrophotometrically at baseline and at the end of the treatment using the clinical chemistry analyzer Cobas c 311, utilizing ready-made kits according to the manufacturer's recommendations.

2.11.1. Determination of hematological parameters

The assessment of the hematological parameters was performed utilizing the Swelab Alfa Plus system which is an automated hematology analyzer for *in vitro* diagnostic use under laboratory conditions. The Swelab Alfa Plus was used for enumeration of white blood cells (WBC); the absolute number and percentage concentration for granulocytes (GRAN), lymphocytes (LYM), mid-sized white cells (MID); red blood cells (RBC); hemoglobin (HGB); mean cell volume of red cells (MCV); hematocrit (HCT); mean cell hemoglobin (MCH); mean cell hemoglobin concentration (MCHC); red cell distribution relative and absolute widths (RDW%, RDWa); platelets (PLT); mean platelet volume (MPV), platelet crit (PCT), platelet distribution relative and absolute widths (PDW%, PDWa).

The measuring principles of the Swelab Alfa Plus analyzer were based on impedance and spectrophotometer principles.

2.11.2. Assessment of Liver Function

2.11.2.1. Determination of serum alanine aminotransferase (ALT): Glutamate Pyruvate Transaminase (GPT)

The quantitative determination of alanine aminotransferase (ALT)/Glutamate Pyruvate Transaminase (GPT) in human serum on Roche/Hitachi cobas c systems was performed by the assay follows the recommendations of the International Federation of Clinical Chemistry (IFCC), but was optimized for performance and stability [222]. ALT catalyzes the reaction between L-alanine and 2-oxoglutarate. The pyruvate formed is reduced by NADH in a reaction catalyzed by lactate dehydrogenase (LDH) to form L-lactate and NAD^+ .

$$\text{L-Alanine} + \text{2-oxoglutarate} \xrightarrow{\text{ALT}} \text{pyruvate} + \text{L} - \text{glutamate}$$

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LDH}} \text{L-lactate} + \text{NAD}^+$$

The rate of the NADH oxidation is directly proportional to the catalytic ALT activity. It is determined by measuring the decrease in absorbance. The serum ALT(GPT) concentration was expressed in U/L.

2.11.2.2. Determination of aspartate aminotransferase (AST): Glutamate Oxaloacetate Transaminase (GOT)

The quantitative determination of aspartate aminotransferase (AST)/ Glutamate Oxaloacetate Transaminase (GOT) in human serum on Roche/Hitachi cobas c systems was performed by an assay follows the recommendations of the IFCC method, but was optimized for performance and stability. [222]. AST in the sample catalyzes the transfer of an amino group between L-aspartate and 2-oxoglutarate to form oxaloacetate and L-glutamate. The oxaloacetate then reacts with NADH, in the presence of malate dehydrogenase (MDH), to form NAD^+. The rate of the NADH oxidation is directly proportional to the catalytic AST activity. It is determined by measuring the decrease in absorbance.

$$\text{L-Aspartate} + \text{2-oxoglutarate} \xrightarrow{\text{AST}} \text{oxaloacetate} + \text{L-glutamate}$$

$$\text{Oxaloacetate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{MDH}} \text{L-malate} + \text{NAD}^+$$

The serum AST(GOT) concentration was expressed in U/L.

2.11.2.3. Determination of Alkaline Phosphatase (ALP)

The quantitative determination of alkaline phosphatase in human serum on Roche/Hitachi cobas c systems was estimated by colorimetric assay in accordance with a standardized method. In the presence of magnesium and zinc ions, p-nitrophenyl phosphate is cleaved by phosphatases into phosphate and p-nitrophenol [223].

$$\text{p- nitrophenyl phosphate} + H_2O \xrightarrow{\text{ALP}} \text{phosphate + p-nitrophenol}$$

The p-nitrophenol released is directly proportional to the catalytic ALP activity. It is determined by measuring the increase in absorbance. The serum ALP concentration was expressed in U/L.

2.11.3. Assessment of Renal Function

2.11.3.1. Determination of Serum Creatinine

The quantitative determination of creatinine in human serum on Roche/Hitachi cobas c systems was estimated by the kinetic colorimetric assay which is based on the Jaffé method [224]. In alkaline solution, creatinine forms a yellow-orange complex with picrate. The rate of dye formation is proportional to the creatinine concentration in the specimen. The assay uses “rate-blanking” to minimize interference by bilirubin. The serum creatinine concentration was expressed in mg/dl.

$$\text{Creatinine} + \text{picric acid} \xrightarrow{\text{Alkaline pH}} \text{yellow-orange complex}$$

2.11.3.2. Determination of Serum Urea

The quantitative determination of urea/urea nitrogen in human serum on Roche/Hitachi cobas c systems was estimated by kinetic test with urease and glutamate dehydrogenase [225]. Urea is hydrolyzed by urease to form ammonium and carbonate.

$$\text{Urea} + 2H_2O \xrightarrow{\text{Urease}} 2NH_4^+ + CO_3^{-2}$$

In the second reaction 2-oxoglutarate reacts with ammonium in the presence of glutamate dehydrogenase (GLDH) and the coenzyme NADH to produce L-glutamate. In this reaction two moles of NADH are oxidized to NAD^+ for each mole of urea hydrolyzed.

$$NH_4^+ + \text{2-oxoglutarate} + \text{NADH} \xrightarrow{\text{GLDH}} \text{L-glutamate} + NAD^+ + H_2O$$

The rate of decrease in the NADH concentration is directly proportional to the urea concentration in the specimen and is measured photometrically. The serum urea concentration was expressed in mg/dl.

2.11.4. Assessment of Lipid profile

2.11.4.1. Determination of Total Serum Cholesterol

The quantitative determination of cholesterol in human serum and plasma was performed on Roche/Hitachi cobas c systems. The principle of the assay was based on Enzymatic-colorimetric method. In this method cholesterol esters are cleaved by the action of cholesterol esterase (CHER) to yield free cholesterol and fatty acids. Cholesterol oxidase (CHOD) then catalyzes the oxidation of cholesterol to cholest-4-en-3-one and hydrogen peroxide. In the presence of peroxidase (POD), the hydrogen peroxide formed affects the oxidative coupling of phenol and

4-aminophenazone to form a red quinone-imine dye. [226].

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{CHER}} \text{cholesterol} + \text{RCOOH}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{cholest-4-en-3-one} + H_2O_2$$

$$2H_2O_2 + 4-\text{APP} + \text{phenol} \xrightarrow{\text{POD}} \text{quinone-imine dye} + 4H_2O$$

The color intensity of the dye formed is directly proportional to the cholesterol concentration. It is determined by measuring the increase in absorbance. The serum cholesterol concentration was expressed in mg/dl.

2.11.4.2. Determination of Triglyceride

The quantitative determination of triglycerides in human serum and plasma was performed on Roche/Hitachi cobas c systems. Test principle was based on Enzymatic colorimetric method [227]. This method utilizes a lipoprotein lipase (LPL) from microorganisms for the rapid and complete hydrolysis of triglycerides to glycerol followed by oxidation to dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide produced then reacts with 4-aminophenazone and 4-chlorophenol under the catalytic action of peroxidase to form a red dyestuff (Trinder endpoint reaction). The color intensity of the red dyestuff formed is directly proportional to the triglyceride concentration and can be measured photometrically.

$$\text{Triglycerides} + 3H_2O \xrightarrow{\text{LPL}} \text{glycerol} + 3\text{RCOOH}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{GK/Mg^{+2}} \text{glycerol-3-phosphate} + \text{ADP}$$

$$\text{glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPO}} \text{dihydroxyacetone phosphate} + H_2O_2$$

$$H_2O_2 + 4-\text{aminophenazone} + 4-\text{chlorophenol} \xrightarrow{\text{Peroxidase}} 4-(\text{p}-\text{benzoquinone}-\text{monoimino})\text{-phenazone} + 2H_2O + HCl$$

Roche/Hitachi cobas c systems automatically calculate the analyte concentration of each sample. The serum triglycerides concentration was expressed in mg/dl.

2.11.4.3. Determination of High density lipoprotein cholesterol (HDL-c)

The quantitative determination of the HDL-cholesterol concentration in human serum was performed on Roche/Hitachi cobas c systems. Test principle was based on homogeneous enzymatic colorimetric test [228]. Non-HDL lipoproteins such as LDL, VLDL and chylomicrons are combined with polyanions and a detergent forming a water-soluble complex. In this complex the enzymatic reaction of cholesterol esterase (CHER) and cholesterol oxidase (CHOD) towards non-HDL lipoproteins is blocked. Finally, only HDL-particles can react with CHER and CHOD. The concentration of HDL-cholesterol is determined enzymatically by CHER and CHOD. The serum HDL-cholesterol concentration was expressed in mg/dl.

2.11.4.4. Determination of Low Density Lipoprotein Cholesterol (LDL-c)

The quantitative determination of LDL-cholesterol in human serum was performed on Roche/Hitachi cobas c systems. Test principle was base on homogeneous enzymatic colorimetric assay [229].

$$\text{LDL} - \text{cholesterol esters} + \text{H}_2\text{O} \xrightarrow{\text{Detergent,Cholesterol esterase}} \text{Cholesterol} + \text{free fatty acids}$$

Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase.

$$\text{LDL} - \text{Cholesterol} + \text{O}_2 \xrightarrow{\text{Cholesterol oxidase}} \Delta^4\text{- cholestenone} + \text{H}_2\text{O}_2$$

In the presence of peroxidase, the hydrogen peroxide generated reacts with 4-aminoantipyrine and sodium N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (HSDA) to form a purple -blue dye. The color intensity of this dye is directly proportional to the cholesterol concentration and is measured photometrically. The serum LDL-c concentration was expressed in mg/dl.

$$2\text{H}_2\text{0}_2 + 4 - \text{aminoantipyrine} + \text{HSDA} + \text{H}_2\text{O} + \text{H}^+ \rightarrow \text{purple} - \text{blue pigment} + 5\text{H}_2\text{O}(\text{Abs.max} = 585\text{nm})$$

2.11.5. Determination of Vitamin D3

The quantitative determination of 25-hydroxyvitamin D3 in human serum was performed on cobas e 411 analyzers. The Elecsys Vitamin D3 (25-OH) assay is used as an aid in the assessment of Vitamin D3 sufficiency in adults. The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and cobas e immunoassay analyzers. The analyzer automatically calculates the analyte concentration of each sample (either in ng/mL or nmol/L) [230].

2.12. Assessment of Adverse Effects

Safety assessment was performed through screening all the patients clinically and monitoring vital signs. A standard adverse-event case report form that formulated based on a Common Terminology Criteria for

Adverse Events (CTCAE) version 4.0 [231] was used at each visit (4 visits) to record all experienced adverse effects, such as skin itching, rash, blisters and skin damage, local pain and discomfort, nausea, gastrointestinal upset.

2.13. Statistical analysis

Analysis of data was performed using GraphPad Prism 5.0.1 program (GraphPad Software Inc., CA, USA). The baseline demographic data and patient characteristics were compared between the treatment groups. Categorical data were analyzed with the chi-square test. Spearman's correlation and ROC analysis were utilized to compare the outcomes of the questionnaires. Continuous variables were analyzed using independent sample *t*-test or the two-way ANOVA and Bonferroni's *post hoc* test. A p value of < 0.05 was considered to indicate statistical significance.

CHAPTER THREE

RESULTS

CHAPTER THREE

RESULTS

3.1 Recruitment and Disposition

A total of one hundred and twenty-four patients were screened for eligibility, of these one hundred and ten patients met the inclusion criteria (Figure 2-1). Only 92 patients completed the study, where 18 patients were dropped out. Of these, 5 patients from the Mlx+Res group and 13 from the Mlx+placebo group not completed the follow-up visits for various reasons.

3.2 Baseline Characteristics

The baseline comparative demographic data between the patients with knee OA in Mlx+Res and Mlx+placebo groups are demonstrated in Table 3-1. There were no significant differences in the demographic and clinical characteristics including gender, age, body weight, body mass index, and disease grade or disease duration between the two study groups. Moreover, the baseline value of total KOOS, total WOMAC and VAS-100 scores were also not significantly different between the two groups.

No effort was made to match the study groups for statistic variables. Mean age of the participants were 58.2±9.1 and 57.6±8.2 years for Mlx+Res, Mlx+placebo respectively. Most of the enrolled patients are women in both groups. Most of the participants were overweight or obese with approximately average BMI of 30.7±5.6, 32.1±4.6 (kg/m^2) for Mlx+Res, Mlx+ placebo, respectively (Table 3-1).

Table 3-1: Demographic data and baseline characteristics of the Knee OA patients treated with Meloxicam+Resveratrol (Mlx+Res) or Meloxicam+placebo (Mlx+placebo)

Parameters	Mlx+Res *n*=50	Mlx+Placebo *n*=42	*P* value
Gender n(%)			
Male	13(26)	10(23.8)	0.07
Female	37(74)	32(76.2)	0.2
Age (year)	58.2±9.1	57.6±8.2	0.77
Body weight (kg)	80.5±16.1	82.4±11.0	0.55
BMI (Kg/m2)	30.7±5.6	32.1±4.6	0.1
Disease duration (year)	3.5±3.2	3.9±2.9	0.45
Disease grade n(%)			
Grade I	7(14)	5(12)	0.12
Grade II	25(50)	18(42.8)	
Grade III	18(36)	19(45.2)	
Baseline KOOS	33.6±9.2	33.5±10.5	0.9
Baseline WOMAC	59.6±13.7	59.3±7.4	0.2
Baseline VAS-100 (mm)	81.0±11.6	85.3±9.2	0.08
Associated diseases n(%)			
Hypertension	20(40.0)	18(42.8)	0.10
Diabetes mellitus	8(16.0)	5(12)	0.12
Smoking habits	3(6.0)	2(4.7)	0.11

Values are presented as percent or mean±S.D; *n*: number of patients; Res: Resveratrol; Mlx: Meloxicam. Chi-square and unpaired *t*-test were utilized to predict significance at $P<0.05$.

3.3. Outcomes Measures

3.3.1. Primary Outcome; Clinical Outcomes Measures

3.3.1.1 KOOS Total

As a primary outcome measure, KOOS total score [ranging from 0 (worst) to 100 (best)] was utilized to evaluate the clinical effect of resveratrol in the present study. Table 3-2 showed that adjuvant use of resveratrol with meloxicam (Mlx+Res) significantly improves total KOOS score after 30 days, compared with both baseline value and that of Mlx+placebo group within the same period ($P<0.05$). Although the effect of resveratrol continues to improve KOOS score after 60 and 90 days, these values are not significantly different ($P>0.05$). The post-intervention KOOS score in Mlx+placebo group were non-significantly different in all time ranges ($P>0.05$). In Figure 3-2, comparison between the effects of Resveratrol (Res) and placebo when administered with meloxicam (Mlx), at baseline and after 90 days revealed that the effect of resveratrol on the total KOOS score was highly significant ($P<0.001$) at the end of treatment, compared with that produced by the placebo at the same time.

3.3.1.2. KOOS Subscale

In table 3-3, the post intervention KOOS subscale score in Mlx+Res group was significantly improved in all KOOS areas [Symptom and stiffness, Pain, Function and daily living (ADL), Sports and Recreational activities (Sport/Rec) and Quality of life (QoL)] compared with both baseline values and those reported in Mlx+placebo group that show non-significant changes in KOOS subscale score all over the treatment period (Figures 3-3 to 3-7).

Table 3-2: Effect of resveratrol (Res) as an adjuvant with meloxicam (Mlx) on the Knee Injury and Osteoarthritis Outcome Score (KOOS), Western Ontario and McMaster Universities Arthritis (WOMAC) index, and Visual Analogue Score (VAS-100) in patients with mild to moderate knee OA.

Score	Mlx+Res, *n*=50				Mlx+Placebo, *n*=42			
	Baseline	30 days	60 days	90 days	Baseline	30 days	60 days	90 days
KOOS	33.6±9.2[a]	81.7±12.9*[b]	87.8±10.2*[b]	89.3±9.5*[b]	33.5±10.5[a]	38.9±15.1[a]	37.2±14.3[a]	38.3±14.8[a]
WOMAC	59.6±13.7[a]	16.2±14.6*[b]	9.5±9.8*[c]	8.1± 8.2*[c]	63.7±11.0[a]	58.6±15.0[a]	59.0±14.4[a]	56.1±15.9[a]
VAS-100	81.0±11.6[a]	29.4±11.5*[b]	22.3±12.5*[c]	18.6±10.8*[c]	85.3±9.2[a]	72.7±17.4[b]	70.9±14.9[b]	65.7±16.8[b]

Values are presented as mean±S.D; *n*: number of patients; * significantly different compared with baseline values within the same group (paired *t*-test); values with different superscripts (a,b,c) are significantly different among different times and different groups (ANOVA; $P<0.05$).

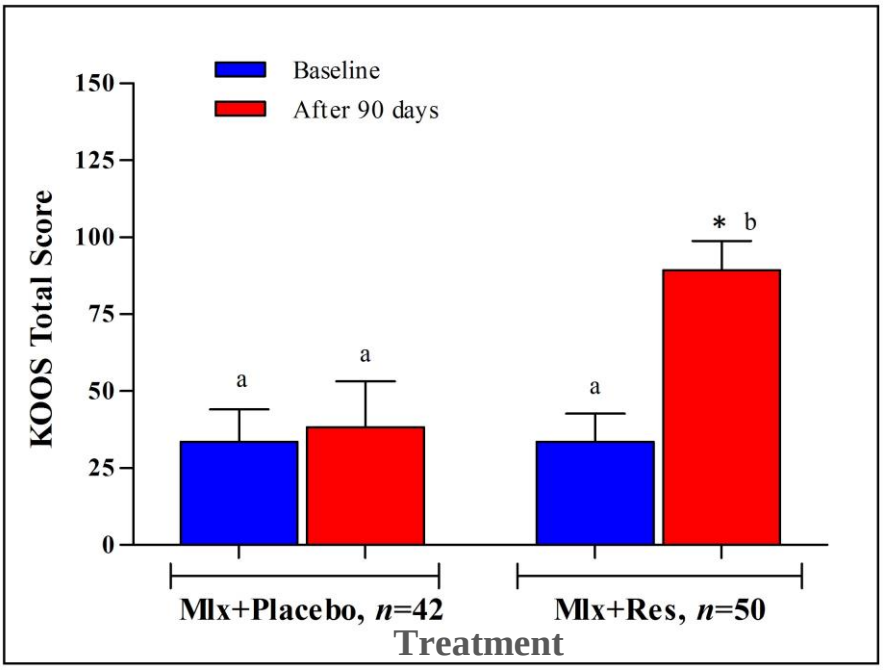


Figure 3-2: Comparison between the effects of resveratrol (Res) and Placebo, when administered with meloxicam (Mlx), on the total KOOS score at baseline and after 90 days; * significantly different compared with baseline values within the same group (paired *t*-test); values with different superscripts (a,b) are significantly different among different times and different groups (ANOVA; $P<0.05$).

Table 3-3: Effect of resveratrol (Res) as an adjuvant with meloxicam (Mlx) on the different areas of Knee injury and Osteoarthritis Outcome Score (KOOS) in patients with mild to moderate knee OA

KOOS area	Mlx+Res, *n*=50				Mlx+Placebo, *n*=42			
	Baseline	30 days	60 days	90 days	Baseline	30 days	60 days	90 days
Symptom & stiffness	49.0±20.2[a]	84.8±12.6*[b]	89.7±11.8*[b]	92.1±9.6*[b]	38.3±17.0[a]	44.1±19.6[a]	43.8±19.9[a]	41.6±22.0[a]
Pain	32.9±14.1[a]	80.2±15.1*[b]	87.9±13.0*[c]	90.3±12.9*[c]	30.3±12.9[a]	39.1±18.8[a]	34.7±17.6[a]	35.5±19.0[a]
Daily living	38.4±14.8[a]	83.7±16.4*[b]	90.3±10.3*[b]	91.6±7.8*[b]	35.1±12.2[a]	39.6±16.2[a]	39.7±15.5[a]	40.9±16.1[a]
Sport/Rec	20.8±15.7[a]	63.0±17.5*[b]	75.2±14.9*[c]	74.6±15.5*[c]	24.5±8.8[a]	30.2±13.4[a]	23.8±14.0[a]	28.6±9.8[a]
QoL	35.3±10.6[a]	77.1±18.5*[b]	85.6±11.1*[c]	88.3±13.9*[c]	29.9±11.2[a]	36.7±14.2[a]	36.3±14.3[a]	39.1±10.9[a]

Values are presented as mean±S.D; *n*: number of patients; Res: Resveratrol; Mlx: Meloxicam; QoL: Quality of life; Sport/Rec: Sport and recreational activities; * significantly different compared with baseline values within the same group (paired *t*-test); values with different superscripts (a,b,c) are significantly different among different times and different groups (unpaired *t*-test and ANOVA; $P<0.05$).

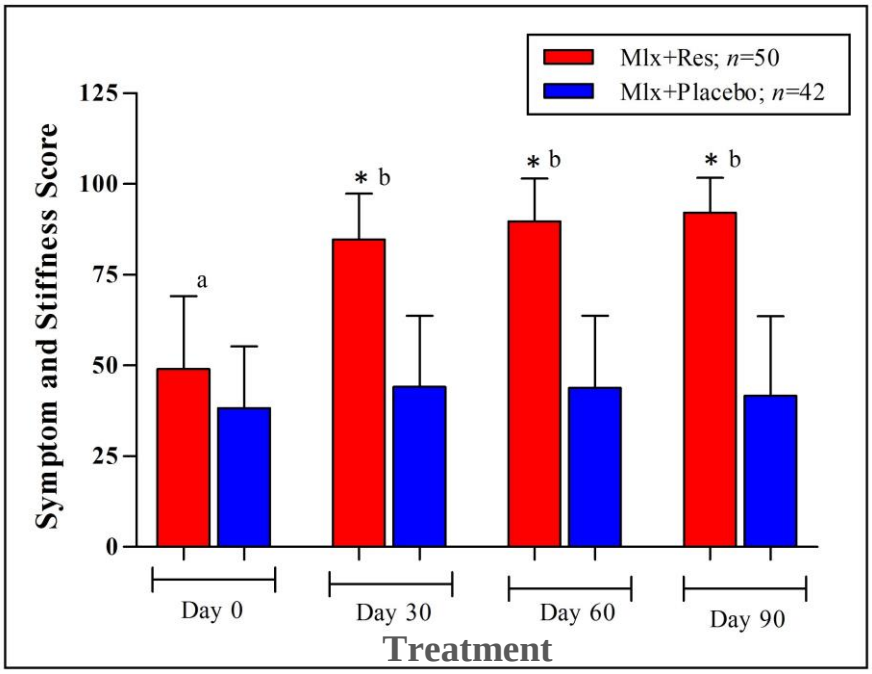


Figure 3-3: Effect of resveratrol (Res) as adjuvant with meloxicam (Mlx) on the symptoms and stiffness (KOOS subscale area) in patients with mild to moderate knee OA. *n*: number of patients; * significantly different compared with baseline values within the same group (paired *t*-test); values with different superscripts (a,b) are significantly different among different times and different groups (ANOVA; $P<0.05$).

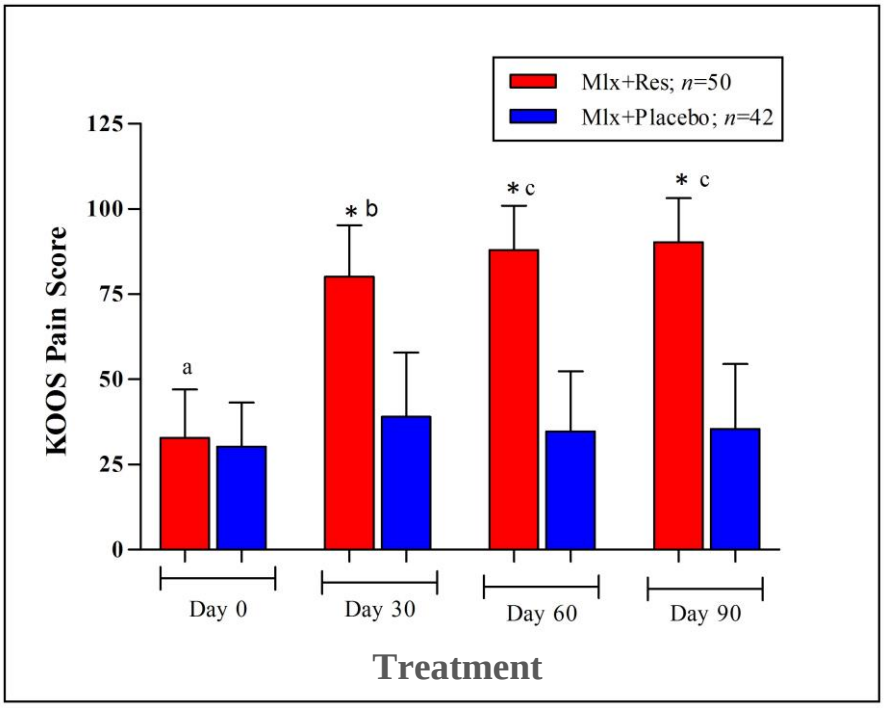


Figure 3-4: Effect of resveratrol (Res) as an adjuvant with meloxicam (Mlx) on the pain subscale area of KOOS in patients with mild to moderate knee OA. *n*: number of patients; * significantly different compared with baseline values within the same group (paired *t*-test); values with different superscripts (a,b,c) are significantly different among different times and different groups (ANOVA; $P<0.05$).

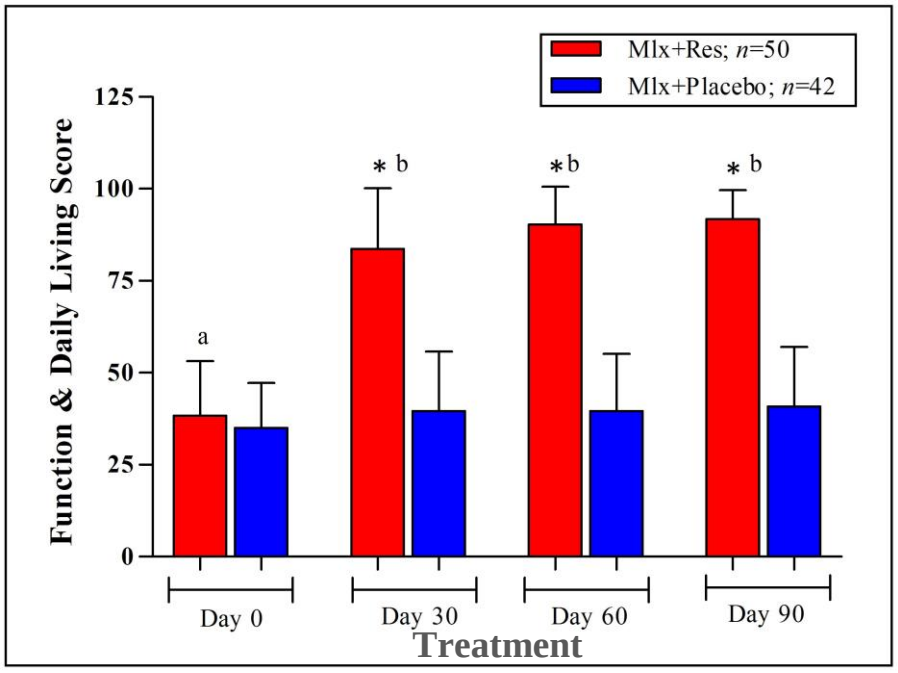


Figure 3-5: Effect of resveratrol (Res) as an adjuvant with meloxicam (Mlx) on the function and daily living score (KOOS subscale area) in patients with mild to moderate knee OA. *n*: number of patients; * significantly different compared with baseline values within the same group (paired *t*-test); values with different superscripts (a,b) are significantly different among different times and different groups (ANOVA; $P<0.05$).

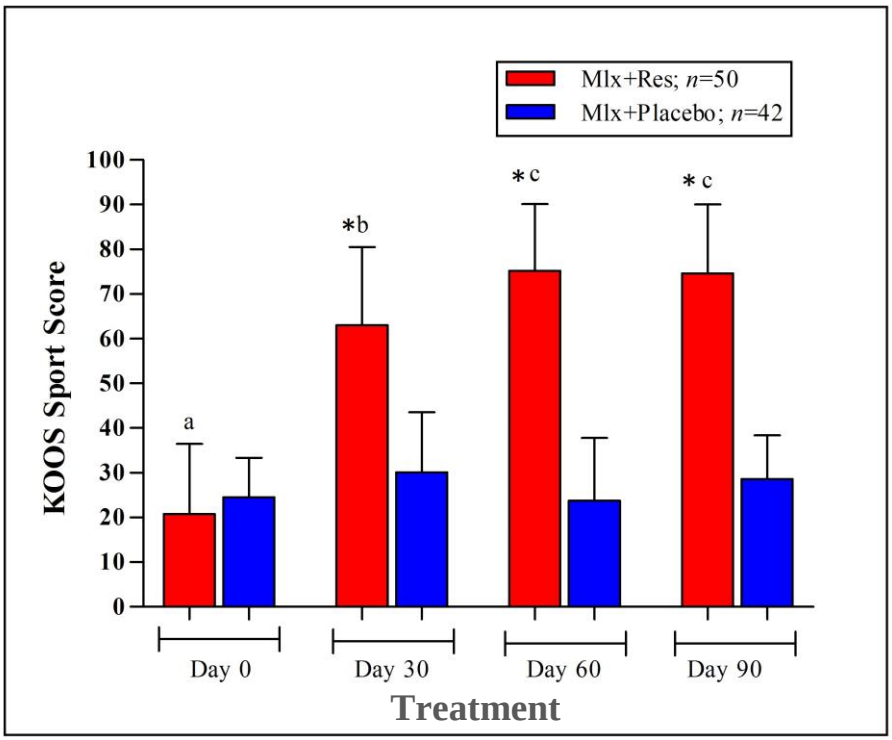


Figure 3-6: Effect of resveratrol (Res) as adjuvant with meloxicam (Mlx) on the sport score (KOOS subscale area) in patients with mild to moderate knee OA. *n*: number of patients; * significantly different compared with baseline values within the same group (paired *t*-test); values with different superscripts (a,b,c) are significantly different among different times and different groups (ANOVA; $P<0.05$).

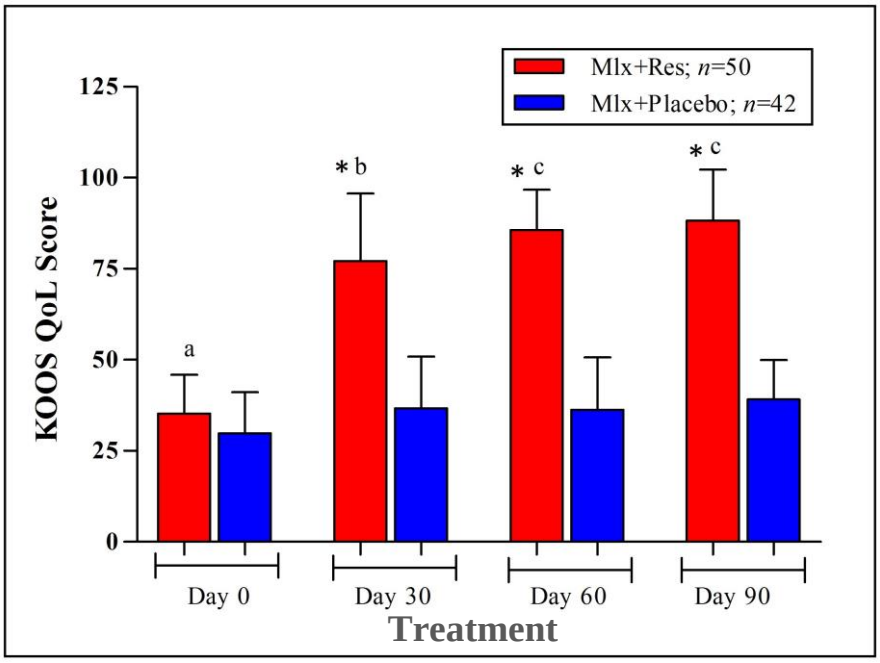


Figure 3-7: Effect of resveratrol (Res) as adjuvant with meloxicam (Mlx) on the Quality of Life score (KOOS subscale area) in patients with mild to moderate knee OA. *n*: number of patients; * significantly different compared with baseline values within the same group (paired *t*-test); values with different superscripts (a,b,c) are significantly different among different times and different groups (ANOVA; $P<0.05$).

3.3.1.3 Total WOMAC score

In addition to the KOOS outcome measure, the WOMAC total score ranging from 0 [best] to 96 [worst] was also used to evaluate the clinical efficacy of resveratrol. The reduction in WOMAC total score at first follow-up, second follow-up and third follow-up are presented in Table 3-2. There was a significant reduction in WOMAC score over the treatment period. The post intervention WOMAC score in Mlx+Res was significantly improved in all time ranges, compared with both baseline value and those of Mlx+placebo group ($P<0.05$); Meanwhile the post-intervention WOMAC score was not significantly changed in Mlx+placebo group among different times of follow-up, i.e., between baseline, day 30, day 60 and day 90. In Figure 3-8, comparison between the effects of resveratrol and placebo at baseline and after 90 days revealed that the effect of Mlx+Res on the total WOMAC score was highly significant ($P<0.001$) at the end of treatment, compared with that produced by the Mlx+placebo at the same time.

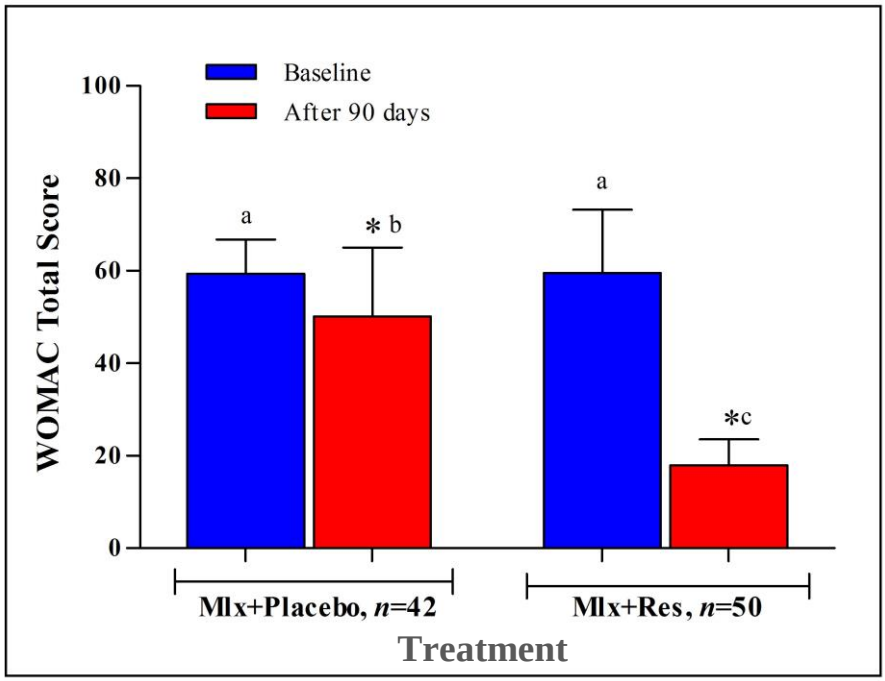


Figure 3-8: Comparison between the effects of resveratrol (Res) and Placebo, when administered with meloxicam (Mlx), on the total WOMAC score at baseline and after 90 days; * significantly different compared with baseline values within the same group (paired *t*-test); values with different superscripts (a,b,c) are significantly different among different groups (ANOVA; $P<0.05$).

3.3.1.4. WOMAC Subscale Areas

Similarly, Table 3-4 indicates that post-intervention WOMAC subscale score in Mlx+Res group was significantly improved in all WOMAC subscale areas [pain (score range 0–20), stiffness (score range 0–8), and physical function (score range 0–68)] compared with baseline values and the results of the corresponding area in Mlx+placebo group, where all the subscale values were non-significantly different during the treatment period (P>0.05).

3.3.1.5. VAS-100 for Pain

In the present study, in addition to the use of KOOS score and WOMAC index for the evaluation of the clinical outcome, the patients' overall pain for the prior 24 hours was assessed using VAS-100mm scale [0 (no pain) and 100 (worst pain)]. Table 3-2 and Figure 3-9 showed that post-intervention VAS-100 score was significantly improved in both groups at the end of the treatment period compared to baseline value; however, the degree of changes in the Mlx+Res group were significantly higher compared to that reported in the Mlx+placebo group after 30,60 and 90 days. Consequently, a significant improvement in VAS was found in Mlx+Res group after 30 days compared with that of Mlx+placebo group within the same period (P<0.05).

Table 3-4: Effect of resveratrol (Res) as an adjuvant with meloxicam (Mlx) on the different areas of Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) in patients with mild to moderate knee OA

WOMAC area	Mlx+Res, *n*=50				Mlx+placebo, *n*=42			
	Baseline	30 days	60 days	90 days	Baseline	30 days	60 days	90 days
Stiffness (0-8)	4.3±2.2[a]	1.1±1.3*[b]	0.8±1.3*[b]	0.7±1.2*[b]	5.8±1.4[a]	5.3±1.8[b]	5.5±1.9[b]	4.6±2.3[b]
Pain (0-20)	13.4±3.3[a]	3.6±3.1*[b]	2.1±2.6*[b]	1.8±2.5*[b]	13.8±2.4[a]	12.1±4.0[a]	12.2±4.0[a]	11.9±4.4[a]
Function (0-68)	41.8±9.3[a]	11.3±11.1*[b]	7.2±7.4*[b]	5.9±5.4*[b]	44.1±8.3[a]	41.1±16.2[a]	39.6±11.6[a]	37.1±12.1[a]

Values are presented as mean±S.D; *n*: number of patients; Res: Resveratrol; Mlx: Meloxicam; * significantly different compared with baseline values within the same group (paired *t*-test); values with different superscripts (a,b,c) are significantly different among different times and different groups (unpaired *t*-test and ANOVA; $P<0.05$

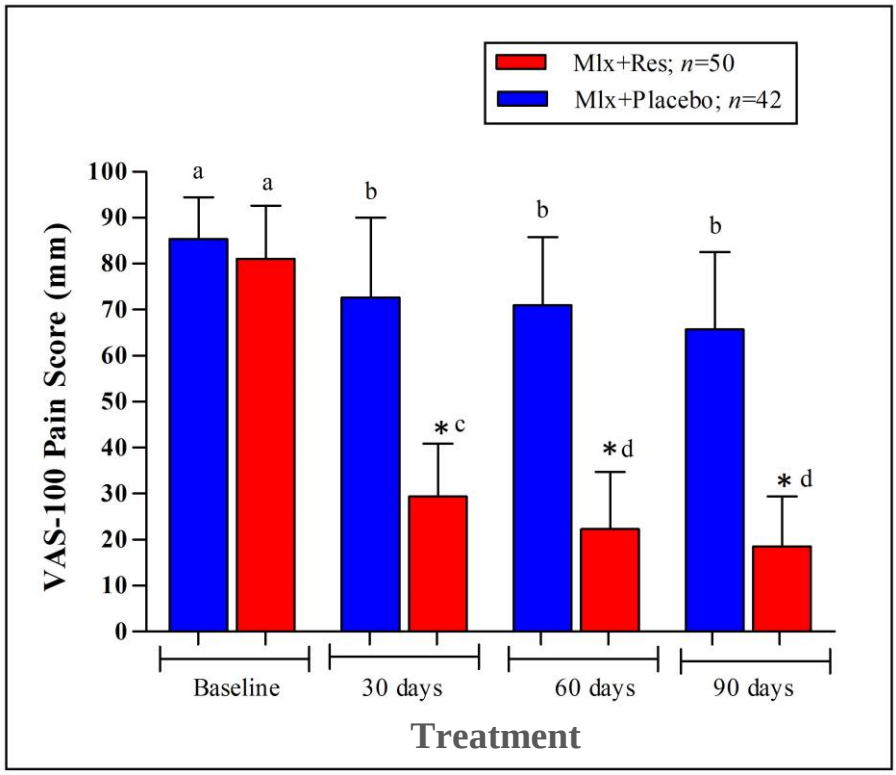


Figure 3-9: Effect of resveratrol, as an adjuvant with meloxicam for 90 days, on the pain severity of patients with mild to moderate knee OA measured by VAS-100. *n*: number of patients; VAS: visual analogue score; * significantly different compared with control group at the same time period ($P<0.05$); values with non-identical letters (a,b,c,d) among the different groups were significantly different ($P<0.05$).

The validity of KOOS at different levels in the evaluation of treatment outcome in both groups compared with WOMAC was evaluated using Spearman's correlation, where a significant negative correlation was detected in the Mlx+placebo group at most of the values greater than 1 (r= - 0.6, *P*= 0.001) (Figure 3-10). The influence of both treatment approaches on the pain score was quite evident. Moreover, Figure 3-11 shows the correlation between KOOS score and VAS-100 score, where high to moderate negative and significant correlation was observed in Mlx+Res and Mlx+placebo groups respectively. In the present study, the receiver operating characteristic (ROC) method was used. This method has the advantage of synthesizing information on the sensitivity and specificity for detecting improvement by both KOOS and WOMAC as shown in Figure 3-12. Accordingly, the evaluation of sensitivity and specificity of the two methods using ROC curve demonstrated a highly significant (*P*<0.0001) pattern.

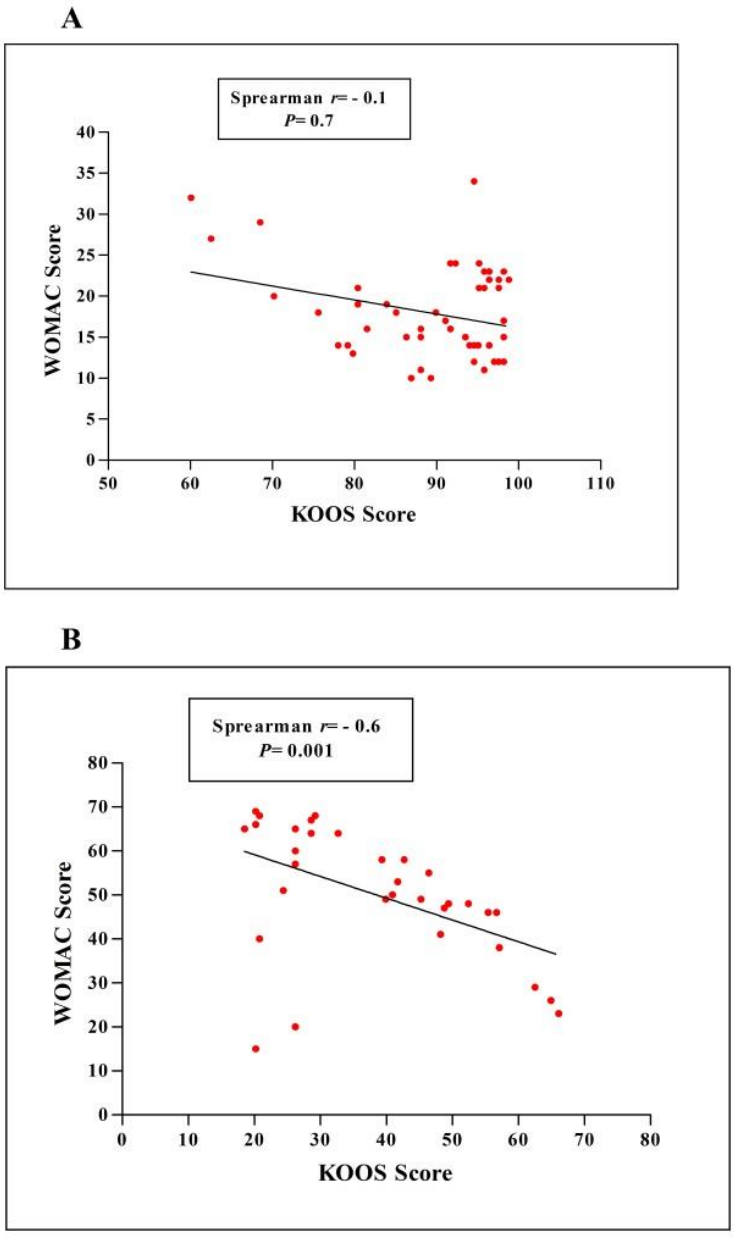


Figure 3-10. Spearman's correlation between KOOS and WOMAC scores in both treatment groups after 90 days. *r*: Spearman's correlation coefficient; A: Mlx+Res group; B: Mlx+placebo group.

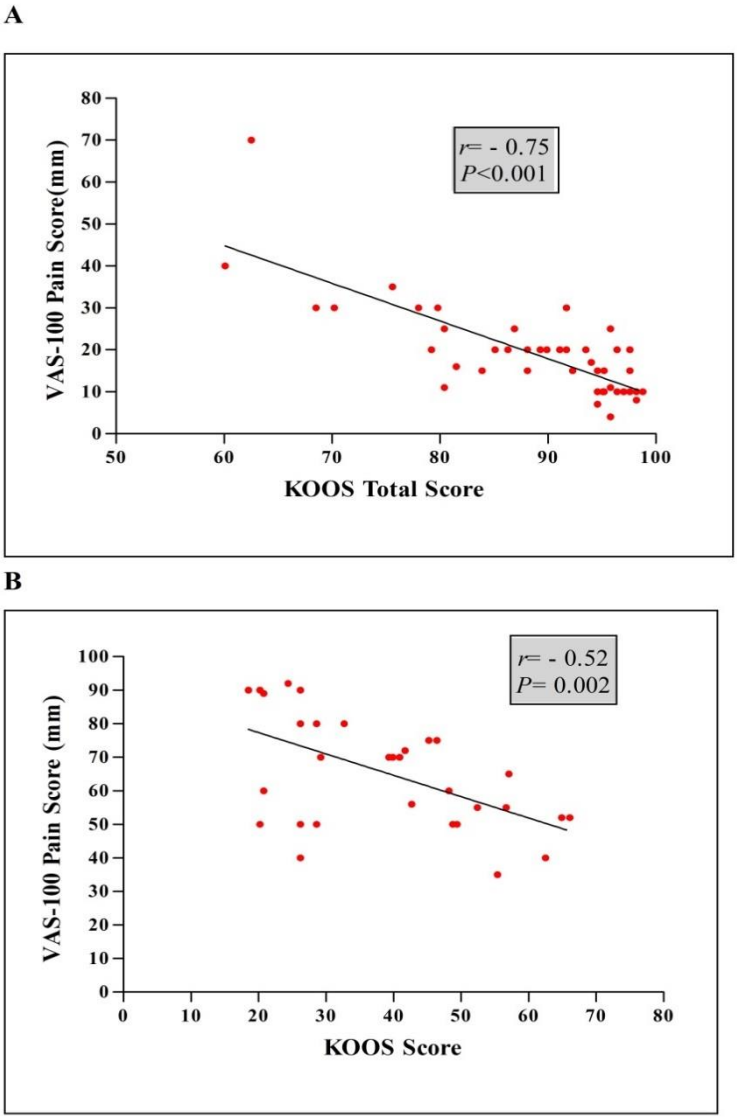


Figure 3-11. Spearman's correlation between KOOS and VAS-100 scores in both treatment groups after 90 days. *r*: Spearman's correlation coefficient; A: Mlx+Res group; B: Mlx+placebo group.

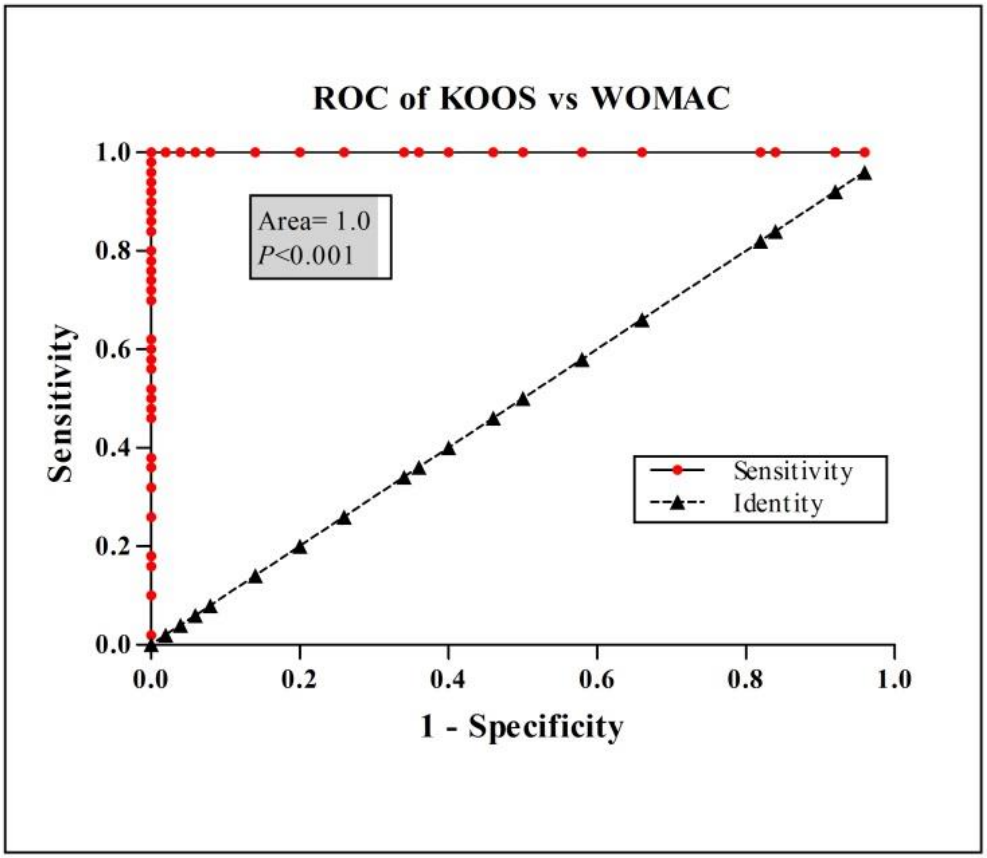


Figure 3-12: ROC curve illustrating the sensitivity and specificity for different values of KOOS corresponding to a WOMAC. ROC: Receiver operating characteristic curve.

3.3.2 Secondary Outcome; **Biochemical Markers**

The baseline values of all the evaluated biochemical markers were not significantly different between the two groups, except for the TNF-α where the baseline level of serum concentration of TNF-α was significantly higher in the group who use resveratrol with meloxicam compared with the group who used meloxicam with placebo.

3.3.2.1 Pro-inflammatory Markers

3.3.2.1.a TNF-α

In Figure 3-13, adjuvant use of resveratrol with meloxicam for 90 days significantly reduces serum levels of the pro-inflammatory cytokine TNF-α in patients with knee OA ($P<0.05$), compared with both the baseline level and that of the group treated with meloxicam and placebo. Meanwhile, analysis of TNF-α in the group treated with meloxicam and placebo showed non-significant elevation of this biomarker within the same period ($P>0.05$).

3.3.2.1.b IL-1β

The use of resveratrol as an adjuvant with meloxicam resulted in significant reduction in the serum levels of IL-1β after 90 days, compared with both the baseline values and those reported in Mlx+placebo group within the same period. However, statistically non-significant elevation was noted in the levels of IL-1β biomarker in Mlx+placebo group after 90 days compared with the corresponding baseline values ($P>0.05$) (Figure 3-14).

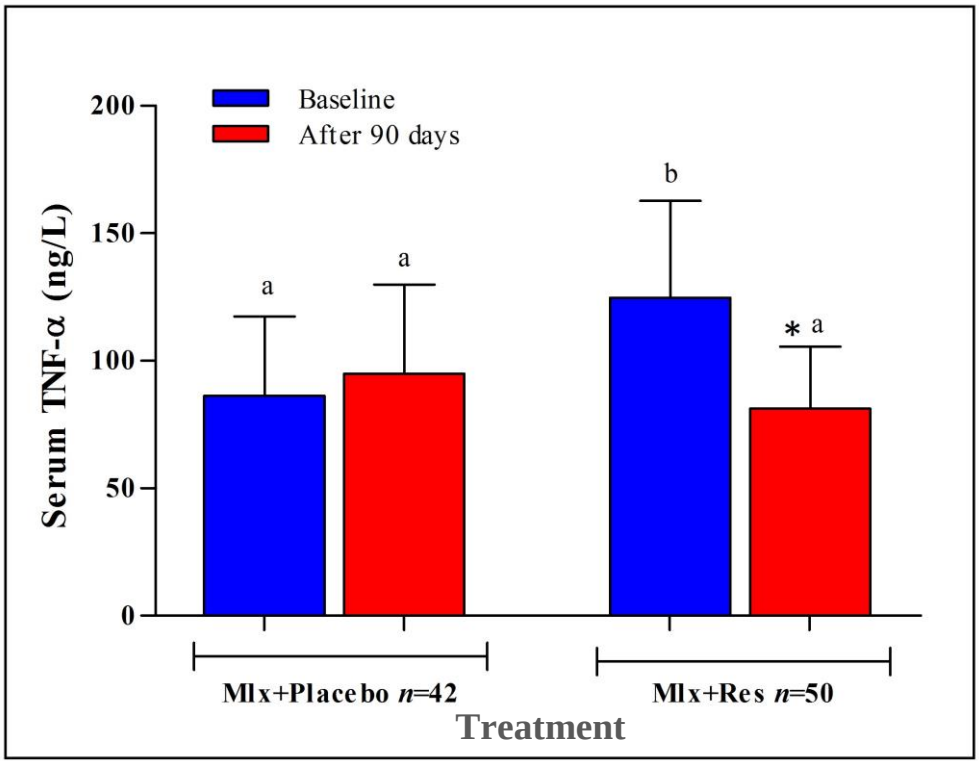


Figure 3-13: Effect of resveratrol, as an adjuvant with meloxicam, on serum level of TNF-α in patients with mild to moderate knee OA. *n*: number of patients; Res: Resveratrol; Mlx: Meloxicam; * significantly different compared with baseline values within the same group ($P<0.05$); values with different letters (a,b) are significantly different within the different groups ($P<0.05$).

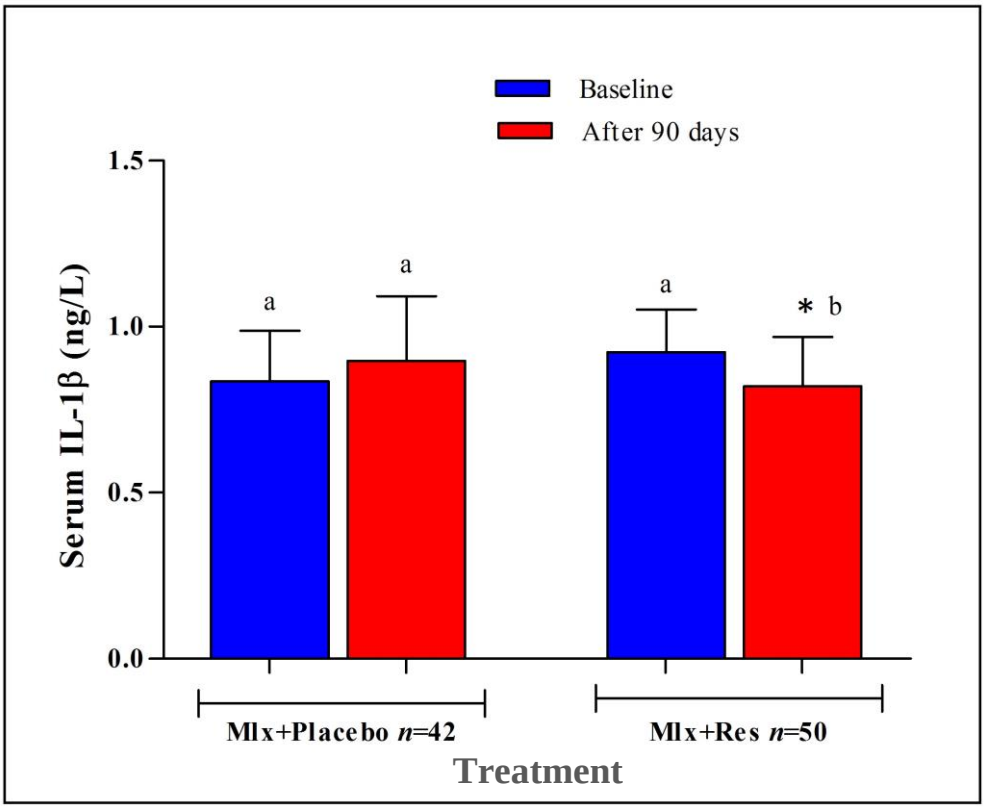


Figure 3-14: Effect of resveratrol, as an adjuvant with meloxicam, on serum level of IL-1β in patients with mild to moderate knee OA. *n*: number of patients; Res: Resveratrol; Mlx: Meloxicam; * significantly different compared with baseline values within the same group ($P<0.05$); values with different letters (a,b) are significantly different within the different groups ($P<0.05$).

3.3.2.1.c IL-6

A 90-day treatment of the knee OA patients with resveratrol as an adjuvant with meloxicam resulted in a significant lowering of the serum level of IL-6 as compared with both the baseline values and those reported in the Mlx+placebo group within the same period. However, this biomarker displays a statistically non-significant increment in the Mlx+placebo group compared with the corresponding baseline values ($P>0.05$) (Figures 3-15).

3.3.2.2. Inflammatory Markers

Among the selected inflammatory biomarkers associated with knee OA, hs-CRP was reported to be significantly lower in the Mlx+Res group compared to the Mlx+placebo group at day 90 ($P<0.05$). Figure 3-16 shows that a 90-day treatment with resveratrol as an adjuvant with meloxicam produced significant decrease in the serum hs-CRP levels compared with both the baseline values and the levels of the hs-CRP in corresponding group that used meloxicam and placebo ($P<0.05$). Meanwhile, minor non-significant elevation of this inflammatory marker has been observed in the Mlx+placebo group compared with the corresponding baseline values ($P>0.05$).

3.3.2.3 Complement Proteins C3 and C4

Co-administration of resveratrol and meloxicam significantly decreases the serum levels of the complement proteins C3 and C4 ($P<0.05$) after 90 days compared with the baseline values of the same group and the values of the group treated with meloxicam plus a placebo formula (Figures 3-17 and 3-18).

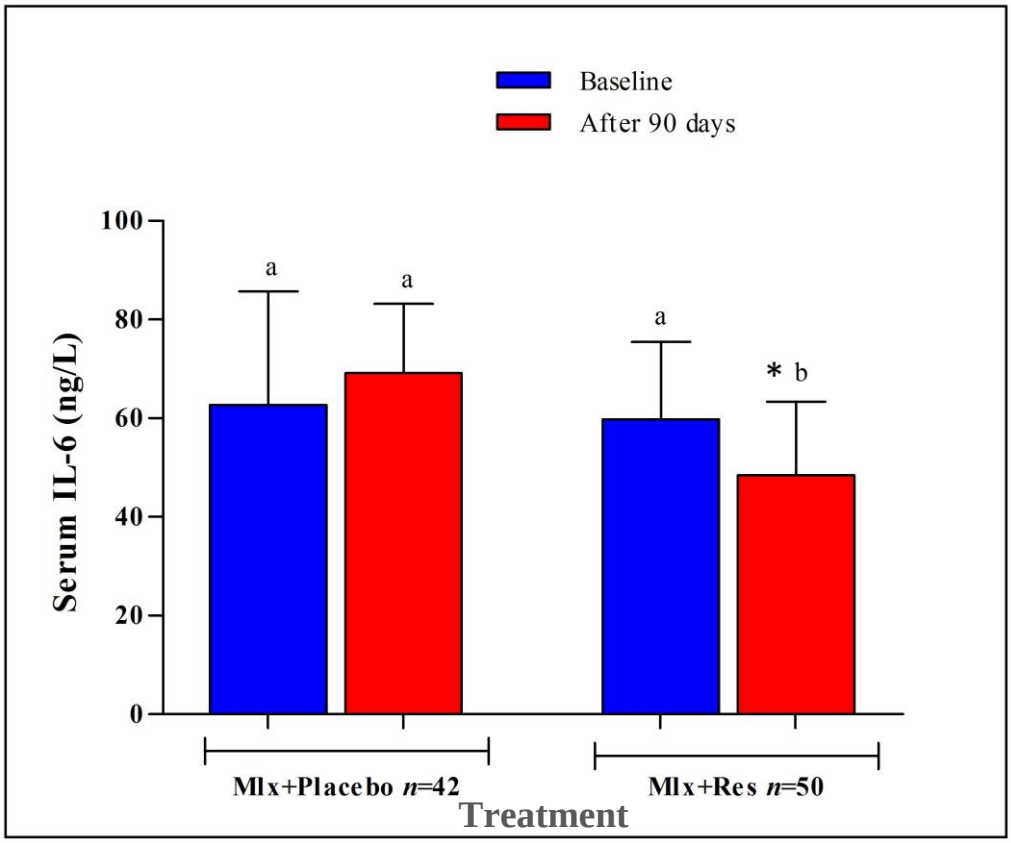


Figure 3-15: Effect of resveratrol, as an adjuvant with meloxicam, on serum level of IL-6 in patients with mild to moderate knee OA. *n*: number of patients; Res: Resveratrol; Mlx: Meloxicam; * significantly different compared with baseline values within the same group ($P<0.05$); values with different letters (a,b) are significantly different among the different groups ($P<0.05$).

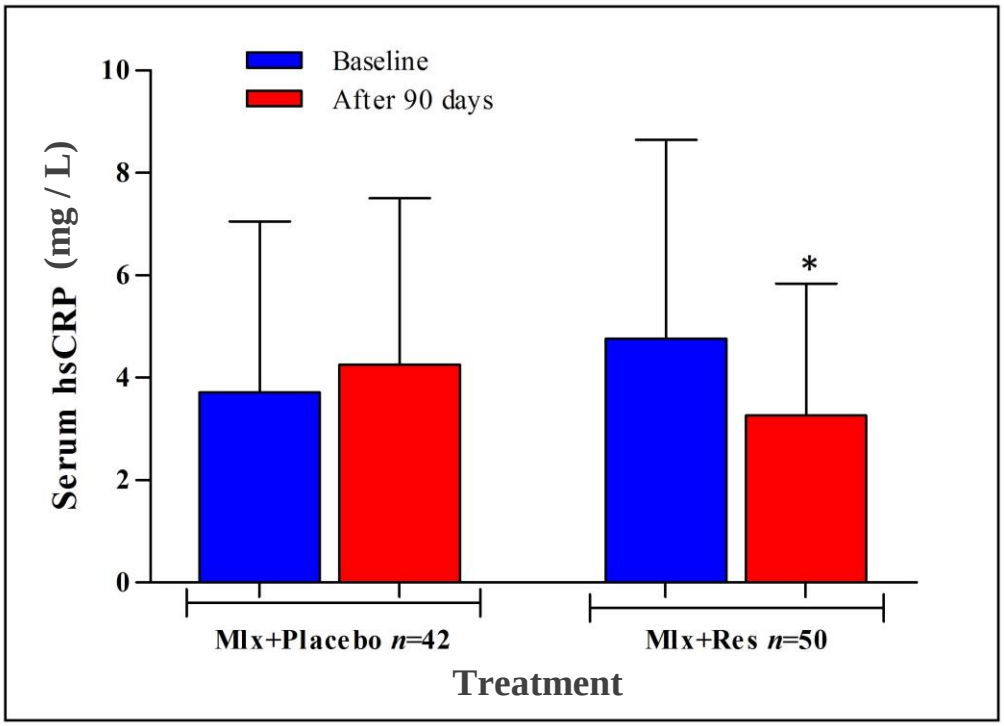


Figure 3-16: Effect of resveratrol, as an adjuvant with meloxicam on serum level of hs-CRP in patients with mild to moderate knee OA. Values are presented as mean±S.D; *n*: number of patients; Res: Resveratrol; Mlx: Meloxicam; * significantly different compared with baseline values within the same group ($P<0.05$).

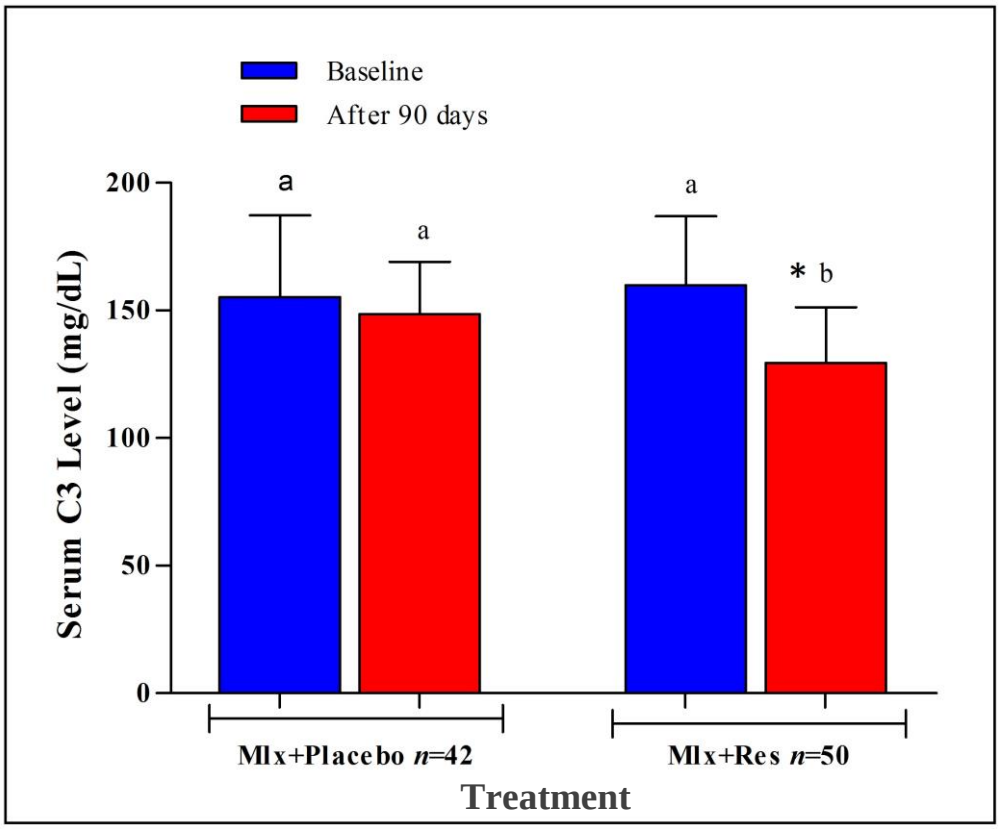


Figure 3-17: Effect of resveratrol, as an adjuvant with meloxicam, on serum level of complement C3 in patients with mild to moderate knee OA. *n*: number of patients; Res: Resveratrol; Mlx: Meloxicam; * significantly different compared with baseline value within the same group ($P<0.05$); values with different letters (a,b) are significantly different within the different groups ($P<0.05$).

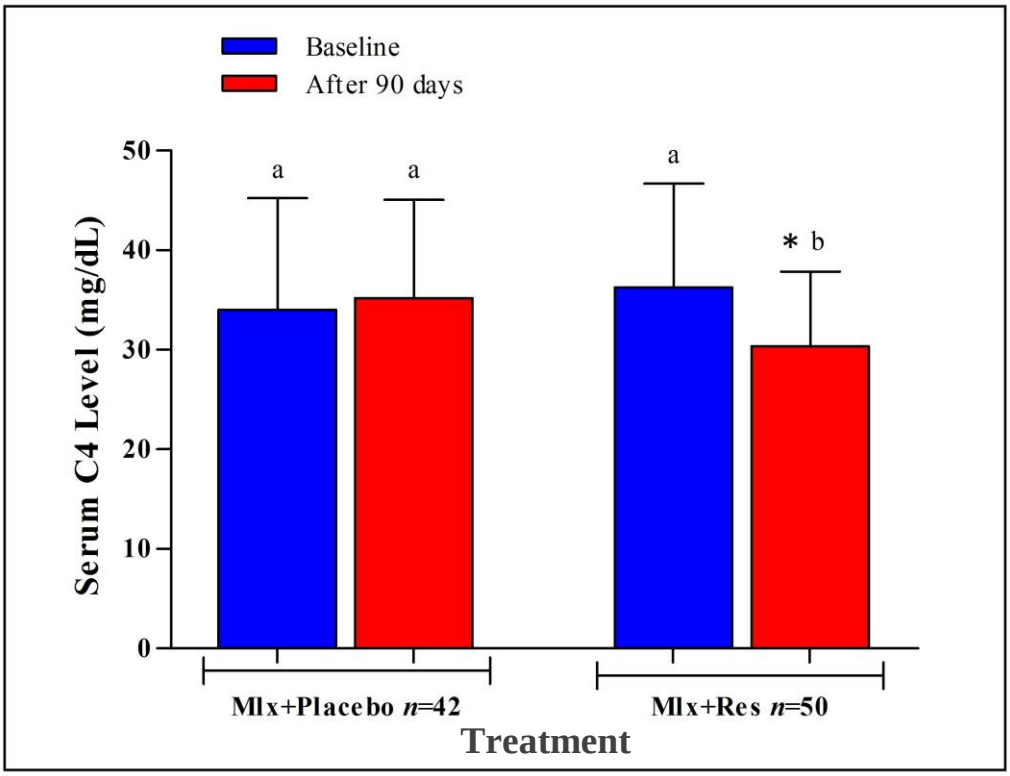


Figure 3-18: Effect of resveratrol, as an adjuvant with meloxicam, on serum level of complement C4 in patients with mild to moderate knee OA. *n*: number of patients; Res: Resveratrol; Mlx: Meloxicam; * significantly different compared with baseline value within the same group ($P<0.05$); values with different letters (a,b) are significantly different within the different groups ($P<0.05$).

3.3.2.4 Correlation between KOOS, WOMAC, and VAS scores and the inflammatory cytokine concentrations

The relationship between OA symptoms and the inflammatory biomarkers was relatively unexplored. In the present study, Spearman's correlation coefficient (non-parametric) test was used to examine correlations between the inflammatory biomarkers and the clinical variables through correlating a panel of serum biomarkers of inflammation (TNF-α, IL-1β, IL-6) with the OA clinical characteristics (pain, stiffness, physical function, function of daily living, sports and recreational activities and quality of life) which were measured by KOOS, WOMAC, VAS instruments in patients with mild to moderately painful knee OA.

3.3.2.4.1. Correlation of TNF-α with the clinical scores (KOOS, WOMAC, and VAS)

Figure 3-19-A shows the correlations between the levels of the inflammatory cytokines and the KOOS, WOMAC, and VAS scores in the Mlx+placebo group at the baseline. TNF-α exhibited a weak non-significant negative correlation with the total KOOS score (r= - 0.3; *P*= 0.11), while a weak non-significant positive correlation was reported with the total WOMAC (r= 0.3; *P*= 0.13) and VAS score (r= 0.3; *P*= 0.08) was seen in the same group at the baseline.

Similar association was obtained for the Mlx+Res group at the baseline (Figure 3-19-B). where serum TNF-α level also shows a weak non-significant association with the clinical symptoms of the patients at the baseline using the mentioned parameters. Total KOOS scores revealed a moderate to severe level of pain and physical impairments, Figure 3-19-B shows a negative weak correlation between serum level of TNF-α and

KOOS scores (r= -0.21; *P*= 0.13), while a weak significant correlation was predicted between serum level of TNF-α and WOMAC scores (r= 0.33; *P*= 0.02), and a very weak non-significant correlation was also obtained between the level of pain measured by VAS and serum TNF-α level (r= 0.15; *P*= 0.28).

The use of meloxicam in Mlx+placebo group for 90 days makes few changes in the association between serum level of TNF-α and the clinical scores as shown in Figure 3-19-C (placebo, day 90), where the KOOS score correlation coefficient shows a weak negative non-significant value with the TNF-α (r= -0.2; *P*= 0.3). Meanwhile, Spearman correlation analysis for WOMAC and VAS scores with serum levels of TNF-α provides a significant weak positive correlation (r= 0.4; *P*= 0.01, r= 0.44; *P*= 0.01) respectively.

Although there was a significant reduction in the serum level of TNF-α at 90 days in the groups Mlx+Res group compared with the baseline, the regression analysis for determining the correlation between such reductions with the improvement of the clinical scores showed non-significant weak correlation between these two variables. Figure 3-19-D depicted the correlation between the serum level of TNF-α and the total clinical scores of KOOS, WOMAC, and VAS (r= 0.21; *P*= 0.14, r= 0.17; *P*= 0.25, r= 0.03; *P*= 0.82) respectively.

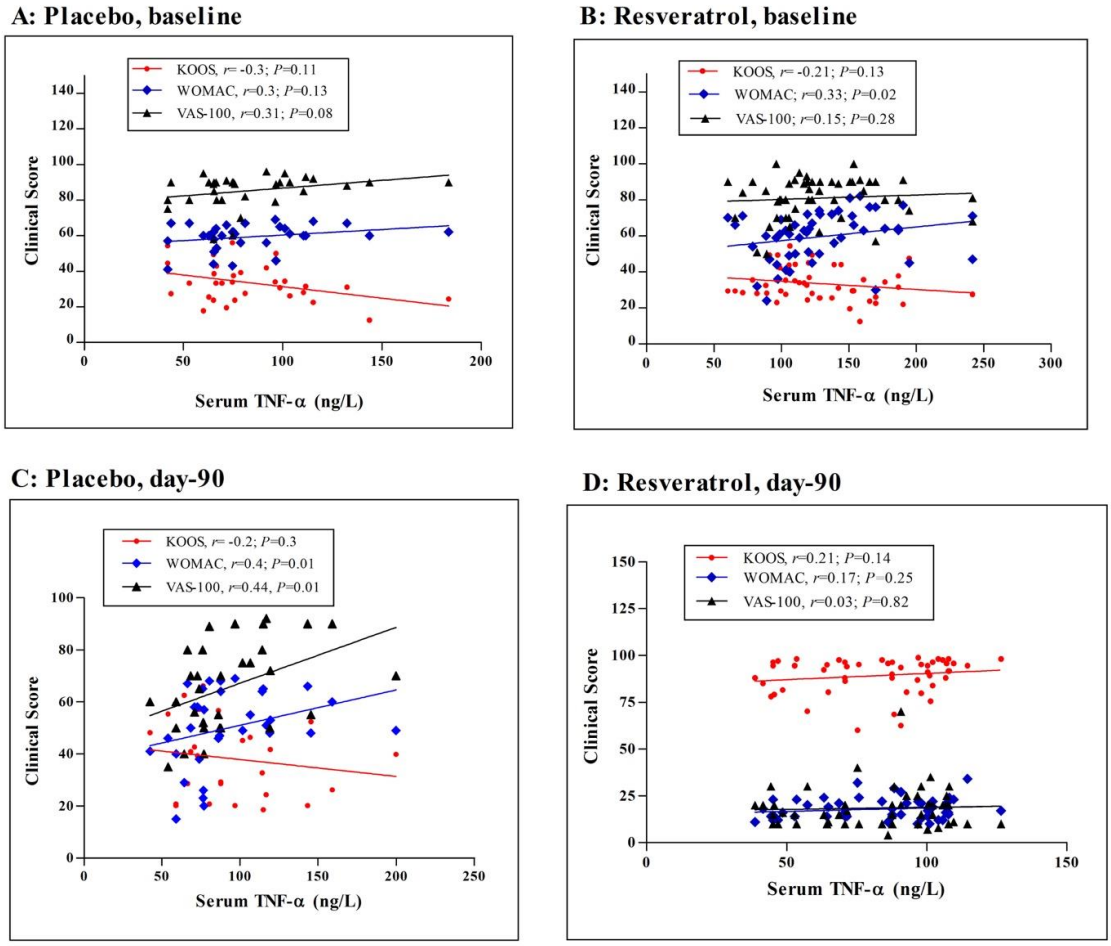


Figure 3-19: Spearman's correlation between serum levels of TNF-α and the clinical scores of KOOS, WOMAC and VAS in both treatment groups at baseline and after 90 days; *r*: Spearman's correlation coefficient; A and C: Mlx+placebo group, B and D: Mlx+Res group

3.3.2.4.2. Correlation between IL-1β and the Clinical scores (KOOS, WOMAC, and VAS)

To determine the association between IL-1β and the improvement in the clinical symptoms, regression analysis was performed using bivariate Spearman's correlation test. In figure 3-20-A, IL-1β exhibited a very weak and non-significant negative correlation with the total KOOS score (r= - 0.16; *P*= 0.39) in the Mlx+placebo group at the baseline, meanwhile a weak and non-significant positive correlation with the total WOMAC (r= 0.3; *P*= 0.09) and VAS score (r= 0.35; *P*= 0.05) was reported in the same group at the baseline.

The association between the inflammatory biomarkers and the clinical scores was also displayed for the Mlx+Res group at the baseline (Figure 3-20-B), where serum IL-1β level shows a non-significant and a very weak negative correlation with the pain, stiffness, physical function, function of daily activities and quality of life, which are reflected by KOOS, WOMAC, and VAS scores at the baseline (r= -0.11; *P*= 0.45), (r= -0.14; *P*= 0.34), (r= -0.13; *P*= 0.35) respectively. After 90 days, the Mlx+placebo group exhibited some non-significant changes in the Spearman's coefficient values (Figure 3-20-C). KOOS score regression coefficient value shows a weak positive non-significant correlation with the serum IL-1β level (r= 0.26; *P*= 0.15), while regression analysis for WOMAC and VAS scores with the serum level of IL-1β displays a very weak negative non-significant correlation (r= -0.06; *P*= 0.75 and r= -0.08; *P*= 0.66, respectively). Figure 3-20-D shows the regression analysis for Mlx+Res group after 90 days. Total clinical scores of each KOOS and WOMAC display a very weak negative and non-significant correlation with the serum level of IL-1β (r= -0.1; *P*= 0.47, r= -0.001; *P*= 0.99), while the VAS pain score showed a weak positive and non-significant

association with the serum level of IL-1β (r= 0.05; *P*= 0.72).

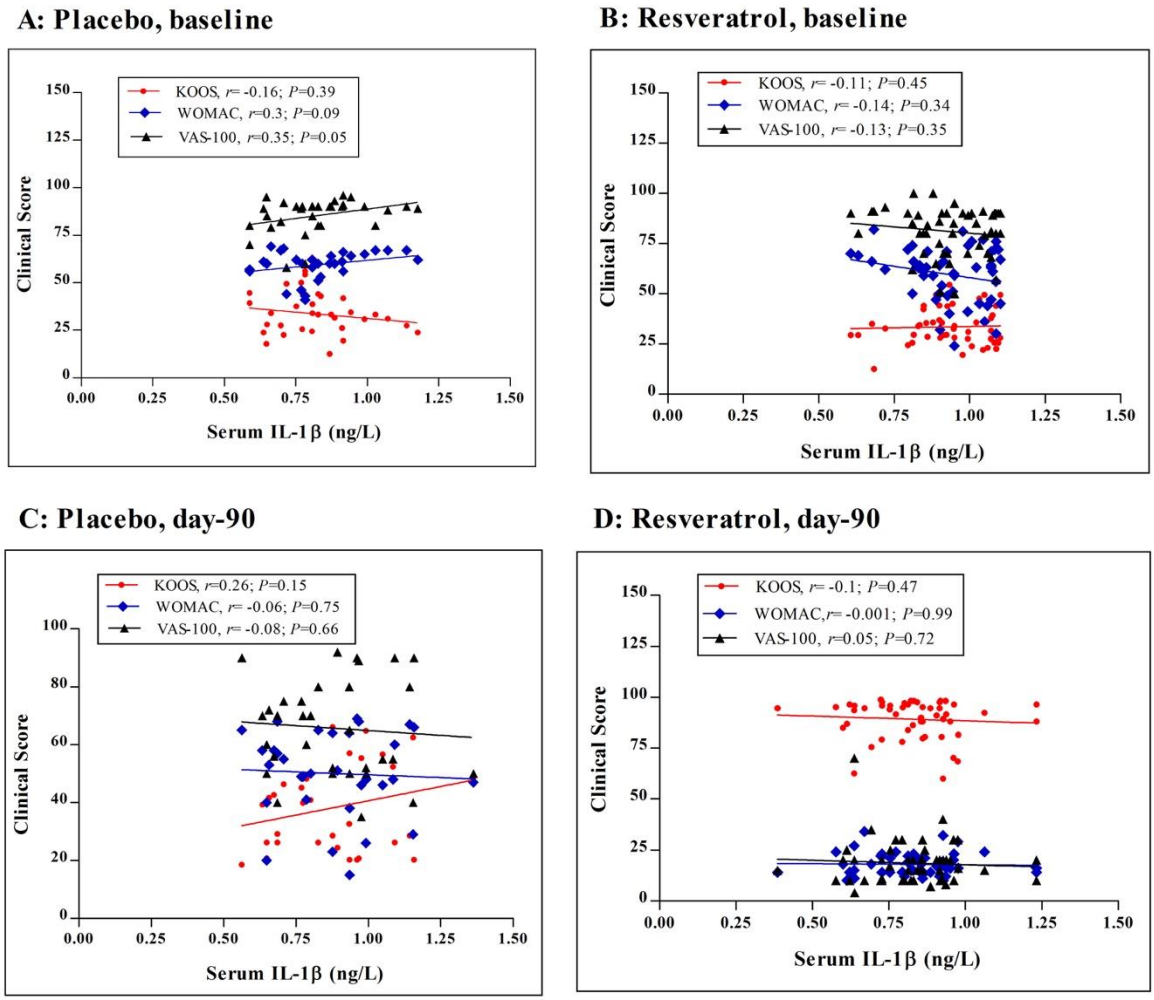


Figure 3-20: Spearman's correlation between serum level of IL-1β and clinical score of KOOS, WOMAC and VAS scores in both treatment groups after 90 days; *r*: Spearman's correlation coefficient; A and C: Mlx+placebo group; B and D: Mlx+Res group.

3.3.2.4.3 Correlation of IL-6 with the Clinical scores (KOOS, WOMAC, and VAS)

Figure 3-21 (A, B, C, and D) shows the regression analysis of the Mlx+placebo and Mlx+Res groups before and after treatment. In figure 3-21-A, adjusting the analysis for IL-6 levels revealed a very weak negative correlation between the serum levels of IL-6 and KOOS score at the baseline (r= -0.02; *P*= 0.9), while the correlation between the serum levels of this biomarker and the other parameters of pain and clinical assessment (WOMAC and VAS) was non-significant with weak positive values at the baseline (r= 0.1; *P*= 0.57, r= 0.15; *P*= 0.4). In comparison with the baseline, the regression analysis of the Mlx+placebo group after 90 days was relatively comparable (Figure 3-21-C), since KOOS and WOMAC scores displayed very weak positive and non-significant correlations with the serum levels of IL-6 (r= 0.1; *P*= 0.56, r= 0.11; *P*= 0.54); meanwhile, VAS exhibits a non-significant negative correlation with the serum concentrations of this cytokine (r= -0.25; *P*= 0.16). Figure 3-21-B and D describe a detectable correlation between the clinical symptoms and the IL-6 levels at the baseline and after 90 days. In both figures, the Spearman's correlation shows a relatively weak and non-significant association between IL-6 level and KOOS, WOMAC, and VAS scores at the baseline (r= 0.23; *P*= 0.11, r= 0.07; *P*= 0.63, r=0.02; *P*=0.9, respectively), and after incorporation of resveratrol as an adjuvant with meloxicam for 90 days (r= 0.13; *P*= 0.11, r= 0.27; *P*= 0.06, r=0.28; *P*=0.05, respectively) in spite of the significant and parallel reduction in the levels of IL-6 and the total clinical scores in Mlx+Res group at day 90.

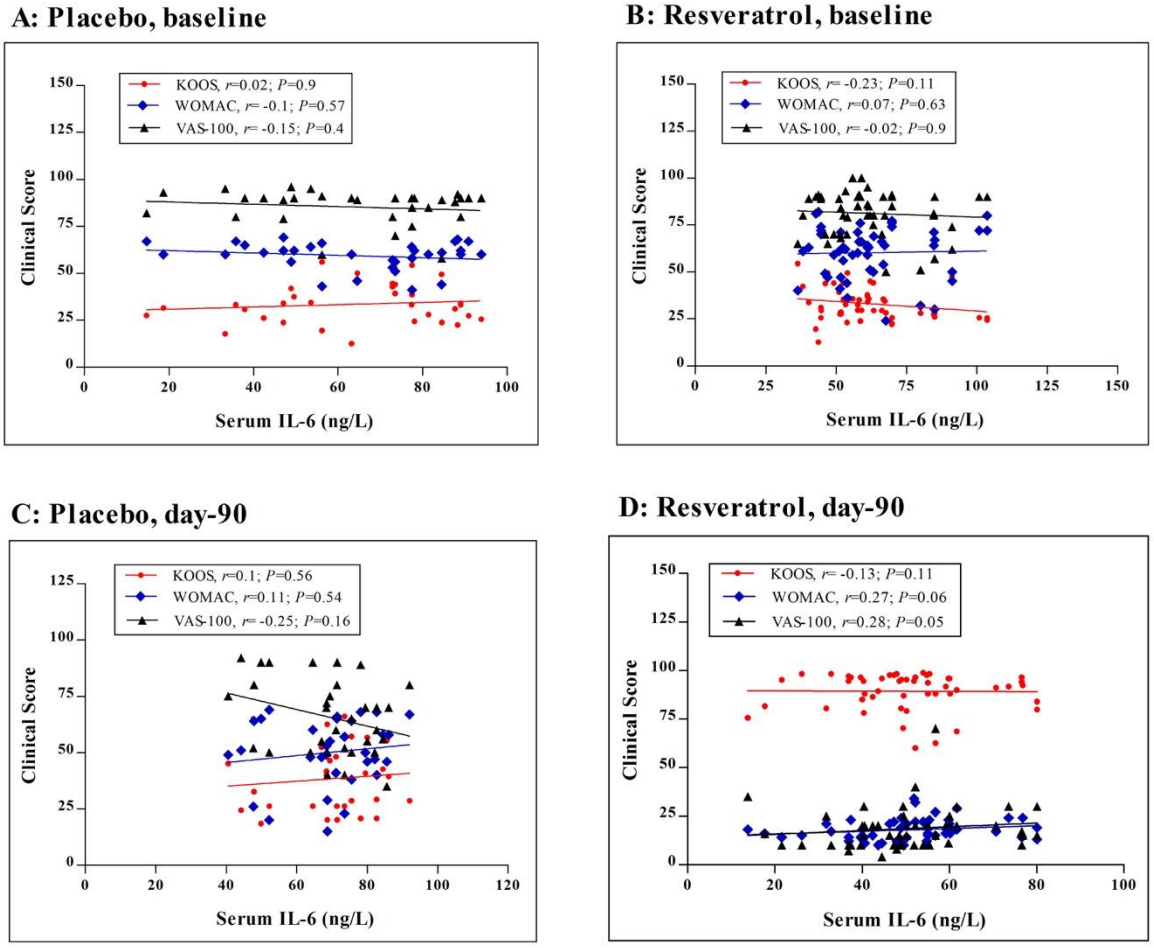


Figure 3-21: Spearman's correlation between serum level of IL-6 and clinical score of KOOS, WOMAC and VAS scores in both treatment groups after 90 days; *r*: Spearman's correlation coefficient; A and C: Mlx+placebo group; B and D: Mlx+Res group.

3.3.3. Safety Assessment

Comprehensive hematological measurements and clinical chemistry studies were carried out on blood samples obtained at baseline and at the end of the treatment to assess the safety and tolerability of resveratrol with in a study period.

3.3.3.1 Hematological Evaluation

As a part of the safety evaluation, hematological tests were performed to evaluate the deleterious effect of resveratrol as an adjuvant therapy on the complete blood count. The value of hematological parameters (Hb g/dL), Hct (%), RBC count, WBC count, and platelets count remained within the normal laboratory range at baseline and at the last day of the treatment (day 90). These findings demonstrate the safety aspect of resveratrol on the hematology profile in humans (Table 3-5).

3.3.3.2 Liver Function

To ensure the safety of the adjuvant use of resveratrol on the liver function, blood was analyzed for liver function markers at baseline and at the end of treatment. In patients of the Mlx-Res group, the serum levels of GOT, GPT and ALP were significantly reduced at day 90 as compared with the value at the baseline. Meanwhile the pronounced elevation of these enzymes have been observed in the Mlx+placebo treated group at day 90, except for serum-ALP level that displayed a significant reduction in both groups (Table 3-6).

Table 3-5: Effect of co-administration of resveratrol (Res) with meloxicam (Mlx) on the Hematological parameters of patients with knee osteoarthritis.

Parameters	Mlx+Placebo (*n*=42)		Mlx+Res (*n*=50)	
	Baseline	After 90 days	Baseline	After 90 days
Hb (g/dL)	13.2±1.3[a]	13.1±1.0[a]	13.2±1.5[a]	13.2±1.4[a]
Hct (%)	40.7±3.8[a]	40.0±3.1[a]	40.3±4.3[a]	40.0±4.0[a]
RBC count x10^6 (cells/µL)	5.0±0.5[a]	4.9±0.4[a]	4.9±0.4[a]	4.9±0.4[a]
WBC count x10^3 cells/µL	7.2±1.8[a]	6.9±1.5[a]	6.9±1.5[a]	6.8±2.1[a]
Platelets count x10^9 cells/L	251±59[a]	227±54*[a]	225±54[a]	224±53[a]

Values were presented as mean±S.D; *n*: number of patients; * significantly different compared with baseline values (paired *t*-test, $P<0.05$); values with different superscripts (a,b) within each parameter were significantly different (ANOVA, $P<0.05$).

Table 3-6 Effects of co-administration of resveratrol (Res) with meloxicam (Mlx) on the liver function markers in patients with mild to moderate knee OA.

Parameters	**Mlx+Placebo (*n*=42)**		**Mlx+Res (*n*=50)**	
	Baseline	After 90 days	Baseline	After 90 days
Serum GOT (U/L)	16.8±5.5	18.3±5.4	19.3±5.3	17.7±4.2*
Serum GPT (U/L)	12.0±5.1[a]	16.2±5.2[b]	16.7±3.8[b]	13.3±4.5*[a]
Serum ALP (U/L)	100.8±31.3	93.2±30.2*	96.0±29.1	86.1±23.3*

Values are mean±STD; *n*: number of patients; * significantly different from baseline in each group (paired *t*-test, $P<0.05$); values with non-identical superscripts (a,b) are significantly different (ANOVA, $P<0.05$); GOT: Glutamate Oxaloacetate Transaminase; GPT: Glutamate Pyruvate Transaminase; ALP: alkaline phosphatase.

3.3.3.3 Renal Function

Our present findings of the kidney function profile showed significant reduction in serum creatinine and urea levels (Table 3-7) in the resveratrol-treated group compared with baseline ($P<0.05$); meanwhile, serum urea significantly elevated in the Mlx+placebo treated group after 90 days.

3.3.3.4 Lipid Profile

Serum total cholesterol, low density lipoprotein cholesterol (LDL-c), high density lipoprotein cholesterol (HDL-c), triglycerides measurement was carried out on blood samples obtained at baseline and at the end of the treatment. Non significant alterations in the serum LDL-c and HDL-c levels were detected in both groups after 90 days of the treatment, as well as non-significant changes were observed in serum level of triglycerides and cholesterol in Mlx+placebo treated group. However, a statistically significant reduction has reported in the total cholesterol level and triglyceride level in Mlx-Res treated group ($P<0.05$) (Table 3-8).

3.3.3.5 Body Mass Index (BMI)

The use of resveratrol did not produce significant alteration in the body weight of the participants at each visit during the 90-day trial. Accordingly, no difference in body mass index (BMI) has been observed between resveratrol-treated group and the Mlx+placebo group (Table 3-9).

Table 3-7: Effects of co-administration of resveratrol (Res) with meloxicam (Mlx) on the renal function markers in patients with mild to moderate knee OA.

Parameters	**Mlx+Placebo (*n*=42)**		**Mlx+Res (*n*=50)**	
	Baseline	After 90 days	Baseline	After 90 days
Serum urea (mg/dL)	31.8±9.8	34.3±10.9	28.0±7.3	25.3±6.7*
Serum creatinine (mg/dL)	0.78±0.2	0.8±0.2	0.84±0.2	0.78±0.19*

Values are mean±STD; *n*: number of patients; * significantly different from baseline in each group (paired *t*-test, $P<0.05$).

Table 3-8: Effect of resveratrol (Res) as adjuvant with meloxicam (Mlx) on the serum lipid profile of patients with knee OA

Parameters	**Mlx+Placebo (*n*=42)**		**Mlx+Res (*n*=50)**	
	Baseline	After 90 days	Baseline	After 90 days
Triglycerides (mg/dl)	176.9±78.5	178.9±88.6	183.0± 87.5	158.1±73.2*
Cholesterol (mg/dl)	202.8±33.9	197.7±32.9	205.1±43.6	195.2±47.3*
LDL-c (mg/dl)	127.7±30.9	129.3±26.9	127.0±34.5	123.3±38.1
HDL-c (mg/dl)	45.8±12.2	46.0±9.0	46.6±12.5	48.7±12.7

Values are presented as mean±S.D; *n*: number of patients; * significantly different compared with baseline within the same group ($P<0.05$). LDL-c: low-density lipoprotein cholesterol; HDL-c: high-density lipoprotein cholesterol.

Table 3-9: Effect of resveratrol (Res) administration with meloxicam (Mlx) on Body Mass Index (BMI) of patients with knee osteoarthritis.

Parameter	**Mlx+Placebo (*n*=42)**				**Mlx+Res (*n*=50)**			
	Day 0	Day 30	Day 60	Day 90	Day 0	Day 30	Day 60	Day 90
Body Mass Index (BMI) kg/m^2	30.47±5.7	30.6±5.8	30.6±5.8	30.5±5.7	32.9±4.5	33.1±4.7	33.1±4.9	32.9±4.9

Values are presented as mean±SD; n: number of patients; no significant differences are reported between all values (P>0.05).

3.3.3.6 Vitamin D level:

To observe the expected effect of resveratrol on vitamin D level, serum vitamin D was measured at baseline and at the end of the treatment; vitamin D intake were allowed within a recommended daily dose for all participants; no significant change in the level of vitamin D has been observed in resveratrol-treated group with baseline value and with the placebo group (Figure 3-22).

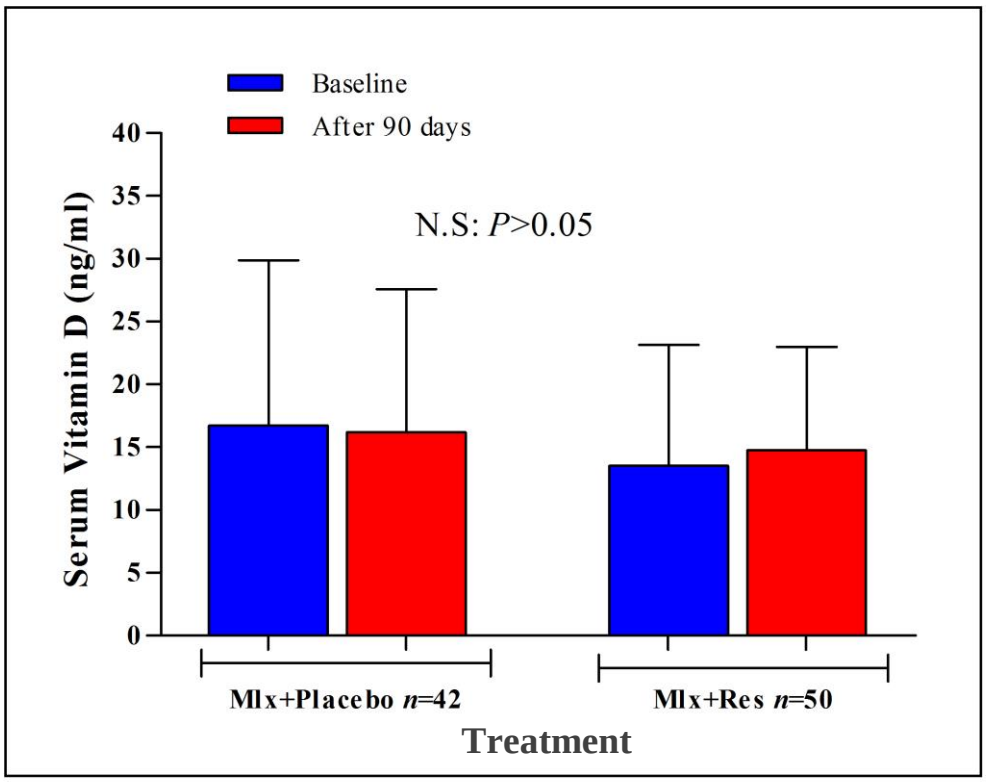


Figure 3-22: Effect of co-administration of resveratrol with meloxicam, on serum level of vitamin D in patients with mild to moderate knee OA. *n*: number of patients; Res: Resveratrol; Mlx: Meloxicam; NS: no significant difference reported (*P*>0.05).

3.3.7. Resveratrol adverse events monitoring

During the course of the treatment (90 days), a standard adverse-event case report form was utilized for each patient to record any adverse effect relevant to the medication protocol. No major adverse events were reported at each follow up visit. Resveratrol was well tolerated in all patients during the entire study period. Short-term follow-up of the clinical and physical examination, vital signs, blood pressure and heart rate, body weight alteration and occurrence of any other drug-related adverse events has revealed no serious adverse events attributed to the coadministration of resveratrol with meloxicam.

CHAPTER FOUR

DISCUSSION

CHAPTER FOUR

DISCUSSION

4.1. Impact of Resveratrol on the Clinical Symptoms of Knee OA

Despite the advances in understanding the mechanisms and treatment of OA-associated pain, many OA patients experienced various levels of acute and chronic pain, which impairs their daily living activities and quality of life [232]. Moreover, untreated OA pain may lead to serious negative impacts, like those reported in the baseline clinical data of the included patients (Table 3-1). The present study is the first randomized controlled clinical trial that provides clinical evidence regarding the effect of resveratrol, when administered with a NSAID, in improving the symptoms and pain associated with knee OA, which can afford the basis for the clinical implementation of oral resveratrol in knee OA pain. In the present study non-selective COX-2 inhibitor; meloxicam has been used because it is a well-recognized NSAID and widely prescribed to treat various types of arthritis due to its properties of reducing pain, swelling, and joint stiffness. The choice of meloxicam was based on its well known gastrointestinal safety, affordability of the dosage form, and the wider use by clinicians during daily clinical practice in our region. Since the symptoms of osteoarthritis were assessed mainly by pain, the outcomes of the present study were quantitatively evaluated by the severity of pain. Commonly, most studies in this regard used VAS-100 of pain or pain index in WOMAC, while others utilized different techniques including frequencies of painkiller use during the study or qualitatively identifies pain descriptors [233,234]. Although knee OA is a chronic musculoskeletal disorder, it is not life-threatening but may seriously influence the emotional, daily physical and social activities of

the patients. Accordingly, it impacts negatively the health-related quality of life. Meanwhile, an effective pharmacological treatment not only improves pain and mobility but also the quality of life as a whole. Due to the high prevalence of OA, its association with a high cost of health care induced by consumption of drugs and loss of workforce and productivity is highly recognized [235]. Therefore, there is an increasing interest in many pharmacological agents that may change the progression of OA, and thereby possibly delay or even prevent the requirement for surgical interventions through total joint replacement. To our knowledge, the present study is the first clinical trial on the effects of the herbal supplement resveratrol as an adjuvant therapy with meloxicam on the pain and associated dysfunction scores in patients with evidence of knee OA. Using KOOS, WOMAC, and VAS-100 questionnaire instruments as an approach, resveratrol supplementation results in significant decreases in total knee pain scores, and an improved disability index and overall health scores compared with placebo. These results support a role for a naturally derived supplement like resveratrol as an alternative or complementary treatment option in pain management, which may also improve the surrogate markers of disease progression in knee OA. These were most evident with pain score; however, we also observed such a type of changes in the KOOS and WOMAC symptom scores. In the present study, 90 days of using resveratrol resulted in a significant improvement of the clinical scores of OA pain and disability in the enrolled knee OA patients. Up to the present time no similar study available to compare our findings. However, in a most recently published clinical trial that uses resveratrol as an effective adjuvant therapy in the management of rheumatoid arthritis, resveratrol produces significant drop in the major clinical and biochemical biomarkers involve in the RA pathogenesis, which is consistent with the finding of our study [236].

Additionally, the clinical observations reported in our study were also in tune with many previously published data showing that resveratrol reduces pain, inflammation, and edema, and articular destruction in experimental models of arthritis [237,238]. Together with the previously reported data, the present finding supports the analgesic activity of dietary polyphenols in patients with mild to moderate knee pain, and be in tune with the results of many reported pilot studies regarding the adjuvant use of polyphenols and other supplements during treatment of inflammatory musculoskeletal disorders like knee OA and RA [239,240]. Although the exact mechanism behind resveratrol's impact on improving OA symptoms in clinical setting is not well described, a series of animal experiments conducted recently or in the past few years demonstrated that oral administration of resveratrol is capable to significantly prevents OA progression [181], and it also exhibits a potent analgesic effect in different animal models [238,241]. Despite the large output of preclinical studies dealing with the analgesic and anti-inflammatory activities of resveratrol, there is a relative lack of clinical trials to investigate the analgesic effect of resveratrol [148,242]. Currently, there are many updated therapeutic regimens available for managing OA, and they mainly focus on relieving pain and stiffness and improving physical function as important goals of therapy [243,244]. The etiology and progression of OA have been well understood to contain inflammatory basis during early stages of the disease [245] which might be associated with the tissue damage, especially the destruction of cartilage. In OA, many important mediators of the altered metabolism and increased catabolism of joint tissues were expressed, based on the cartilage damage and the increased circulating inflammatory factors including pro-inflammatory cytokines [246]. Moreover, inflammation and pain are firmly related to humans' health, and there are always a strong interplay

between pain and inflammation. Thus, inflammation is regarded as an important part of OA pathogenesis and relatively correlated with the deterioration of the clinical subscales targeted through using KOOS and WOMAC. In tune with previous reports, our findings support the analgesic effects of dietary polyphenols in adults with mild to moderate knee pain.

Assessment of OA pain has been largely determined by questionnaires, such as those based on the quality of life indicators. Meanwhile, radiographic imaging and physical examination have been used to stage the disease. In the present study, we used KOOS, WOMAC and VAS-100 questionnaires, which have been widely employed to assess knee pain, quality of life, and disability in patients with mild to moderate knee OA [247]. Based on the present findings, resveratrol consistently improved pain scores as observed across all the sub-scales of KOOS and WOMAC that evaluate constant, intermittent and total pain. It also lowers the associated scores reflecting a functional improvement. We observed a difference in pain scores assessed by VAS-100 in our participants, which could be explained by the visual expression of general pain intensity used in VAS-100 scoring compared to the knee OA-specific magnitude of knee pain numerically rated by the KOOS and WOMAC questionnaires. Other notable finding of the present study is the high level of agreement between clinical outcomes when assessed by either KOOS or WOMAC in Iraqi patients with knee OA (Figure 3-12), which was in tune with previously reported data that indicates KOOS as instrument with improved validity and may be at least as responsive as WOMAC [248].

4.2 Biochemical Changes and Anti-inflammatory Action of Resveratrol in OA

It has been implied that development and progression of OA involve inflammation even in the primary phases of the disease. The excessively expressed inflammatory mediators such as pro-inflammatory cytokines are critical factors of the disturbed metabolism and enhanced catabolism of the joint tissue involved during OA pathogenesis [246]. Among these mediators, IL-1β, IL-6, TNF-α, hs-CRP and complement proteins have a key role in inducing apoptosis, inflammation, and matrix destruction through stimulating proteolytic enzymes secretion from the chondrocytes and synovial fibroblasts [67,249]. Following these biochemical changes, the early inflammatory phase of OA occurs [249] since inflammation is an important source of pain [250], and it is the main predictor of cartilage loss [251]. Therefore, it seems logical to propose that supplements with defined analgesic and anti-inflammatory activities may be of benefit in the management of OA.

The primary outcome of the present study was to evaluate the clinical effect of resveratrol in patients with knee OA. Simultaneously, changes in serum level of the inflammatory biomarkers of the disease were investigated as a secondary outcome. This may provide a potential correlation between severity of the disease and the levels of the evaluated biomarkers. The clinical improvement in KOOS, WOMAC score and VAS may be subjective; however, the levels of the evaluated biomarkers may represent the status of the anti-inflammatory activity. It has been established that there is a rise in these markers in various inflammatory diseases, and lowering of these markers was strongly correlated with the disease severity and/or progression [252]. Based on the principal findings of the present study, administration of 500 mg/day resveratrol as an adjuvant with meloxicam for 90 days reduces the pain severity in patients

with mild-to-moderate radiological evidence of knee OA, in addition to the significant decreases of the serum levels of many inflammatory mediators like TNF-α, IL-1β, IL-6, hs-CRP and complement proteins compared with placebo. In many experimental models of OA, resveratrol was found to significantly decrease serum levels of many inflammatory mediators [180,253]. However, no clinical study yet has determined the effect of resveratrol on serum levels of the inflammatory mediators in knee OA patients. Thus, the available clinical data on the influence on the inflammatory mediators are scarce in previous reports of using dietary supplements for the management of OA. To our knowledge, this study is the first pilot clinical trial that focuses on the effect of resveratrol on the pain severity and its association with the expression of the inflammatory biomarkers in patients with mild to moderate knee OA. The presented data support the suggested role for many natural polyphenols, such as resveratrol, as a complementary treatment option in the management of pain and inflammation, the markers of disease progression in knee OA. Accordingly, it can be considered as an effective "add-on" treatment and/or as an alternative to the currently used pharmacological agents for OA. The pharmacological effects of resveratrol have been well-defined in various kinds of literature. Referring to its well-recognized cardioprotective and neuroprotective properties [138,254] the clinical benefits of resveratrol can be attributed to its antioxidant, anti-apoptotic and anti-inflammatory properties [188,255]. Various studies have been done on the clinical efficacy of dietary polyphenol supplementation in knee OA [256,257,258,233], only a few of them presented data on the effects on the inflammatory biomarkers. In the present study, 90 days of treatment with resveratrol resulted in significant decrease in the serum levels of TNF-α, IL-1β, IL-6, and hs-CRP of knee OA patients, and seems to be consistent with the anti-inflammatory effects of many dietary polyphenols

in OA management. These clinical findings were in tune with data showing that blueberry and raspberry extracts lowered the pain, inflammation, and articular destruction in experimental arthritis [259,260]. In addition to the pro-inflammatory cytokines, complement activation also plays a significant role in the initiation of cartilage destruction and synovitis [261] and this surrogate marker was found also to be improved in the present study. During pathogenesis of OA, the chondrocytes synthesize the complement components and the process can be up-regulated by many pro-inflammatory cytokines such as IL-1β and TNF-α [262]. Dysregulation of complements in the synovial joints has a key role in the pathogenesis of OA [34].

Moreover, C-reactive protein is a marker of low-grade systemic inflammation that can be up-regulated by the excessive liberation of IL-6 [263]. In early OA, elevated serum levels of high sensitivity C-reactive protein (hs-CRP) can be detected [264], and currently hs-CRP, as a marker of low-grade systemic inflammation, was correlated with pain severity in OA [265] while low-grade synovitis is now recognized as a common finding in OA [50]. In the present study, there was no significant difference between the two groups once assigned for the study in terms of the hs-CRP and the complement proteins (C3 and C4) prior to the intervention. However, after 90 days of treatment, the serum concentrations of C3, C4, and hs-CRP were decreased significantly in the resveratrol-supplemented group. Resveratrol in combination with meloxicam significantly lowered serum levels of the complement proteins C3 and C4 ($P<0.05$) after 90 days of treatment compared with the baseline values. Moreover, this combination produced a significant decrease in serum hs-CRP levels compared with both the baseline values and the levels of the corresponding group that used meloxicam alone ($P<0.05$). To date, there is no clinical trial on the efficacy of resveratrol in

alteration of inflammatory biomarkers such as C3, C4, and hs-CRP. However, many clinical reports on non-musculoskeletal diseases demonstrated the value of this dietary polyphenol in attenuating these inflammatory biomarkers [266].

The exact mechanism to elucidate how resveratrol could decrease knee OA associated pain is not fully evaluated. It is believed that it may be attributed to cumulative effects including the chondroprotective anti-inflammatory activities, in addition to its ability to reduce cartilage destruction and loss of matrix proteoglycan content in the cartilage tissue with a decrease in the apoptosis rate of the chondrocytes [190]. Resveratrol also has an inhibitory role on the activated NF-κB [185]. Furthermore, resveratrol is a direct inhibitor of COX-2 that produces leukotrienes and prostaglandins, the pro-inflammatory lipid mediators responsible for mediation of pain sensation [267]. Accordingly, the anti-inflammatory effect of resveratrol can be clearly explained based on resveratrol's ability to target crucial molecules of inflammation such as COX-2 and NF-κB [268]. While the pharmacological action of resveratrol has been an area of controversy, most evidence now points to AMPK as the major target of resveratrol. Previous in vitro findings provided novel evidence linking resveratrol's anti-allodynic effects in the periphery to ERK and mTOR inhibition via activation of AMPK [269].

In addition to the previous reports, the recently emerged evidence that resveratrol has the ability to attenuate production of COX-2 and PGE2 in the rat model of adjuvant arthritis might be the most convincing mechanism, among others, to explain its pain relieving action [270]. Similar to most polyphenols, we cannot rule out the intrinsic antioxidant capacity of resveratrol which might contribute to its anti-osteoarthritic properties. It also induces the expression of many antioxidant enzymes [271] making it difficult to interpret the precise contribution of each

mechanism to an overall reduction in pain intensity of the OA patients enrolled in the present study.

4.3. Correlation between the Clinical Scores of KOOS, WOMAC, and VAS with the Inflammatory Biomarkers

In this part of the present study, we examined whether the improvement of the clinical symptoms by the use of resveratrol as an adjuvant therapy with meloxicam is correlate with the reduction in the serum level of the pro-inflammatory biomarkers in participants with mild to moderate painful knee OA. We analyzed the correlation between TNF-α, IL-1β, and IL-6 as pro-inflammatory biomarkers in the serum with the pain and OA clinical manifestations (KOOS, WOMAC and VAS scores) in order to support the pivotal role of the biomarkers in interventional studies.

Currently, many studies provide pieces of evidence on the potential correlation between the circulating inflammatory cytokines and proteins that reflect changes in joint remodeling during OA associated pain [68]. Many authors have described the association of disability, measured by the WOMAC scores, with the elevated levels of pro-inflammatory cytokines [272,273]. In particular, some researchers identified the correlation of higher levels of serum cytokines like IL-6, TNF-α with knee OA [272]. Our major findings indicated that both baseline and post-treatment levels of TNFα, IL-1β, and IL-6 were weakly associated with the obtained clinical relief of knee pain assessed by the total KOOS, WOMAC and VAS scores. Generally, studies that have investigated the potential mechanisms of joint pain relief in the intervention trials are lacking. However, many *in vitro* studies documented that inflammation plays a substantial role in the development of pain and the relevant clinical manifestation in OA. One of the sources of knee pain in OA is

believed to be related to local chronic inflammation of the knee joints, which involves the production of inflammatory cytokines such as TNF-α, IL-1B, and IL-6. Currently, there are limited clinical investigations on the role of inflammatory markers in knee pain severity, development or progression. Several cross-sectional studies have found inconsistent associations between knee pain and IL-6, TNF-α and CRP [274,275,265]. It has been reported that the locally expressed pro-inflammatory cytokines, such as IL-1B, TNF-α, and IL-6, produced by inflammatory cell infiltrated to the synovium were detectable even in early OA [250,251,276 ,277] and they were associated with the radiographic OA and knee cartilage loss in middle-aged to older people with or without knee pain. These findings suggest that the inflammatory factors may play a role in the structural changes in OA. Although our finding reported a significant reduction in the serum levels of TNF-α, IL-1B, IL-6 alongside with a significant improvement in the clinical scores of each KOOS, WOMAC and VAS post-treatment compared with the baseline values, the regression analysis using Spearman test displays a very weak and non-significant correlation between those pro-inflammatory biomarkers and the reported relief of pain and improvement of the clinical manifestations. It is therefore likely that there is involvement of some other proximate causes or mechanisms alongside with inflammation associated with the pathogenesis of osteoarthritis. Furthermore, resveratrol may engage other target molecule rather than pro-inflammatory cytokines to alleviate OA associated pain as reported by Tillu D *et al* who stated that resveratrol activates AMPK to attenuate ERK and mTOR signaling, the two important pathways involved in the sensitization of peripheral receptors [269]. In this regard our finding seems inconsistent with that of a longitudinal study conducted by Stannus et al [250] who have reported that baseline and change in hs-CRP and TNFα over 2.7 years were

associated with the increases in knee pain assessed by the total WOMAC score.

Moreover, it has been previously reported that serum IL-6 levels were not significantly associated with the improvement of WOMAC scores [278] while significantly associated with the pain severity on a VAS scale [279]. In many randomized clinical trials, IL-6 levels have been found to decrease alongside with the decreases in pain score and improvements in physical function [280,281] Similarly, baseline IL-6 levels have been associated with a change in pain while standing [250]. Meanwhile, Penninx et al reported that in patients with knee OA, serum levels of TNF-α were not associated with WOMAC knee pain, stiffness and radiographic scores [275]. However, in another clinical study, TNF-α was significantly associated with the WOMAC score while IL-6 had a weakly significant correlation with the subscale of stiffness. The results of these studies suggest that these cytokines play a role in the pathogenesis of synovitis in osteoarthritic knees in different ways, where TNF-α is well correlated with pain, whereas IL-6 is correlated with joint function [282]. Also another meta-analysis study provides evidence showing that a change in cytokine levels including TNF-α is correlated with pain relief in patients with painful total knee arthroplasty who had a clinically meaningful pain relief after an intra-articular injection [283]. Moreover, assessment of the effects of intraarticular hyaluronic acid treatment in patients with knee OA, in which IL-1β has been used as a marker of efficacy of intervention, a moderate negative correlation between changes in synovial fluid IL-1β and a reduction in pain severity over a 6-month period was observed [284]. To our knowledge, the present study will be the first clinical trial that provides a correlation data between cytokines and clinical scores obtained by different types of validated instruments, including KOOS. These inconsistencies between the present study and

the previous studies are most likely due to variations in sample size, study population, design, and a short period of follow-up, furthermore, in the present study the associations were not adjusted for age, sex, BMI, and other potential confounding factors.

4.4 Safety and Tolerability of Resveratrol

The increasing demand for safe and effective alternative therapy to treat OA can be addressed by identifying promising nutraceutical candidates. In this regard, resveratrol has demonstrated a great potential in preventing and/or slowing the breakdown of articular cartilage and extracellular matrix in preclinical studies [180]. In human articular chondrocytes, resveratrol also offers an anti-apoptotic, anti-inflammatory and antioxidant effects [141,148]. Similarly, other preclinical study demonstrated potent anabolic and anti-catabolic potential of resveratrol in human adult articular chondrocytes via the inhibition of matrix-degrading enzymes [285].

In light of these promising preclinical findings and previously reported evidence, resveratrol appears to be a potential anti-osteoarthritic agent. The present trial will be the first study to investigate the potential therapeutic use and safety of resveratrol for the management of OA, based on short-term follow up of a group of patients with mild to moderate knee OA. In the present study, the patients in the resveratrol-treated group were administered 500mg resveratrol/day for 90 days, and to determine the safety of resveratrol supplementation we screened blood of the patients for many safety-related biomarkers. We confirmed the safety of resveratrol by carrying out a 3-month follow up monitoring of the liver and renal functions, in addition to lipid profile and hematological examination. Additionally, clinical examination was performed for each patient to observe any deleterious effect of the orally administered

resveratrol. The clinical and physical examination was carried out by the designated site investigator and frequency of occurrence of adverse events, as a measure of assessment of safety, in each trial group was recorded throughout the study period. No major adverse effects have been recorded. This result may be considered as an additional parameter supporting resveratrol tolerability and safety.

The outcomes of the present study may help to determine the safety of short-term resveratrol treatment in OA patients and provide an extension and confirmation to the previous clinical observations conducted in the healthy volunteers or cancerous individuals, which assessed the safety of resveratrol utilizing higher and lower doses for different duration. Taken together, the findings of the previous studies suggest that the adverse reactions to resveratrol in doses of less than 1,000 mg/day are uncommon or mild, and short-term resveratrol supplementation is well tolerated. These findings could be comparable with the results obtained in the present study which used resveratrol in a dose less than 1000 mg/day [206,134,286]. In the present study resveratrol did not change the hematological parameters of the patients after 3 months. Thus, no deleterious effect of resveratrol on blood has been observed. The present study was consistent with the findings of Timmers *et al* who observed no hematological, coagulation, general biochemical alteration in the obese participants who used 150 mg of pure resveratrol for 30 days [210]. A previous report also verified the safety of resveratrol in an experimental animal model, where the administration of high-dose resveratrol did not change the hematological parameters in rats [287]. Furthermore, the protective effect of resveratrol on the fluoride-induced hematological alterations in animal models was also determined by Atmaca *et al*. [288]. Renal function tests demonstrated a significant reduction in the level of serum creatinine and urea in Mlx+Res group that may represent the

nephroprotective effect of resveratrol, as shown previously in experimental model of cisplatin induced-kidney injury [289]. To ensure the safety of resveratrol (adjuvant with meloxicam) on the liver function, the serum has been screened for GOT, GPT, and ALP activities as indicators of hepatic integrity. The results displayed a significant reduction in enzyme activities in the Mlx+Res treated group; while the pronounced elevation of these enzymes has been observed in the Mlx+placebo treated group except for ALP at day 90. This finding was in tune with a study that defines resveratrol as a safe supplement without marked harmful effects [287]. Furthermore, the hepatoprotective role of resveratrol was reported in different models of hepatitis, which linked this action with its antioxidant property. On the other hand, hepatic steatosis was reduced by resveratrol through modulating the insulin resistance and lipid profile in animal models [290]. In our study, no significant alteration has been observed in the lipid profile of both groups, except for total cholesterol and triglyceride levels, which display a significant reduction in resveratrol-treated group; most of the enrolled patients have normal lipid profile at the baseline and resveratrol has preserved this range during the entire period of the study. This finding is consistent with the results of several clinical trials that show the unaltered levels of plasma HDL-c or LDL-c levels [291,292,293] the classical markers in the evaluation of the cardiovascular risks.

The tolerability and safety of resveratrol were demonstrated by many clinical studies, including those enrolled cancer patients who administered high doses of resveratrol [205,294].

The 90-day oral administration of 500mg/day resveratrol did not affect the body weight or the BMI of the patients treated with resveratrol; this result was in agreement with the *in vivo* studies conducted by Turner *et al*. and Juan *et al*. [295,287]; they reported no alteration in the body

weight due to resveratrol intake in growing rats. Moreover, it has been reported that the use of resveratrol monotherapy in obese individuals did not show a significant difference compared with placebo in term of weight reduction [296] On the other hand, our finding was inconsistent with that demonstrated by Asghari *et al* in which significant reductions in weight and BMI were found in the resveratrol-treated NAFLD patients compared to the placebo [297].

Taken altogether, there is currently insufficient evidence to support the recommendation of resveratrol supplements in the management of obesity, and most of them indicated no significant change in the BMI or body weight [298].

Previous pre-clinical, epidemiological and clinical reports have hypothesized that resveratrol and vitamin D have important potential interactions that could seriously affect the biological system [221]. In this regard, in order to quantitatively evaluating the possibility of such important interactions between resveratrol and vitamin D, we measured serum vitamin D level before and at the end of the interventional period, and vitamin D intake was permitted in our study within the recommended daily allowance. The results showed no alteration in the level of vitamin D after resveratrol treatment for three months.

4.5 Limitations and Strength of the Study

Based on the strengths and limitations of the utilized pain assessment tool, the use of more than one tool was highly suggested to cover the multi-dimensional aspects of OA pain. The present study has many limitations that may affect interpretation and generalizability of its findings. These include the small sample size (n=110) and short duration of treatment (3 months). The included patients had mild-to-moderate radiographic knee OA at baseline (Kellgren–Lawrence scores of < 3) and relatively moderate

knee pain. Whether patients with more severe knee OA (Kellgren–Lawrence score of 4) and severe pain would benefit from resveratrol intervention requires further investigation. Moreover, other accurate and reliable markers of OA pathogenesis, such as biomarkers of collagen degradation, aggrecan metabolites, and other non-collagenous proteins were not evaluated. In addition to that, the present study did not include assessment of these markers in the synovial fluid, which may provide more accurate determination of changes specific to the knee OA. Additionally, we did not evaluate the radiological outcomes at the end of the trial.

The strengths of the present study include a randomized, placebo-controlled study design, which accounts most of the variations in parallel arm studies. Also, based on the treatment of the control group with a placebo formula that matched the resveratrol dosage form in sensory qualities, we were able to keep the patients and evaluators blinded to the identity of the test agents. In addition, we excluded knee OA patients who were taking any other kind of supplements, like antioxidant vitamins, fish oil and other herbal supplements for any purpose.

The other strong point of the present study is that this clinical trial included measurement of both the clinical benefit and safety of resveratrol which provides a convincing evidence for an optimal therapeutic application of resveratrol in a musculoskeletal disorder like knee OA. Using rescue medication, which is not allowed in our study, may be prone to bias. However, the similarity of the type and amount of pain medication (Meloxicam) used between the two groups make this unlikely.

4.6. Conclusions

According to the results of the present study, we conclude:

1. The patients with mild to moderate painful knee OA who used 500 mg resveratrol as an adjuvant therapy with meloxicam daily for three months showed significant improvements in pain scores and physical function.

2. Co-administration of resveratrol with meloxicam significantly decreases the serum levels of the inflammatory biomarkers IL-1β, IL-6, TNF-α, hs-CRP and the complement proteins C3 and C4, compared with the administration of placebo, and provide a relatively convincing evidence for the anti-inflammatory action of resveratrol in mild to moderate knee OA cases.

3. The present study demonstrates weak and non-significant correlations between serum biomarkers and the clinical outcome scores in patients with mild to moderate OA knee pain.

4. Adult patients with mild to moderate knee OA may benefit from the supplementary intake of resveratrol with their conventional therapy.

5. The daily use of resveratrol, as an "add-on" treatment with meloxicam, was superior in terms of safety, tolerability and efficacy to the use of meloxicam alone for the treatment of pain and improvement of physical function in patients with knee OA. However, larger sample size and longer duration studies are required to validate the resveratrol as a complementary OA treatment.

4.7. Recommendation for future work

Further studies are required for:

1. Long-term evaluation of the clinical efficacy of resveratrol in severe cases of knee OA.
2. Evaluation of the chondroprotective effect of resveratrol utilizing more specific and sensitive cartilage biomarkers, such as collagenous and non-collagenous biomarkers in both serum and synovial fluid.
3. Evaluation of the analgesic and anti-inflammatory effects of resveratrol in other musculoskeletal inflammatory degenerative diseases such as rheumatoid arthritis and fibromyalgia.
4. Evaluation of the clinical efficacy of resveratrol in certain gynecological disorders-associated pain such as primary and secondary dysmenorrhea.
5. Evaluation of the adjuvant effect of resveratrol in other orthopedic conditions such as fracture healing and repair.
6. Multicenter studies to assess the effect of resveratrol in different groups of patients.

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APPENDICES

Appendix A

University of Sulaimani
College of Medicine
Pharmacology Department

CONSENT FORM

This consent form is for patients (female and male) with knee osteoarthritis admitted to Shar teaching Hospital and Rehabilitation and Rheumatology center in Sulaimani and who we are inviting to participate in research on the specific herbal extract (Resveratrol) formulated as capsules by the researcher, the title of the research is:

Adjuvant Use of Resveratrol in Patients with Knee Osteoarthritis: Comparative Study with Meloxicam

Below are the points that declared by the participant:

1. I agree to take part in the above study.
2. I confirm that I have understood my role for the above study and have had the opportunity to ask questions.
3. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving reason.
4. I have been informed about the duration of the research and the relevant follow up (3 months), time of visits and amount of blood that will be taken from my arm during each visit.
5. The researcher clarified the unwanted effects of the tested drug that I will be taken for this research.

Name of Participant	Date	Signature
Name of Researchers	Date	Signature

Appendix B

زانیاری نەخۆش و فۆرمی پەسەندکردن

بە ڕێز .....

پێخۆشحالین بە بەشداربوونت لە لێکۆلینەوەیەکی تایبەت لەسەربەکارهێنانی سەرچاوەی گیایی (ریسڤیراترۆڵ) وەك یاریدەدەری دەرمانی لە و نەخۆشانەدا کە هەوکردنی جومگەی ئەژنۆ یان هەیە : لێکۆلینەوەیەکی بەراوردکاری یە لەگەڵ دەرمانی میلۆکسیکام .

پێش ڕازی بوونت بە بەشداری کردن لەم لێکۆلینەوەیە پێویستە ئەم زانیاری یانەی خوارەوە بە ووردی بخوێنیتەوە :

- پرۆژەی ئەم لێکۆلینەوەیە پەسەند کراوە لەلایەن لیژنەی زانستی و ئیتیکی کۆلیجی پزیشکی زانکۆی سلێمانی.
- لەم لێکولینەوەیەدا نزیکەی سەدو پە نجا (130) نەخۆش بەشداری ئەکات .
- بەشداربوونت لەم لێکۆلینەوەیە خۆبەخشی یە , واتە ئەتوانیت داواکاری یەکەمان پەسەند نەکەیت.
- ماوەی بەشداربوونت لە م تویژینەوەیە سێ مانگە.
- ئەتوانیت پرسیار بکەیت لەسەر هە ر شتێك کە لە لات ڕوون نی یە و ئاشکرا نی یە .
- مەبەست لە ئە نجام دانی ئەم تویژینەوەیە هەلسەنگاندنی توانای کاریگەری سەرچاوەی گیایی (ریسڤیراترۆڵ) ە لە و نەخۆشانەدا کەداخورانی کرکراگە و هەوکردنی جومگەی ئەژنۆیان هەیە بە هیوای کەم کردنەوەی هەوکردن و نارەحەتی لە جومگەکانیاندا.
- لە کاتی بەشداربوونت لە م تویژینەوەیەدا ,ئەم سەرچاوە گیایی یە و دەرمانی تر وەك میلۆکسیکام یان میلۆکسیکام بە تەنها وەردەگریت بۆ ماوەی سێ مانگ لە ژێر چاودێری تیمی لێکۆلەرەوەکان و هەموو مانگێك بەدوواداچوون و شیکاری تاقیگەییت بۆ دەکرێت لە ڕێگەی سەردانی کرد نمان .
- ئەم سەرچاوە گیایی یە زیانی لابەلای لەو بڕەدا کە بەکاردێت لەم لێکۆلینەوەیەدا زۆر کەمە کەلەوانەیە سك چوونێکی کەم بکات یا ئارەزووی خواردن کەم بکاتەوە.

ناوی بەشداربوو:

واژووی بەشداربوو:

ناوی تویژەر : بشری حسن معروف

بەروار:

PUBLICATIONS:

1. Bushra Hassan Marouf, Saad Abdulrahman Hussain, Ziyad Serdar Ali, Runj Simko Ahmmad. Clinical efficacy of resveratrol as adjuvant with meloxican in treatment of knee osteoarthritis patients: A double-blind, randomized, placebo-controlled trial. It has been accepted for publication in Brazilian Journal of Pharmaceutical Sciences (listed in Thomson Reuters -Clarivates Analytics), Impact factor 0.47, Vol 54, No: 4, 2018.

2. Bushra Hassan Marouf, Saad Abdulrahman Hussain, Ziyad Serdar Ali, Runj Simko Ahmmad. Resveratrol Supplementation Reduces Pain and Inflammation Experience in Knee Osteoarthritis Patients Treated with Meloxicam: A Randomized Controlled Study. It has been accepted for publication in Journal of Medicinal Food (listed in Thomson Reuters-Clarivates Analytics), Impact Factor 1.955, Vol 21 No 12 ,December, 2018
3. Bushra Hassan Marouf, Saad Abdulrahman Hussain, Ziyad Serdar Ali, Runj Simko Ahmmad. Effect of Resveratrol co-administration with Meloxicam on the lipid-hematological profile, liver and kidney functions in Knee Osteoarthritis patients in journal Clinical intervention in aging it has been accepted for publication in Clinical interventions in aging, (listed in Thomson Reuters -Clarivates Analytics) Impact factor: 2.505

PRESENTATION:

ORAL:

- Seminar on the PhD-progress work: Nutraceuticals as an alternative medicine in Osteoarthritis.

هەرێمی کوردستانی عێراق
وەزارەتی خوێندنی باڵاو توێژینەوەی زانستی
زانکۆی سلێمانی
کۆلیجی پزیشکی

کاریگەری ریسقیراتۆر وەك یاریدەدەری دەرمانی لەو نەخۆشانەدا کەداخورانی کرۆکراگە و هەوکردنی جومگەی ئەژنۆیان هەیە : لێکۆلینەوەیەکی بەراوردکاری یە لەگەل میلۆکسیکام

لێکۆلینەوەیەکە پێشکەش بە بەشی دەرمانزانی و خوێندنی باڵای کۆلیجی پزیشکی/ زانکۆی سلێمانی کراوە وەك بەشێك لە پێداویستیەکانی بە دەستهێنانی بڕوانامەی دکتۆرا لە دەرمانزانی و ژەهرزانی

لە لایەن
بشری حسن معروف
ماستەر لە دەرمانزانی

سەرپەرشتیار
پ.د. سعد عبدالرحمن حسین
دکتورا لە دەرمانزانی و ژەهرزانی

گەلاوێژ 2018

پوختە

پێشەکی و ئامانجی توێژینەوەکە: داخورانی جومگەی ئەژنۆ یەکێکە لە نەخۆشی یە درێژخایەنەکانی جومگە کە بە هێواشی داخورانی کرکراگەی جومگەی ئەژنۆ ڕووەدات و بە هۆیەوە جومگەی ئەژنۆ تووشی کەفتەیی و پەککەوتن دەکات. هەوکردن و ئازار گرنگترین ڕۆڵیان هەیە لە هۆکاری پەرەسەندنی نەخۆشی یەکە کە دەبنە هۆی لەکەڵک خستنی شانەکەو ناساغی جەستەیی. لە ئێستادا خولیایەکی زۆر هەیە بۆ پەرەپێدانی ستراتیجی یەکی چارەسەرکردن بۆ ئەم نەخۆشی یە بۆ کەم کردنەوەی ئەو داخورانە بەردەوامەی جومگە کە کاریگەری یەکی زۆری هەبێت لەگەڵ کەمترین زیانی لابەلا . هەوڵەکان تیشک دەخاتە سەر ئەو چارەسەرە سروشتیانەی کە هەوکردن و داخوران کەم دەکاتەوە یان دەیوەستێنێت لەگەڵ کەم کردنەوەی نیشانەکانی نەخۆشی یەکە. ریسڤراترۆڵ یەکێکە لە ماددە دژە ئۆکساندنە بەهێزەکان کە سیفەتی دژە هەوکردنی هەیە کە لەوانەیە دابنرێ بە یەکێك لەو ماددانەی کە بەهایەکی چارەسەری هەیە لە نەخۆشی یەکانی جومگە دا. ئامانج لە داڕشتنی پلانی ئەم توێژینەوەیە هەڵسەنگاندنی سووە پزیشکی یەکان و بێ وەیی و ئەو گۆڕانکاری یە بایوکیمیکی یانەی کە ریسڤراترۆڵ لە خوێندا دروستی دەکات کە بەکاردەهێنرێت وەك یاریدەدەری چارەسەری لەگەڵ میلۆکسیکام لەو نەخۆشانەی کە داخورانی جومگەی کەم یان مام ناوەندیان هەیە.

ڕێگای کارکردن: ئەم توێژینەوەیە توێژینەوەیەکی دووڵایەنە-کوێرانەی بەڕێکەوت ئەنجام دراوە کە لە چەند سەنتەرێکی جۆراو جۆر کە دەرمانە درۆزنەی کۆنترۆڵ کراوی تیادا بەکارهێنرا. لەم لێکۆڵینەوەیەدا سەدو دە نەخۆش کە داخورانی جومگەی ئەژنۆیان هەیە بەشداریان کرد لە نەخۆشخانەکانی شاری فێرکاری و هەناوی و سەنتەری ڕاهێنان و ڕۆماتیزمی لە شاری سلێمانی کە لە مانگی کانونی یەکەمی ساڵی 2016 دەست پێکرا تا مانگی ئەیلولی ساڵی 2017 . بەشداربووان 15 ملگم میلۆکسیکامیان وەرگرت لەگەڵ 500 ملگم ریسڤراترۆڵ یان دەرمانە درۆزنە بۆ ماوەی نەوەد ڕۆژ. کاریگەری چارەسەرکردنەکە هەڵسەنگێنرا بە پێوانەکردنی ئەو گۆڕانکاری یانەی ڕووی دا لە پێوەری پزیشکی کە بۆ ئازارو نیشانەکانی ئەم نەخۆشی یە بە کاردێت ئەوانیش نمرەی KOOS, WOMAC, VAS-100 لە خاڵی دەست پێکردنەوە تا ڕۆژی نەوەد لە بەکارهێنانی چارەسەرکردنەکە. هەروەها ئینتەرلیوکین β1 و 6 و فاکتەری TNF-α و پرۆتینی تەواوکەری C3,C4 پێوانە کرا لە خوێندا. بۆ لێکۆڵینەوە لە سەلامەتی و بێ وەیی ریسڤراترۆڵ شیکاری خوێن ئەنجام درا بۆ کاریگەری ئەم ماددەیە لەسەر چەوری خوێن و پێکهاتەکانی خوێن و کاری جگەر و گورچیلە لە نەخۆشەکان دا.

ئەنجام: بەکارهێنانی ریسڤراترۆڵ وەك ماددەیەکی یاریدەدەر لەگەڵ میلۆکسیکام بە شێوەیەکی سەرنج ڕاکێش بووە هۆی کەم کردنەوەو بەرەو باش بردنی ئاستی پێوانەیی کلینیکی دوای 90,60,30 ڕۆژ بە بەراورد لەگەڵ خاڵی دەست پێ کردن و ئەو نەخۆشانەش کە میلۆکسیکام و دەرمانە درۆزنەیان بە کارهێنا بوو بۆ هەمان ماوە ($P<0.05$) . هەروەها ئاستی نیشانکەرە بایوکیمیکی یەکان بە شێوەیەکی سەرنج ڕاکێش کەمی کرد($P<0.01$).

سەرئەنجام: بە پێ ی ئەو ئەنجامانەی کە دەرکەوت لە کۆتایی توێژینەوەکە دا دەتوانرێت بووترێت کە ریسڤراترۆڵ کاریگەری یەکی باشی هەیە وەك ماددەیەکی یاریدەدەر لەگەڵ میلۆکسیکام لە چارەسەرکردنی داخورانی جومگەی ئەژنۆی کەم یان مام ناوەند.

أقليم كردستان –العراق
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تأثير أستخدام ريسفراترول كعلاج إضافي للمرضى الذين يعانون من سوفان الركبة: مقارنة مع ميلوكسيكام

رسالة مقدمة الى فرع الأدوية و الدراسات العليا في كلية الطب / جامعة السليمانية كجزء من متطلبات نيل شهادة الدكتوراه في علم الأدوية و السموم

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آب 2018

الخلاصة

المقدمة وأهداف الدراسة: سوفان الركبة من الامراض المزمنة التي تصيب مفصل الركبة، و يتميز بفقدان الغضاريف والخلايا بشكل بطيء ومستمر مما يؤدي الى العوق في المفصل. التهابات وآلام المفاصل لها تأثير قوي و مهم في التطور السلبي للمرض. استخدام العلاج الفعال الذي يقلل من عملية التآكل الغضروفي والذي عادة ما ترافقه مضاعفات جانبية شائعة. وحاليا توجد اهتمامات لإيجاد علاجات متطورة للحد من التآكل المستمر لأنسجة المفاصل مع أقل مضاعفات جانبية . هناك جهود عديدة نحو أستخدام منتجات طبيعية لوقف تآكل الغضاريف و تخفيف الأعراض المرافقة لها. ريسفراترول مضاد تاكسدي والتهابي قوي و يعد من المواد الفعالة في علاج أمراض المفاصل . لقد تم تصميم هذه الدراسة لتقييم الفوائد السريرية والسلامة والتغيرات البيوكيميائية المرتبطة بأستخدام ريسفراترول كعلاج إضافي مع ميلوكسيكام للمرضى الذين يعانون من سوفان الركبة من الدرجة الخفيفة و المعتدلة.

طريقة العمل: كانت الدراسة عبارة عن تجربة سريرية-عشوائية- ثنائية التعمية شملت مائة وعشرة مرضى يعانون من سوفان الركبة، أجريت الدراسة في مستشفى شار التعليمي و المستشفى العام و مركز امراض الروماتيزم المتخصص في مدينة السليمانية من بداية شهر كانون الأول 2016 لغاية شهر أيلول 2017. لقد تناول المشاركون 15 ملغم ميلوكسيكام مع 500 ملغم ريسفراترول أو دواء وهمي لمدة تسعين يوما. تم تقييم فعالية العلاج من خلال قياس التغيرات التي تحصل بين خط القاعدي وبعد تسعين يوما من العلاج في كل من .KOOS, WOMAC, VAS-100 . ولقد تم قياس انترليوكين β-1 و 6 في المصل و عامل TNF-α و البروتينات المكملة C3 , C4 ايضا . و تم تقييم سلامة ريسفراترول من خلال تقييم صورة الدهون في المصل اضافة للمعايير الدموية ووظائف الكبد والكلى في المرضى المشاركين .

النتائج: أدى أستخدام مادة ريسفراترول مع ميلوكسيكام الى تحسن كبير في النتائج السريرية بعد 30 ,60 و 90 يوما مقارنة مع خط القاعدي و مع نتائج المرضى الذين أستخدموا ميلوكسيكام مع الجرعة الوهمية في نفس الفترة ($P<0.05$) و كذلك أدى الى تقليل العلامات البوكيميائية بشكل ملحوظ ($P<0.01$) .

ألأستنتاجات: وفقا للنتائج التي تم الحصول عليها في هذه الدراسة يمكن الأستنتاج بأن مادة ريسفراترول لها قدرة فعالة كمادة إضافية مع ميلوكسيكام في علاج سوفان الركبة من الدرجة الخفيفة و المعتدلة.