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Biological activities of *Phoenix dactylifera* and Treg in Rheumatoid arthritis induced by hyperhomocysteinemia and formalin and on tumoral process

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Dedication

This thesis is dedicated to my family, my father RACHID and my mother AKILA who gave me the courage and the support I needed to continue. To my fiancé Asma, my brother Youcef and my second mother my dear sister Lamia, for their moral support and encouragement during my studies.

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CHAPTER III: RESULTS

List of publications

International publications

1. **Kehili H E, Zerizer S, Kabouche Z. (2014).** Immunostimulatory activity of *Phoenix dactylifera* "AZARZA". International Journal of Pharmacy and Pharmaceutical Sciences, 6: 73-76.
2. **Kehili H E, Zerizer S, Beladjila K A, Kabouche Z. (2016).** Anti-inflammatory effect of Algerian date fruit (*Phoenix dactylifera*). Food and Agricultural Immunology. http://dx.doi.org/10.1080/09540105.2016.1183597.

International communications

1. **Kehili H E, Zerizer S, Kabouche Z. (2013).** Immunostimulatory activity of *Phoenix dactylifera* AZARZA. International days of biotechnology, Hamamat Tunisia, 21-24 December 2013.
2. **Kehili H E, Zerizer S, Kabouche Z. (2014).** Immunostimulatory and antioxidant activities of *Phoenix dactylifera* AZARZA. 2nd international congress of biotechnology and valorization of bio-resources, Tabarka Tunisia, 18-20 Mars 2014.
3. **Kehili H E, Boudebouz S, Zerizer S, Kabouche Z. (2014).** Biological activity of *Phoenix dactylifera* in Rheumatoid arthritis induced by formalin. 31 International Medico-Surgical and dental Seminar, Mila Algeria, 18-20 June 2014.
4. **Messaoudi S, Kehili H E. (2014).** Etude des complications cardiovasculaires et rénales dans le diabète de type 2. 31 International Medico-Surgical and dental Seminar, Mila Algeria, 18-20 June 2014.

5. **Kehili H E, Zerizer S, Kabouche Z. (2014).** Immunostimulatory activity of *Phoenix dactylifera* "Homayra". 1st International congress of Immunology and Molecular Immunopathology. Tlemcen Algeria, 17-20 October 2014.

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1. Participation at Second workshop of Cellular Immunology and Molecular Pathology, Tlemcen, 20-22 June 2013.

- 1st class of the department (animal biology) and 2nd class of the university. Doctoral days 2013-2014 level D2. University of Constantine, 30 April 2014.
- 1st class of the department (animal biology) and 2nd class of the university. Doctoral days 2014-2015 level D3. University of Constantine, 17 April 2015.

2. **Kehili H E, Zerizer S, Kabouche Z. (2014).** Immunostimulatory activity of *Phoenix dactylifera*. 2nd Scientific days of Valorisation of Algerian medicinal plants, Constantine, 24-25 February 2014.
3. **Kehili H E, Zerizer S, Kabouche Z. (2015).** Anti-inflammatory effect of Algerian date fruit in Rheumatoid arthritis induced by formalin. 1st National seminar of the valorisation of natural resources and environment, El oued, 10-11 November 2015.

List of abbreviations

ACR: American College of Rheumatology

AIF: Apoptosis Inducing Factor

ANOVA: Analysis of Variance

Anti CCP: Anti-Cyclic Citrullinated Proteins

aP2: Adipocyte Fatty Acid Binding Protein 2

Apaf‑1: Apoptosis Protease Activating Factor-1

APC: Antigen Presenting Cells

APRIL: Proliferation-Inducing Ligand

AZ: Azarza variety

BHMT: Betaine Homocysteine Methyltransferase

BLyS: B-Lymphocyte Stimulator

C/EBP-α: CCAAT/Enhancer Binding Protein- α

CBS: Cystathionine β-Synthase

CD4: Cluster of Differentiation 4

cMOAT: Canalicular Multispecific Organic Anion Transporter

CRP: C-Reactive Protein

CTH: Cystathionine γ-Lyase

CXC: Chemokines

DCs: Dendritic Cells

DMARDs: Disease Modifying Anti-Rheumatic Drugs

List of abbreviations

DMSO: Dimethyl Sulfoxid

DPBS: Dulbecco's Phosphate-Buffered Saline

DTNB: 5,5′-dithiobis-(2 nitrobenzoic acid)

EBV: Epstein - Barr virus

ERK: Extracellular Signal Regulated Kinases

EULAR: European League Against Rheumatism

FAS: Fatty Acid Synthase

FoxP3: Forkhead Box P3

FR: Rheumatoid Factor

GM-CSF: Granulocyte–Macrophage Colony-Stimulating Factor

GNMT: Glycine N-Methyl Transferase

GP39: Proteoglycans 39

GSH: Glutathione Reduced

h NHEPS: Human Normal Hepatocytes

H.E: Hematoxylin Eosin

Hcy: Homocysteine

HM: Homayra variety

Hsp70: Heat Shock Proteins 70

HUVEC: Human Umbilical Vein Endothelial Cell

i.p: Intraperitoneal

List of abbreviations

IFN-γ: Interferon γ

IL-1: Interleukin 1

LD50: Medial Lethal Dose

LPL: Lipoprotein Lipase

MAT: Methionine-Adenosyltransferase

MBL: Manose Binding Lectin

MHC II: Major Histocompatibility Complex II

MMPs: Matrix Metallo-Proteinases

MTT: Microculture Tetrazolium

NADPH: Nicotinamide Adenine Dinucleotide Phosphate

NF-κB: Nuclear Factor κB

NK: Natural Killer

NLRs: (NOD)-Like Receptors

NSAIDs: Non-Steroid Anti-Inflammatory Drugs

OECD: Organisation for Economic Cooperation and Development

PAD: Peptidylarginine Deiminase

PGE2: Prostaglandin E2

PPAR-γ: Peroxisome Proliferation-Activity Receptor- γ

RA: Rheumatoid Arthritis

RANKL: Receptor Activator of Nuclear factor-κB Ligand

List of abbreviations

RASFs: Rheumatoid Arthritis Synovial Fibroblasts

RES: Reticuloendothelial Systems

SD: Standard Deviation

SPSS: Statistical Package for Social Science

TCR: T Cell Receptor

TGF-β: Transforming Growth Factor-β

TLRs: Toll-Like Receptors

TNF-RI: Tumor Necrosis Factor- Receptor 1

TNFα: Tumor Necrosis Factor α

Treg: Regulatory T cell

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Introduction

Primarily inflammation is essential to protect the body against pathogens or dangerous cells. However, abnormal regulation of this process can result in destruction of cells or disturbed cellular metabolism, which often contributes to chronic diseases characterized by a low grade of inflammation. Normally inflammation lasts within days because of the short half-life of some immune cells and inflammatory mediators, and the delayed production of anti-inflammatory cytokines. This type of inflammation is referred to as acute inflammation. On the other hand, longer lasting inflammation is referred to as chronic inflammation, which can last weeks to years. The chronic inflammation is known to be the cause of many chronic diseases such as atherosclerosis, cardiovascular disease, autoimmune disease, rheumatoid arthritis, cancer (Park *et al.*, 2013).

Rheumatoid arthritis (RA) is an autoimmune disease afflicting numerous joints throughout the body. RA is a chronic inflammatory disease characterized by progressive joint destruction. Joint degradation involves damage to the articular cartilage caused by inflammatory cells, activated fibroblasts in the synovial membrane and chondrocytes (Cojocaru *et al.*, 2013). RA as an autoimmune disease has a major characteristic which is the absence of the immune tolerance which is insured by the lymphocyte Treg. This tolerance protects the self-antigens from the immune system's reaction.

In the case of the rheumatoid arthritis some studies suggest that the lymphocyte Treg are not able to suppress the secretion of major inflammatory cytokines, the tumor necrosis factor α (TNF- α) and interferon γ (IFN-γ) (Cojocaru *et al.*, 2013).

Homocysteine (Hcy) is an intermediate amino acid formed during the metabolism of the essential amino acid methionine. Abnormal elevations of plasma Hcy levels have been reported in patients with severe hyperhomocysteinemia. Recent research on homocysteine has implicated abnormal homocysteine metabolism in a wide range of important disease processes, including developmental birth defects, neurodegenerative diseases like Alzheimer's disease, autoimmune diseases like rheumatoid arthritis (Roubenoff *et al.,* 1997, Provenzano *et al.,* 2003 and Wu, 2007), hormonal imbalances, renal failure, cancer and degenerative diseases of aging (Shaker *et al.,* 2013), it have been also associated with cardiovascular risk (Wald *et al*., 2002).

In another side the mediators like pro-inflammatory cytokines and cellular effectors of inflammation are important constituents of the local environment of tumours. In some types of cancer, inflammatory conditions are present before a malignant change occurs. Conversely, in other types of cancer, an oncogenic change induces an inflammatory microenvironment that promotes the development of tumours. Regardless of its origin, 'smouldering' inflammation in the tumour microenvironment has many tumour-promoting effects. It aids in the proliferation and survival of malignant cells, promotes angiogenesis and metastasis, subverts adaptive immune responses, and alters responses to hormones and chemotherapeutic agents (Mantovani *et al.,* 2008).

In normal case, the human body has normal mechanism to resist and to fight against the cancer cells development called apoptosis or programmed cell death. Apoptosis is a regulatory process of self-stabilization of the body that can inhibit tumor growth. The Apoptosis depends on the apoptotic proteins (Bcl2 and BAX) dominance. Bcl-2 can prevent damaged cells from undergoing apoptosis and lead to

cancer progression. In the other hand, Bax promotes cell apoptosis by the stimulation of an apoptosis signal or the inhibition of the Bcl-2 expression (Yue *et al.,* 2015).

The knowledge of the inflammatory mechanisms and mediators has allowed modification of the treatment strategy for patients with inflammatory diseases like RA, reinforcing the rationale for early intervention to prevent joint damage. Indeed, results from several studies indicate that therapeutic intervention with so-called disease-modifying anti-rheumatic drugs (DMARDs) and the non-steroid anti-inflammatory drugs (NSAIDs) can halt or retard the progression of the inflammation. The current mode of treatment of various inflammatory diseases based on synthetic drugs is expensive, alters genetic and metabolic pathways and also shows adverse side effects. Thus, safe and effective approach is needed to prevent the diseases development and progression. From this view, Natural products are good remedy in the treatment/management of diseases and they are affordable and effective without any adverse effects (Rahmani *et al*., 2014).

Plants have always been the source of medicine and of direct use to the mankind. All systems of traditional medicine have their roots in folk medicine and household remedies. However some of those earliest remedies were subjected to certain refinements, revisions and improvements through practices by trained medicine men. The history of early civilization reveals that a considerable number of drugs in modern medicine figured in ancient manuscripts such as the Quran and the Bible (Panda *et al., 2011).*

This study investigated the mode action of the jam dates of two *Phoenix dactylifera* L varieties: Azarza and Homayra.

The date palm (*Phoenix dactylifera* L.) is one of mankind's oldest cultivated plants. It has been used as food for 6000 years. It could be used for generations to

come due to its remarkable nutritional, health and economic value, in addition to its aesthetic and environmental benefits. Every part of the date palm is useful. Dates offer useful prospects for fighting hunger and diseases (Sulieman *et al*., 2012).

The *Phoenix dactylifera* is a monocotyledonous woody perennial belonging to the Arecaceae family, which comprises 200 genera and 3000 species (Boukouada and Yousfi, 2009), commonly known as date is the only species of the genus *Phoenix* which is cultivated for its fruit (Shafi Bhat and Al-Daihan, 2012). *Phoenix dactylifera* is an important source of energy, fibers and potential natural source of bioactive phytochemicals such as phenolic compounds and flavonoids (Louaileche *et al*., 2015).

In this thesis we have attempted to focus these objectives:

- Testing the safety use of *Phoenix dactylifera* Azarza and Homayra varieties extracts and determining the lethal doses using toxicity tests in animals.
- Evaluation of immunomodulatory effect of *Phoenix dactylifera* Azarza and Homayra varieties extracts using carbon clearance assay.
- Evaluation of antioxidant effect of *Phoenix dactylifera* Azarza and Homayra varieties extracts using the GSH reduced dosage from liver.
- Evaluation of the anti-inflammatory activity of *Phoenix dactylifera* Azarza and Homayra varieties extracts in acute model of inflammation induced by formalin and hyperhomocysteinemia.
- Evaluation of the antiarthritic and anti-inflammatory effects of *Phoenix dactylifera* Azarza and Homayra varieties extracts in formalin induced arthritis comparing to standard drug.

- Examination of the effect of *Phoenix dactylifera* Azarza and Homayra varieties extracts on inflammation and arthritis through the measurement of plasma hs-CRP, homocysteine and Anti-CCP.
- Confirmation of the action of formalin induced arthritis and plant extract on joint inflammation by histological study.
- Evaluation of the anti-proliferative activity of *Phoenix dactylifera* Azarza and Homayra varieties extracts on liver cancer (hepatocarcinoma cell line HepG2) and breast cancer cell line MCF7using cell culture techniques.
- Study the effect of extracts *Phoenix dactylifera* Azarza and Homayra varieties extracts on cell viability of healthy human cells: endothelial cells of blood vessels HUVEC, h NHEPS liver cells).
- Test of the effect of the *Phoenix dactylifera* Azarza and Homayra varieties extracts on some apoptosis-related gene expression (Bcl2, BAX).
- Test the effect of *Phoenix dactylifera* Azarza and Homayra varieties extracts on the differentiation of lymphocytes from naïve lymphocyte T CD4 to lymphocytes Treg.

Chapter I

Literature Review

I.1.Rheumatoid Arthritis

Rheumatoid Arthritis (RA) is an autoimmune disease of unknown etiology that leads to pain, stiffness and physical disability (Elshafie, 2013) and characterized by chronic inflammation of the synovial lining of the joint (Suurmond *et al.,* 2011). RA is a symmetric polyarticular arthritis that primarily affects the small diarthrodial joints of the hands and feet. In addition to inflammation in the synovium, which is the joint lining, the aggressive front of tissue called pannus invades and destroys local articular structures. The synovium is normally a relatively acellular structure with a delicate in timal lining (Firestein, 2003) (**Figure 1**).

The first description of RA acknowledged by modern medicine is found in the dissertation of **Augustin Jacob Landré-Beauvais** from the year **1800**. He first noticed the symptoms and signs of what we now know to be RA. He examined and treated a handful of patients with severe joint pain that could not be explained by other known maladies at the time (such as "rheumatism" or osteoarthritis) and he hypothesized that the patients were suffering from a previously uncharacterized condition, which he named "*Goutte Asthénique Primitive"*, or "Primary Asthenic Gout" (Entezami *et al.,* 2011).

The next important contributor to the study of RA was **Alfred Garrod** in **1859** He named it "Rheumatoid Arthritis" to distinguish it from the two well-known forms arthritis, rheumatic fever and gout. By the early 20th century, RA was viewed as separate from osteoarthritis. In **1957**, **Charles Short** described RA definitively and clearly set it apart as a defined clinical entity distinct from the seronegative spondyloarthropathies, crystal induced disease, osteoarthritis, systemic lupus erythematosus, and many other conditions (Firestein, 2003).

In the last few decades, the recognition of the central role of an early diagnosis and the early administration of disease modifying antirheumatic drugs (DMARDs), greatly improved the management of RA. The more effective diagnostic and treatment strategies led to a better control of the disease with a deeper suppression of synovitis and the prevention of radiological progression of bone erosions in the joints (Sakellariou *et al.,* 2013).

For these reasons, the early recognition of RA has become a central issue in clinical practice, although the absence of a single and reliable test to identify the disease does not always allow an immediate diagnosis. The American College of Rheumatology (ACR) proposed in 1987 a set of classification criteria, developed in patients with longstanding disease with the aim to be specific rather than sensitive (Sakellariou *et al.,* 2013). The 1987 American College of Rheumatology classification criteria for RA have been criticized for

their lack of sensitivity in early disease. This work was undertaken to develop new classification criteria for RA (Aletaha *et al.,* 2010). In 2010 the ACR and the European League Against Rheumatism (EULAR) jointly developed new classification criteria, aiming to allow earlier patient classification, treatment and inclusion in clinical trials (Sakellariou *et al.*, 2013).

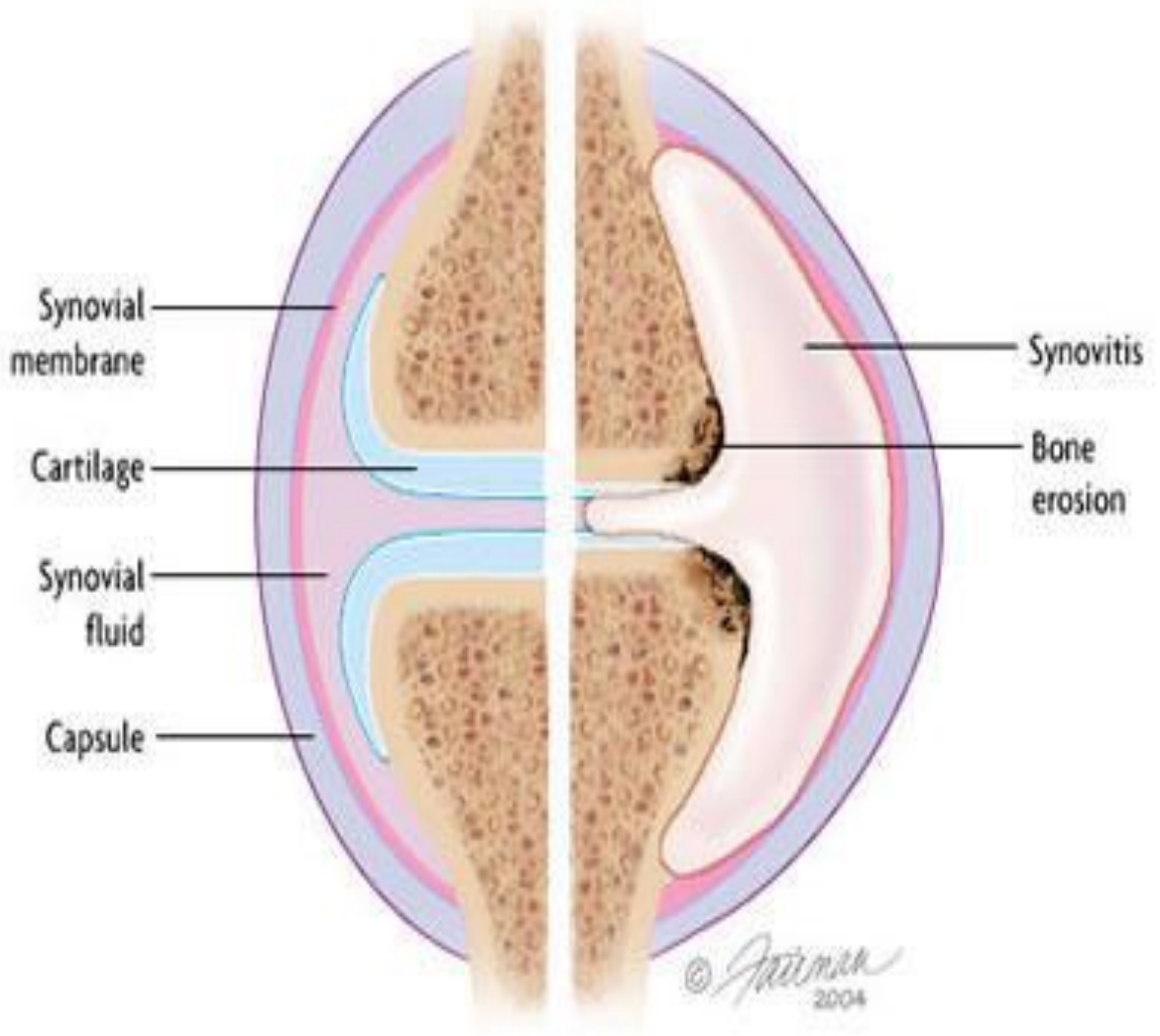

Figure 1: The Rheumatoid Arthritis joint (Ruderman and Tambar, 2012).

The normal joint structure appears on the left. On the right is the joint with rheumatoid arthritis.

I.2. Epidemiology of Rheumatoid arthritis

Several epidemiological studies of RA have been published. They show variations in the incidence and prevalence of RA across populations. Further variation include occurs as a result of differences in statistical methods and case-ascertainment criteria. Several large prospective studies have improved our knowledge of the risk factors for RA (Tobón *et al.,* 2010). The majority of prevalence studies curried out in Northern European and North American areas estimate a prevalence of 0.5– 1.1%. Studies from Southern European countries report a prevalence of 0.3–0.7%. Studies from developing countries also report a relatively lower prevalence of the disease (between 0.1% and 0.5%). A higher prevalence has been reported in certain Native Americans, and a very low frequency of RA in some areas of rural Africa (Alamanos and Drosos, 2005) **(Table 1).**

The prevalence of RA in Algeria has been estimated at 0.15 % of the adult population (≈ 30.000 of adults with RA in Algeria) (Slimani *et al.,* 2014).

I.3. Etiology of Rheumatoid arthritis

Rheumatoid arthritis is one of the complex immune-mediated diseases for which an understanding of the etiology is dependent on the definition of environmental triggers that, in a restricted genetic context, may initiate immune reactions having the potential to contribute to disease development (Klareskog *et al.,* 2006). To increase understanding of the etiology and pathogenesis of RA, environmental and genetic risk factors were investigated over the last decades.

I.3.1. Environmental factors

Several environmental factors have been suspected and studied as possibly related to an increased risk of RA, as well as to a worse or improved prognosis of the disease. However, the impact of most of these factors on the risk of developing RA and the expression of the disease remains still uncertain (Alamano and Drosos, 2005). These environmental factors can be classified as regional environmental factors, including geography, climate (Perricone *et al.,* 2011), endemic microbes including potential candidates implicated in an infectious etiology included Epstein-Barr virus (EBV), *Mycobacterium tuberculosis*, *Escherichia coli*, *Proteus mirabilis*, retroviruses and parvovirus B19 (Edwards and Cooper, 2005).Also socio-cultural environmental factors such as lifestyle, smoking and dietary habits (Perricone *et al.,* 2011).

Table 1: Prevalence and incidence rates of Rheumatoid arthritis worldwide (cases per 100 inhabitants) (Alamanos and Drosos, 2005).

Population		Prevalence rates	Incidence rates
North America	• USA (general population)	0.9–1.1	0.02–0.07
	• USA (native-Americans)	5.3–6.0	0.09–0.89
North Europe	• England	0.8–1.10	0.02–0.04
	• Finland	0.8	0.03–0.04
	• Sweden	0.5–0.9	
	• Norway	0.4–0.5	0.02–0.03
	• Netherlands	0.9	0.05
	• Denmark	0.9	
	• Ireland	0.5	
South Europe	• Spain	0.5	
	• France	0.6	0.01
	• Italy	0.3	
	• Greece	0.3–0.7	0.02
	• Yugoslavia	0.2	
South America	• Argentina	0.2	
	• Brazil	0.5	
	• Colombia	0.1	
Asia	• Japan	0.3	0.04–0.09
	• China	0.2–0.3	
	• Taiwan		0.3
	• Indonesia	0.2–0.3	
	• Philippines	0.2	
	• Pakistan	0.1	
Middle East	• Egypt	0.2	
	• Oman	0.4	
	• Turkey	0.5	
Africa		0–0.3	

I.3.2. Genetics factors

Rheumatoid arthritis was one of the first inflammatory diseases where MHC class II genes, notably HLA-DRB1 01 and variations in HLA-DQ and DP were exclusively associated with the risk of the disease (Klareskog *et al.*, 2011). Only decades later where it was possible to define additional susceptibility genes such as: PTPN22, STAT4 and TRAF1-C5 (Imboden, 2009).

I.4. Immunopathology of Rheumatoid arthritis

The nature of the inflammatory infiltrate in the joints has been the subject of detailed investigation. But the precise pathogenesis of RA remains unclear, T cells, B cells, macrophages, neutrophils, synovial fibroblasts, the inflammatory cytokines the complement proteins are central to the mechanisms of joint inflammation and disease progression (Andersson *et al.,* 2008).

The inflammatory process of RA resembles a symphony orchestra playing a piece of music--not a song that anyone wants to hear, but a painful song. Each cellular player has a distinct role, and all must coordinate in order to play their discordant "music" successfully (Weyand, 2007). Rheumatoid synovitis consists of resident cells and invading immune cells that together arrange the inflammatory process in RA. There are 3 major types of synovitis that RA can comprise: germinal center synovitis, aggregate synovitis, and diffuse synovitis. Germinal centers are highly organized complex structures that are functionally competent. Aggregates are B cells and T cells arranged in defined follicles, yet they lack germinal center reactions. Diffuse synovitis is the least organized but can still cause significant damage. For each of these types of synovitis, the cellular players and their molecular instruments vary significantly. Differences in lymphoid micro organizations draw attention to the process of lymphoid organogenesis as a fundamental pathway of rheumatoid synovitis, a process that lends stability and sustainability to dysfunctional immune responses (Weyand, 2007).

Many hypotheses have been proposed to explain the pathogenesis of RA that can be divided into 3 phases, which are not mutually exclusive: the initiation phase, the inflammatory phase and destruction phase (Zeisel, 2004).

I.4.1. Initiation of rheumatoid synovitis

The mechanisms by which rheumatoid synovitis is initiated remain unclear, but they may involve both antigen-specific and non–antigen-specific mechanisms. The non–antigen-specific hypothesis of the initiation of rheumatoid synovitis is that an early and episodic

release of TNFα and granulocyte–macrophage colony-stimulating factor (GM-CSF) from synovial macrophages and fibroblasts may be induced by minor trauma, infections, allergic responses, vaccinations, or local immune complex deposition. Then these cytokines differentiate resident dendritic cells into potent antigen-presenting cells, which may selectively present self-antigens for the induction of specific T cell responses. The presence of the shared epitope may decrease the threshold for the transformation of a mild reactive synovitis into a rapidly destructive synovial reaction by increasing the presentation of self-antigens by the dendritic cells (DCs) (Arend, 2001).

In recent studies, the hypothesis of an initiation of activation of innate and adaptive immunity in the periphery (outside the joint) was advanced. Under the action of hormonal factors or environment such as tobacco, charcoal and infections, lung macrophages are activated and cause activation of the peptidylarginine deiminase (PAD) (**Figure 2**). This enzyme generates neo-antigens from self-antigens such as type II collagen, proteoglycans 39 (GP39), matrix proteins, IgG, fibrin and the aggrecans (Essakalli *et al.*, 2011) by inducing a post transcriptional modifications that consist in changing their arginine residues to citrulline (Morel *et al.*, 2004).

These citrullinated proteins recognized as non-self, are collected by the APC (Antigen presenting cells). Their processing and presentation to lymphocytes are facilitated by the presence of the shared epitope in MHC II.

In the initiation phase involves the stimulation and maturation of APC (DC, macrophages) (Zeisel, 2004) leading to the migration and activation of T and B lymphocytes and involving antibody synthesis cars which will locate at the joint where they contribute to the onset of inflammation (Essakalli *et al.*, 2011).

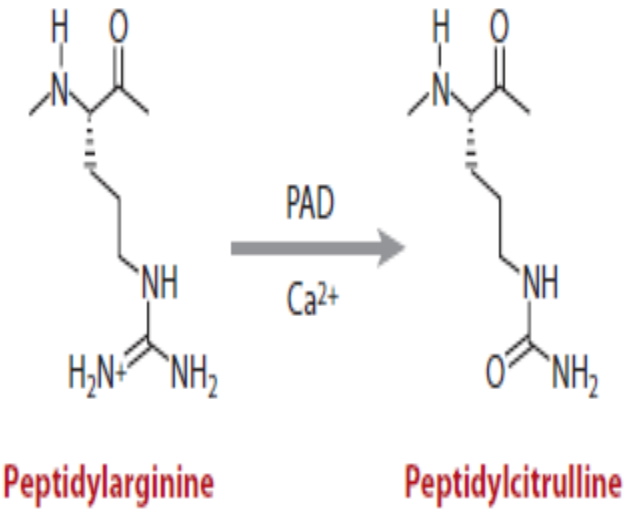

Figure 2: Citrullination of proteins (Imboden, 2009).

I.4.2. Inflammatory phase

Immune cells and soluble inflammatory mediators play a crucial role in RA pathogenesis. Various leucocyte populations, orchestrated by several cytokines, chemokines, growth factors and hormones, infiltrate rheumatoid tissues and increase injury. These inflammatory processes resemble to those in other chronic inflammatory diseases, such as atherosclerosis. The activation of monocytes, T and B cells, vascular endothelial cells and the elevation of circulating inflammatory factors and markers characterizing both diseases, suggest that different inflammatory disorders can be induced by common inflammatory processes (Montecucco and Mach, 2009).

I.4.2.1. Innate immune system

Recent data indicate that the innate immune system might be involved in early events leading to clinically active disease. Potential mediators in these events are macrophages, DCs, rheumatoid arthritis synovial fibroblasts (RASFs), chondrocytes which can all detect, process, and react on contact with ‘non-self ’antigens without the support of effector cells of the adaptive immune system, especially T cells and B cells (Müller-Ladner *et al.,* 2005), the complement system proteins, autocrine and paracrine-acting cytokines as well as chemokines that have inflammatory, homeostatic, and even anti-inflammatory properties (Gierut *et al.,* 2010). Mast cells and natural killer (NK) cells are also found in rheumatoid synovial tissue.

Neutrophils seem to be predominant in the synovial fluid, but they are also present in the synovial membrane (Boissier *et al.*, 2012).

Macrophages (predominantly M1 phenotype) one of the major effectors of synovitis, act via cytokines secretion, mainly TNF-α, IL-1β, IL-6; they produce also short half-life mediators of inflammation, metalloproteinases and are turned toward phagocytosis and enhanced antigen-presentation. Macrophage activation is driven by cytokines, cell contact (with activated T cells), toll-like receptors (TLRs) (mainly TLR 2/6, 3, 8) and nucleotide binding oligomerization domain (NOD)-like receptors (NLRs). Microorganisms or endogenous ligands can activate these TLRs and NLRs (Boissier *et al.*, 2012).

DCs may be involved in the initiation of synovial inflammation. These cells are enriched in rheumatoid synovial fluid and tissue and are derived from circulating immature precursors. Synovial DCs exhibit markers of differentiation such as CD86 expression. It is hypothesized that these fully differentiated DCs in the synovial tissue are capable of stimulating lymphocyte activation locally. In contrast, less-differentiated DCs in the synovial fluid, containing ingested antigens, migrate to regional lymph nodes, where they undergo further maturation and initiate T cell responses (Arend, 2001).

The rheumatoid synovial harbors a special cell population, known as activated RASFs (Rheumatoid Arthritis Synovial Fibroblasts) that is engaged in the initiation and perpetuation of RA and thus distinguishes RA from other inflammatory disorders of the joints. These cells appear to be in the center of the local pathogenic events, and there is growing evidence that activation of RASFs (e.g. by responses of the innate immune system) is an early step in the development of RA. Once activated, RASFs produce a variety of cytokines, chemokines and matrix degrading enzymes that mediate the interaction with neighboring inflammatory and endothelial cells and are responsible for the progressive destruction of articular cartilage and bone. In this scenario, the production of cytokines and chemokines within the rheumatoid synovium would help to recruit T cells, macrophages and neutrophils, which in turn, attract more inflammatory cells (Huber *et al.*, 2006).

There is increasing evidence for an important role of components of the complement cascade in the pathophysiology of RA. In general, the complement cascade is involved in the induction and progression of inflammation reactions and is a major defense system against various pathogenic agents, including bacteria, viruses, and other antigens. Complement is traditionally considered to be mainly activated by bacteria or immune complexes. RA patients have increased levels of circulating immune complexes. Part of these complexes contains

rheumatoid factors (RFs), which are autoantibodies against human IgG. RF-containing immune complexes are capable of activating complement via the classical pathway, with IgM-RF being considerably more effective in complement activation than IgG-RF. Inappropriate activation, however, can lead to tissue damage and manifestation of disease. Although increased complement activation is potentially related to the occurrence and/or augmentation of inflammation in RA, complement deficiency may induce RA. C1q deficiency and suppression are related to the development of RA as well as C2 deficiency. Association with autoimmune diseases, including RA, has been shown for deficiencies of other complement components, including C1r and C1s, C4, C7, C9 and factor I. In the lectin pathway, Manose binding lectin (MBL) deficiency is not directly associated with the occurrence of RA but it is associated with the disease severity (Ballanti *et al.*, 2011).

I.4.2.1.a *Cytokines and Rheumatoid arthritis*

The cytokines form a tangled and redundant network whose overall balance depends on multiple interactions (**Figure 3**). Regarding inflammation, the result at a given point in time is either progression or resolution of the inflammatory process. However, none of the cytokines has a single effect and none of the phases of the inflammatory process depends on a single cytokine. Thus, the cytokine network is both pleiotropic and redundant. The inflammation that characterizes RA is ascribed to a predominance of the effects of pro-inflammatory cytokines over those of anti-inflammatory cytokines (Boissier, 2011).

These cytokines are implicated in each phase of the pathogenesis of rheumatoid arthritis, by promoting autoimmunity (during the pre-articular phase, at least in animal models of arthritis), by maintaining chronic inflammatory synovitis and by driving the destruction of adjacent joint tissue.

Cytokines therefore integrate the immune-regulatory and tissue-destructive events that underlie the clinical presentation and progression of RA (McInnes and Schett, 2007).

- **TNF-*α***

TNF-α was identified in 1975 as the factor in serum isolated from endotoxin-treated mice that induced necrosis of a methylcholanthrene- induced murine sarcoma. It soon became apparent that TNF-α had other effects, including the ability to induce signs and symptoms of shock and multi-organ damage via pro-inflammatory effects on vascular endothelium. The demonstration that TNF-α played a key role in RA followed from the demonstration of its potential to degrade cartilage and bone (Brennan and McInnes, 2008).

TNF-α is produced by activated macrophages and T-cells, exerts pro-inflammatory effects after binding to one of its receptors, p55 (TNF-RI) or p75 (TNF-RII). The p55 receptor is expressed in most tissues and the p75 receptor on immune system cells (Boissier, 2011).

TNF is clearly of primary importance in the pathogenesis of rheumatoid arthritis. TNF is present in most synovial biopsies, and its inhibition suppresses various arthritis models, whereas overexpression of a TNF transgene induces spontaneous erosive inflammatory arthritis. TNF induces leukocyte and endothelial-cell activation, synovial-fibroblast activation and survival, contributes to failure of Treg to suppress the proliferation of effector cells by lowering Foxp3 mRNA expression and this can be reversed by treatment with anti-TNF therapy (Nistala and Wedderburn, 2009), pain-receptor sensitization and angiogenesis, which together represent key pathological features of rheumatoid arthritis (McInnes and Schett, 2007) (**Figure 4**).

- **Il-1**

IL-1 is the prototypical example of pro-inflammatory cytokines (Boissier, 2010). It is mainly produced by monocytes and synoviocytes macrophagic type. As TNF, IL-1 levels are greatly increased in patients with RA and many experimental models demonstrate its importance. This cytokine is implicated in both the maintenance of the inflammation and the joint destruction, IL-1 stimulates the expression of prostaglandin E2 (PGE2) and matrix metallo-proteinases (MMPs) and aggrecanases responsible for cartilage destruction. IL-1 alters the articular reparation process by inhibiting the synthesis of collagen and proteoglycans that form the cartilage. In addition, IL-1 increases bone destruction by activating the differentiation and survival of osteoclasts (Audo, 2008).

- **IL-6**

In addition to IL-1 and TNF-α synovial fluid and synovium from RA patients contains IL6 activity that is significantly elevated compared to control patients with RA.

Moreover, increased IL-6 activity correlates with elevations of acute phase reactants, as well as other signs of inflammation, including fever and anemia. Additionally, IL-6 has been implicated as a trophic factor for the generation of autoantibodies (including rheumatoid factor). Recently it has been known, that the blockade of IL-6 has become a focus of growing scientific and clinical interest in the treatment of RA (Park and Pillinger, 2007) (**Figure 5**).

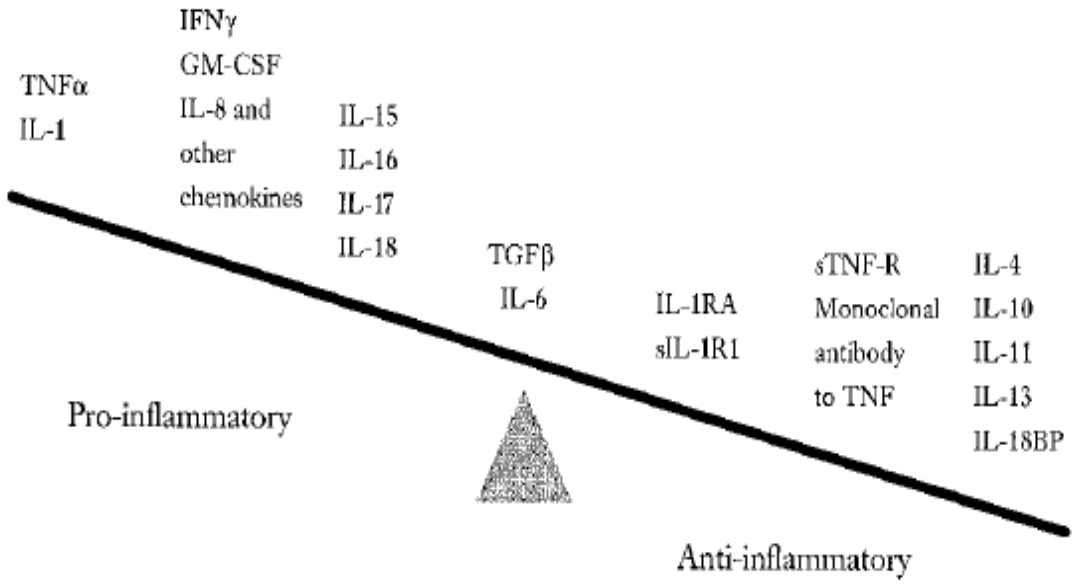

Figure 3: Restoration of equilibrium in rheumatoid synovitis (Arend, 2001).

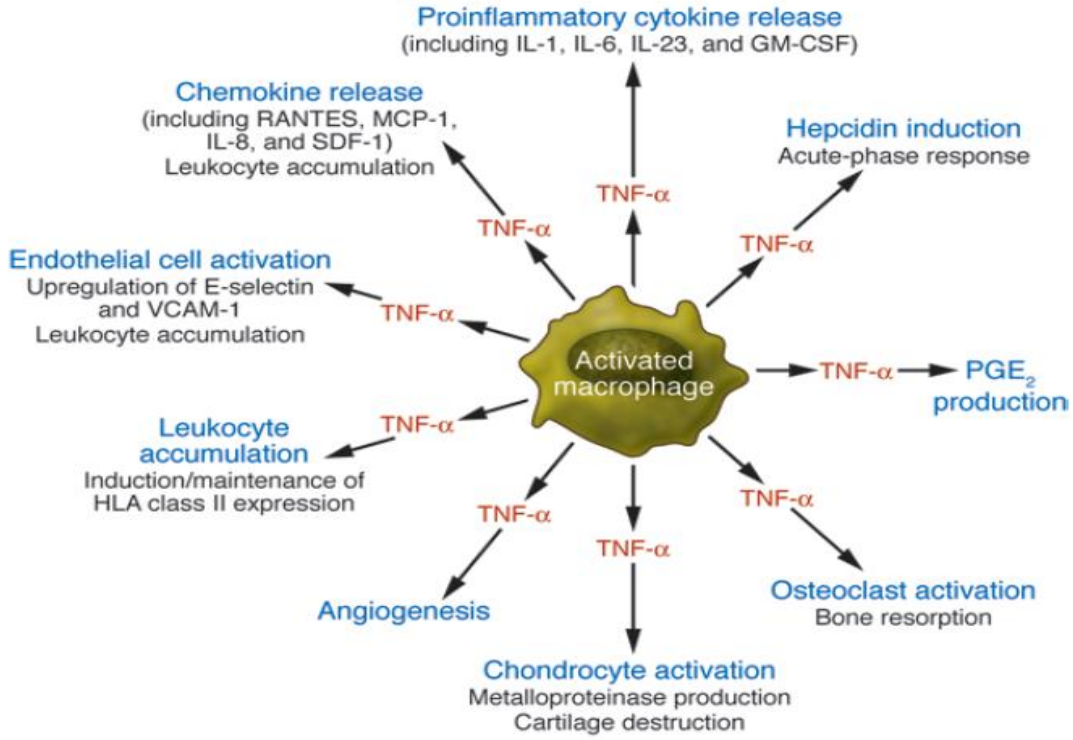

Figure 4: TNF-α action relevant to the pathogenesis of Rheumatoid arthritis (Brennan and McInnes, 2008).

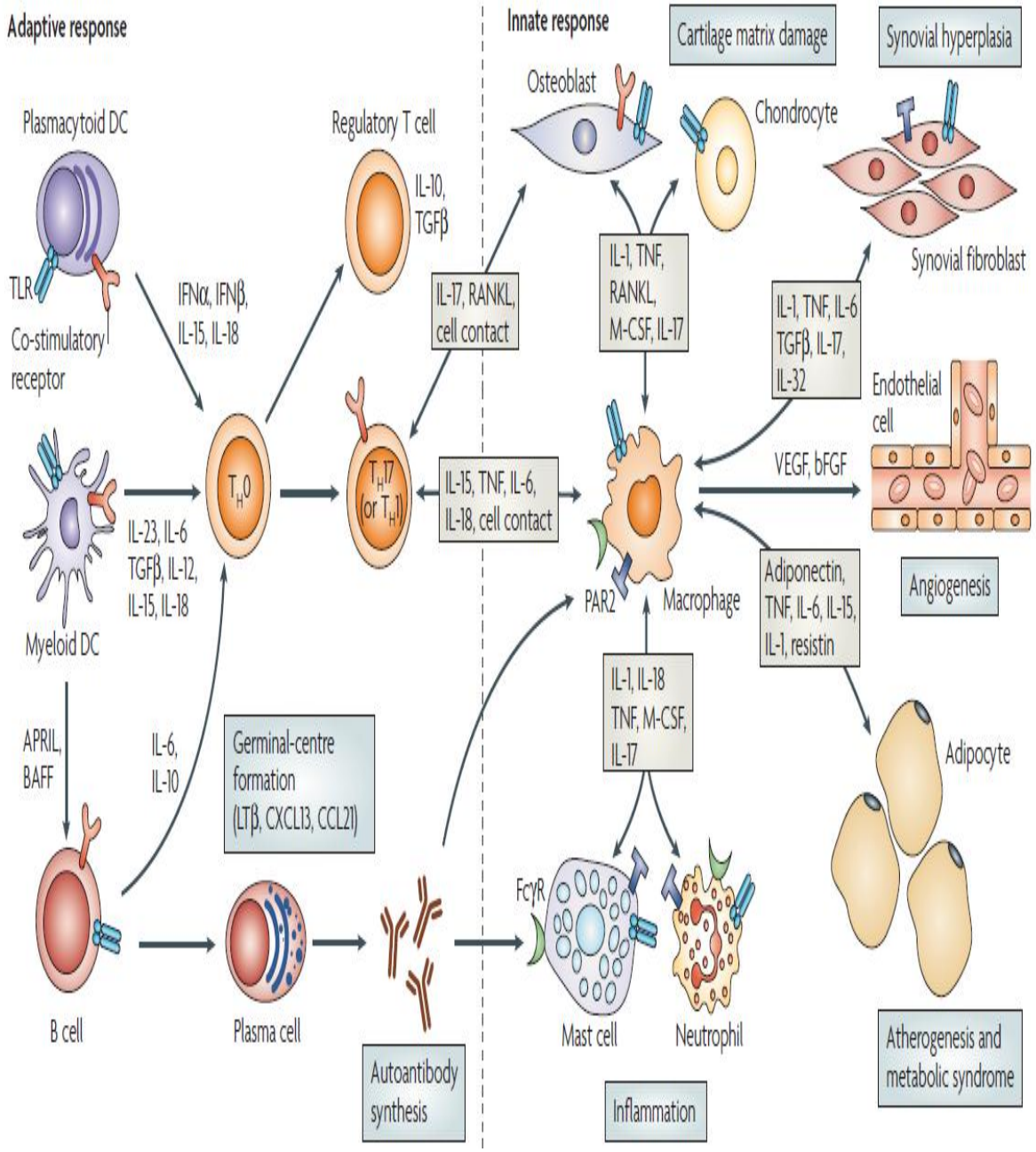

Figure 5: An overview of the cytokine-mediated regulation of synovial interactions

(McInnes and Schett, 2007).

I.4.2.1.b *C-reactive protein in Rheumatoid arthritis*

C-reactive protein (CRP) is a phylogenetically highly conserved plasma protein, with homologs in vertebrates and many invertebrates, that participates in the systemic response to inflammation. Its plasma concentration increases during inflammatory states, a characteristic that has long been employed for clinical purposes. CRP is a pattern recognition molecule, binding to specific molecular configurations that are typically exposed during cell death or found on the surfaces of pathogens. Its rapid increase in synthesis within hours after tissue injury or infection suggests that it contributes to host defense and that it is part of the innate immune response (Black *et al.,* 2004).

Mainly produced by the liver, CRP was considered for many decades as a low, specific systemic marker of inflammation (Montecucco and Mach, 2009). The CRP gene is located on the short arm of chromosome 1, contains only one intron, which separates the region encoding the signal peptide from that encoding the mature protein. Induction of CRP in hepatocytes is principally regulated at the transcriptional level by the cytokine interleukin-6 (IL-6), an effect which can be enhanced by interleukin-1β (IL-1β) (Black *et al.,* 2004).

In the inflamed tissues, CRP directly activates immune cells with the secretion of other inflammatory molecules, by initiating a vicious circle that maintains and increases the inflammatory state. This experimental evidence strongly supports CRP as an active inflammatory mediator with both systemic and local effects. In addition, this may suggest that inflammatory disorders, characterized by high levels of CRP, can develop a secondary immune cell activation, which may result in the increase of atherogenesis. Therefore, the chronic increased CRP serum levels in RA patients can directly induce an acceleration of atherosclerosis and its complications. Numerous prospective epidemiological studies showed that in healthy subjects, serum CRP predicts myocardial infarction mortality, stroke and arrhythmias, including sudden cardiac death. A meta-analysis of 14 prospective long-term studies showed that after correction for age, smoking and other cardiovascular risk factors, CRP was strongly related to coronary heart disease. These studies show that CRP should be considered a direct pro-inflammatory factor in the pathogenesis of inflammatory diseases such as RA (Montecucco and Mach, 2009) (**Figure 6**).

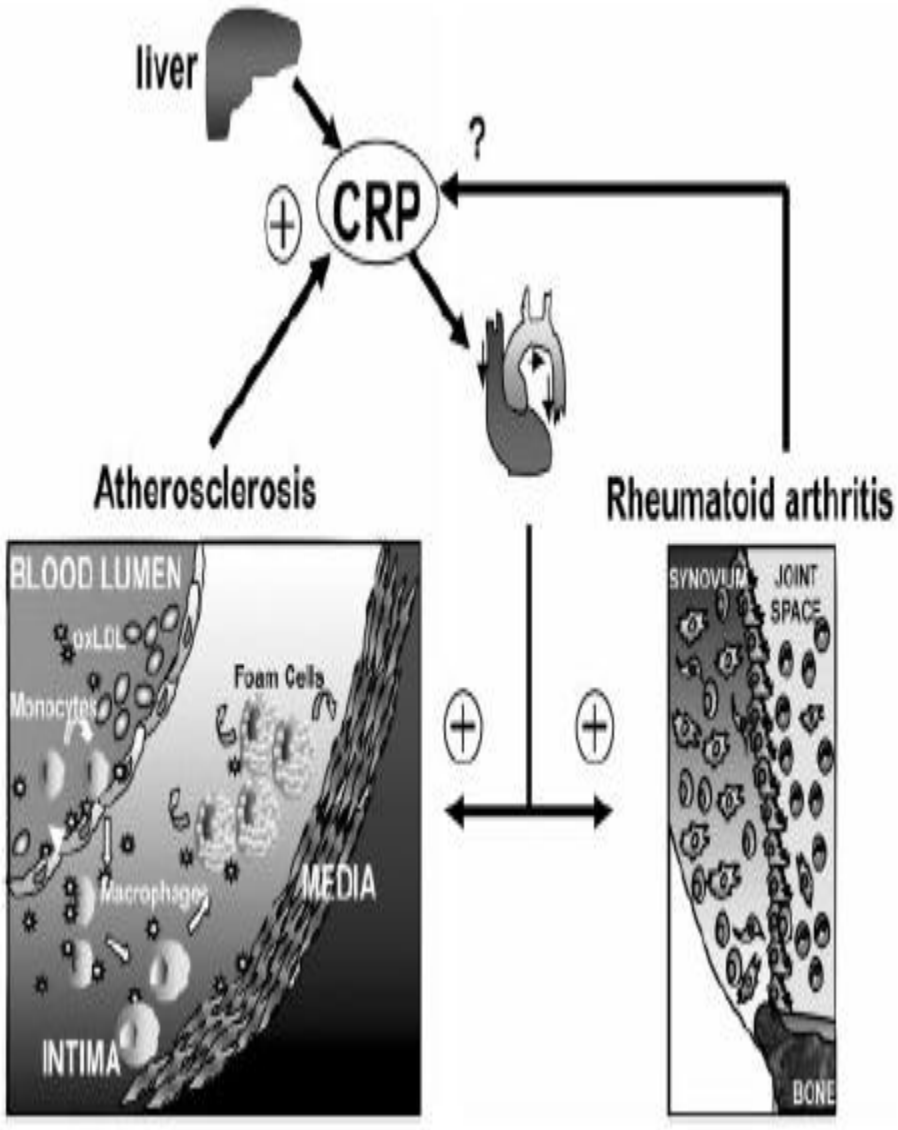

Figure 6: C-reactive protein increases both atherosclerosis and Rheumatoid arthritis (Montecucco and Mach, 2009).

I.4.2.2. Adaptive immune system

Rheumatoid arthritis is a prototypic example of a disease in which the relative contribution of adaptive immunity to disease pathogenesis is incompletely understood. Although numerous markers have been identified that reflect an activated adaptive immune system (Scherer and Burmester. 2011).

One of the striking characteristics of patients with moderate to severe RA is the presence in the synovial membrane of inflammatory infiltrates that resemble tertiary lymphoid structures, including follicular or germinal center like reactions. These structures likely play a key role in lymphocytes T and B cell cooperation and the local generation of specific autoantibodies. While this pattern of lymphocytic infiltration is found in a subset of patients, it provides robust evidence for ongoing immune reactions at the site of joint inflammation. It is likely that these structures harbor the core cell-to-cell interactions between T cells and B cells essential for immunoglobulin synthesis, as well as those between T cells and macrophages and resident stromal fibroblasts (Cope *et al.,* 2007).

I.4.2.2.a *Lymphocyte T and Rheumatoid arthritis*

T lymphocytes are involved at multiple check points in the immune pathogenesis RA. T helper and regulatory cells play a key role in the formation and maintenance of chronic inflammatory lesions in the synovial membrane, and the depletion of tissue-infiltrating T cells promptly disrupts the production of pro-inflammatory cytokines as well as tissue-injurious metalloproteinases. Besides the accumulation of activated T cells, B cells, macrophages, and dendritic cells in rheumatoid synovium, mechanisms of T cell homeostasis affecting the entire pool of T lymphocytes are also abnormal in patients with RA. While normal individuals have a highly diverse compartment of T cells, T cell diversity is contracted in RA patients and is accompanied by the emergence of grossly expanded clonotypes (Warrington *et al.,* 2001).

Over recent years, it has become clear that autoimmune inflammatory arthritis can't be explained simply in terms of a classical antigen-driven expansion of effector T-cell clones that target synovial joints. Furthermore, pathways of differentiation do not appear to conform to the traditional polarized pathways of T-cell differentiation, as early studies of rodent arthritis models (Cope *et al*., 2007) (**Figure 7**).

RA synovial T cells infiltrating affected synovial joints would express a cell surface phenotype that is compatible with prior antigen experience, is indicative of extensive proliferative activity, is suggestive of clonal expansions of subsets of antigen-specific T cells,

is consistent with enhanced migratory competence, and favors survival in situ. Current paradigms of adaptive immunity would also predict differentiation of T-helper cell subsets along a distinct effector T-cell lineage, whereas histological analysis of synovial tissue explants might reveal the presence of T cells as components of diffuse inflammatory infiltrates, with a prevalence of CD4+ over CD8+ T cells, in close association with APCs (Cope, 2008).

The recent excitement following the identification of a third Th cell subset, characterized by the expression of IL-17A, as well as IL-22, IL-21 and TNF, has raised the possibility that Th17 cells may be an important effector T-cell subset in diseases such as RA, multiple sclerosis, and inflammatory bowel disease. Certainly, expression of IL-17, IL-17RA, and IL-17RC has been described in RA synovial tissue and juxta-articular bone by immunohistochemistry, and IL-17 protein production has been detected in synovial fluid and culture supernatants of synovial mononuclear cell explants. Indeed, the cytokine milieu in the joint, with IL-1β, IL-6, and IL-23 in particular, would certainly support Th17 differentiation.

Nonetheless, IL-17 can stimulate the production of IL-1β and TNFα from macrophages and triggers human synoviocytes to produce IL-6, IL-8, granulocyte macrophage colony-stimulating factor, and prostaglandin E2, suggesting that IL-17 could be an upstream mediator in the pathogenesis of arthritis. Early neutralization of endogenous IL-17 prior to the development of arthritis in the experimental arthritis model suppresses the onset of disease. Furthermore, IL-17 may be involved in tissue destruction. IL-17 has biologic activities similar to those of IL-1β, and additive/ synergistic effects with IL-1β and TNFα. *In vitro*, IL-17 suppresses matrix synthesis by articular chondrocytes through enhancement of nitric oxide (NO) production (Lubberts *et al.*, 2004) (**Figure 8**).

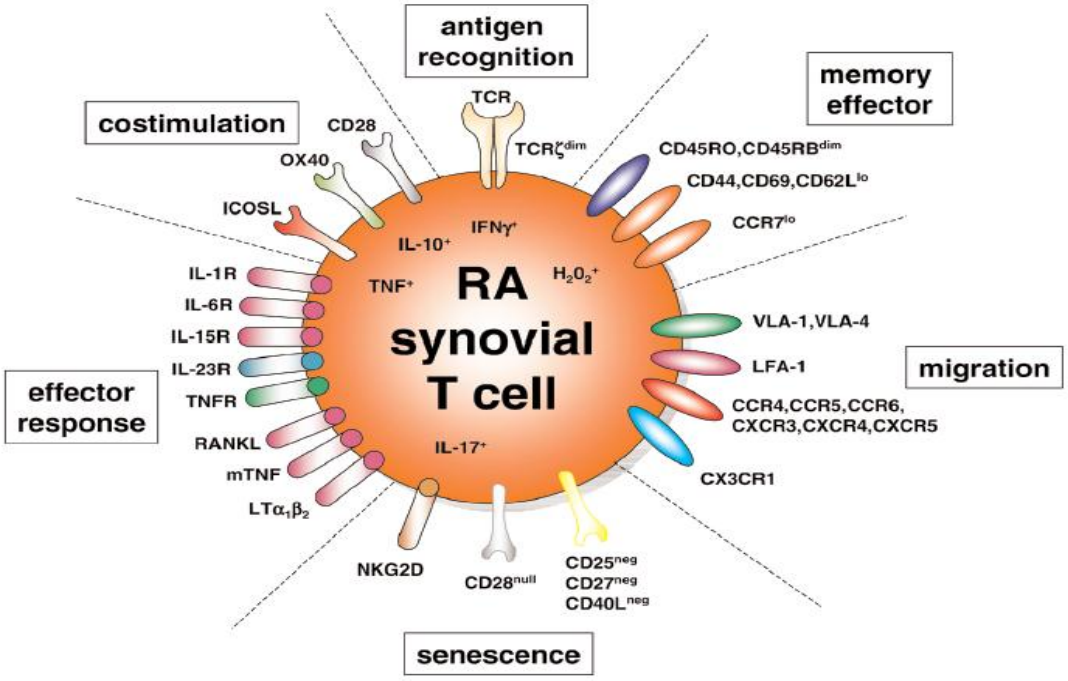

Figure 7: The phenotype of rheumatoid arthritis synovial T cells (Cope, 2008).

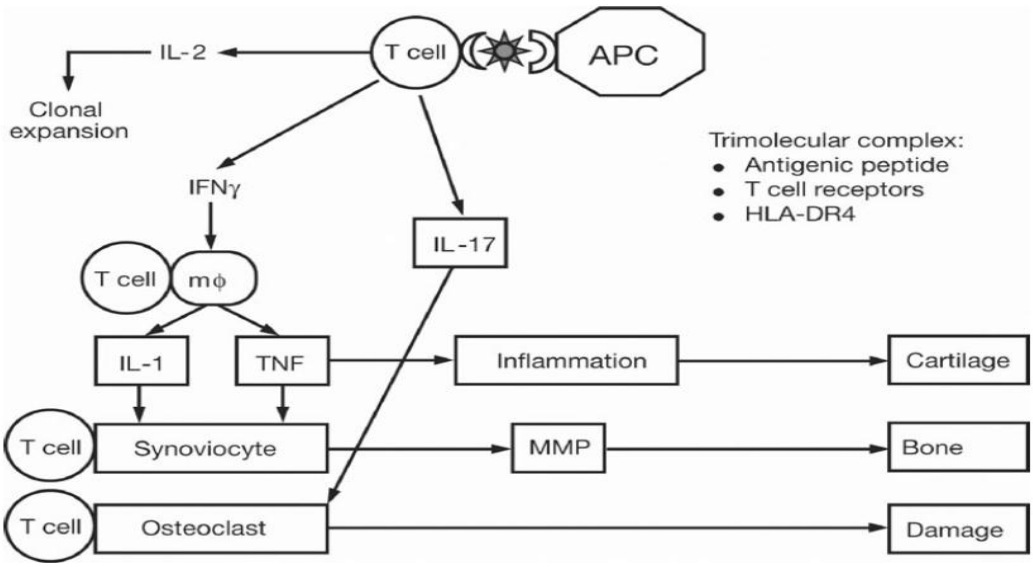

Figure 8: T cell-mediated pathways in Rheumatoid arthritis (Panayi, 2005).

I.4.2.2.b *Immune regulation in Rheumatoid arthritis*

Regulatory mechanisms in the peripheral immune system are required to protect against both the generation of self-directed immune responses and the consequence there of the initiation of autoimmune-mediated pathology. One such mechanism of peripheral tolerance involves the by CD4+ T cells with potent regulatory capacity. A major subset of these T cells is the CD4+, CD25+ regulatory T cell (Treg) subset. Tregs are characterized by low proliferative capacity upon triggering of the TCR with polyclonal or allogeneic stimulation *in vitro*, and by their ability to suppress CD4+ and CD8+ T-cell immune responses via cell-contact dependent mechanisms (Cope *et al.*, 2007). A major challenge to our understanding of the pathogenesis of immune-mediated diseases is to elucidate why pathways of peripheral tolerance might fail. To this end, efforts have been invested in evaluating the number and function of regulatory cell subsets, in order to test the hypothesis that at sites of synovial inflammation there may be defects in pathways of peripheral tolerance (Cope, 2008).

There remains controversy as to whether there are genuine defects in the numbers of circulating Tregs in RA. This may have as much to do with their selective migration and accumulation in synovial joints compared with peripheral blood. Whether the inflammatory milieu influences their survival and function remains a topic of great debate. This is because, despite expression of transforming growth factor-β (TGF-β), which might promote the expression and function of Treg subsets, inflammatory cytokines such as IL-7, IL-15, and TNF have been shown to suppress Treg activity (Cope, 2008).

These divergent results could reflect differences in the populations of patients, the methods used to purify Treg, or how the suppression assays were performed. Some evidence suggests that Treg from RA patients are defective in their ability to suppress the production of two principal pro-inflammatory cytokines IFN-γ and tumor necrosis factor TNF-α by effector T cells (Esensten *et al.*, 2009). Evidence from the Ehrenstein group (Ehrenstein *et al.*, 2004) has suggested that Treg from the peripheral blood of RA patients with active disease could suppress proliferation but were unable to suppress IFN-γ production, although their ability to suppress IL-17 production by T cells has not yet to be tested. This functional defect can be restored by TNF-α blockade by infliximab, possibly through the TGF-β dependent generation of a new population of Treg in the periphery (Nistala and Wedderburn, 2009).

Although existing drugs significantly reduce the morbidity and mortality associated with RA, these therapies are not curative. Several drugs that affect Treg numbers or function have shown efficacy in the treatment of RA. These results imply that direct administration of Treg could be an ideal therapy to induce durable remission of RA, as these cells persist *in vivo* and act in an antigen- specific manner. Thus, approaches that bolster Treg numbers and functions could be a fruitful means of selectively and durably inhibiting pathologic inflammation without blocking protective immune responses against infection (Panayi, 2005) **(Figure 9)**.

I.4.2.2.c *Lymphocyte B and Rheumatoid arthritis*

Humoral adaptive immunity is integral to rheumatoid arthritis. Synovial B cells are mainly localized in T-cell–B-cell aggregates indeed, some tissues have ectopic lymphoid follicles that are supported by the expression of factors that include a proliferation-inducing ligand (APRIL), B-lymphocyte stimulator (BLyS), and CC and CXC chemokines (e.g., CXC chemokine ligand 14 and CC chemokine ligand 21) (McInnes and Schett, 2011).

One of the main cellular components of the follicular infiltrates is B cells, which can differentiate into plasma cells. Approximately 30% of patients with RA have synovia that shows follicular B cells infiltrates (Panayi, 2005).

B cells can play a number of potentially critical roles in the pathogenesis of RA. They may function as APCs by processing and presenting antigenic peptides to the T cells. The T cells then proliferate and exert pro-inflammatory activities. It is well known that B cells can bind antigens through their immunoglobulin receptor. The immunoglobulin receptor lies on the surface of the B cell and can bind a very low level of antigen from the environment. The antigen is degraded by the B cell into antigenic peptides. These antigenic peptides are then presented in the groove of the HLA-DR4 molecule to activate the T cells, which in turn undergo various processes, including proliferation, cytokine production and cell-to-cell interaction, which contribute to the pathogenic process in RA (Panayi, 2005).

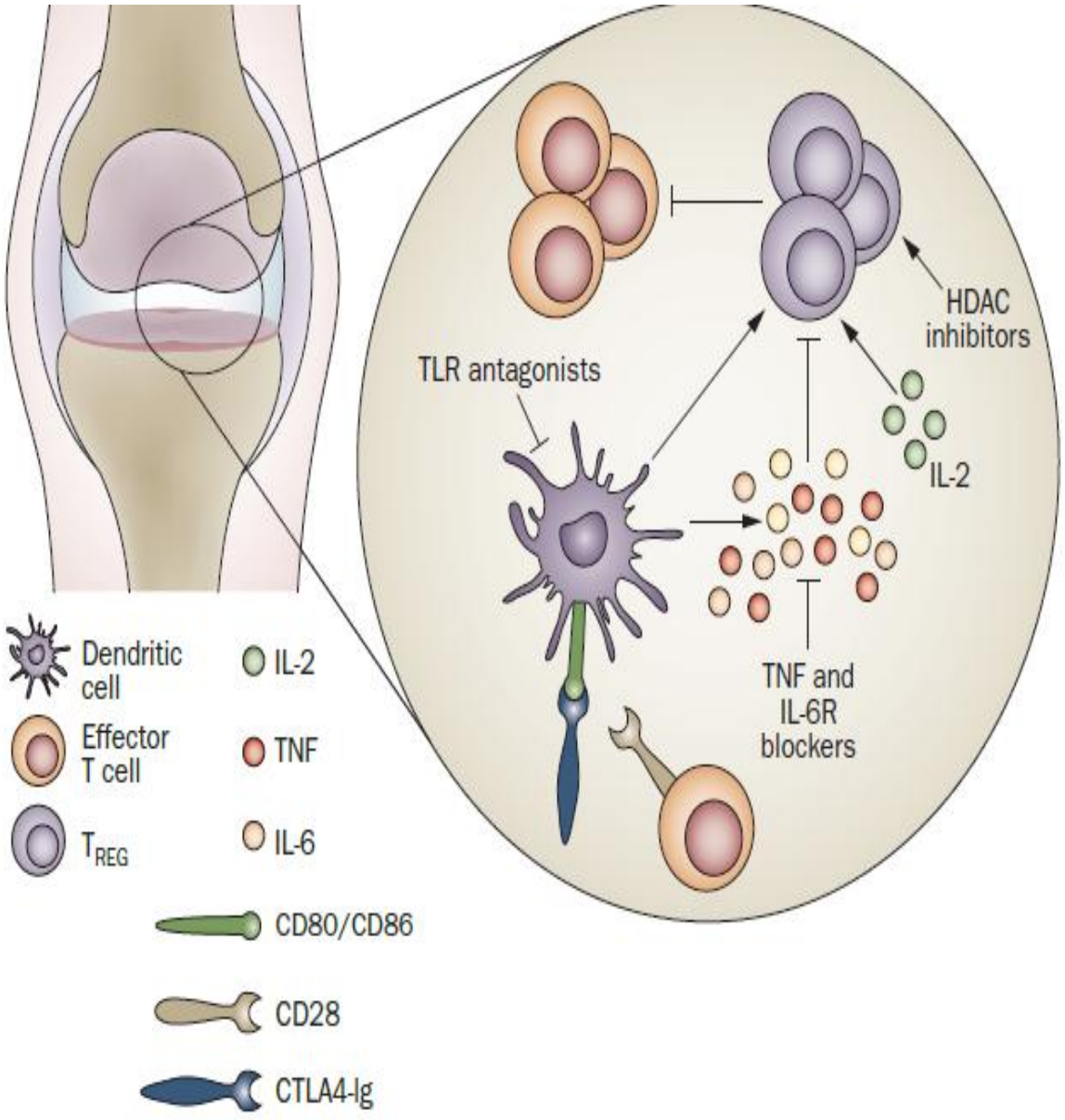

Figure 9: Effects on Treg of various therapies for Rheumatoid arthritis (Esensten *et al.*, 2009).

In 80 % of patients with RA, B cells produce autoantibodies that are specific for the constant region of IgG. These antibodies are called Rheumatoid factors (RFs) and the most common isotype is IgM, but IgG and IgA also exist (Rehnberg, 2012). Furthermore, the severity and activity of RA has been correlated with RF levels, and 'seropositive' RA is associated with more aggressive articular disease, a higher frequency of extra-articular manifestations and increased mortality and morbidity (Panayi, 2005). However, the presence of RF is not specific for RA but is rather a general consequence of immune activation in the context of immune complex formation and no experimental studies have demonstrated any pro-arthritogenic effects of RF (Klareskog *et al.,* 2008).

Patients with RA also often have antibodies against cyclic citrullinated proteins (CCP) like type II collagen, heat shock proteins, proteoglycans, cartilage link proteins and heavy chain binding proteins (Rehnberg, 2012). Collagen type II (CII) is a relevant joint-specific auto-antigen in the pathogenesis of (RA). Whereas the reasons for the breakage of self-tolerance to this major cartilage component are still enigmatic, T cell responses to glycosylated CII determinants in RA patients indicate that post-translational modifications play a role. Since the conversion of arginine into citrulline by peptidylarginine deiminases (PAD) in some non-joint-specific antigens such as filaggrin or fibrin has been shown to give rise to RA-specific humoral immune responses by anti CCP (Burkhardt *et al.*, 2005).

Antibodies to citrullinated protein antigens are detected in ~70% of RA patients using second-generation anti-CCP. These IgG autoantibodies recognize citrulline in the context of neighboring amino acids. Serum antibodies from a CCP-positive individual RA patient typically recognize multiple citrullinated epitopes, and there is considerable patient-to-patient variation in the reaction patterns (Imboden, 2009) **(Figure 10).**

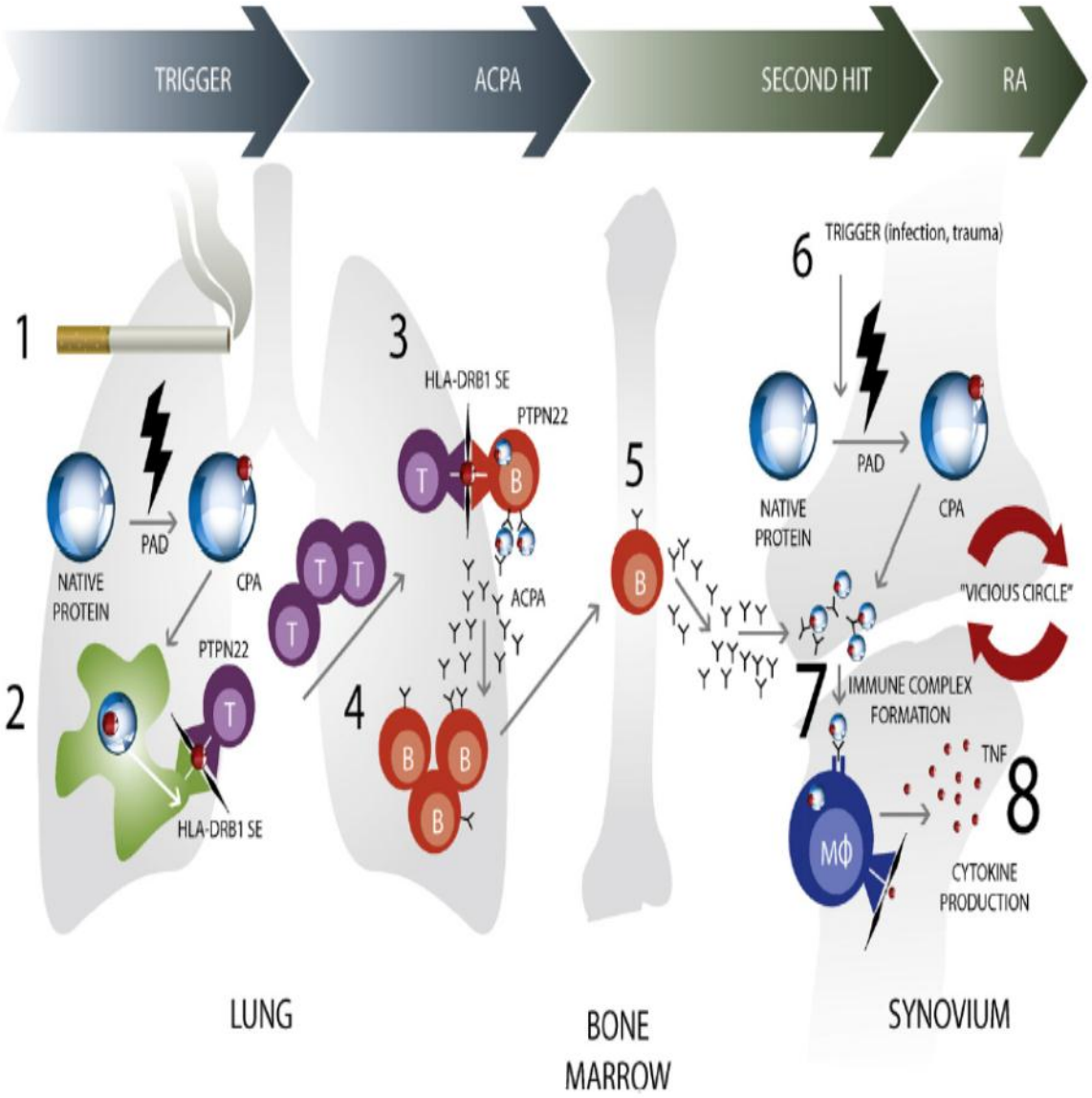

Figure 10: Illustration of the hypothetical evolution of ACPA-positive RA from a pre-disease state into the chronic polyarthritis that fulfills criteria for Rheumatoid arthritis (Klareskog *et al.*, 2011)

I.4.3. Bone and cartilage destruction

The normal joint comprises a thin synovial membrane, which spans between the joint ends and constitutes the inner layer of the joint capsule. The inner layer of the synovium, which is directed to the synovial space containing the synovial fluid, is a fine mesodermal membrane composed of one to two cell layers. The synovial membrane inserts at the periosteum of both joint ends and is in close connection with neighbouring ligaments and tendons. In the case of arthritis this synovial membrane faces a dramatic structural change, which is based on the influx of immune cells such as monocytes/macrophages and neutrophils, as well as T and B lymphocytes. In addition, proliferation of resident synovial fibroblasts occurs, contributing to synovial hyperplasia. Based on the close relationship of the synovial membrane to cartilage and bone, these structures are severely damaged during arthritis and face structural remodeling during the course of the disease (Schett, 2007). Knowledge of this complex destructive process is predominantly driven by findings of radiographic examinations, which have identified local bone erosions as well as joint space narrowing as key monitoring parameters in RA. From these findings it is apparent that inflamed synovial tissue has the capacity to invade neighboring structures such as bone and cartilage (Jimenez-Boj *et al.,* 2007), which has a relatively simple structure compared to other tissues, cartilaginous injuries can be extremely unforgiving. Its limited blood supply and lack of neural innervations often result in incomplete and inadequate repair by local physiologic measures (Daher *et al.,* 2009).

Two important questions can be raised with respect to the pathogenesis of focal bone erosions in RA. The first relates to the cell type responsible for bone resorption and the second relates to the definition of the mechanisms that underlie the disturbance in bone remodeling that accounts for the progressive bone loss (Goldring, 2003).

I.4.3.1. Phase of bone and cartilage destruction

I.4.3.1.a *Osteoclasts*

In rheumatoid arthritis, osteoclasts are the primary bone reabsorbing cells, accumulate and degrade the periarticular bone as well as the mineralised cartilage. Osteoclasts are specialized cells that reabsorb bone and their local accumulation in the joint by far outweighs bone formation and reflects a catabolic state. Osteoclasts form locally in the joint and differentiate from mononuclear precursor cells abundantly present in the inflamed joint due to the influx of monocytes in the context of inflammation. Further studies indicated that synovial fibroblasts express membrane-bound factors that stimulate osteoclastogenesis and induce the differentiation of synovial macrophages into osteoclasts; however, it was not until RANKL was cloned (Goldring, 2003).

Remarkable progress has been made in recent years in the field of osteoclast research primarily due to the finding of the receptor activator of nuclear factor κB (NF-κB) ligand (RANKL)/RANK system. RANKL binds to its receptor RANK, a transmembrane receptor belonging to the TNF receptor superfamily, which is expressed in monocyte-macrophage lineage which is the osteoclast precursor cells as well as in mature osteoclasts and dendritic cells (Takayanagi, 2009). Binding of RANKL to RANK induces intracellular signals including NF-κB activation and c-Jun N-terminus kinase activation resulting in differentiation and activation of osteoclasts and play an essential role in osteoclast development and bone destruction in RA (Tanaka, 2013) **(Figure 11).**

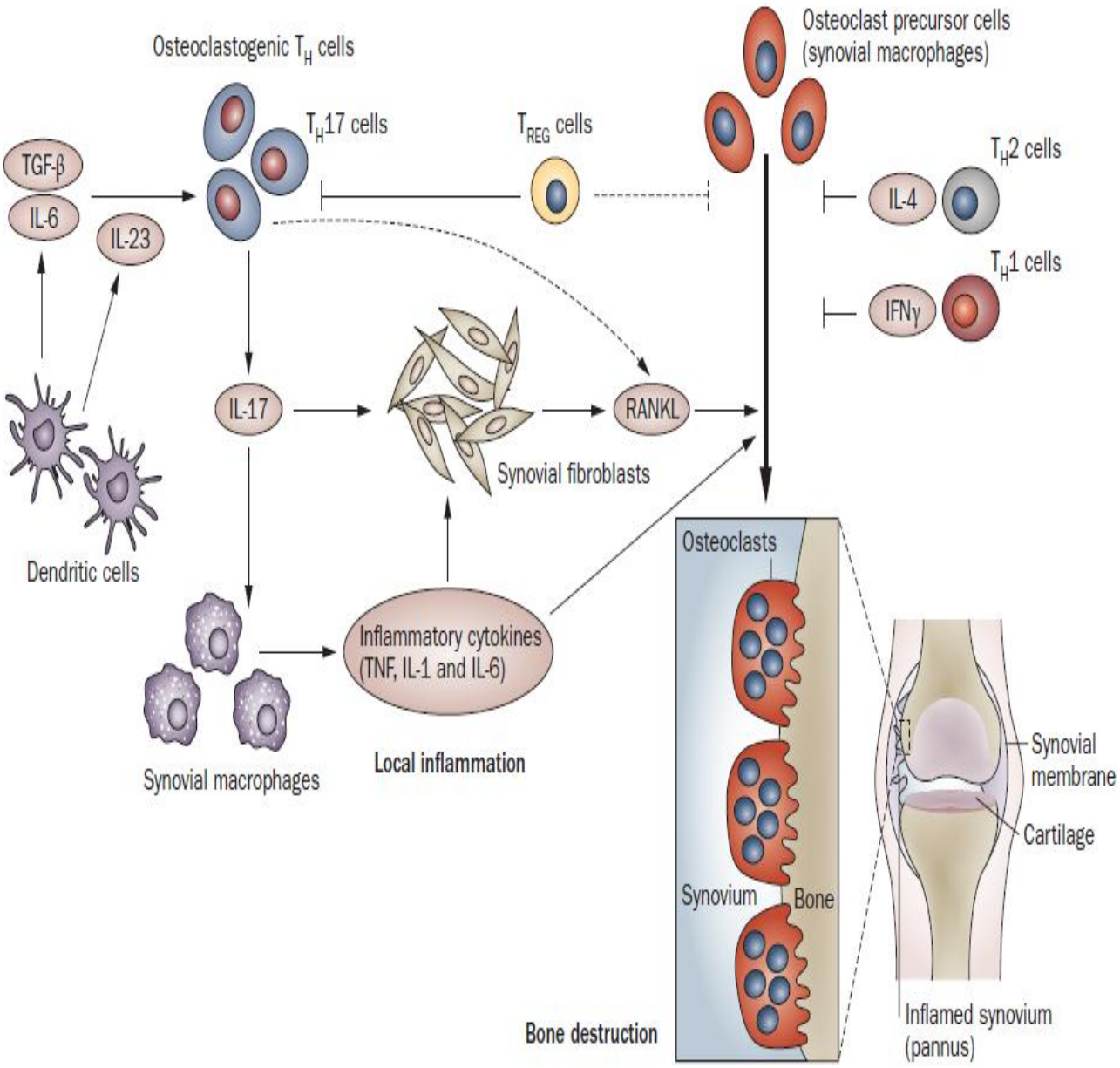

Figure 11: Mechanism of bone destruction in Rheumatoid arthritis (Takayanagi, 2009).

Chapter II

Materials and Methods

II.1. Materials

II.1.1. Plant material

II.1.1.1. Collection

The jam was prepared from the date palms (*Phoenix dactylifera* AZ and HM varieties) which were collected from Ghardaïa and Adrar (Algerian septentrional Sahara).

II.1.1.2. Preparation of the extracts

II.1.1.2.a *Preparations of the date jam "Roub"*

The preparation was realized following the protocol used in the laboratory of Pr: KABOUCHE Zahia (Laboratoire d'obtontion des substances thérapeutiques "LOST").

The first step of the preparation was to boil the total of the date fruit with enough water until it appears a white colour on the seeds as a sign of being well cooked. Then the mixture was squeezed and clearly separated from the solution which recooked one more time with a round pieces made of wheat for the absorption of the extra water.

II.1.1.2.b *Preparation of Acetone and Methanol extracts*

The preparation was realized following the protocol used in the laboratory of Dr: D'hallewin Guy (Institute of sciences of food production; Sassari, Italy)

1. ***Acetone extraction***

1.5 g of the date extract was solubilised with 10 ml of Acetone (70%, 30%) and centrifuged for 15 minutes. Then the supernatant was obtained and the pit was solubilized another time twice in 10 ml of acetone. After that, the collected supernatant was put under a rota-vap for ≈ 45 min. After we put the bottles of the acetone extract in the ice dryer (after we freeze them in the -80°C) we recuperated the dried extract and conserved it in glass containers.

2. ***Methanol extraction***

1.5 g of the date extract was solubilised with 10 ml absolute Methanol and centrifuged for 15 minutes. Then the supernatant was obtained and the pit was solubilized another time twice in 10 ml of acetone. After that, the collected supernatant was put under a rota-vap for ≈ 45 min.

After 1 night in the freezer we took the simples extracted with the methanol and we obtained the extract in glass containers.

- Before the cell culture, extracts were diluted in medium and sterilized using Ministart® Single use filter unit.

II.1.2. Animals

Adult male Mus Musculus albinos mice (2-3 month old) were procured from central pharmacy Algeria. The animal experiments weighing (20–33 g) were used for all the *in vivo* study part. The animals were kept in polyacrylic cages and maintained under standard housing conditions and water adlibitum. Food was provided in the form of dry pellets (SARL Production Locale, Bouzareah. Algeria).

II.2. Methods

II.2.1. Acute Oral Toxicity

The present study was conducted according to the guideline proposed by the Organisation for Economic Cooperation and Development (OECD) revised up-and-down procedure for acute toxicity testing. This guideline is based on the procedure of (Bruce, 1985).

A dose limit of 2000 mg/kg of *Phoenix dactylifera* extracts was used in five healthy male adult mice. Mice were fasted overnight from food, but not water, prior to dosing and weighed before the extract was administered orally. A dose of 2000 mg/kg was given to the first mouse, and this mouse was observed for mortality and clinical signs (behaviours: unusual aggressiveness, unusual vocalization, restlessness, sedation and somnolence) for the first hour, then hourly for 3 h and, finally periodically until 48 h. If the animal survived, then four additional animals were given the same 2000 mg/kg dose sequentially at 48-h intervals. All of the experimental animals were maintained under close observation for 14 days, and the number of mice that died within the study period was noted. The LD50 was predicted to be above 2000 mg/kg if three or more mice survived.

II.2.2. Evaluation of the plant extracts immunostimulatory activity

The immunostimulant potential of the plant extract of Phoenix *dactylifera* on the phagocytic activity of reticuloendothelial systems (RES) was assayed by carbon clearance test. Phagocytic index was calculated as a rate of carbon elimination of reticuloendothelial systems by carbon clearance test determined by a reported method (Biozzi *et al.*, 1955).

Animals were divided into four groups, GI, GII, GIII and GIV. Group I (Control) was given by intra-peritoneal (i.p) injection 0.9% NaCl (0.5 ml/mouse.), groups II, III and IV were administered with different concentrations of the *Phoenix dactylifera* varieties **(Table 2 and 3)**.

After 48 h of i.p injection, Carbon ink suspension was injected via the tail vein to each mouse at a dose of 0.1 ml/10g, the mixture consisted of black carbon ink 3 ml, saline 4 ml and 3% gelatin solution 4 ml. Blood samples (≈14 drops or 25µl) were then withdrawn from the retro-orbital plexus at 5 and 15 minutes after injection of colloidal carbon ink via heparin glass capillaries and lysed in 0.1% sodium carbonate solution (4ml). The optical density was measured spectrophotometrically at 676nm.

Table 2: Treatment of mice in carbon clearance rate test *Phoenix dactylifera* AZARZA

Experimental groups	**Treatment**	**Number of mice**	**Dose**
GI	Nacl 0,9%	5	0.5 ml/mouse
GII	*Phoenix dactylifera* AZ	5	30mg/kg
GIII	*Phoenix dactylifera* AZ	5	50mg/kg
GIV	*Phoenix dactylifera* AZ	5	100mg/kg

Table 3: Treatment of mice in carbon clearance rate test *Phoenix dactylifera* HOMAYRA

Experimental groups	**Treatment**	**Number of mice**	**Dose**
GI	Nacl 0,9%	5	0.5 ml/mouse
GII	*Phoenix dactylifera* HM	5	50mg/kg
GIII	*Phoenix dactylifera* HM	5	150mg/kg
GIV	*Phoenix dactylifera* HM	5	200mg/kg

The phagocytic activity is expressed by the phagocytic index **K** which measures all the reticuloendothelial system function in the contact with the circulating blood and by corrected phagocytic index **α** which expresses this activity by unit of active weight organs: liver and spleen. The clearance rate is expressed as the half-life period of the carbon in the blood (**t½**, min) (Biozzi *et al.*, 1970). These parameters are calculated using the following formulas:

$$K = \frac{\log OD\ 1 - \log OD\ 2}{t2 - t1} \qquad t½ = \frac{0.693}{K}$$

$$\alpha = \sqrt[3]{K}\,\frac{Body\ weight\ of\ animal}{Liver\ weight + spleen\ weight}$$

OD1 and OD2 are the optical densities at time t1 and t2, respectively.

II.2.3. Evaluation of the plant extracts anti-oxidant activity

The anti-oxidant activity was measured by spectrophotometric determination of glutathione from liver's homogenate following the method of Weckbeker *et al*, 1988 (Rahman *et al.*, 2006).
At the end of the experience for the Immunostimulatory activity the animals were sacrificed and the liver and spleen dissected and weighted immediately in the wet state**.**

II.2.3.1. Preparation of the homogenate

The weight of 0,5g of the liver was homogenized in 2ml of TBS (Tris 50 mM, NaCl 150 mM, pH 7.4). Then the homogenates were centrifuged at 9000 g for15 min at 4˚C after that the supernatant was used for determination of glutathione reduced (GSH).

II.2.3.2. GSH dosage

The glutathione reduced content in the liver was measured spectrophotometrically by using 5,5′-dithiobis-(2 nitrobenzoic acid) (DTNB) as a coloring reagent.
The experimental procedure for the glutathione dosage is the following:

- 0.8 ml of the homogenate was added to 0.2 ml of the sulfo-salicylique acid (0.25%) solution, Then the mixture was incubated in an ice bath for 15 min.

- Next a centrifugation at 1000 tours/min for 5 min was realized. After that, 0.5 ml of the supernatant and 1 ml of the buffer Tris-EDTA (PH 9.6) were added to 0.025 ml of DTNB of 0.01 M and after 5 min the optical density was measured at 412 nm.

The glutathione concentration was calculated by the following formula:

$$\boldsymbol{GSH}\ (\mathbf{nmol}\frac{\mathbf{GSH}}{\mathbf{mg}}\mathbf{protein}) = \frac{\boldsymbol{DO} \times \mathbf{1} \times \mathbf{1.525}}{\mathbf{13100} \times \mathbf{0.8} \times \mathbf{0.5} \times \mathbf{mg\ protein}}$$

- DO: Optical Density.
- 1: total volume of the protein decomposition solutions (0.8 ml the homogenate + 0.2 sulfo-salicylique acid).
- 1.525: volume of the total solutions used for the GSH dosage at the supernatant level (0.5 ml supernatant + 1 ml Tris-EDTA + 0.025 ml DTNB).
- 13100: group –SH absorbance coefficient at 412 nm.
- 0.8: homogenate volume.
- 0.5: supernatant volume.

The proteins concentration was determinated by method of Bradford (1976) by adding 0.1 ml of the homogenate with 5 ml of the Bradford reagent and after 5 min the optical density was measured at 595 nm.

The proteins concentration was calculated by a comparison to the BSA calibration line realized in the same conditions.

II.2.4. Evaluation of the plant extracts anti-inflammatory activity

Anti-inflammatory activity of the *Phoenix dactylifera* extract was analyzed *in vivo* using the formalin induced paw edema test in mice during 5 hours (Uma *et al.*, 2014).

The inflammation level was measured by the measure of the edema size, also the dosage of C - reactive protein (CRP) and homocysteine (Hcy) levels in the blood.

Animals were divided into five groups of 5 mice each (**Table 4**): “N” negative control “F” (control group) was given flour balls orally and three treated groups: “P” (for AZARZA variety) was given the plant extract (HM for HOMAYRA variety), “M” was given methionine 400 mg/kg

and "MP" (for AZARZA variety and MHM for HOMAYRA variety) was given both plant extract and methionine (Sakhri, 2014), (Boudebouz, 2013).

Table 4: Treatment of mice in formalin induced inflammation and hyperhomocysteinemia test *Phoenix dactylifera*

Experimental groups	Flour	Formalin	Dose	Methionine
N	+++ 0.1 g	/		/
F	+++	+++ 100 µl		/
P	+++	+++ 100 µl	50mg/kg	/
HM	+++	+++ 100 µl	150 mg/kg	/
M	+++	+++ 100 µl	400 mg/kg	+++
MP, MHM	+++	+++ 100 µl	50mg/kg,150mg/kg	+++ 400 mg/kg

The administration was realised orally by mixing the plant extract and the methionine of the treated groups into a flour balls.

II.2.4.1. Formalin test in mice

100 µl of formalin (2%) was injected into the sub-plantar of the right hind paw and the edema size was measured by a digital caliper in the moment of the injection and after every 1 hour for 5 hours.

After the 5th hour blood was withdrawn from the retro-orbital plexus for the CRP and Hcy dosage.

II.2.5. Evaluation of the plant extracts anti-arthritis activity

Experimental arthritis was induced in mice according to the methods described by (Mazumder *et al.*, 2012)

After acclimatization of one week, adult mice were divided into four groups: negative control group treated only with flour (N), group treated with 0.1 ml of formalin (2%) (FF), group treated by the *Phoenix dactylifera* extracts (P) and the last group was treated by 10 mg/kg of an anti-inflammatory drug (Diclofenac of Sodium 50mg) (D) **(Table 5)**.

Table 5: Treatment of mice in formalin induced arthritis test *Phoenix dactylifera*

Experimental groups	Flour	Formalin	Dose
N	+++	/	0.1 g
FF	+++ 0.1 g	+++	100 µl
AZ	+++ 0.1 g	+++ 100 µl	50mg/kg
HM	+++ 0.1 g	+++ 100 µl	150 mg/kg
D	+++ 0.1 g	+++ 100 µl	10 mg/kg

One of the most known symptoms of arthritis is the chronic inflammation. In this study the chronic inflammation was detected *in vivo* by measuring the edema size of right paw hind using a digital caliper all the period of treatment (10 days). At the end of the study, mice were fasted overnight, and the blood samples were collected from the retro orbital vein into Heparin tubes by using glass capillaries. They were centrifuged immediately, and the plasma was used to realize some specific lab tests for arthritis such us anti citrullinated cyclic proteins (Anti-CCP) and CRP.

The body weight was measured every day of the experiment period

II.2.6. Biochemical analysis

At the end of the treatment, mice were fasted overnight, and the blood samples were collected from the retro orbital vein into Heparin tubes by using glass capillaries. They were centrifuged immediately, and the plasma was stored at -30°C.

II.2.6.1. Homocysteine test

The total Hcy test was done by IMMULITE analyser. The main principal repose on the technic of chimioluninescent enzymatic immunodoge with competition on liquid phase.

The test started by a pre-treatment step consist to free the disulphate thiol (–SH) function by S-adenosyl-L-homocystéine (SAH), hydrolase and dithiothreitol solutions. After 30 min, the pre-treated simples were transferred into another tube contain a specific antibody for SAH and a polystyrene ball covered of SAH.

During the 30 min incubation, the modified SAH come from the pre-treated simples enter in a completion with the fixed to be linked to the SAH antibody marked with alkaline phosphatase. The conjugated not linked was eliminated by washing and separated by ultracentrifugation. The substrate was added and the rest of the technic is just the classic immunodosage.

II.2.6.2. C-Reactive Protein test

The plasma hs-CRP values were measured by by an immunoturbidimetric method on a Cobas integra 400 plus analyser (Roche). The analysis was performed in the medical laboratory IBN SINA, Constantine.

II.2.6.3. Anti-Citrullinated Cyclic Peptides test (Anti-CCP)

The test was effectuated at the IBN SINA medical analysis laboratory by ELIZA technic using "EUROIMMUN ANALYSER -1-" (Germany) multi-parametric automate and EUROIMMUN KIT as reagent.

II.2.7. Histological study of the joints

At the end of the *in vivo* study, the animals were sacrificed and the joints were taken to been used for the Histological study.

II.2.7.1. Dissection protocol

- The animal was pinned down with the belly facing up. Then, it was wet with ethanol by washing the carcass in order to disinfect the tissues.
- The animal was cut along the ventral midline from the groin to the chin, PS: be careful to only cut the skin and not the muscle wall underneath.
- An incision was made from the start of the first incision downward to the knee on both sides of the animal and pull the skin back on the sides.
- After opening both the abdominal cavity and the rib cage, the organs were collected and cleaned with phosphate buffer saline (PBS) pH 7.4.

II.2.7.2. Histological sections preparation

II.2.7.2.a *Joint's bone softness*

After the dissection of the animals, the joints were cut and kept in small containers filled with diluted formol 10%. The first step in protocol is to incubate the organs in the formic acid (2%) for 48 h.

II.2.7.2.b *Tissue fixation*

As a second step, the dehydration is performed through a series of ethanol solution (50%, 70%, and 96%), each step was placed for approximately 30 min (3×30 min=1h 30min). The organs were then kept in small containers filled with butanol for an entire week. After that, they were immersed twice in a xylene solution, for 10 min each time.

II.2.7.2.c *Infiltration and embedding in paraffin*

The organs were immersed in paraffin twice, for 2 hours each time. The sectioning is performed with a microtome.

Paraffin slices of 5µm thick were stained following the haematoxylin eosin staining protocol.

II.2.7.2.d *Hematoxylin eosin staining*

- Dip slides in alcohol for 5minutes.
- Rinse with water.
- Stain slides in hematoxylin for 4 minutes.
- After rinsing stain with eosin for 10 minutes.
- Rinse with water.
- Dip slides in alcohol for 1 minute.
- After rinsing and drying, the editing is done using xylene.

II.2.8. Evaluation of the plant extracts anti-proliferative activity

To evaluate the anti-proliferative effect of the plant extracts, hepatocarcinoma cells line HepG2, Breast cancer cell line MCF7 from the Anatomia pathologica (Civil hospital of Cagliari, Italy) and two normal human cells, normal Human Umbilical Vein Endothelial Cell (HUVEC) (ATCC®, PCS-100-010™) and normal human hepatocytes (h NHEPS) (LONZA, CC-2591S) were used following this protocol for MTT test following the protocol of American Type Culture

Collection (ATCC), MTT Cell Proliferation Assay ATCC® 30-1010K kit (the protocol was realized under the Thermo scientific "MAXISAFE 2020" laminar flow hood)

II.2.8.1. MTT test

Measurement of cell viability and proliferation forms the basis for numerous *in vitro* assays of a cell population's response to external factors. The reduction of tetrazolium salts is now widely accepted as a reliable way to examine cell proliferation. The yellow tetrazolium MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) is reduced by metabolically active cells, in part by the action of dehydrogenase enzymes, to generate reducing equivalents such as NADH and NADPH. The resulting intracellular purple formazan can be solubilized and quantified by spectrophotometric means. The number of assay steps has been minimized as much as possible to expedite sample processing. The MTT Reagent yields low background absorbance values in the absence of cells. For each cell type the linear relationship between cell number and signal produced is established, thus allowing an accurate quantification of changes in the rate of cell proliferation.

II.2.8.1.a *Preparation of cell culture medium*

The cell culture medium was prepared by using, 200 ml of SIGMA MEM (Minimal Essential Medium), 20 ml of FBS (Fetal Bovine Serum) (10%), 4 ml of antibiotic made of penicillin and streptomycin, 2 ml of glutamine and 2 ml of SIGMA MEM nonessential amino acid.

II.2.8.1.b *Trypsinization and cell counting*

Human breast cancer cell line (MCF7), hepatocarcinoma cells (HepG2), normal Human Umbilical Vein Endothelial Cell (HUVEC) and normal human hepatocytes (h NHEPS) from one T75 flask were used in this part of the study. First, the medium was removed and the flask was washed by 2ml of Dulbecco's Phosphate-Buffered Saline (DPBS) (gibco® by Life Technologies™). After removing the DPBS, 1.5 ml of trypsin (gibco® by Life Technologies, cascade biologics™) was added to the flask (cells + trypsin) and then incubated for 5 min in CO2 incubator (Thermo scientific "FORMA STERI-CYCLE"). After that, 4.5 ml of medium was added to flask (cells + trypsin + medium) and with a smooth pipetting to mix the solutions which then had been transferred in conic tubes with 3 ml of DPBS used also to wash the cells from the

flask. Then the tubes transferred for 5min/300g centrifugation (Thermo scientific SL40). After the centrifugation, the supernatant was removed and the cells were solubilized in 2ml of the medium.

The next step was to know the number of cells in the 2 ml of cell solution. For that objective, 10 µl of trypan bleu was added to 10 µl of the cell solution. Then, 10 µl of the mixture was transferred to each side of Countess™ cell counting chamber slides. The last step was the cell counting using Invitrogen™ "Contess automated cell counter".

II.2.8.1.c *MTT protocol*

MCF7, HepG2, HUVEC and h NHEPS cells were seeded in 96 well plates (5000 Cells/100µl for well) and then incubated at 37°C for 24 hours. After 24 hours the medium was removed and 6 different concentrations of the methanol and acetone extract from each variety **(Table 6)** were added and incubated at 37°C for 24 hours. After that, the extracts were removed and 100µl of MTT (0.65 mg/ml) was added to each well and incubated at 37°C for 2 hours. The last step in the protocol was to remove carefully the medium and 100µl of DMSO (Dimethyl Sulfoxide, Sigma D2650 5x5 ml) was added to each well and the absorbance was read at 570nm with background subtraction at 630-690nm.

The percentage of cell growth was calculated using the following formula (Patel *et al*, 2009):

$$\% \text{ cell proliferation } = \frac{\text{At} - \text{Ab}}{\text{Ac} - \text{Ab}} \times 100$$

Where,

At= Absorbance value of test compound (*Phoenix dactylifera*).

Ab= Absorbance value of blank (Medium alone).

Ac= Absorbance value of control.

Table 6: Treatment of cells in the MTT viability test by *Phoenix dactylifera*

Code	Concentration	Treatment	Code	Concentration	Treatment
A1.1	2µg/100µl	Acetone AZ	**B1.1**	2µg/100µl	Acetone HM
A1.2	4µg/100µl	Acetone AZ	**B1.2**	4µg/100µl	Acetone HM
A1.3	7,5µg/100µl	Acetone AZ	**B1.3**	7,5µg/100µl	Acetone HM
A1.4	8µg/100µl	Acetone AZ	**B1.4**	8µg/100µl	Acetone HM
A1.5	16µg/100µl	Acetone AZ	**B1.5**	16µg/100µl	Acetone HM
A1.6	20µg/100µl	Acetone AZ	**B1.6**	20µg/100µl	Acetone HM
A2.1	2µg/100µl	MeOH AZ	**B2.1**	2µg/100µl	MeOH HM
A2.2	4µg/100µl	MeOH AZ	**B2.2**	4µg/100µl	MeOH HM
A2.3	7,5µg/100µl	MeOH AZ	**B2.3**	7,5µg/100µl	MeOH HM
A2.4	8µg/100µl	MeOH AZ	**B2.4**	8µg/100µl	MeOH HM
A2.5	16µg/100µl	MeOH AZ	**B2.5**	16µg/100µl	MeOH HM
A2.6	20µg/100µl	MeOH AZ	**B2.6**	20µg/100µl	MeOH HM
CTR+	100µl	Control (Medium + cells)			
CTR-	100µl	blank (Medium)			

II.2.8.2. Gene expression in breast cancer cells

MCF7 cells were seeded in 24 well plates (60.000 Cells/500µl for well) and then incubated at 37°C for 24 hours. After 24 hours the medium was removed and 3 different concentrations of the methanol and acetone extract from each variety **(Table 7)** were added and incubated at 37°C for 24 hours. After that, the extracts were removed and the wells were washed with 500µl of DPBS. Then the DPBS was removed and 200µl of trypsin was added to each well and the plate was incubated at 37°C for 5min. Next, the wells were washed by 600µl of medium and 500µl of DPBS and the cells solution was transferred for centrifugation (5min, 2000g RPM). At the end the supernatant was removed and the pellet was conserved in eppendorf tubes.

Table 7: cells Treatment during the gene expression test by *Phoenix dactylifera*.

Code	Concentration	Treatment	Code	Concentration	Treatment
A1.1	4µg/100µl	Acetone AZ	**B1.1**	4µg/100µl	Acetone HM
A1.2	8µg/100µl	Acetone AZ	**B1.2**	8µg/100µl	Acetone HM
A1.3	16µg/100µl	Acetone AZ	**B1.3**	16µg/100µl	Acetone HM
A2.1	4µg/100µl	MeOH AZ	**B2.1**	4µg/100µl	MeOH HM
A2.2	8µg/100µl	MeOH AZ	**B2.2**	8µg/100µl	MeOH HM
A2.3	16µg/100µl	MeOH AZ	**B2.3**	16µg/100µl	MeOH HM
CTR	500µl	Control (Medium + cells)			

After that the cells were used for the RNA extraction for the apoptotic genes expression by two steps RT-PCR (reverse transcription-polymerase chain reaction) which is a sensitive method for the detection of mRNA expression levels. Traditionally RT-PCR involves two steps: the RT reaction and PCR amplification. RNA is first reverse transcribed into complementary DNA (cDNA) which is then used as templates for subsequent PCR amplification using primers specific for the BAX, BCL2) (Orrù *et al*., 2006).

The RNA extraction was realized following this protocol:

- 500µl of Trizol was added to each eppendorf with 100µl of chloroform (agitated for 15 sec with vortex) and incubated for 10 min. Then, the solution was centrifuged for 15min (4°C, 12000g). Next, the supernatant was recuperated (the transparent phase) and added with 250µl of isopropanol (shake and put for 10 min in ice). Then the tubes were centrifuged for 10 min (4°C, 12000g). After removing the supernatant, 500µl of ethanol was added and then centrifuged for 5min (7500g). Next, the ethanol was removed (wait ≈ 5min to evaporate all the ethanol) and 20µl of water was added.

- The RNA extracted was then used for the reverse transcription (RT reaction) of the cDNA by a first-stand cDNA protocol.

At the beginning of this protocol, a mix was made for each simple by adding 5µl of water (RNAse and DNAse free) with 4 µl of the Reaction Mix (Invitrogen,5X VILO™ Reaction Mix11754-050), 2 µl of the SuperScript (Invitrogen, 10X SuperScript™ Enzyme Mix 11754-

050) and 9 µl of RNA (total volume is 20 µl for each simple). Then, the mixtures was transferred into a thermocycler (Biometra "Tpersonal") for 25 cycles using three different temperature 25°C for 10 min, then 42°C for 60 min and terminate the reaction at 85°C for 5 min. The next step is to cool it down at 4°C in the thermocycler for ≈ 1h.

The second step was the evaluation of gene expression by a Real Time PCR (RT-PCR) using a PCR thermocycler "Light cycler 2.0". The primers used were from Eurofin MWG Operon.

- 10 µl of the gene solution were diluted in 90 µl of water.

The protocol for the gene expression started by a mix preparation by adding 0.6 µl of water with 1.4 µl of Magnesium chloride (MgCl2, Light cycler DNA master HybProb), 5 µl of Premix ex taq (Premix ex taq™, TAKARA: RR039A), 1 µl of Sybr Green (SIGMA, Sybr Green nucleic Acid gel strain: S9430), 0.5 µl of the forward and the reverse primer of each gene and 1 µl of the cDNA of each simple. Then the mixtures were centrifuged for 30 sec: 1.8 rpm using an eppendorf "Centrifuge 5415D".

II.2.9. Lymphocytes differentiation experiment

In this part of the study we used Normal Human Cord Blood Cryopreserved NAIVE CD4+ T Helper Cells from Zenbio® (Catalog #: SER-CBCD4+ TH-N-F. Lot #: CD4T052915A) and following the protocol of (Zhang *et al.*, 2009).

II.2.9.1. *In vitro* cell activation

CD4+ T Helper cells (2500 cells) were seeded in a twenty-four-well cell culture plates and added to ImmunoCult™ Human CD3/CD28 T Cell Activator from Stem cell technologies™ (25µl/1ml of cell suspension) and human TGF-β1 from Thermo Fisher Scientific™ (2 ng/ml). Then the wells were divided into three groups: group 1 did not receive any other chemicals, group 2 was added with *Phoenix dactylifera* extracts at 3 different concentrations (1µg/100µl, 2µg/100µl and 4µg/100µl), and group 3 was treated with 100 ng/ml of cyclosporine A (CsA). All cells were further cultured at 37 ◦C for 72 h.

II.2.9.2. Flow cytometric analysis

Cells were stained with two human antibodies from Biolegend®: anti human CD4 (Clone: RPA-T4) human anti CD25 (Clone: M-A251). Then the cells were fixed and permeabilized with Cytofix/Cytoperm (BD Pharmingen) and stained with APC antihuman FoxP3 (eBioscience, Clone: PCH101). Samples were collected on a FACSort (BD Biosciences, Mountain View, CA) and data analysis was conducted using CellQuest software (BD Biosciences).

Statistical analysis

Data from *in vivo* and *in vitro* studies were analyzed using Statistical Package for Social Science (SPSS) program, version 20. In each study the experiment data were presented as mean ± SD, and the number of assays (n) is mentioned under every result. Results were analyzed by one way analysis of variance (ANOVA) followed by Tukey-test for multiple comparisons. The significant difference was considered at $P<0.05$.

Chapter III

Results

III.1. Acute Oral Toxicity

In the acute toxicity study, the *Phoenix dactylifera* extracts at a dose of 2000 mg/kg caused neither visible signs of toxicity or mortality. A total of five male adult mice were treated orally with the same extract at the same dose and observed for 14 days. All five mice survived until the end of the observation period.

III.2. Evaluation of the *Phoenix dactylifera* immunomodulatory and antioxidant activities

III.2.1. *Phoenix dactylifera* "Azarza variety"

The present data showed that there is a significant difference in the means for the phagocytic index (K) between groups (NaCl, 30 mg, 50 mg and 100 mg) P= 0.003 and the group 50 mg has the Highest significantly difference (**K**= 0.0806 ±0.01) from the other three groups: NaCl (**K=** 0.019 ±0.0026), 30 mg (**K**= 0.053 ±0.014) and 100 mg (**K**= 0.06 ±0.008) at P=0.002. This indicates that *Phoenix dactylifera* Azarza variety enhanced the phagocytic activity by stimulating the reticuloendothelial system **(Figure 12).**

As shown in the **figure 13**, the half time of colloidal carbon was decreased significantly between groups P= 0.003 however at the concentration of 50mg/kg was faster (**t½=** 9.22 ±1.28 min) when it is compared to the other groups: NaCl (**t½=** 40.62 ±9.10), 30 mg (**t½=** 18.58 ±5.51), 100 mg (**t½=** 11.37 ±1.30) P= 0.004.

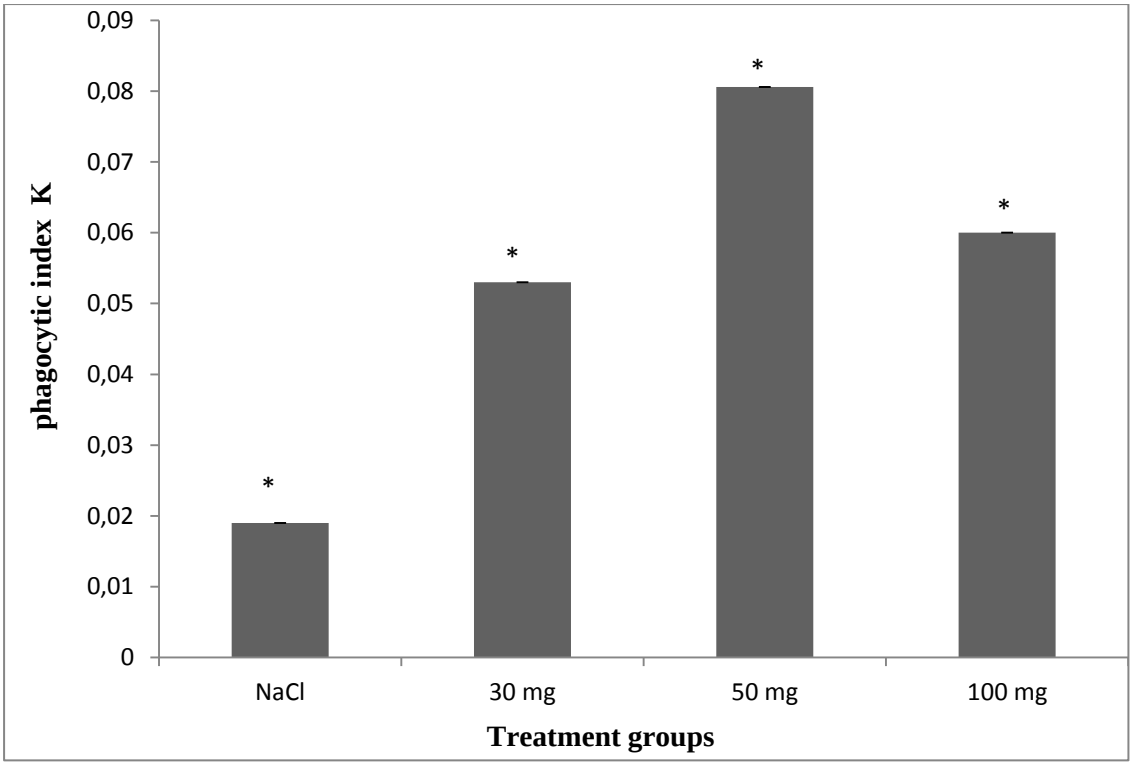

Figure 12: Effect of Phoenix dactylifera "AZARZA variety" extract on phagocytic activity. Results are shown as mean ± SD (n=5) and significant difference from the control group is shown as *p<0.05, **p<0.01, ***p<0.001

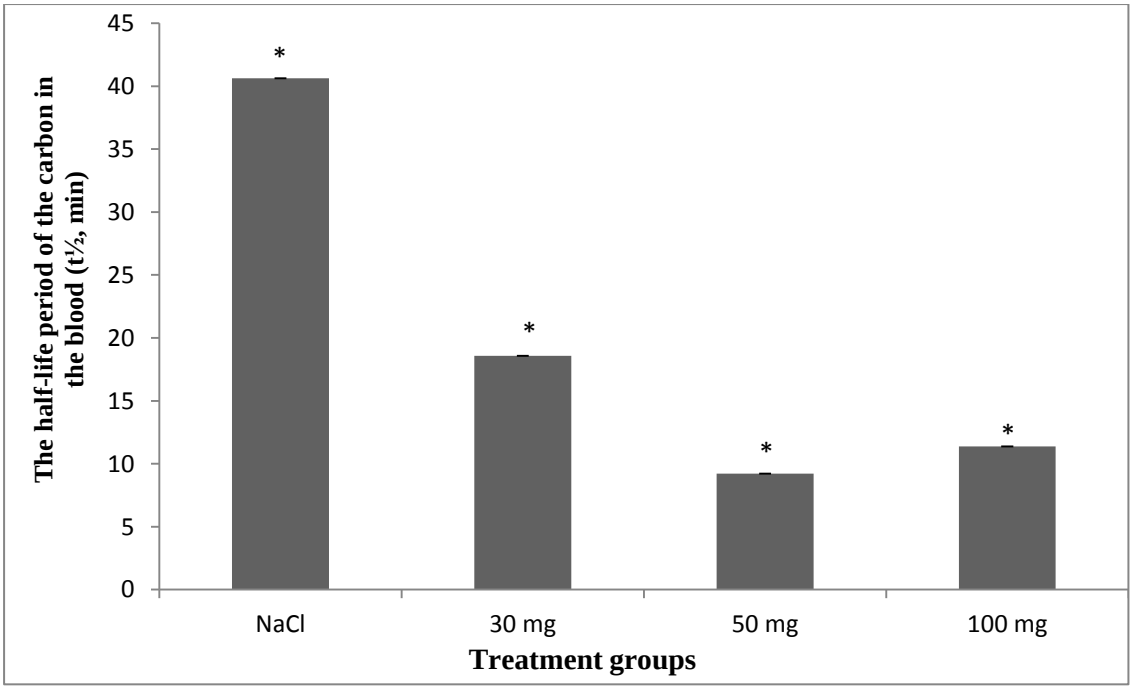

Figure 13: Effect of *Phoenix dactylifera* Azarza extract on half time t1/2 of carbon in blood. Results are shown as mean ± SD (n=5) and significant difference from the control group is shown as *p<0.05, **p<0.01, ***p<0.001

The results of this study showed that there is a significant difference in the means for the corrected phagocytic index α between groups (NaCl, 30 mg, 50 mg and 100 mg) P= 0.004 and the corrected phagocytic index α was increased significantly in groups: 30 mg (**α=** 7.15 ± 0.78), 50 mg (**α=** 7.73 ±0.30) and 100 mg (**α=** 7.52 ±0.54) when it is compared to the control group NaCl (**α=** 4.83 ±0.29) P<0.05 but at the concentration of 50mg /kg the corrected phagocytic index α was higher than the other groups P= 0.006 **(Figure 14)**.

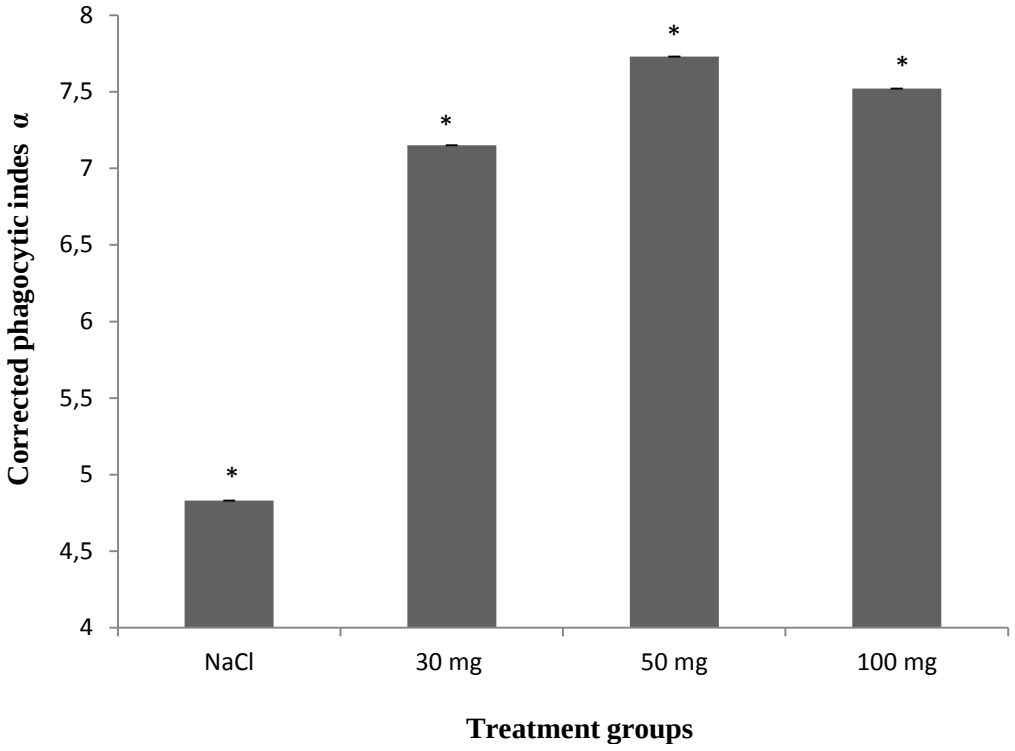

Figure 14: Effect of *Phoenix dactylifera* Azarza extract on corrected phagocytic index α. Results are shown as mean ± SD (n=5) and significant difference from the control group is shown as *p<0.05, **p<0.01, ***p<0.001

The last part of this study showed that there is a significant difference in the means for the Glutathione values between groups (NaCl, 30 mg, 50 mg and 100 mg) P= 0.002 and the Glutathione values was decreased highly and significantly in groups 30 mg (**GSH=** 1.35 ±0.35), 50 mg (**GSH=** 0.73 ±0.08), and 100 mg (**GSH=** 0.76 ±0.14) when it is compared to the control group NaCl (**GSH=** 2.39 ±0.40) P<0.05 **(figure 15)**. This indicates that the extract reduces the glutathione particles from liver and affirms that Phoenix dactylifera "AZARZA variety" extracts enhanced the anti-oxidant activity.

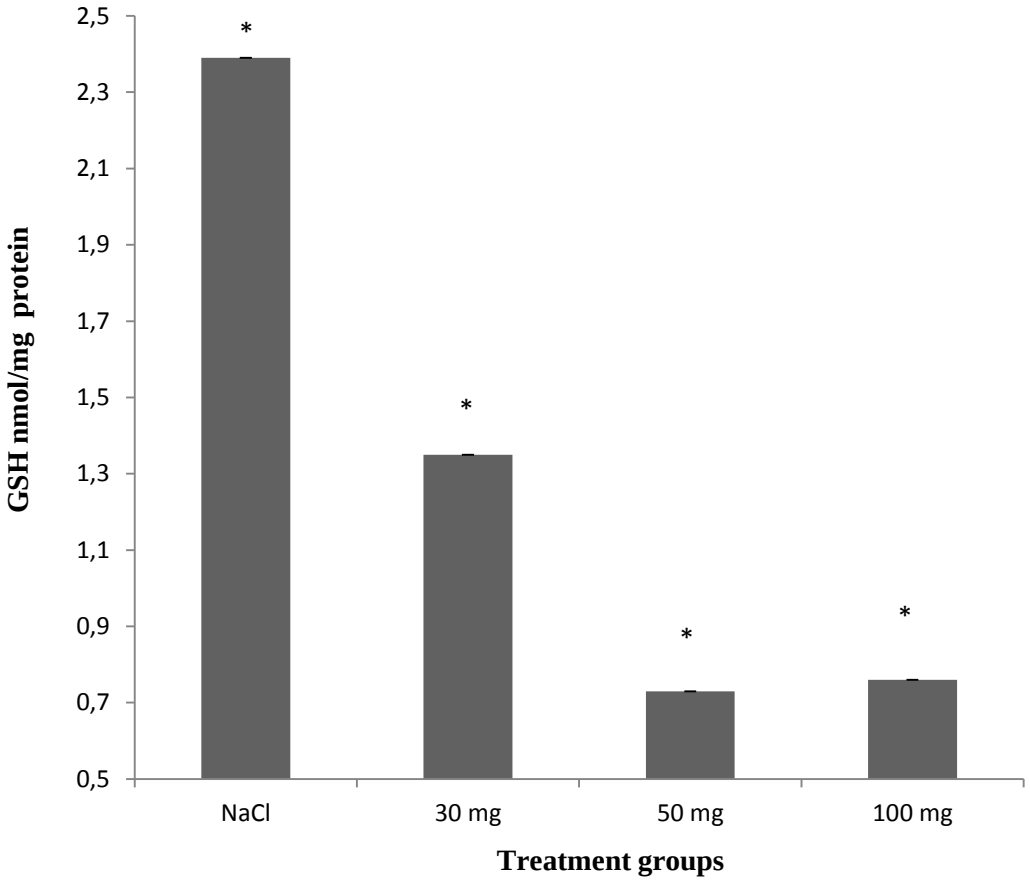

Figure 15: Effect of *Phoenix dactylifera* Azarza on Glutathione GSH values.
Results are shown as mean ± SD (n=5) and significant difference from the control group is shown as *p<0.05, **p<0.01, ***p<0.001

III.2.2. *Phoenix dactylifera* "Homayra variety"

The results showed that there is a significant difference in the means for the phagocytic index (K) between groups (NaCl, 50 mg, 150 mg and 200 mg) P= 0.01 and the group 150 mg (**K=** 0.076 ±0.019) has the Highest significantly difference from the other groups: NaCl (**K=** 0,018 ±0.004), 50 mg (**K=** 0.06 ±0.025) and 200 mg (**K=** 0.048 ±0.0148) at P=0.01.This indicates that *Phoenix dactylifera* Homayra variety enhanced the phagocytic activity by stimulating the reticuloendothelial system **(Figure 16).**

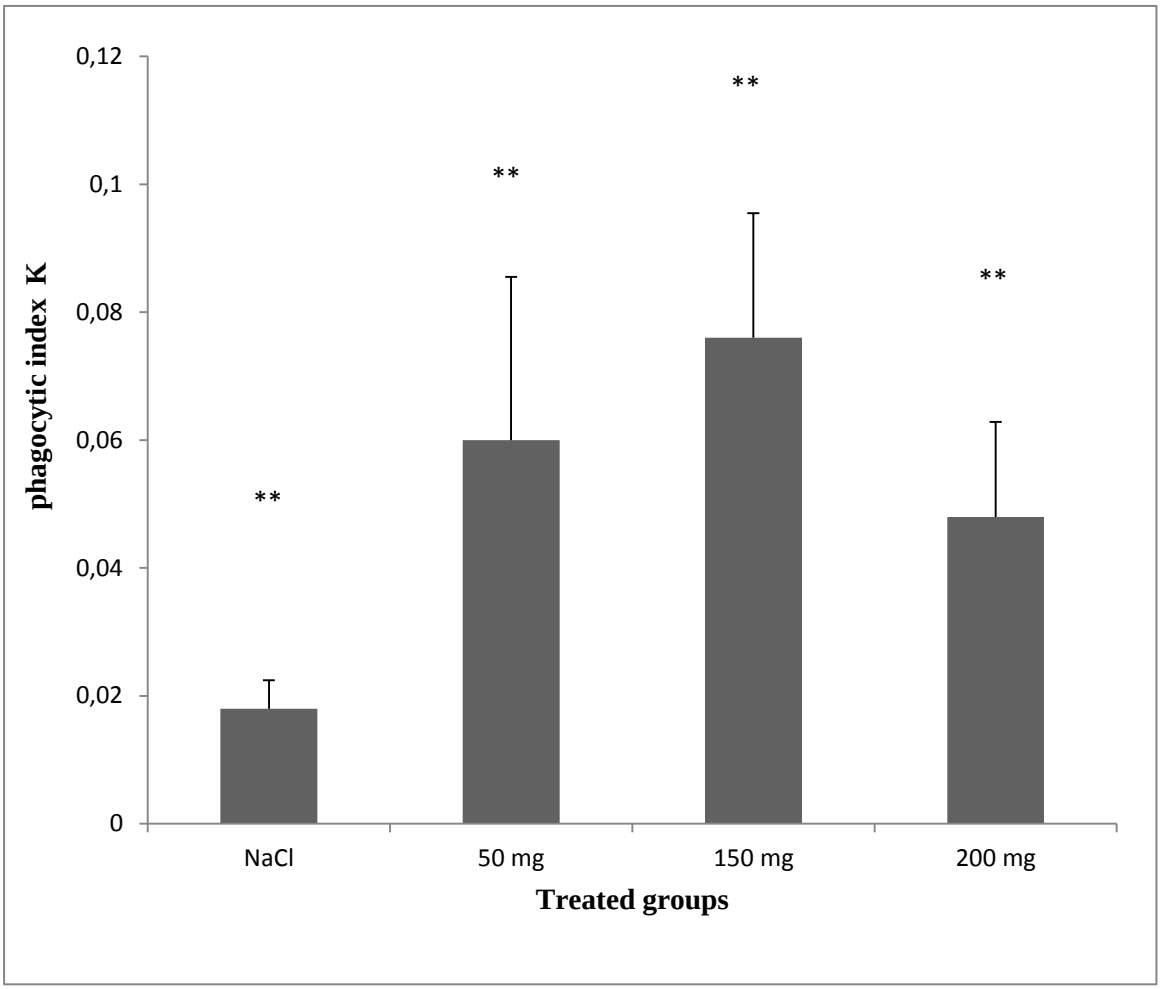

Figure 16: Effect of Phoenix dactylifera "Homayra variety" extract on phagocytic activity. Results are shown as mean ± SD (n=5) and significant difference from the control group is shown as *p<0.05, **p<0.01, ***p<0.001

As shown in the **figure 17**, the half time of colloidal carbon was decreased significantly between groups P= 0.002 however at the concentration of 150 mg/kg (**t½** = 9.788 ±2.596 min) was faster when it is compared to the other groups: NaCl (**t½** = 40.65 ±20.345 min), 50 mg (**t½** = 13.586 ±4.964 min), 200 mg (**t½** = 16.652 ±4.946 min) P= 0.002.

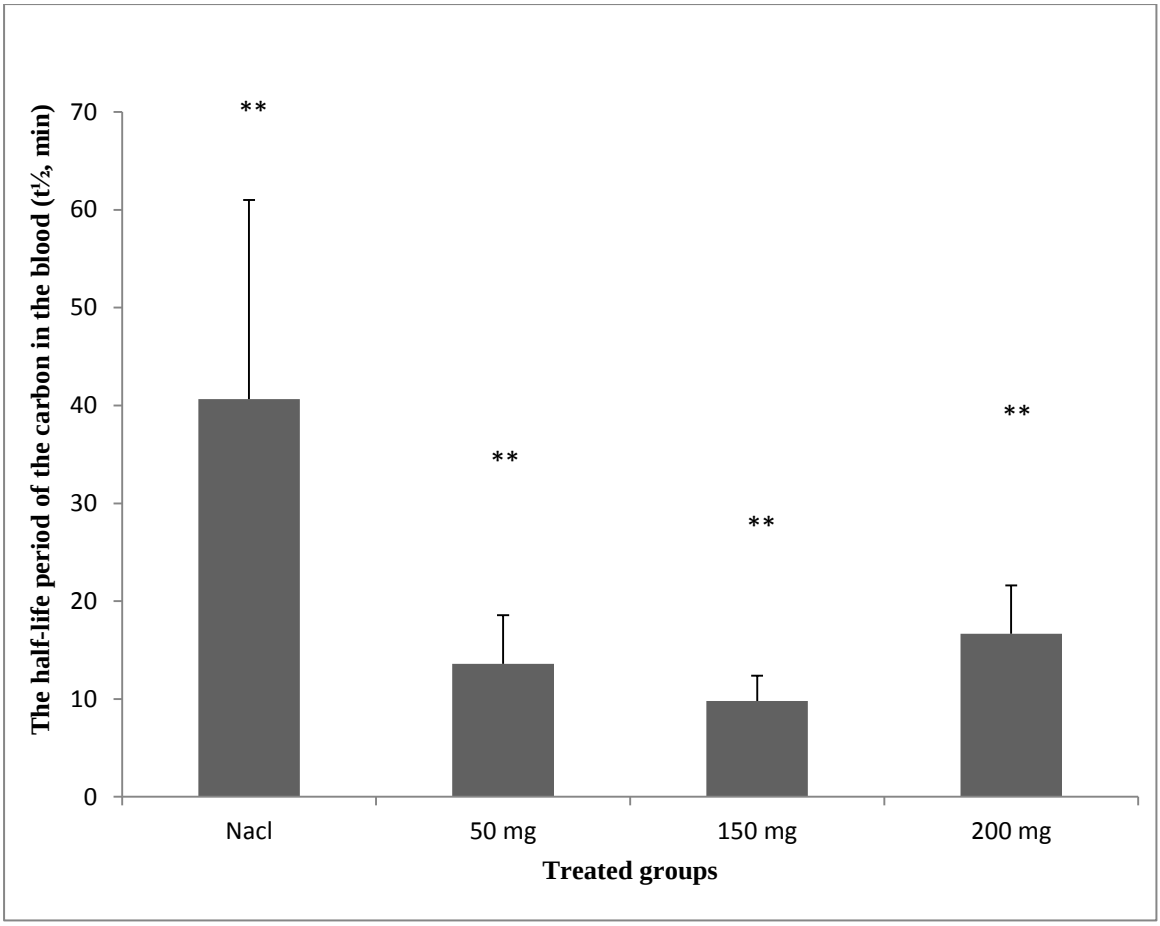

Figure 17: Effect of *Phoenix dactylifera* Homayra extract on half time t1/2 of carbon in blood. Results are shown as mean ± SD (n=5) and significant difference from the control group is shown as *p<0.05, **p<0.01, ***p<0.001

The results of this study showed that there is a high significant difference in the means for the corrected phagocytic index α between groups (NaCl, 50 mg, 150 mg and 200 mg) P= 0.000 and the corrected phagocytic index α was increased significantly in groups: 50 mg (**α** =6.126 ±0.616) , 150 mg (**α** =7.172 ±0.731) and 200 mg (**α** =6.128 ±0.418) when it is compared to the control group NaCl (**α** =4.83 ±0.652) P<0.05 but at the concentration of 150mg /kg the corrected phagocytic index α was higher than the other groups P= 0.000 **(Figure 18)**

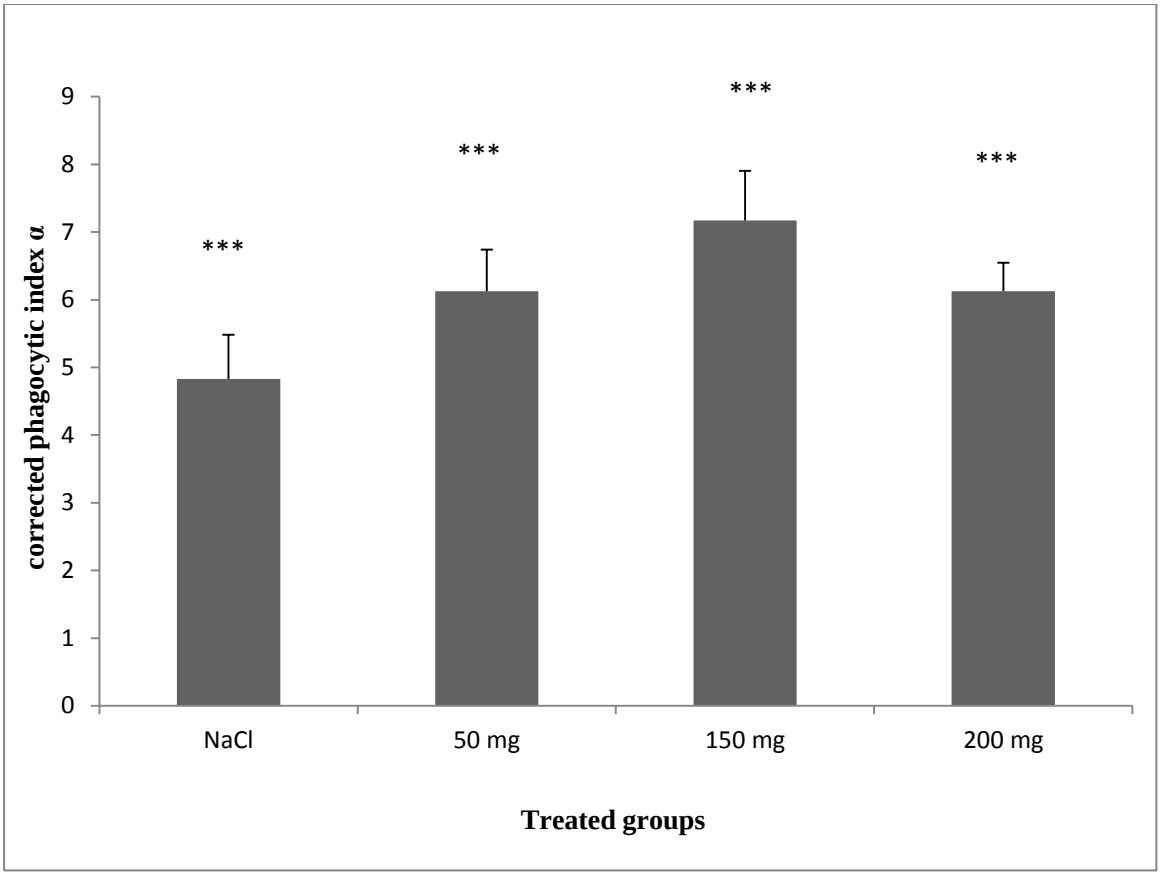

Figure 18: Effect of *Phoenix dactylifera* Homayra extract on corrected phagocytic index α. Results are shown as mean ± SD (n=5) and significant difference from the control group is shown as *p<0.05, **p<0.01, ***p<0.001

The last part of this study showed that there is a significant difference in the means for the Glutathione values between groups (NaCl, 50 mg, 150 mg and 200 mg) P= 0.01 and the Glutathione values was decreased significantly in groups 50 mg (**GSH=** 1.652 ±0.386), 150 mg (**GSH=** 1.57 ±0.128) and 200 mg (**GSH=** 1.83 ±0.088) when it is compared to the control group NaCl (**GSH=** 2.578 ±0.79) P<0.05 **(figure 19)**. This indicates that the extract reduces the glutathione particles from liver and affirms that Phoenix dactylifera "Homayra variety" extracts enhanced the anti-oxidant activity.

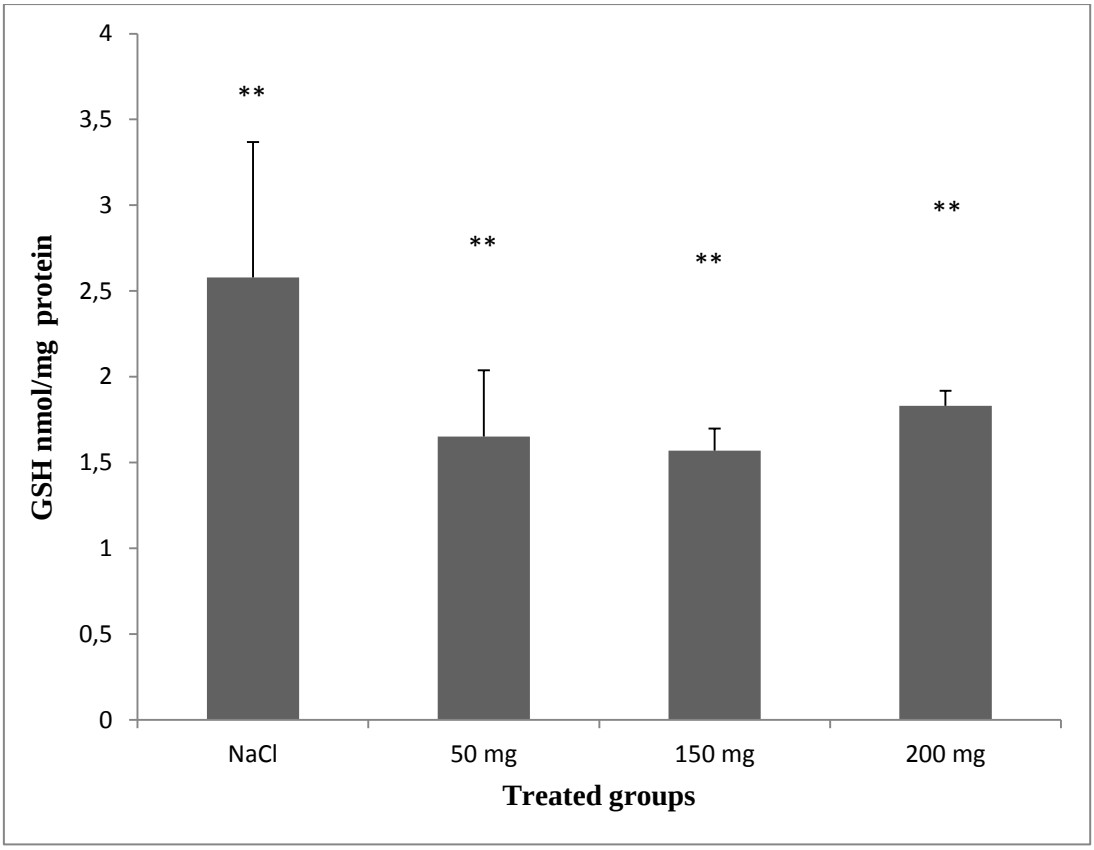

Figure 19: Effect of *Phoenix dactylifera* Homayra on Glutathione GSH values. Results are shown as mean ± SD (n=5) and significant difference from the control group is shown as *p<0.05, **p<0.01, ***p<0.001

III.3. Evaluation of the *Phoenix dactylifera* Anti-inflammatory activity

III.3.1. *Phoenix dactylifera* "Azarza variety"

The results showed a highly significant decrease of the edema size (P = 0.000) in the groups "P" (*Phoenix dactylifera* "Azarza variety") and "MP" with the best result in the group "P" (*Phoenix dactylifera* "Azarza variety") and a highly significant increase of the edema size (P = 0.000) in the group M when it is compared to the control "F" **(figure 20).**

As shown in **figure 21** there was a highly significant decrease in the edema size between groups (P = 0.000) during the 5 hours of the experiment. Also a highly significant decrease in the "P" (*Phoenix dactylifera* "Azarza variety") and "MP" groups edema size (P = 0.000) after one hour from induction of inflammation until it reaches the lowest

value in the 5th hour with a very close results between the two groups treated with (Phoenix dactylifera "Azarza variety" (H1: P= 2.682 ± 0.36 mm, MP= 3.944± 0.34 mm) (H5: P= 1.837± 0.23 mm, MP= 2.15± 0.052 mm) comparing to the control "F". Also a highly significant increase in the edema size of the group treated by methionine "M" (P = 0.000) from the 1st hour (H1= 4.57± 0.51 mm, H3= 5.761± 0.71 mm) comparing to the control "F"

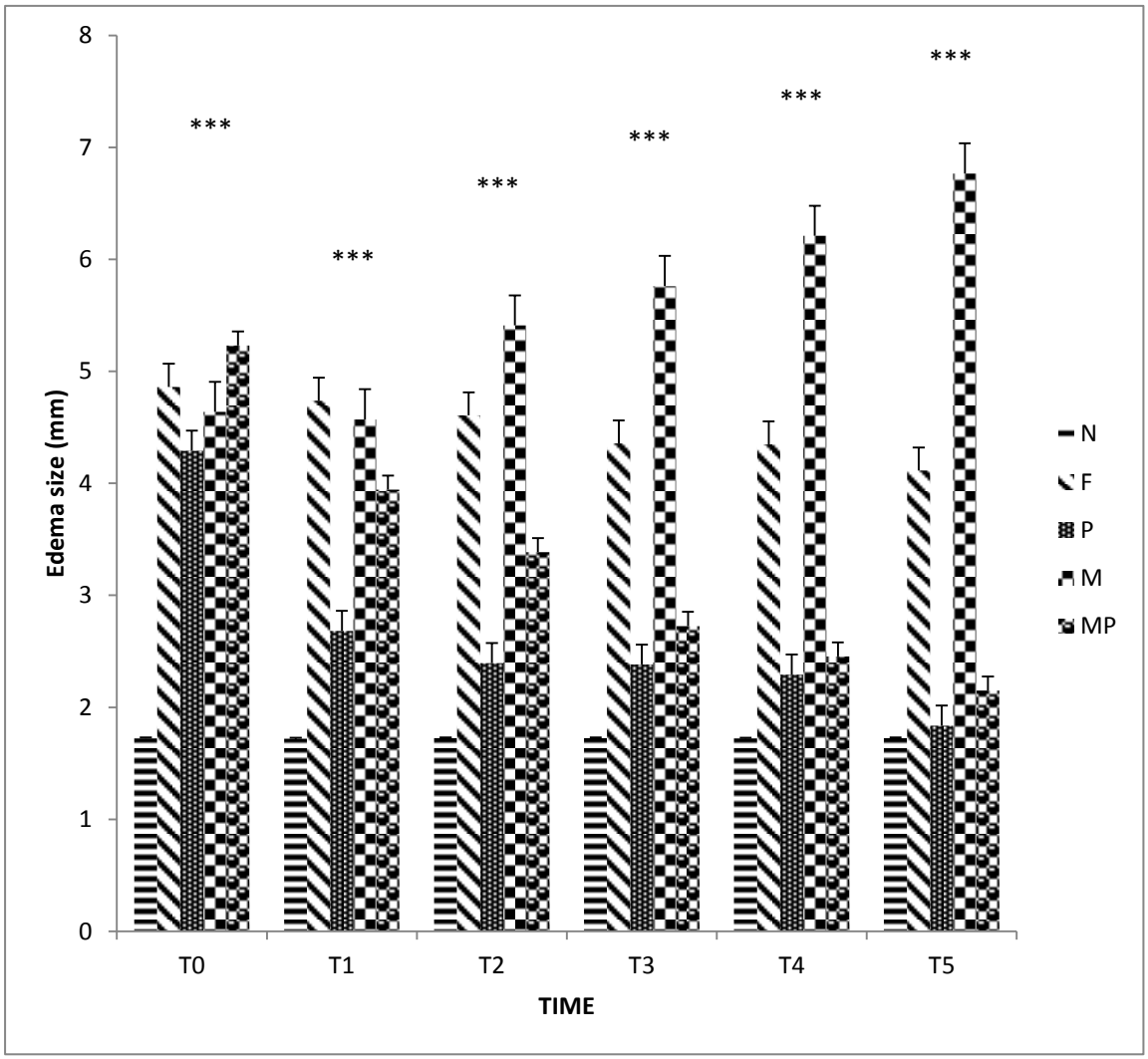

Figure 20: Comparison of paw edema size between the different groups in each hour (Azarza). Results are shown as mean ± SD (n=5) and significant difference from the control group is shown as *p<0.05, **p<0.01, ***p<0.001

N: negative control; **F:** was given flour balls orally; **P:** (for AZARZA variety) was given the plant extract at dose of 50 mg/kg; **M:** was given methionine at dose of 400 mg/kg; **MP**: was given both plant extract at dose of 50 mg/kg and methionine at dose of 400 mg/kg.

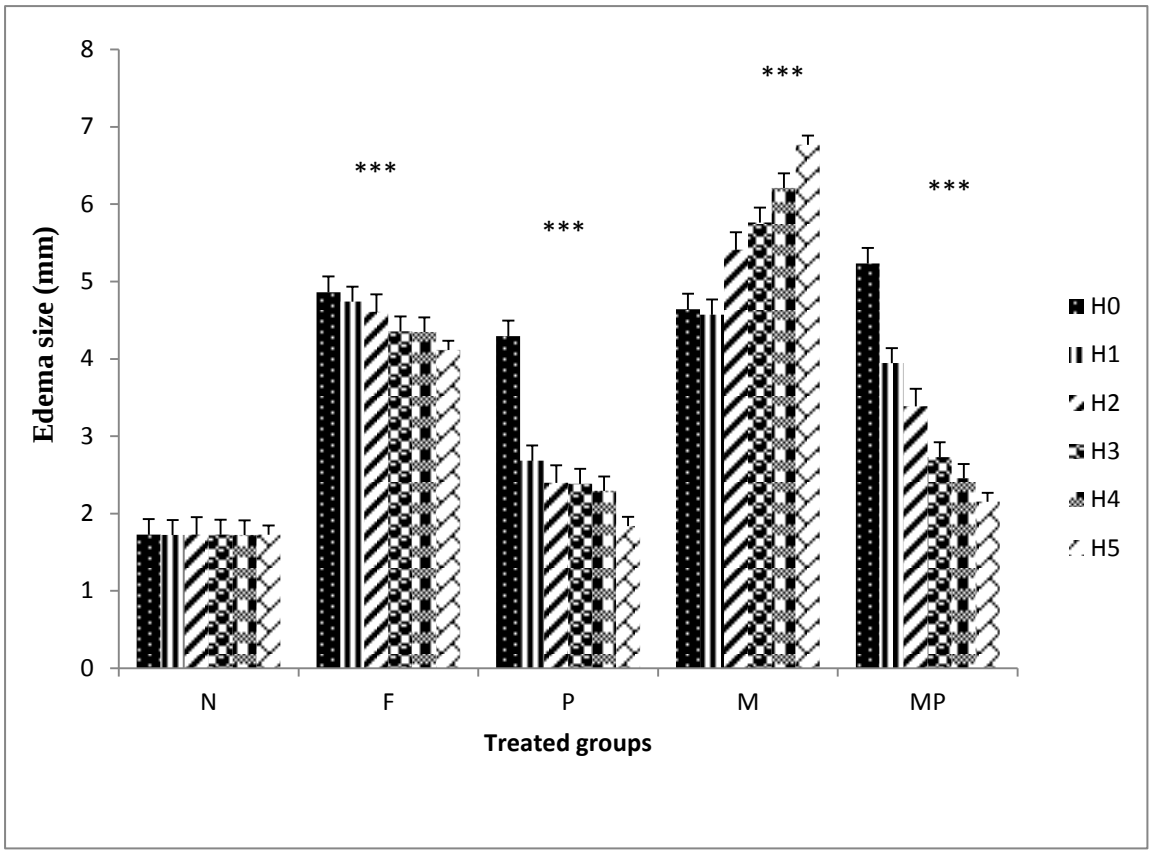

Figure 21: Comparison of paw edema size between the different hours in each group (Azarza). Results are shown as mean ± SD (n=5) and significant difference from the control group is shown as *p<0.05, **p<0.01, ***p<0.001.

The last part of this study showed that there is a significant decrease in the means for the CRP values in the group "P" (*Phoenix dactylifera* "Azarza variety") (0.452± 0.546 mg/l) and group "MP" (0.84 ± 0.322 mg/l) (P<0.05) when it is compared to the group treated by methionine "M" (1.76± 0.868 mg/l) and the control group "F" (1.31± 0.857 mg/l) **(figure 22)**. Also a highly significant decrease in the means for the Hcy values in the group "P" (Phoenix dactylifera "Azarza variety") (7.122± 2.34 µmol/L) and group "MP" (6.124± 3.94 µmol/L) (P= 0.000) when it is compared to the group treated by methionine "M" (22.344± 3.27 µmol/L) and the control "F" (7.85± 0.87 µmol/L) as showed in **figure 23**.

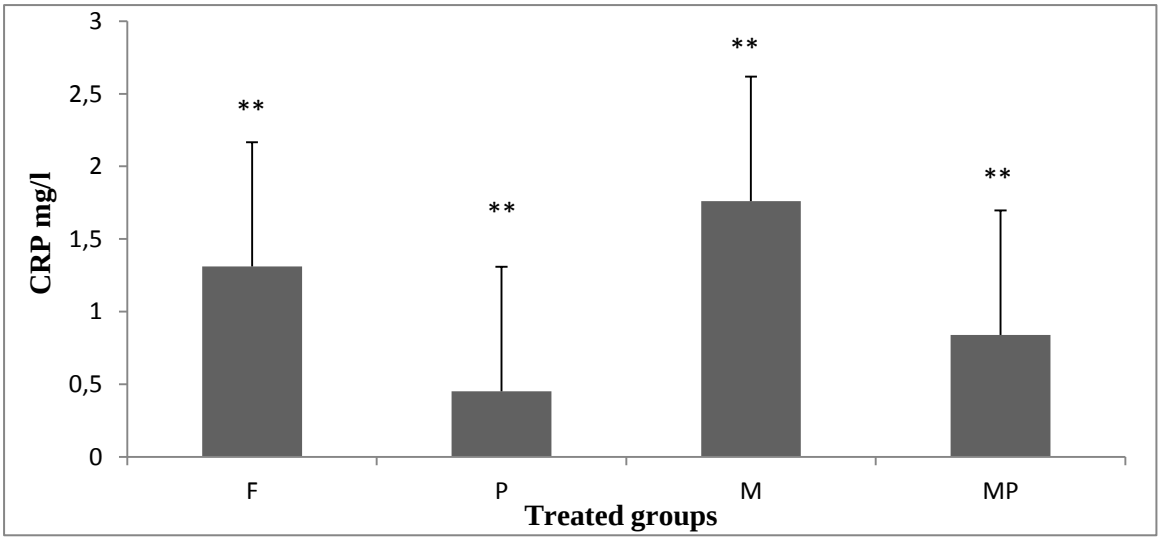

Figure 22: The effect of (*Phoenix dactylifera* "Azarza variety") extract on the CRP values. Results are shown as mean ± SD (n=5) and significant difference from the control group is shown as *p<0.05, **p<0.01, ***p<0.001

F: was given flour balls orally; **P:** (for AZARZA variety) was given the plant extract at dose of 50 mg/kg; **M:** was given methionine at dose of 400 mg/kg; **MP:** was given both plant extract at dose of 50 mg/kg and methionine at dose of 400 mg/kg.

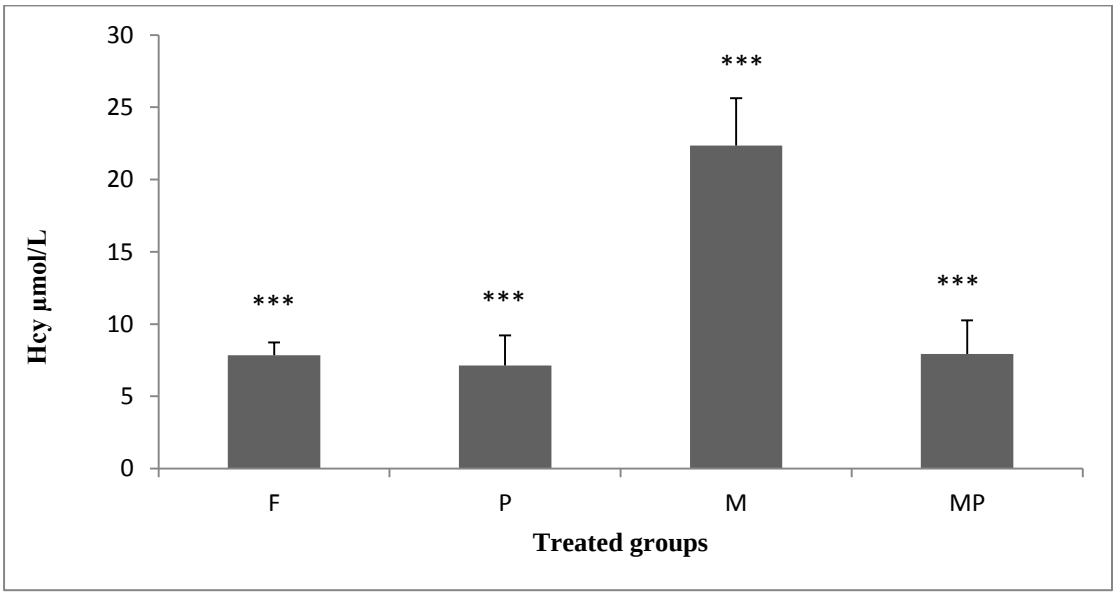

Figure 23: The effect of (*Phoenix dactylifera* "Azarza variety") extract on the Hcy values. Results are shown as mean ± SD (n=5) and significant difference from the control group is shown as *p<0.05, **p<0.01, ***p<0.001

F: was given flour balls orally; **P:** (for AZARZA variety) was given the plant extract at dose of 50 mg/kg; **M:** was given methionine at dose of 400 mg/kg; **MP:** was given both plant extract at dose of 50 mg/kg and methionine at dose of 400 mg/kg.

III.3.1. *Phoenix dactylifera* "Homayra variety"

The results showed a highly significant decrease of the edema size (P = 0.000) in the groups "P" (*Phoenix dactylifera* "Homayra variety") and "MP" with the best result in the group "P" (*Phoenix dactylifera* "Homayra variety") and a highly significant increase of the edema size (P = 0.000) in the group M comparing to the control "F" **(figure 24)**.

As shown in **figure 25** there was a highly significant decrease in the edema size between groups (P = 0.000) during the 5 hours of the experiment. Also a highly significant decrease in the "HM" (*Phoenix dactylifera* "Homayra variety") and "MHM" groups edema size (P = 0.000) after one hour from induction of inflammation until it reaches the lowest value in the 5th hour with a very close results between the two groups treated with (*Phoenix dactylifera* "Homayra variety" (**H1**: HM= 3.691 ± 0.087mm, MHM= 3.908 ± 0.047 mm) (**H5**: HM= 3.066 ± 0.058 mm, MHM= 3.149 ± 0.101 mm) comparing to the control "F". Also a highly significant increase in the edema size of the group treated by methionine "M" (P = 0.000) from the 1st hour (H1= 4.57± 0.51 mm, H3= 5.761± 0.71 mm) comparing to the control "F".

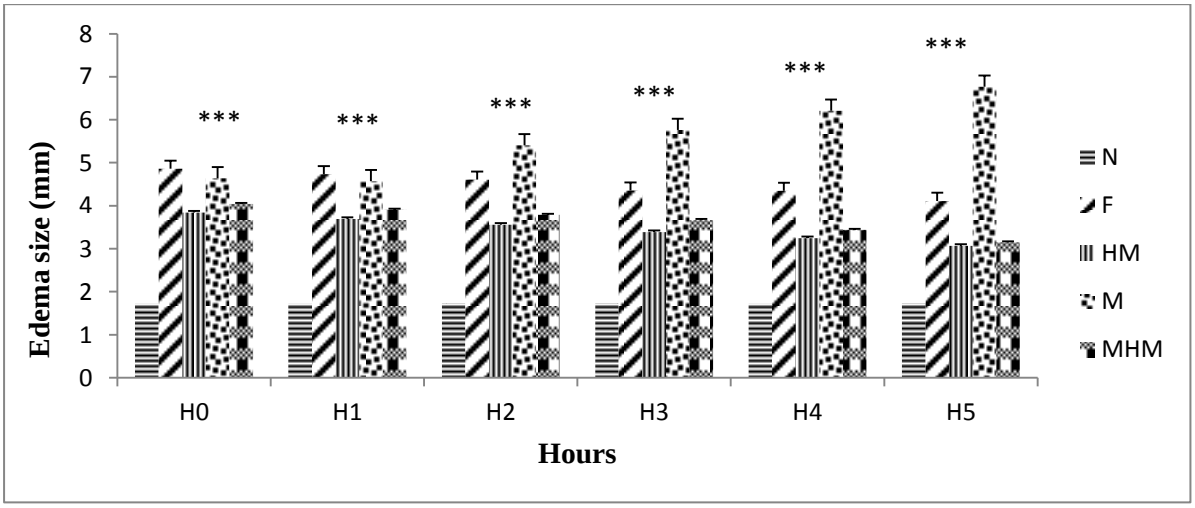

Figure 24: Comparison of paw edema size between the different groups in each hour (Homayra). Results are shown as mean ± SD (n=5) and significant difference from the control group is shown as *p<0.05, **p<0.01, ***p<0.001

N: negative control; **F:** was given flour balls orally; **HM:** (for HOMAYRA variety) was given the plant extract at dose of 150 mg/kg; **M:** was given methionine at dose of 400 mg/kg; **MHM:** was given both plant extract at dose of 150 mg/kg and methionine at dose of 400 mg/kg.

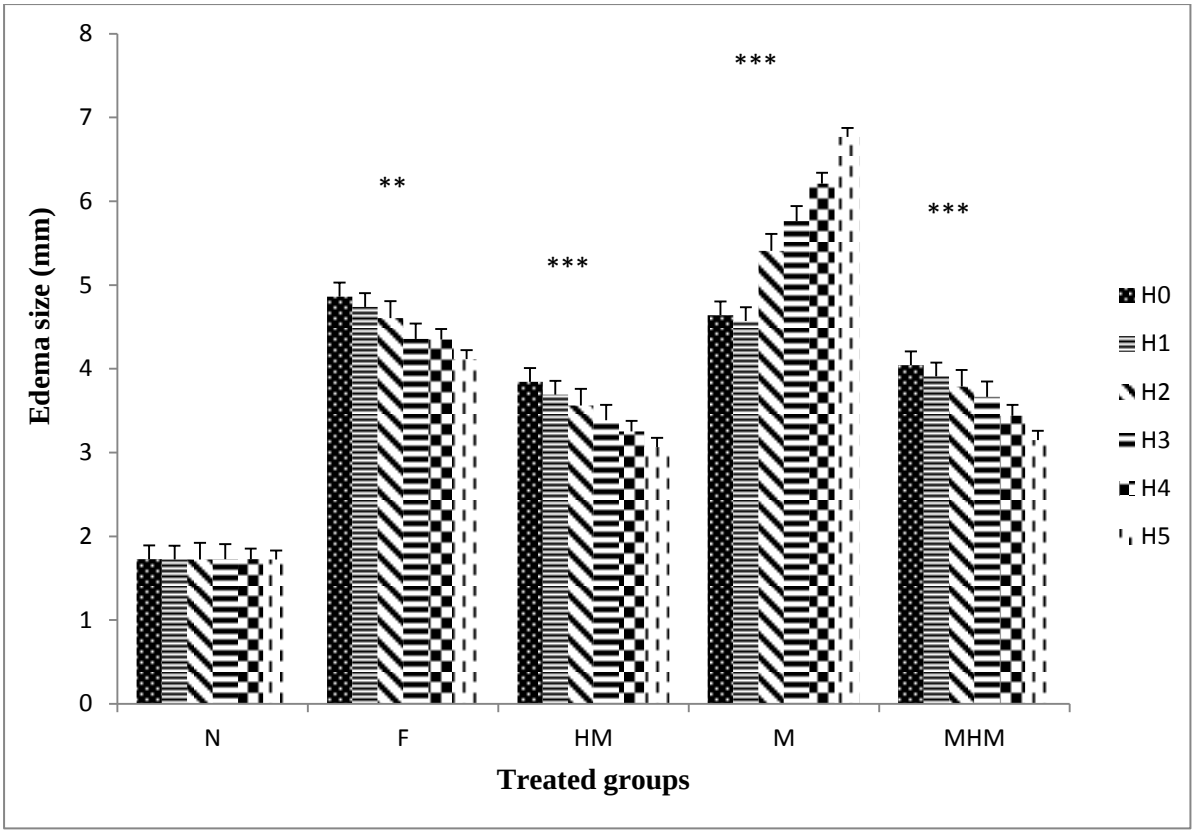

Figure 25: Comparison of paw edema size between the different hours in each group (Homayra). Results are shown as mean ± SD (n=5) and significant difference from the control group is shown as *p<0.05, **p<0.01, ***p<0.001

The last part of this study showed that there is a significant decrease in the means for the CRP values in the group "HM" (*Phoenix dactylifera* "Homayra variety") (0.688 ± 0.313 mg/l) and group "MHM" (1.248 ± 0.765 mg/l) (P<0.05) when it is compared to the group treated by methionine "M" (1.99 ± 0.542 mg/l) and the control group "F" (1.31± 0.857 mg/l) **(figure 26).** Also a highly significant decrease in the means for the Hcy values in the group "HM" (*Phoenix dactylifera* "Homayra variety") (7.234 ± 2.567 µmol/L) and group "MHM" (6.956 ± 4.942 µmol/L) (P= 0.000) when it is compared to the group treated by methionine "M" (22.344± 3.27 µmol/L) and the control "F" (7.85± 0.87 µmol/L) as showed in **figure 27.**

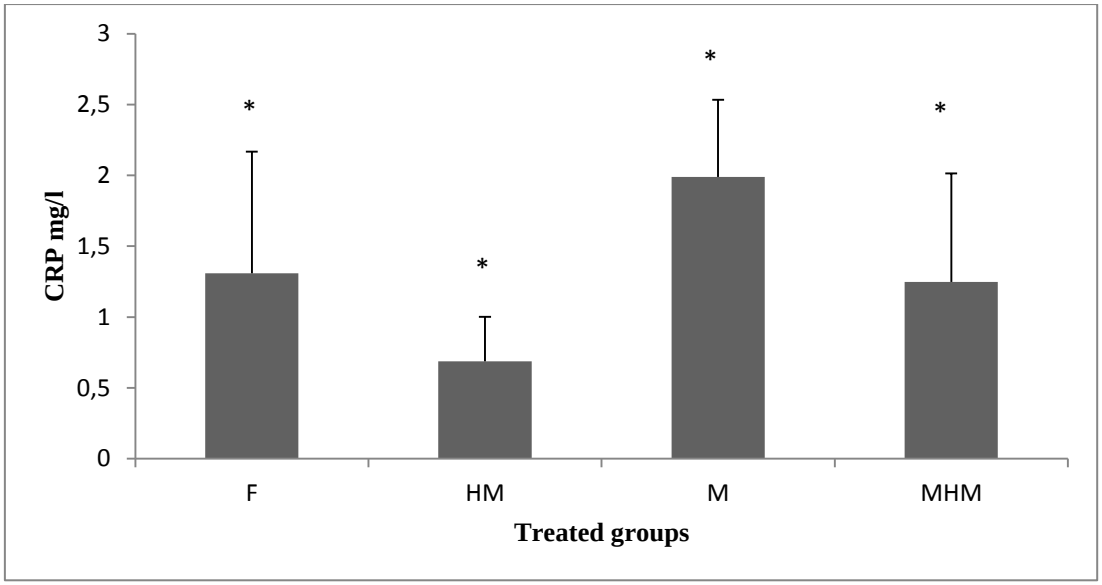

Figure 26: The effect of (*Phoenix dactylifera* "Homayra variety") extract on the CRP values. Results are shown as mean ± SD (n=5) and significant difference from the control group is shown as *p<0.05, **p<0.01, ***p<0.001

F: was given flour balls orally; **HM:** (for HOMAYRA variety) was given the plant extract at dose of 150 mg/kg; **M:** was given methionine at dose of 400 mg/kg; **MHM:** was given both plant extract at dose of 150 mg/kg and methionine at dose of 400 mg/kg.

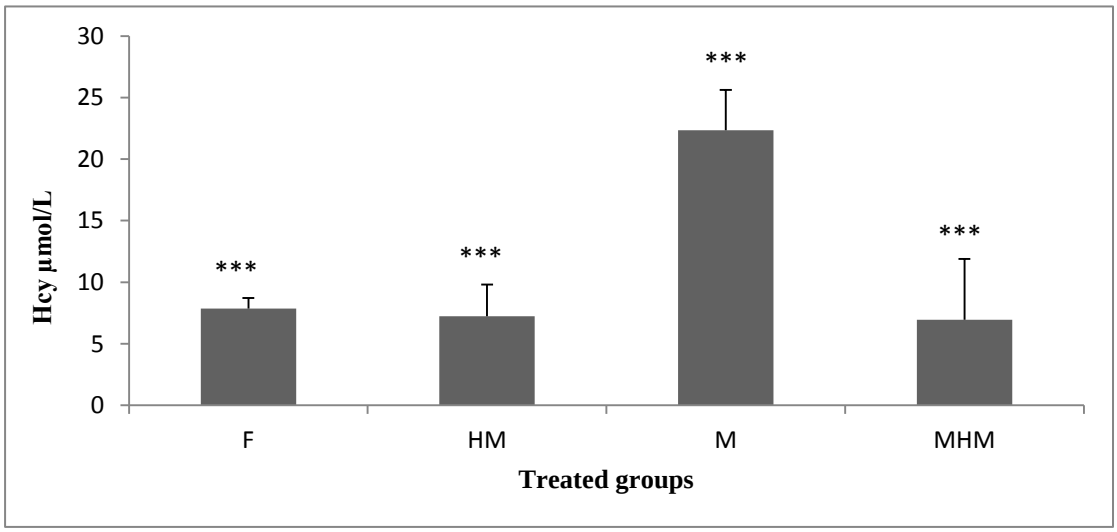

Figure 27: The effect of (*Phoenix dactylifera* "Homayra variety") extract on the Hcy values. Results are shown as mean ± SD (n=5) and significant difference from the control group is shown as *p<0.05, **p<0.01, ***p<0.001

F: was given flour balls orally; **HM:** (for HOMAYRA variety) was given the plant extract at dose of 150 mg/kg; **M:** was given methionine at dose of 400 mg/kg; **MHM:** was given both plant extract at dose of 150 mg/kg and methionine at dose of 400 mg/kg.

III.4. Effect of *Phoenix dactylifera* on food consumption and mice body weight

III.4.1. *Phoenix dactylifera* "Azarza variety"

The results from the food consumption and mice body weight pursuing showed a highly significant difference between the control groups and the treated groups in all the experiment period for the food consumption (P = 0.000) **(figure 28)** but for the mice body weight was only from the 1st to the 8th day and a significant difference for the last two days (P< 0.05) **(figure 29).**

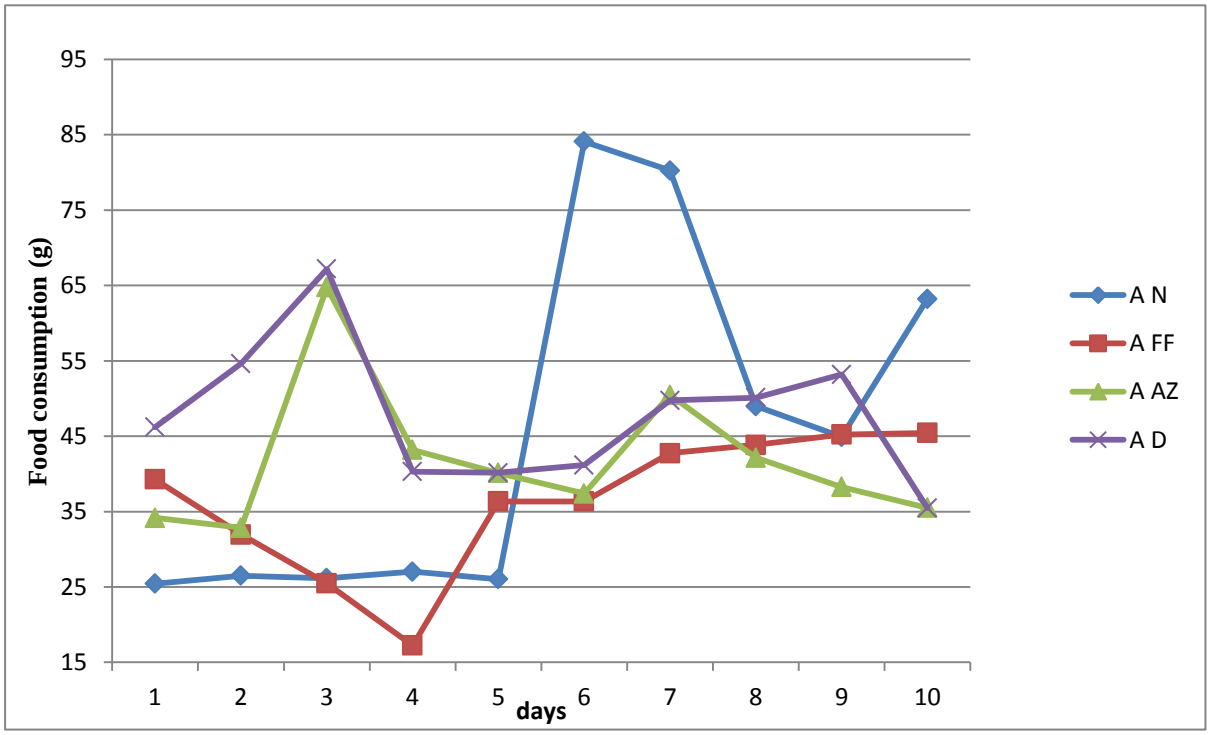

Figure 28: The effect of *Phoenix dactylifera* "Azarza variety" extracts on the Food consumption P< 0.05. Results are shown as mean ± SD (n=5) and significant difference from the control group is shown as *p<0.05, **p<0.01, ***p<0.001

A N: Aliment consumed by negative control; **A FF:** Aliment consumed by the group treated with Formalin; **A AZ:** Aliment consumed by the group treated with Azarza variety; **A D:** Aliment consumed by the group treated with Diclofenac of sodium (10 mg/kg).

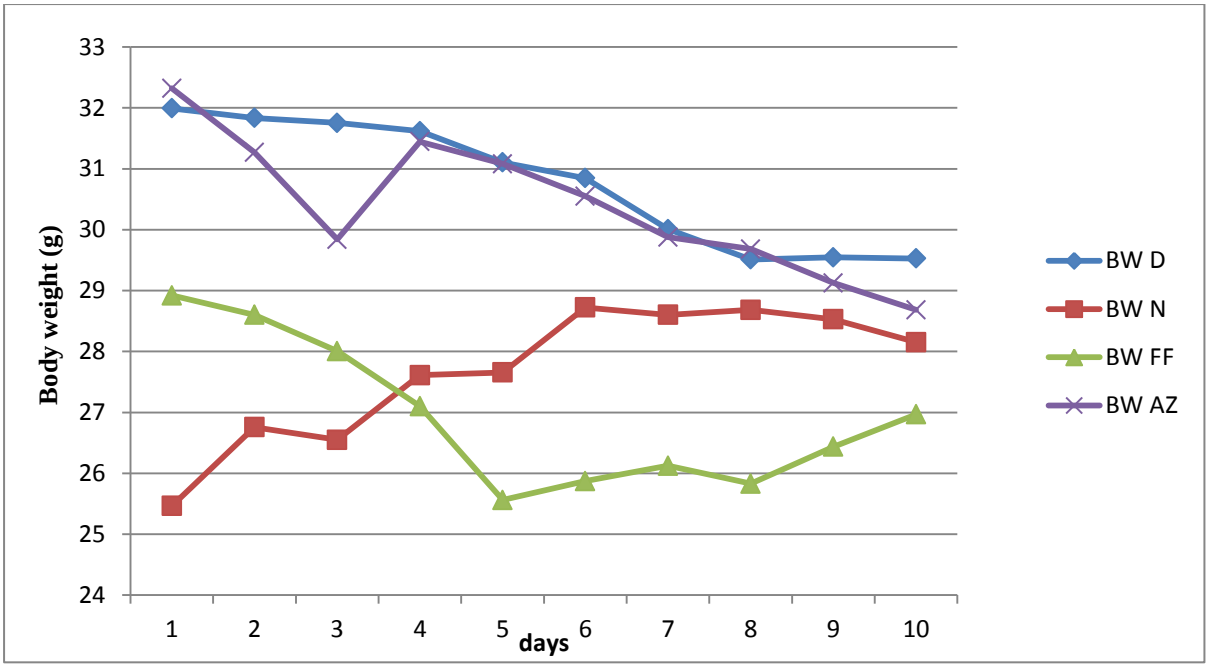

Figure 29: The effect of *Phoenix dactylifera* "Azarza variety" extracts on mice body weight P< 0.05. Results are shown as mean ± SD (n=5) and significant difference from the control group is shown as *p<0.05, **p<0.01, ***p<0.001

BW N: Body weight of the negative control; **BW FF:** Body weight of the group treated with Formalin; **BW AZ:** Body weight of the group treated with Azarza variety; **BW D:** Body weight of the group treated with Diclofenac of sodium (10 mg/kg).

III.4.2. *Phoenix dactylifera* "Homayra variety"

The results from the food consumption and mice body weight pursuing showed a highly significant difference between the control groups and the treated groups in all the experiment period for the food consumption (P = 0.000) **(figure 30)** but for the mice body weight was only from the 1st to the 8th day and a significant difference for the last two days (P< 0.05) **(figure 31).**

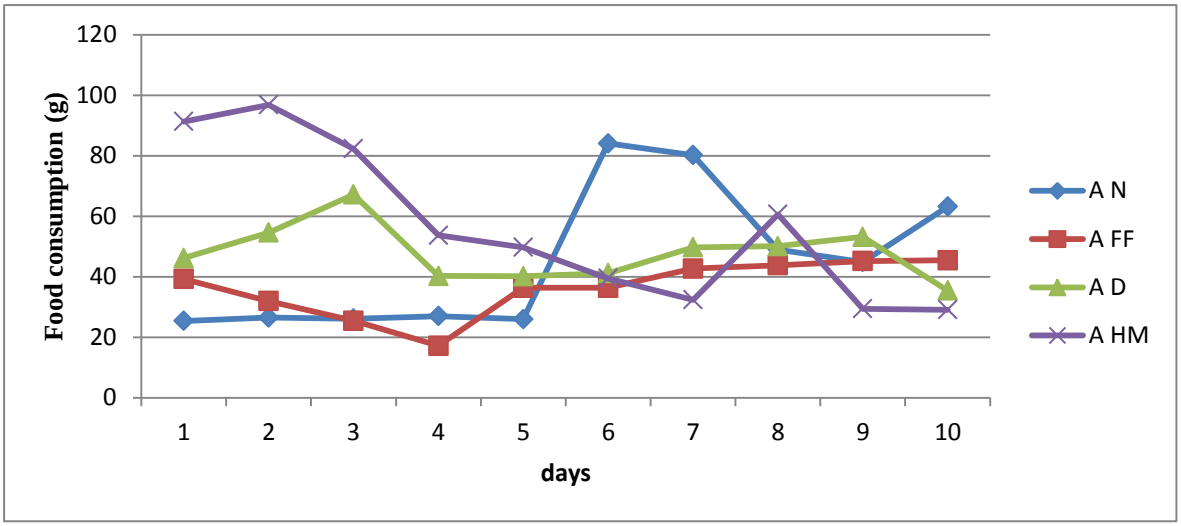

Figure 30: The effect of *Phoenix dactylifera* "Homayra variety" extracts on the Food consumtion. Results are shown as mean ± SD (n=5) and significant difference from the control group is shown as *p<0.05, **p<0.01, ***p<0.001

A N: Aliment consumed by negative control; **A FF:** Aliment consumed by the group treated with Formalin; **A HM:** Aliment consumed by the group treated with Homayra variety; **A D:** Aliment consumed by the group treated with Diclofenac of sodium (10 mg/kg).

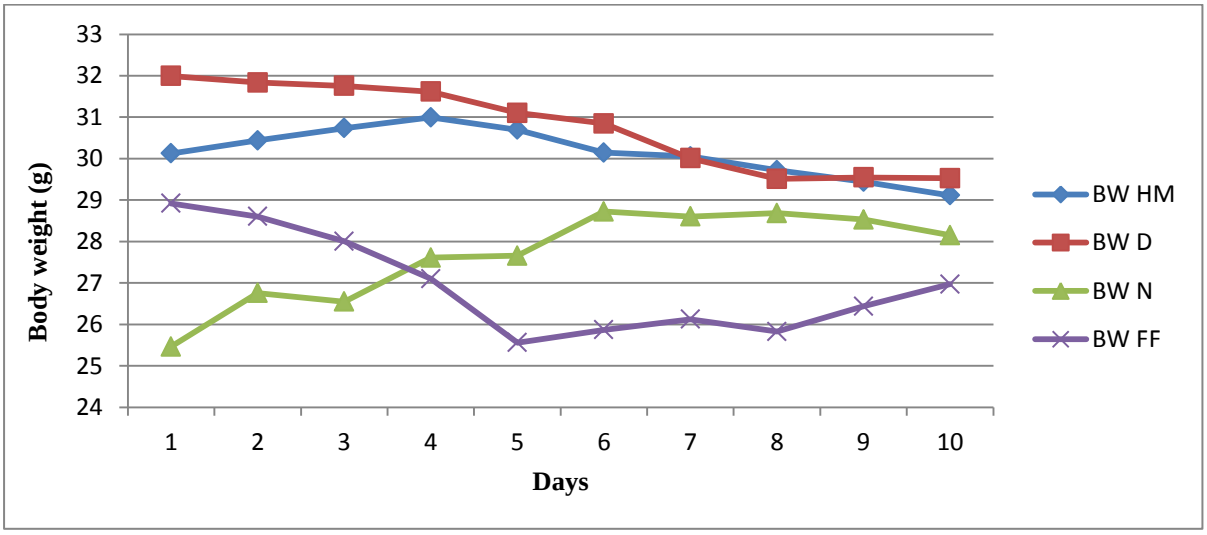

Figure 31: The effect of *Phoenix dactylifera* "Homayra variety" extracts on mice body weight P< 0.05 Results are shown as mean ± SD (n=5) and significant difference from the control group is shown as *p<0.05, **p<0.01, ***p<0.001

BW N: Body weight of the negative control; **BW FF:** Body weight of the group treated with Formalin; **BW HM:** Body weight of the group treated with Homayra variety; **BW D:** Body weight of the group treated with Diclofenac of sodium (10 mg/kg).

III.5. Evaluation of the *Phoenix dactylifera* Anti-arthritis activity

III.5.1. *Phoenix dactylifera* "Azarza variety"

The results showed a highly significant decrease of the edema size (P = 0.000) in the groups AZ (*Phoenix dactylifera* "Azarza variety") and D with a highly significant increase of the edema size (P = 0.000) in the group FF comparing to the negative control group N**.**

As shown in **figure 32** there was a highly significant decrease in the edema size between groups (P = 0.000) during the 10 days of the experiment. Also a highly significant decrease in the AZ (*Phoenix dactylifera* "Azarza variety") and D groups edema size (P = 0.000) from the 3rd day of inflammation until it reaches the lowest value in the 10th day with a very close results between the group treated with (*Phoenix dactylifera* "Azarza variety" and the group treated by Diclofenac (day 3: AZ= 3.42 ±0.27 mm, D= 3.415 ±0.17 mm) (day 10: AZ= 2.583 ±0.04 mm, D= 2.473 ±0.14 mm). Also a highly significant increase in the FF group edema size (P = 0.000) from the 3rd day (3.646 ±0.12 mm), 6th day (4.057 ±0.14 mm) then it started to decrease in the 9th day (3.578 ±0.37 mm) and the 10th day (3.27 ±0.44 mm) comparing to the negative control group N.

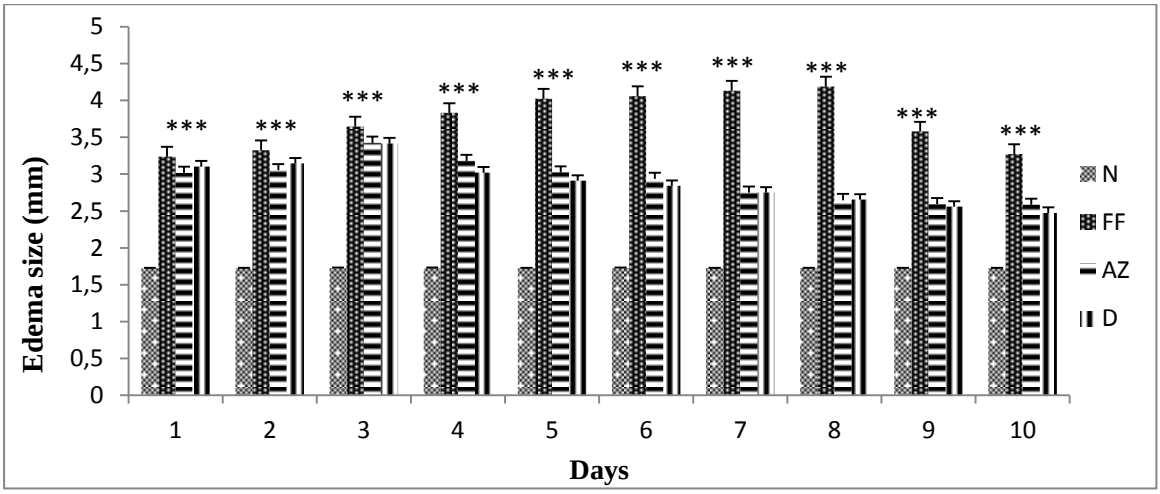

Figure 32: Comparison of paw edema size between the different groups in each day (Azarza). Results are shown as mean ± SD (n=5) and significant difference from the control group is shown as *p<0.05, **p<0.01, ***p<0.001

N: negative control; **FF:** group treated with Formalin; **AZ:** group treated with Azarza variety; **D:** group treated with Diclofenac of sodium (10 mg/kg).

The results showed a highly significant decrease of the Anti-CCP antibodies values (P = 0.000) in the groups AZ (*Phoenix dactylifera* "Azarza variety") and D with approximately similar results between the two groups (**AZ Anti-CCP=** 1.774 ±0.268 UI/ML; **D Anti-CCP=** 1.57 ±0.56 UI/ML), and a highly significant increase of the Anti-CCP antibodies values (P = 0.000) in the group FF (**Anti-CCP=** 3.13 ±0.96 UI/ML) when it is compared to the negative control group N **(figure 33).**

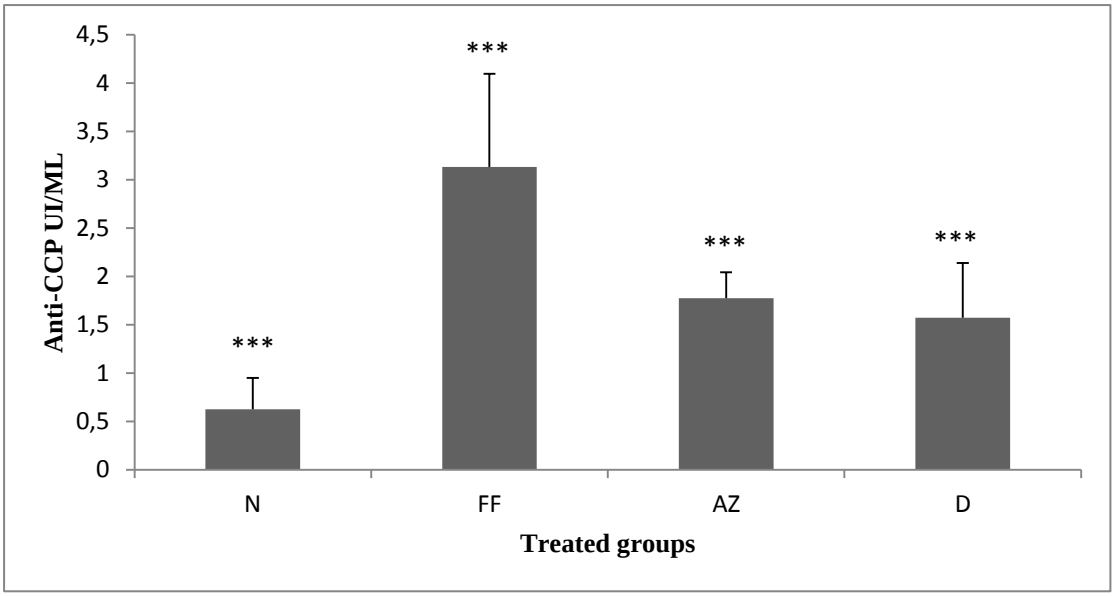

Figure 33: The effect of (*Phoenix dactylifera* "Azarza variety") extract on the Anti-CCP values. Results are shown as mean ± SD (n=5) and significant difference from the control group is shown as *p<0.05, **p<0.01, ***p<0.001

N: negative control; **FF:** group treated with Formalin; **AZ:** group treated with Azarza variety; **D:** group treated with Diclofenac of sodium (10 mg/kg).

The last part of the results showed a high significant decrease of the CRP (P< 0.05) in the groups AZ (**CRP=** 0.37 ±0.33 mg/l) (*Phoenix dactylifera* "Azarza variety") and D (**CRP=** 0.68 ±0.26 mg/l) with the best results in the group treated by the plant extract, and a highly significant increase CRP values (P = 0.000) in the group FF (**CRP=** 1.62 ±0.82 mg/l) comparing to the negative group N **(figure 34).**

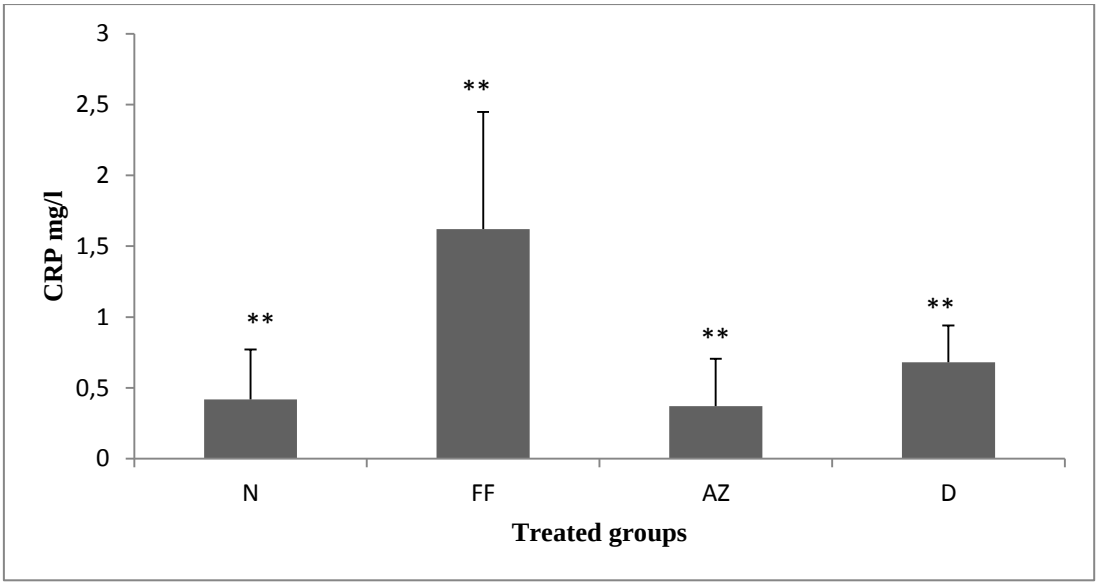

Figure 34: The effect of (*Phoenix dactylifera* "Azarza variety") extract on the CRP values. Results are shown as mean ± SD (n=5) and significant difference from the control group is shown as *p<0.05, **p<0.01, ***p<0.001

N: negative control; **FF:** group treated with Formalin; **AZ:** group treated with Azarza variety; **D:** group treated with Diclofenac of sodium (10 mg/kg).

III.4.2. *Phoenix dactylifera* "Homayra variety"

The results showed a highly significant decrease of the edema size (P = 0.000) in the groups HM (*Phoenix dactylifera* "Homayra variety") and D with a highly significant increase of the edema size (P = 0.000) in the group FF comparing to the negative control group N**.**

As shown in **figure 35** there was a highly significant decrease in the edema size between groups (P = 0.000) during the 10 days of the experiment. Also a highly significant decrease in the HM (*Phoenix dactylifera* "Homayra variety") and D groups edema size (P = 0.000) from the 3rd day of inflammation until it reaches the lowest value in the 10th day with a very close results between the group treated with (*Phoenix dactylifera* "Homayra variety" and the group treated by Diclofenac (day 3: HM= 3.665 ±0.16 mm, D= 3.415 ±0.17 mm) (day 10: HM= 2.885 ± 0.155 mm, D= 2.473 ±0.14 mm). Also a highly significant increase in the FF group edema size (P = 0.000) from the 3rd day (3.646 ±0.12 mm), 6th day (4.057 ±0.14 mm) then it started to decrease in the 9th day (3.578 ±0.37 mm) and the 10th day (3.272 ±0.44 mm) comparing to the negative control group N.

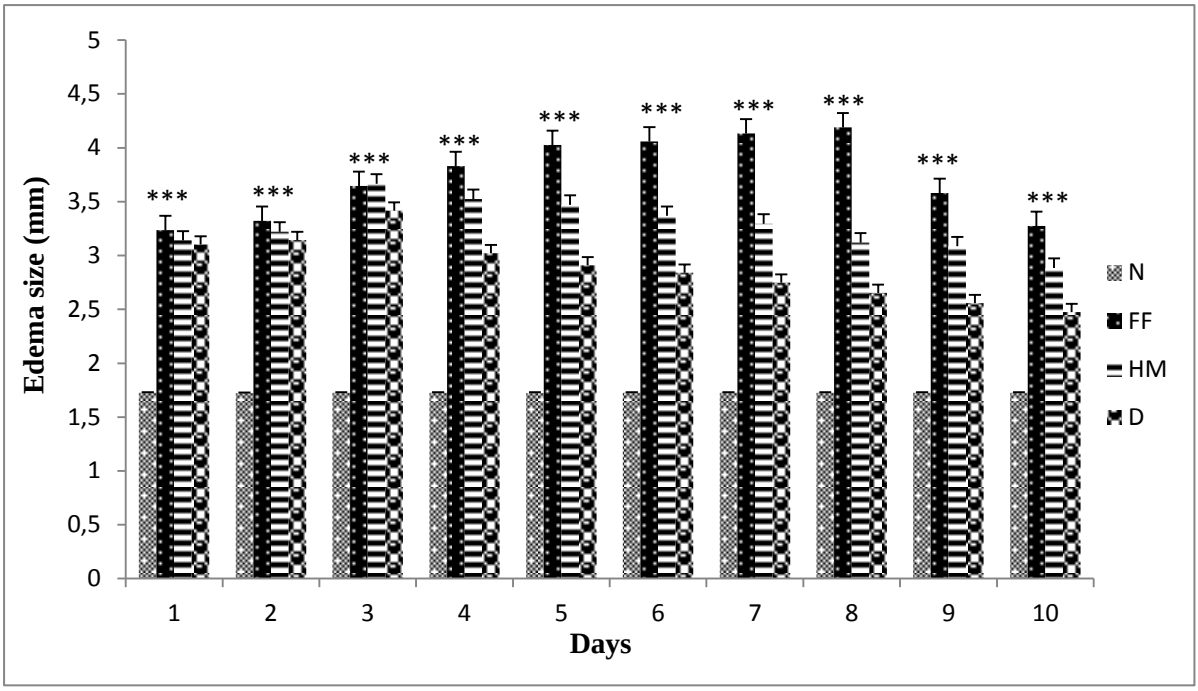

Figure 35: Comparison of paw edema size between the different groups in each day (Homayra). Results are shown as mean ± SD (n=5) and significant difference from the control group is shown as *p<0.05, **p<0.01, ***p<0.001

N: negative control; **FF:** group treated with Formalin; **HM:** group treated with Homayra variety; **D:** group treated with Diclofenac of sodium (10 mg/kg).

The results showed a highly significant decrease of the Anti-CCP antibodies values (P = 0.000) in the groups HM (*Phoenix dactylifera* "Homayra variety") and D with approximately similar results between the two groups (**HM Anti-CCP=** 2.036 ±0.54 UI/ML; **D Anti-CCP=** 1.57 ±0.56 UI/ML), and a highly significant increase of the Anti-CCP antibodies values (P = 0.000) in the group FF (**Anti-CCP=** 3.13 ±0.96 UI/ML) comparing to the negative control group N **(figure 36).**

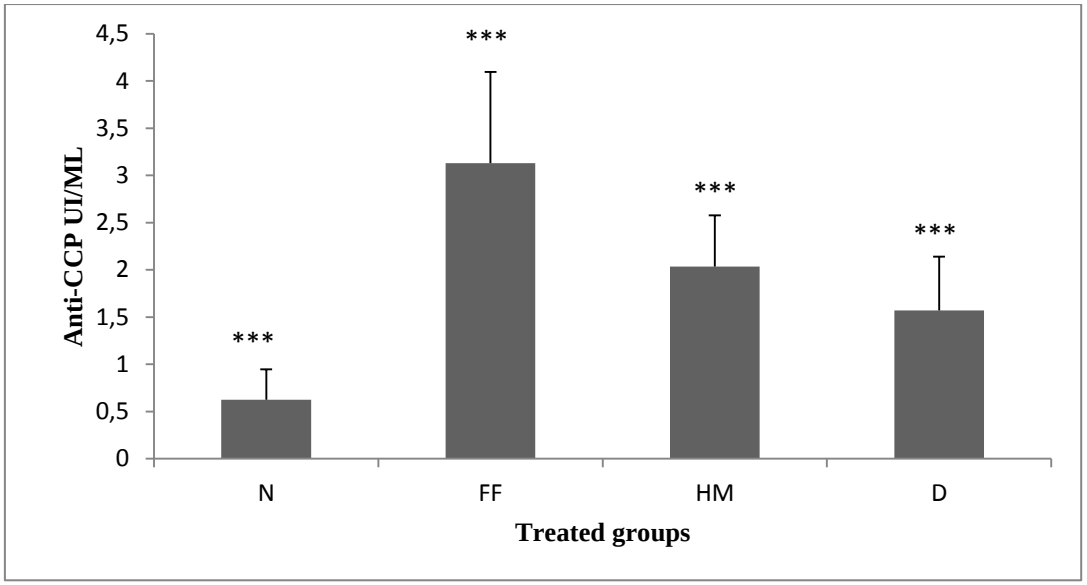

Figure 36: The effect of (*Phoenix dactylifera* "Homayra variety") extract on the Anti-CCP values. Results are shown as mean ± SD (n=5) and significant difference from the control group is shown as *p<0.05, **p<0.01, ***p<0.001

N: negative control; **FF:** group treated with Formalin; **HM:** group treated with Homayra variety; **D:** group treated with Diclofenac of sodium (10 mg/kg).

The last part of the results showed a high significant decrease of the CRP (P< 0.05) in the groups HM (**CRP=** 1.078 ±0.66 mg/l) (*Phoenix dactylifera* "Homayra variety") and D (**CRP=** 0.68 ±0.26 mg/l) with the best results in the group treated by the plant extract, and a highly significant increase CRP values (P = 0.000) in the group FF (**CRP=** 1.62 ±0.82 mg/l) when it is compared to the negative group N **(figure 37).**

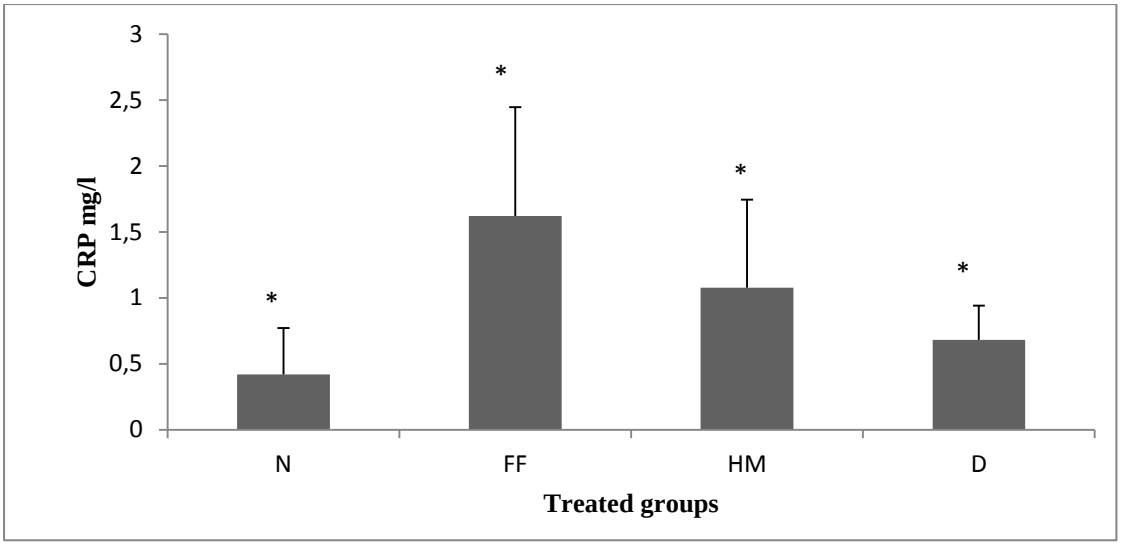

Figure 37: The effect of (*Phoenix dactylifera* "Homayra variety") extract on the CRP values. Results are shown as mean ± SD (n=5) and significant difference from the control group is shown as *p<0.05, **p<0.01, ***p<0.001

N: negative control; **FF:** group treated with Formalin; **HM:** group treated with Homayra variety; **D:** group treated with Diclofenac of sodium (10 mg/kg).

III.6. Histological study of the mice joint

In the group (FF) which received subplantar injection of formalin) showed degeneration of the knee joint and Cellular desquamation of synovial membrane **(Figure 38A and 38B)** respectively. However, in the normal group (N) , the joint sections have intact structure where we have detected the three parts of the joint ; the first part which consists of hyaline cartilage where we find the chondrocytes located in three layers (Tangential , transitional and radial),the second part is the calcified cartilage and the third one is the spongy bone also we have observed the tide line between the uncalcified and calcified cartilage. Meanwhile we have observed intact synovial membrane with collagenous fiber, blood vessels and synovial cells (type A and type B) **(Figure 39A and 39B)** respectively.

Histological sections of the joint paws of the groups of mice treated by *Phoenix dactylifera* with both varieties AZ and HM demonstrated intact articular cartilage with chondrocytes with intact articular surface **(Figures 40A,40B and 41)** respectively. On the other hand , the joint section of the group (D) with diclofenac of sodium showed intact hyaline cartilage with chondrocytes and no erosion was observed on the articular surface but we have localize a formation of granuloma in the synovial membrane **(Figure 42).**

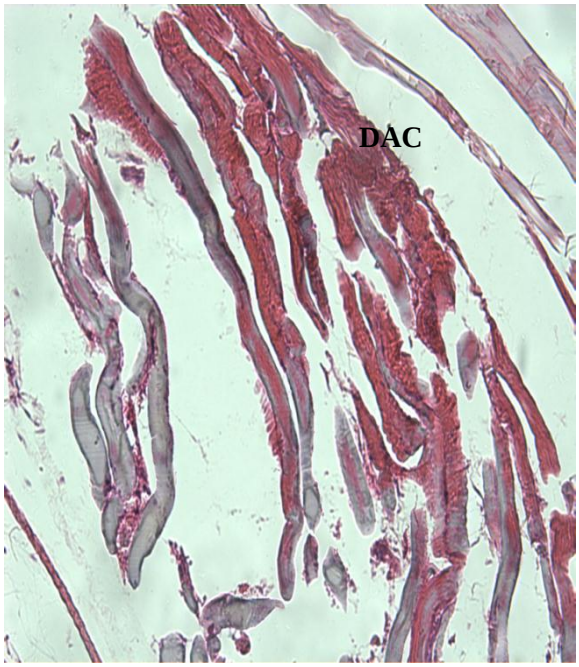

Figure 38A: Histological section of mouse joint (10 days) flour + formalin (FF) application H.E staining (X100).

DAC: Degeneration of articular cartilage.

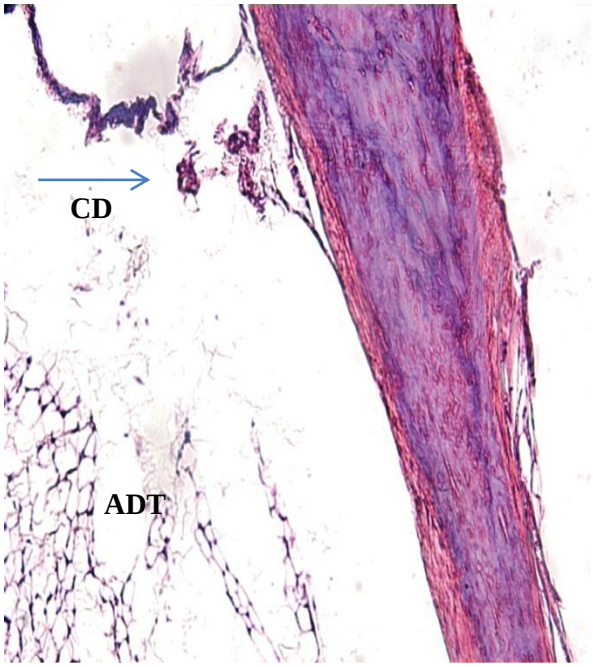

Figure 38B: Histological section of mou joint (10 days) flour + formalin (FF) application H.E staining (X100).

CD: Cellular desquamation of synovial membrane; **ADT:** Adipose tissue.

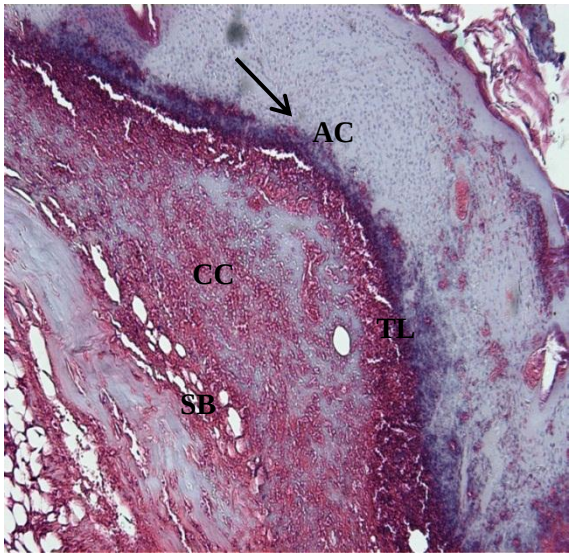

Figure 39A: Histological section of mouse normal joint (N) (10 days) oral flour application H.E staining (X100).

AC: Articular Cartilage; **TL:** Tide Line **CC:** Calcified Cartilage; **SB:** Spongy Bone.

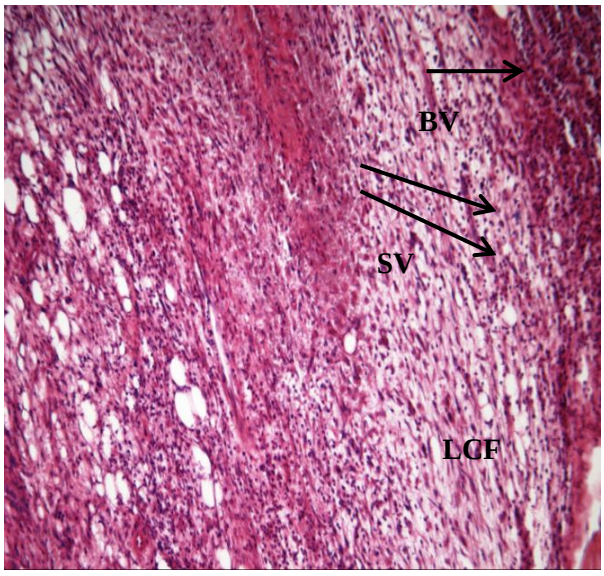

Figure 39B: Histological section of synovial membrane mouse normal joint (N) (10 days) oral flour application H.E staining (X100).

LCF: Layer of collagenous fiber; **BV:** Blood vessels; **SV:** Synoviocytes.

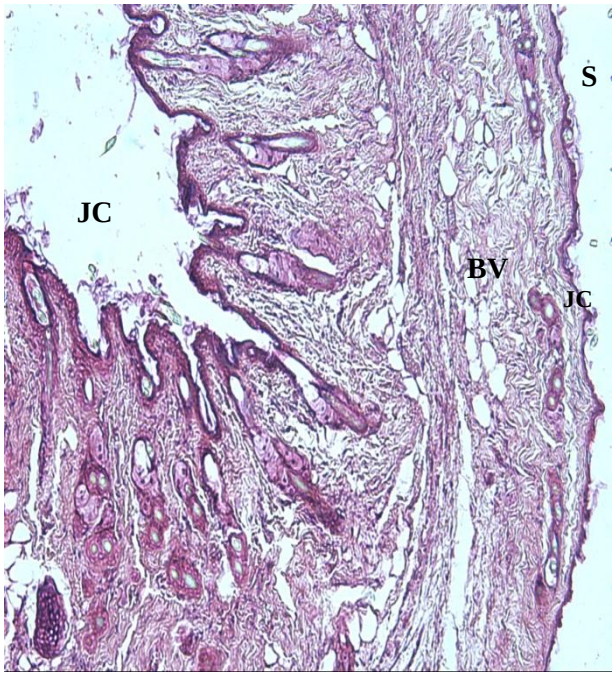

Figure 40A: Histological section of mouse joint (10 days) flour + *Phoenix dactylifera* (AZ) application H.E staining (X100).

S: Surface of the synovial membrane; **JC:** Joint Cavity; **BV:** Blood Vessels.

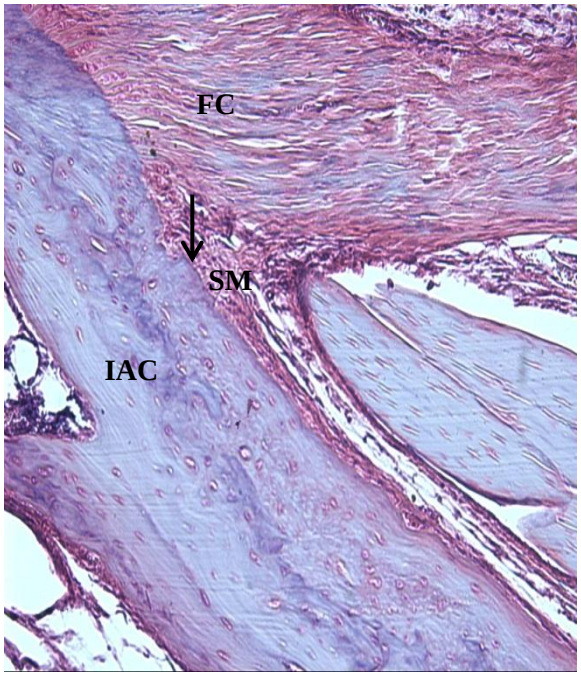

Figure 40 B: Histological section of mouse joint (10 days) flour + *Phoenix dactylifera* (AZ) application H.E staining (X100).

IAC: Intact articular cartilage with chondrocytes; **FC:** Fibrous Capsule; **SM:** Synovial Membrane.

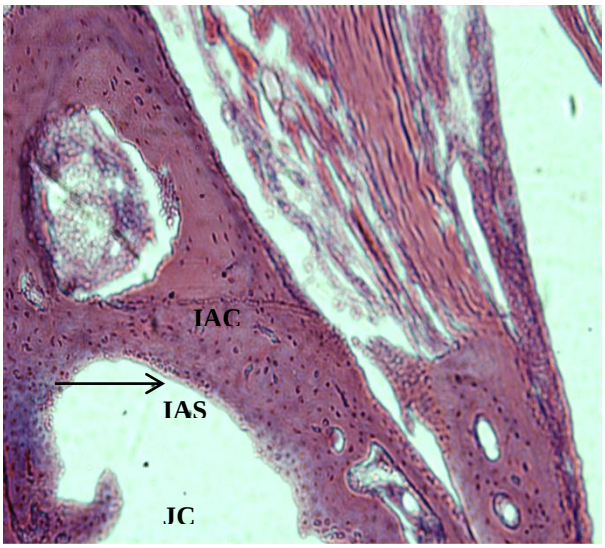

Figure 41: Histological section of mouse joint (10 days) flour + Phoenix dactylifera (HM) application H.E staining (X100).

IAC: Intact articular cartilage; **IAS:** Intact Articular Surface; **JC:** Joint Cavity.

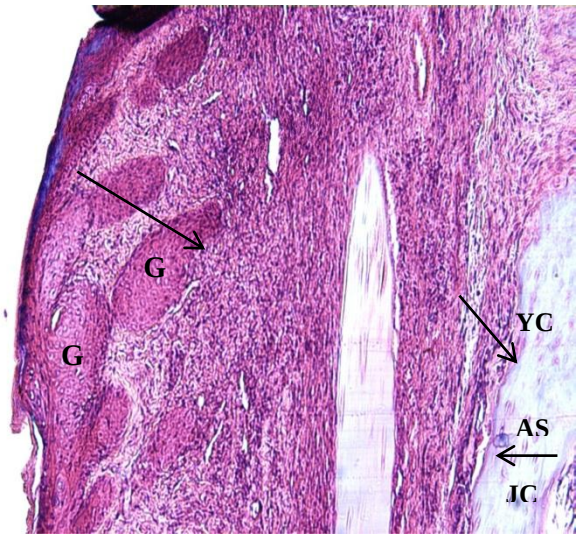

Figure 42: Histological section of mouse joint (10 days) flour + Diclofenac (D) application H.E staining (X100).

YC: Hyaline Cartilage with chondrocytes; **JC:** Joint Cavity; **G:** Granuloma; **AS:** Articular surface.

III.7. Anti-proliferative activity of *Phoenix dactylifera*

III.7.1. Effect of *Phoenix dactylifera* on breast cancer cells (MCF7)

III.7.1.1. *Phoenix dactylifera* "Azarza variety"

The results in **(figure 43)** concerning the effect of *Phoenix dactylifera* "AZ variety" with both Acetone (A1) and methanol (A2) extract on the proliferation of breast cancer cells showed a high decrease in the cancer cells viability (P=0.000). The decrease in cells viability was a concentration dependent decrease (more the concentration increased more the viability decreased). By comparing between the two extracts the methanolic extract was more effective than the acetone extract with low concentrations (**4 µg/100ml A2** = 18.72% ± 0.20, **4 µg/100ml A1** = 42.87% ±0.16) and high concentrations (**20 µg/100ml A2** = 7.68% ± 0.034, **20µg/100ml A1** = 18.37% ±0.26).

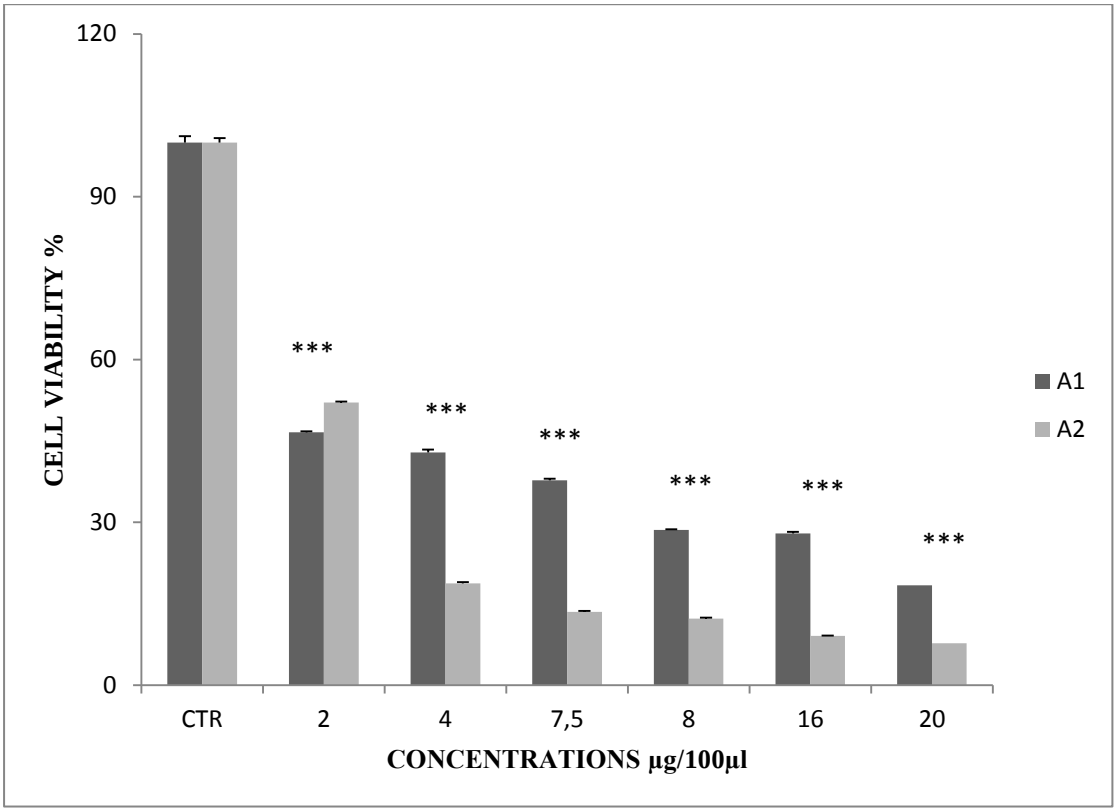

Figure 43: The effect of *Phoenix dactylifera* "AZ" methanolic and acetone extracts MCF7 cells viability. Results shown in the figure are expressed as the means average deviations of three separate experiments and error bars indicate SD ***P = 0.000**

A1: The Acetone extract of *Phoenix dactylifera* "AZARZA variety".

A2: The Methanolic extract of *Phoenix dactylifera* "AZARZA variety".

III.7.1.2. *Phoenix dactylifera* "Homayra variety"

In figure 44, the results concerning the effect of *Phoenix dactylifera* "HM variety" with both Acetone (B1) and methanol (B2) extract on the proliferation of breast cancer cells showed a high decrease in the cancer cells viability (P=0.000). The decrease in cells viability was a concentration dependent decrease (more the concentration increased more the viability decreased). By comparing between the two extracts the methanolic extract was more effective than the acetone extract with low concentrations (**4 µg/100ml B2** = 22.882% ± 0.68, **4 µg/100ml B1** = 35,298% ±0.96) and high concentrations (**20 µg/100ml B2** = 9.43% ± 0.131, **20µg/100ml B1** = 11.90% ±0.159).

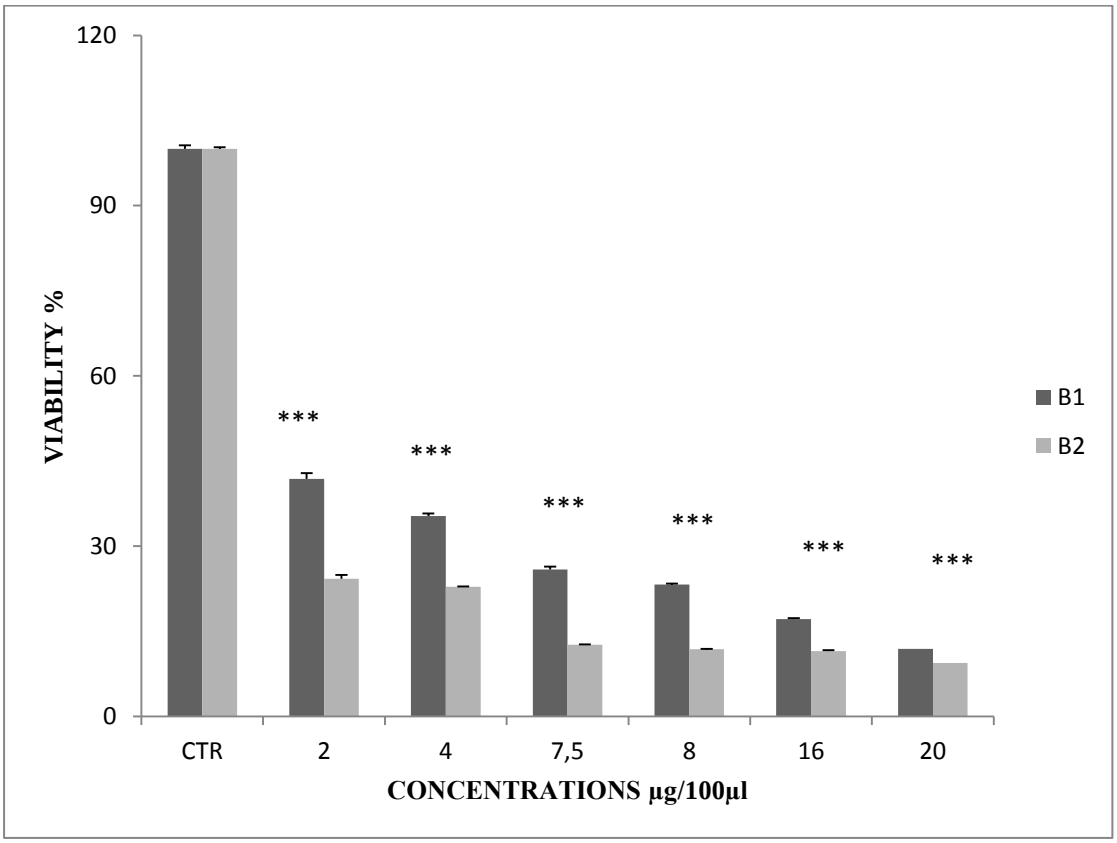

Figure 44: The effect of *Phoenix dactylifera* "HM" methanolic and acetone extracts MCF7 cells viability. Results shown in the figure are expressed as the means average deviations of three separate experiments and error bars indicate SD *****P = 0.000**

B1: The Acetone extract of *Phoenix dactylifera* "HOMAYRA variety".

B2: The Methanolic extract of *Phoenix dactylifera* "HOMAYRA variety".

The result in the **(Table 8)** represents a comparison between the effects of the two *Phoenix dactylifera* varieties extracts. The results showed that with the low concentrations Homayra variety extracts (B1, B2) has more effect on cancer cells viability comparing to Azarza variety extracts (A1, A2). But in the high concentrations the Methanolic extract of Azarza variety (A2) became more effective than the methanolic extract of Homayra variety (B2) (**16µg/100µl: A2**= 9.061% ±0.19, **B2**= 11.54% ±003), (**20µg/100µl: A2**= 7.68% ±0.03, **B2**= 9.43% ±0.13) and the acetone extract of Homayra variety (B1) was more effective than the acetone extract of Azarza variety (A1) (**16µg/100µl: A1**= 27.96% ±0.13, **B1**= 17.19% ±0.16), (**20µg/100µl: A1**= 18.370% ±0.26, **B1**= 11.90% ±0.15)

Table 8: Comparison between the effects of the two *Phoenix dactylifera* varieties extracts on MCF cells viability

Concentrations	A1	B1	A2	B2
2µg/100µl	46,56% ±1,16	41,89% ±0,61	52,07% ±0,77	24,26% ±0,26
4µg/100µl	42,87% ±0,16	35,29% ±0,96	18,72% ±0,20	22,88% ±0,68
7.5µg/100µl	37,71% ±0,53	25,89% ±0,47	13,51% ±0,26	12,63% ±0,06
8µg/100µl	28,59% ±0,29	23,25% ±0,51	12,23% ±0,13	11,88% ±0,05
16µg/100µl	27,96% ±0,13	17,19% ±0,16	9,061% ±0,19	11,54% ±0,03
20µg/100µl	18,370% ±0,26	11,90% ±0,15	7,68% ±0,03	9,43% ±0,13

A1: Acetone AZ A2: Methanol AZ B1: Acetone HM B2: Methanol HM

III.7.2. Effect of *Phoenix dactylifera* on Liver cancer cells (HepG2)

III.7.2.1. *Phoenix dactylifera* "Azarza variety"

The results in **(figure 45)** concerning the effect of *Phoenix dactylifera* "AZ variety" with both Acetone (A1) and methanol (A2) extract on the proliferation of hepatocarcinoma cells showed a high decrease in the cancer cells viability (P=0.000). The decrease in cells viability was a concentration dependent decrease (more the concentration increased more the viability decreased). By comparing between the two extracts the methanolic extract was more effective than the acetone extract with low concentrations (**2 µg/100ml A2** = 27.58% ± 1.172, **2µg/100ml A1** = 49.93% ±3.604)

and high concentrations (**20 µg/100ml A2** = 16.55% ± 0.399, **20µg/100ml A1** = 32.58% ±2.199).

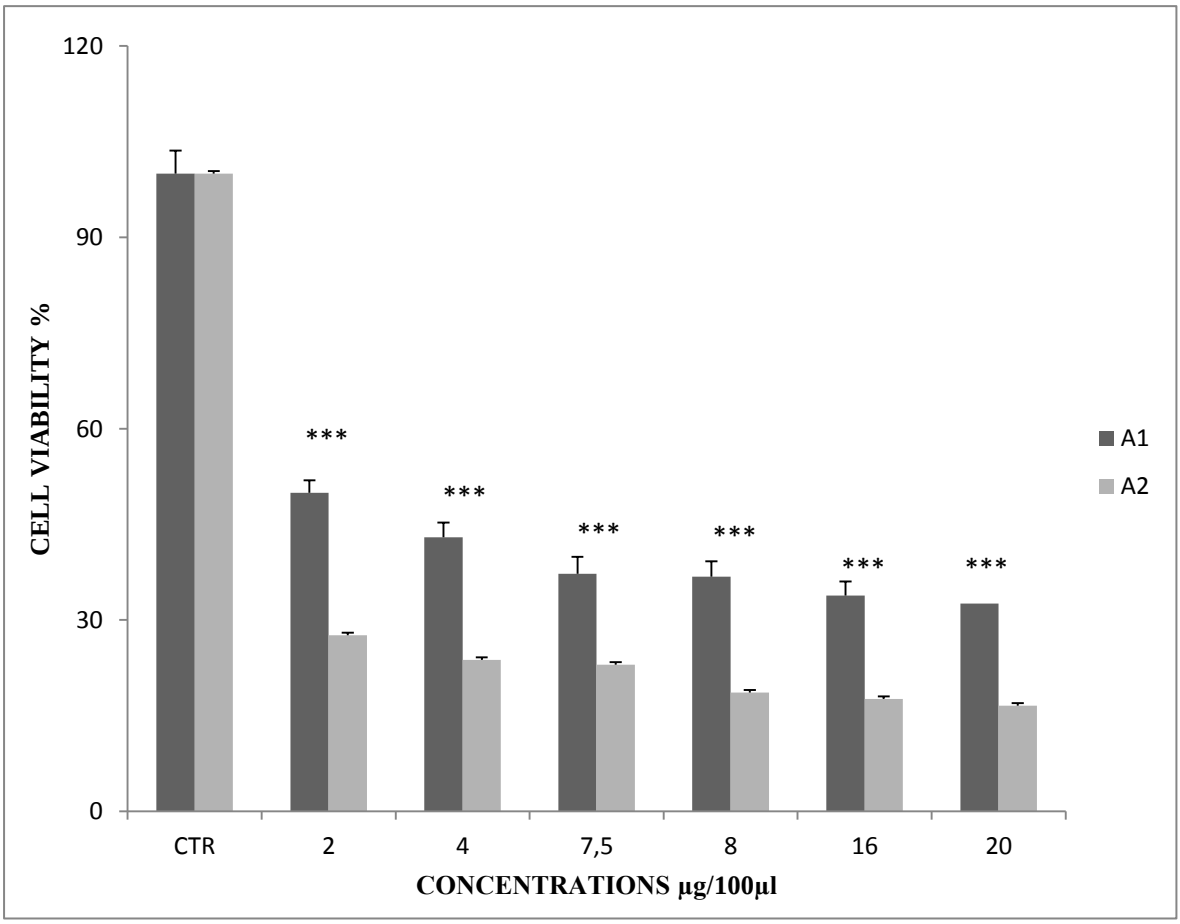

Figure 45: The effect of *Phoenix dactylifera* "AZ" methanolic and acetone extracts HepG2 cells viability. Results shown in the figure are expressed as the means average deviations of three separate experiments and error bars indicate SD *****P = 0.000**

A1: The Acetone extract of *Phoenix dactylifera* "AZARZA variety".

A2: The Methanolic extract of *Phoenix dactylifera* "AZARZA variety".

III.7.2.2. *Phoenix dactylifera* "Homayra variety"

In **figure 46**, the results concerning the effect of *Phoenix dactylifera* "HM variety" with both Acetone (B1) and methanol (B2) extract on the proliferation of breast cancer cells showed a high decrease in the cancer cells viability (P=0.000). By comparing between the two extracts the methanolic extract was more effective than the acetone extract with low concentrations (**2 µg/100ml B2** = 22.35% ± 2.214, **2µg/100ml B1** = 44.59% ±2.096) and high concentrations (**20 µg/100ml B2** = 4.29% ± 0.231, **20µg/100ml B1** = 13.32% ±0.435).

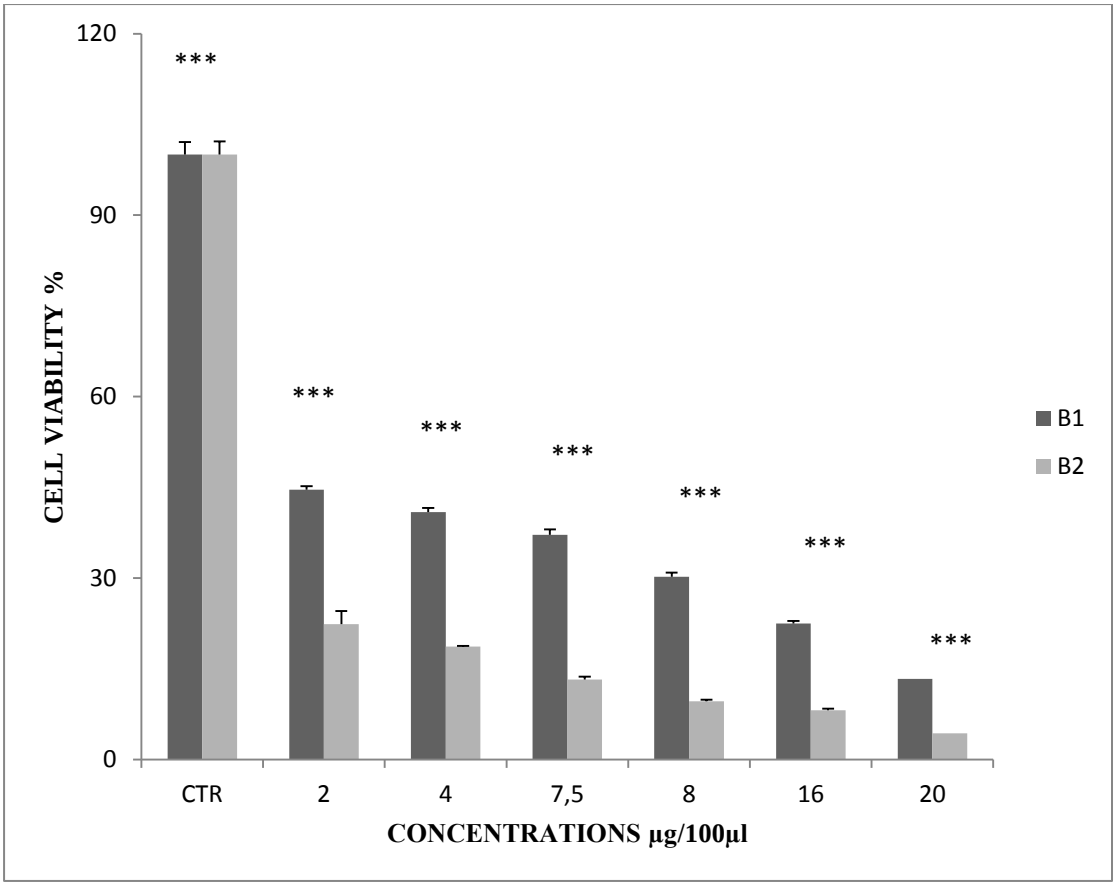

Figure 46: The effect of *Phoenix dactylifera* "HM" methanolic and acetone extracts HepG2 cells viability. Results shown in the figure are expressed as the means average deviations of three separate experiments and error bars indicate SD *****P = 0.000**

B1: The Acetone extract of *Phoenix dactylifera* "HOMAYRA variety".

B2: The Methanolic extract of *Phoenix dactylifera* "HOMAYRA variety".

The result in the **(Table 9)** represents a comparison between the effects of the two *Phoenix dactylifera* varieties extracts. The results showed that with the low and the high concentrations Homayra variety extracts (B1, B2) has more effect on cancer cells viability comparing to Azarza variety extracts (A1, A2). (**2µg/100µl: A1**= 49.93% ±3.604, **B1**= 44.59% ±2.096), (**2µg/100µl: A2**= 27.58% ± 1.172, **B2**= 22.35% ±2.214) (**20µg/100µl: A1**= 32.58% ±2.199, **B1**= 13.32% ±0.435), (**20µg/100µl: A2**= 16.55% ±0.399, **B2**= 4.29% ±0.231)

Table 9: Comparison between the effects of the two *Phoenix dactylifera* varieties extracts on HepG2 cells viability

Concentrations	A1	B1	A2	B2
2µg/100µl	49,93% ±3,604	44,59% ±2,096	27,58% ± 1,172	22,35% ±2,214
4µg/100µl	42,93% ±1,985	40,88% ±0,587	23,713% ±0,521	18,67% ±2,179
7.5µg/100µl	37,23% ±2,312	37,15% ±0,690	22,97% ±0,784	13,21% ±0,107
8µg/100µl	36,78% ±2,674	30,17% ±0,907	18,59% ±0,537	9,58% ±0,492
16µg/100µl	33,83% ±2,411	22,45% ±0,729	17,62% ±0,226	8,14% ±0,292
20µg/100µl	32,58% ±2,199	13,32% ±0,435	16,55% ±0,399	4,29% ±0,231

A1: Acetone AZ A2: Methanol AZ B1: Acetone HM B2: Methanol HM

III.7.3. Effect of *Phoenix dactylifera* on normal Human Umbilical Vein Endothelial Cell (HUVEC)

III.7.3.1. *Phoenix dactylifera* "Azarza variety"

The results in **(figure 47)** concerning the effect of *Phoenix dactylifera* "AZ variety" with both Acetone (A1) and methanol (A2) extract on normal Human Umbilical Vein Endothelial Cell (HUVEC) showed no toxic effect, and by comparing between the two extracts the methanolic extract had more influence than the acetone extract with low concentrations and high concentrations (**A1**; **2µg/100µl**: 99.14% ± 0.23, **8µg/100µl**: 90.32% ± 0.69, **20µg/100µl**: 84.25% ±1.43), (**A2**; **2µg/100µl**: 96.87% ± 0.41, **8µg/100µl**: 84.25% ± 1.59, **20µg/100µl**: 81.14% ± 1.02).

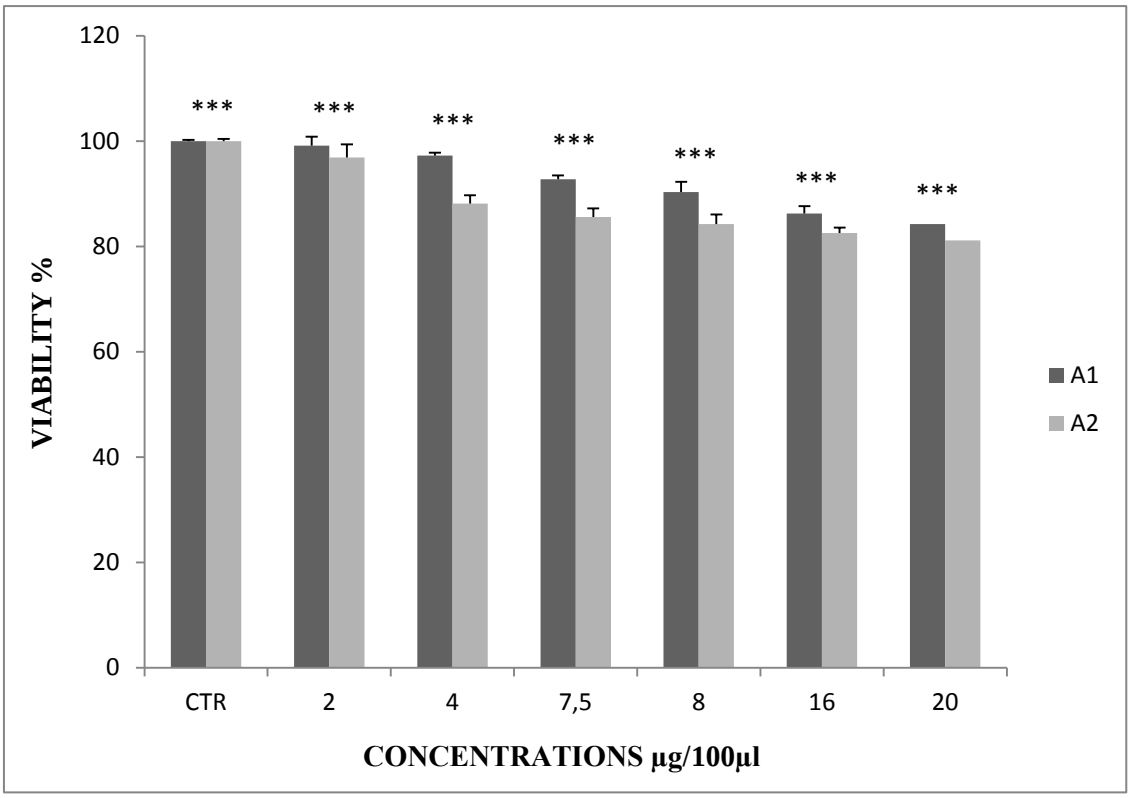

Figure 47: The effect of *Phoenix dactylifera* "AZ" methanolic and acetone extracts on HUVEC cells viability. Results shown in the figure are expressed as the means average deviations of three separate experiments and error error bars indicate SD *****P = 0.000**

A1: The Acetone extract of *Phoenix dactylifera* "AZARZA variety".

A2: The Methanolic extract of *Phoenix dactylifera* "AZARZA variety".

III.7.3.2. *Phoenix dactylifera* "Homayra variety"

The results in **(figure 48)** concerning the effect of *Phoenix dactylifera* "HM variety" with both Acetone (B1) and methanol (B2) extract on normal Human Umbilical Vein Endothelial Cell (HUVEC) showed no toxic effect, and by comparing between the two extracts the methanolic extract had more influence than the acetone extract with low concentrations and high concentrations (**B1**; **2µg/100µl**: 97.58% ± 1.32, **8µg/100µl**: 89.91% ± 4.17, **20µg/100µl**: 86.50% ± 3.55), (**B2**; **2µg/100µl**: 95.50% ± 0.29, **8µg/100µl**: 82.79% ± 1.69, **20µg/100µl**: 79.60% ± 0.83).

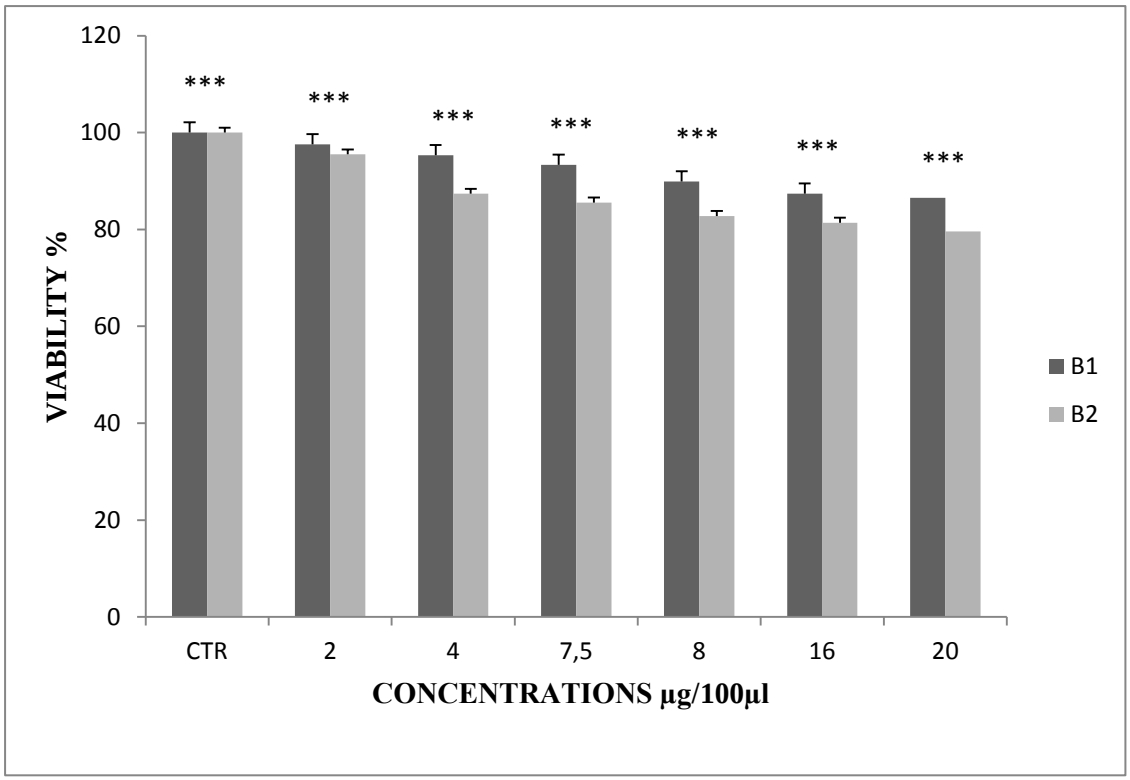

Figure 48: The effect of *Phoenix dactylifera* "HM" methanolic and acetone extracts on HUVEC cells viability. Results shown in the figure are expressed as the means average deviations of three separate experiments and error error bars indicate SD *****P = 0.000**

B1: The Acetone extract of *Phoenix dactylifera* "HOMAYRA variety".

B2: The Methanolic extract of *Phoenix dactylifera* "HOMAYRA variety".

The result in the **(Table 10)** represents a comparison between the effects of the two *Phoenix dactylifera* varieties extracts. The results showed that with the low and the high concentrations Homayra variety extracts (B1, B2) had more influence on HUVEC cells viability comparing to Azarza variety extracts (A1, A2). (**2µg/100µl: A1**= 99.14% ± 0.23, **B1**= 97.58% ± 1.32), (**2µg/100µl: A2**= 96.87% ± 0.41, **B2**= 95.50% ± 0.29) (**20µg/100µl: A1**= 84.25% ±1.43, **B1**= 86.50% ± 3.55), (**20µg/100µl: A2**= 81.14% ± 1.02, **B2**=79.60% ± 0.83).

Table 10: Comparison between the effects of the two *Phoenix dactylifera* varieties extracts on HUVEC cells viability

Concentrations	A1	B1	A2	B2
2µg/100µl	99,14% ± 0,23	97,58 % ±1,32	96,87% ±0,41	95,50% ±0,29
4µg/100µl	97,21% ± 1,71	95,32 % ±1,92	88,15% ±2,50	87,37% ±1,90
7.5µg/100µl	92,76 % ± 0,57	93,31 % ±1,38	85,59% ±1,55	85,54 % ±1,69
8µg/100µl	90,32% ± 0,699	89,91% ±4,17	84,25% ±1,59	82,79% ±1,69
16µg/100µl	86,22% ± 1,93	87,41% ±3,11	82,55% ±1,81	81,38% ±0,94
20µg/100µl	84,25% ± 1,43	86,50% ±3,55	81,14% ±1,02	79,60% ±0,83

A1: Acetone AZ A2: Methanol AZ B1: Acetone HM B2: Methanol HM

III.7.4. Effect of *Phoenix dactylifera* on normal human hepatocytes (h NHEPS)

III.7.4.1. *Phoenix dactylifera* "Azarza variety"

The results in **(figure 49)** concerning the effect of *Phoenix dactylifera* "AZ variety" with both Acetone (A1) and methanol (A2) extract on normal human hepatocytes (h NHEPS) showed no toxic effect, and by comparing between the two extracts the methanolic extract had more influence than the acetone extract with low concentrations and high concentrations (**A1**; **2µg/100µl**: 98.01% ± 0.13, **8µg/100µl**: 91.56% ± 1.03, **20µg/100µl**: 85.74% ± 0.09), (**A2**; **2µg/100µl**: 96.28% ± 0.19, **8µg/100µl**: 86% ± 0.41, **20µg/100µl**: 77.93% ± 2.75).

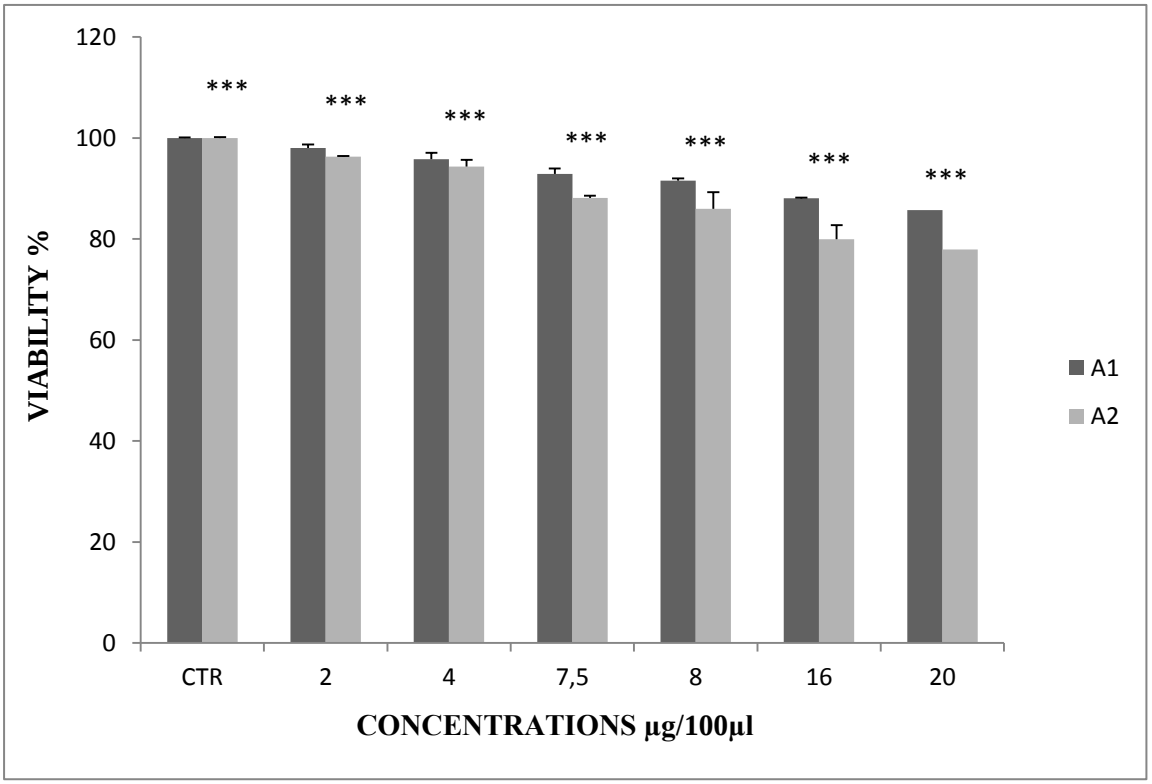

Figure 49: The effect of *Phoenix dactylifera* "Azarza variety" methanolic and acetone extracts h NHEPS cells viability. Results shown in the figure are expressed as the means average deviations of three separate experiments and error bars indicate SD *****P = 0.000**

A1: The Acetone extract of *Phoenix dactylifera* "AZARZA variety".

A2: The Methanolic extract of *Phoenix dactylifera* "AZARZA variety".

III.6.4.2. *Phoenix dactylifera* "Homayra variety"

The results in **(figure 50)** concerning the effect of *Phoenix dactylifera* "Homayra variety" with both Acetone (B1) and methanol (B2) extract on normal human hepatocytes (h NHEPS) showed no toxic effect, and by comparing between the two extracts the methanolic extract was more effective than the acetone extract with low concentrations and high concentrations (**B1**; **2µg/100µl**: 98.22% ± 0.66, **8µg/100µl**: 90.99% ± 0.96, **20µg/100µl**: 84.88% ± 1.06), (**B2**; **2µg/100µl**: 96.69% ± 0.28, **8µg/100µl**: 86.92% ± 2.29, **20µg/100µl**: 79.56% ± 4.74).

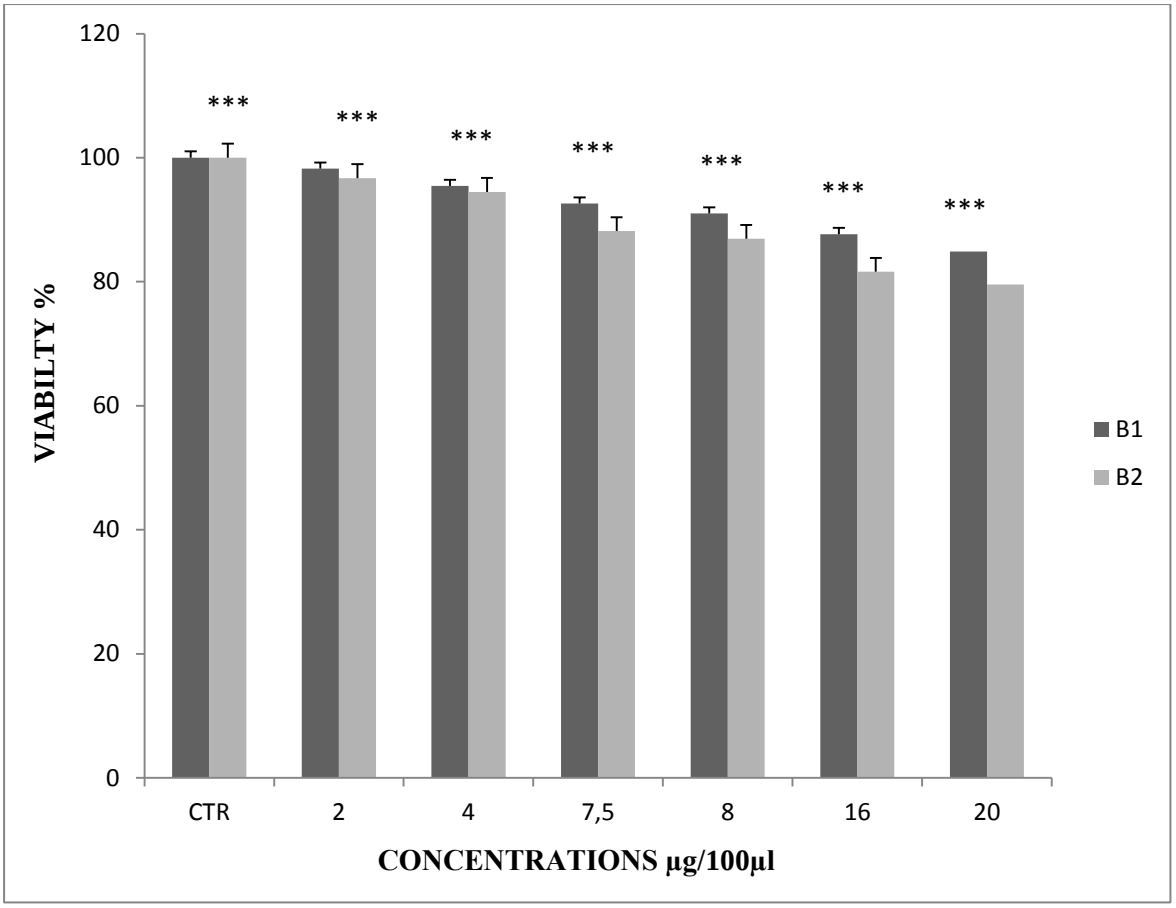

Figure 50: The effect of *Phoenix dactylifera* "Homayra variety" methanolic and acetone extracts on h NHEPS cells viability. Results shown in the figure are expressed as the means average deviations of three separate experiments and error bars indicate SD *****P = 0.000**

B1: The Acetone extract of *Phoenix dactylifera* "HOMAYRA variety".

B2: The Methanolic extract of *Phoenix dactylifera* "HOMAYRA variety".

The result in the **(Table 11)** represents a comparison between the effects of the two *Phoenix dactylifera* varieties extracts. The results showed that with the low and the high concentrations Homayra variety extracts (A1, A2) had more influence on HUVEC cells viability comparing to Azarza variety extracts (B1, B2). (**2µg/100µl: A1**= 98.01% ± 0.13, **B1**= 98.22% ± 0.66), (**2µg/100µl: A2**= 96.28% ± 0.19, **B2**= 96.69% ± 0.28) (**20µg/100µl: A1**= 85.74% ± 0.09, **B1**= 84.88% ± 1.06), (**20µg/100µl: A2**= 77.93% ± 2.75, **B2**=79.56% ± 4.74).

Table 11: Comparison between the effects of the two *Phoenix dactylifera* varieties extracts on h NHEPS cells viability

Concentrations	A1	B1	A2	B2
2µg/100µl	98,01% ±0,13	98,22% ±0,66	96,28 % ±0,19	96,69 % ±0,28
4µg/100µl	95,82% ±0,68	95,43% ±1,32	94,36% ±0,18	94,47% ±0,13
7.5µg/100µl	92,91% ±1,269	92,60% ±1,37	88,16% ±1,32	88,16% ±2,13
8µg/100µl	91,56% ±1,03	90,99% ±0,96	86,00% ±0,41	86,92% ±2,29
16µg/100µl	88,10% ±0,42	87,65% ±2,16	79,97% ±3,28	81,61% ±4,55
20µg/100µl	85,74% ±0,09	84,88% ±1,06	77,93% ±2,75	79,56% ±4,74

A1: Acetone AZ A2: Methanol AZ B1: Acetone HM B2: Methanol HM

III.8. Effect of *Phoenix dactylifera* on genes expression in Breast cancer (MCF7)

III.8.1. RNA Quantification

The results from this part of the study showed low quantities of the RNA in all the samples treated by *Phoenix dactylifera* compared to the control (Acetone extracts: **A1.1** = 0.37 ±0.248 µg/µl, **B1.1**= 0.4448 ±0.284 µg/µl, **A1.3** = 0.28 ±0.196 µg/µl) (Methanol extracts: **A2.1** = 0.282 ±0.169 µg/µl, **B2.1** = 0.2244 ±0.20 µg/µl, **A2.3** = 0.346 ±0.40 µg/µl, **B2.3** = 0.148 ±0.054 µg/µl) (**CTR** = 2.657 ±0.62 µg/µl)

The results showed also that the amount of the RNA between the 3 concentrations of each variety's extracts was higher with the 8µg/100µl when it is compared to the other two concentrations (**A1 (4µg/100µl)** = 0.37 µg/µl ±0.24, **A1 (8µg/100µl)** = 0.40 µg/µl ±0.16, A1 **(16µg/100µl)** = 0.28 µg/µl ±0.19). (**B2 (4µg/100µl)** = 0.224 µg/µl ±0.20, **B2 (8µg/100µl)** = 0.469 µg/µl ±0.33, **B2 (16µg/100µl)** = 0.148 µg/µl ±0.05) (**Figure 51).**

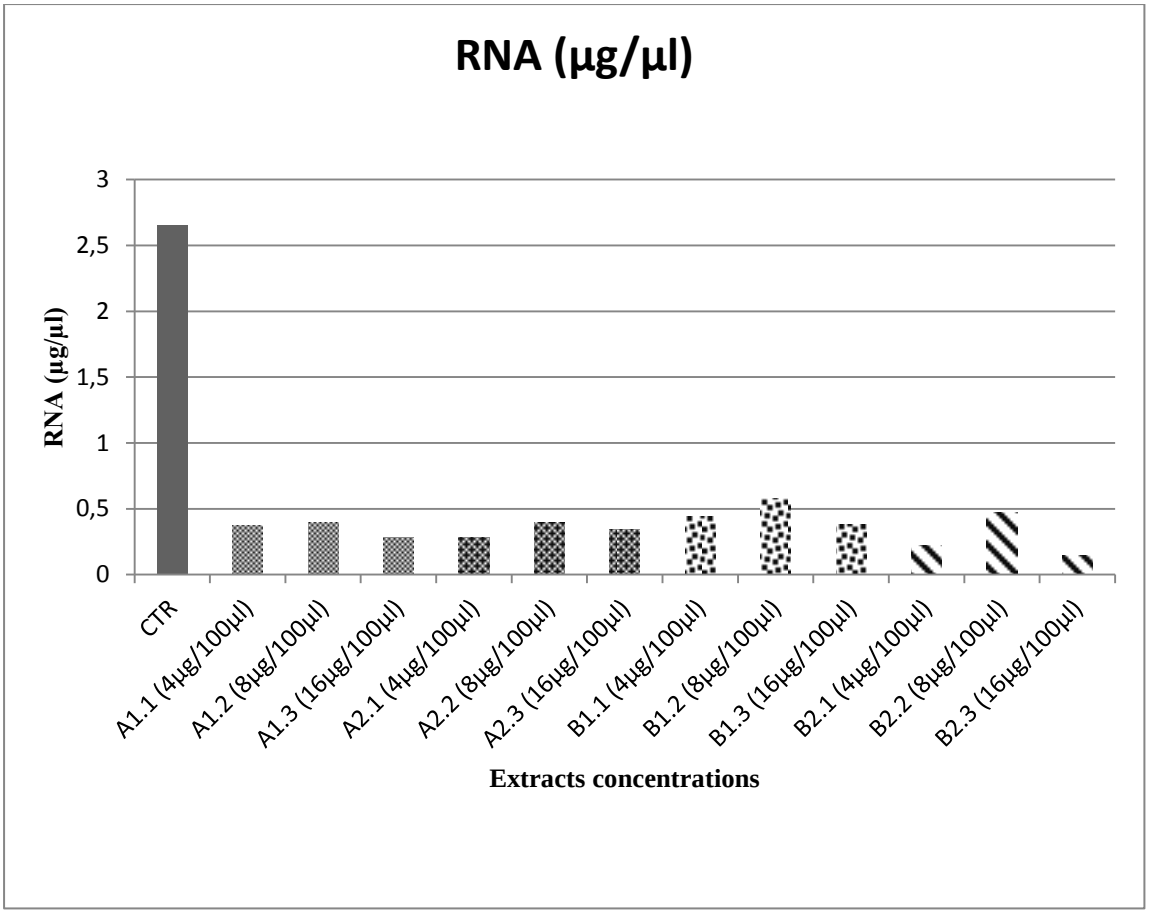

Figure 51: Comparison between the effects of the two *Phoenix dactylifera* varieties extracts on RNA quantities

III.9. Effect of *Phoenix dactylifera* on the lymphocytes differentiation

During the experimental study of Treg differentiation we have obtained that all cells were died, due to technical problems.

Chapter IV

Discussion

Discussion

The natural resistance of the body against infection can be enhanced by the use of herbal drugs. Several herbal preparations that can enhance the body's immune system status are extensively being used in the indigenous system of medicines. There is an upsurge in the clinical use of indigenous drugs as they are free from serious side effects (Chandua *el al*., 2011). Medicinal plants and plant products have been reported to modulate the innate as well as humoral immune responses by interacting with the immunoregulatory cascade and thereby altering the immune cell proliferation, phagocytic function, cytotoxic activity, expressions of cytokines, cellular co-receptors and immunoglobulins (Maji *et al.*, 2013) In this study we aimed to evaluate some of the biological effects of the Algerian dates *Phoenix dactylifera* (AZ and HM varieties).

In the acute toxicity study represented in our study by up and down method, our extracts used from *Phoenix dactylifera* (AZ and HM varieties) are toxically safe by oral administration in mice.

The use of herbal medicines as alternative treatments has been increasing worldwide and gaining popularity in developing countries. Although medicinal plants may have biological activities that are beneficial to humans, the potential toxicity of these bioactive substances has not been well established. Thus, the safety and efficacy of these plants must be studied thoroughly to maximise their benefits. To achieve this objective, a toxicological evaluation is performed using an experimental animal to provide guidelines for selecting a "safe" dose for human uses. To evaluate the toxicity of plant extracts, experimental animals, are treated at specific doses for a defined period of time. At this period, the mice were controlled and survived (Almança *et al.*, 2011) and (Aribi, 2015). This result agrees with those of (Nath and Yadav, 2015) who reported that the use of *Hibiscus rosa-sinensis* L. leaf extract at 2000 mg/kg as a limit dose is devoid of any toxic effects in mice.

The second part of the study was to evaluate the immunomodulatory effect of the Algerian dates *Phoenix dactylifera* extract (AZ and HM varieties). The immunostimulants are substances, which can stimulate either innate or adaptive arms of immune system. Many synthetic immunostimulants are launched by pharmaceutical companies but with many side effects (Talmale *et al.*, 2014). In the other side some of plant products are believed to enhance the natural resistance of the body to infection, on the basis of their constituents like polysaccharides, lectins, saponins and flavonoids etc. Some of these stimulate both 'humoral and cell mediated immunity', while others activate only the cellular components of the

immune system (Compton and Jones, 1985). Immunostimulatory molecules intensify and modify the lymphocyte mediated immune response and its duration. Such molecules can, therefore, be potentially applied as adjuvants in vaccines and allergy preparations (Ranta *et al.*, 2012).

The immunomodulation effect of these substances relates to the non-specific activation of the immune system, primarily implies a non-antigen dependent stimulation of the function and efficiency of macrophages, granulocytes, complement, natural killer cells, lymphocytes and also the production of various effector molecules. Non-specific immunomodulation using medicinal plants can provide protection against foreign invaders like bacteria, fungi, viruses etc. and constitutes an alternative to conventional chemotherapy for variety of diseases, especially when host defence mechanism has to be activated or selectively suppressed (Maji *et al.,* 2013). To evaluate this effect a carbon clearance test was done to evaluate the effect of date extract on the reticuloendothelial system (RES), which is a diffuse system consisting of phagocytic cells. The moment when the carbon particles in the form of ink are injected directly into the systemic circulation, the macrophage and the other phagocytic cells start the clearance of carbon from the blood by phagocytosis.

The results showed that the concentrations of *Phoenix dactylifera* extracts used in the treated groups had an effect on the immune system by stimulating the phagocytic system to execute a fast elimination of the carbon from the blood with a best result at the concentration of 50 mg/kg (AZ) and 150 mg/kg (HM) when it is compared to the control group. The increase in the rate of carbon clearance from the circulatory system indicated the augmentation of the phagocytic activity and competency of granulopoetic system in the removal of foreign particles. These results agree with the results of (Bharani *et al.,* 2010) who reported that the cells of the reticuloendothelial systems play important role in the clearance of particles from the blood stream. When colloidal carbon particles in the form of ink are injected directly into the systemic circulation, the rate of clearance of carbon from the blood by macrophage is increased during the treatment of rats by the methanolic extract of *Morus Alba* Linn (Mulberry) leaves.

Also we found that these results are compatible with those of (Patel *et al*, 2010) who reported that both low dose (100 mg/kg, *p.o*) as well as high dose (500 mg/kg, *p.o*) of *Aegle marmelos* stimulates immune system and (Tang *et al.*, 2012) who demonstrated that *Tenebrio molitor Linnaeus* has a dose dependent effect to potentiate the capability of carbon granule clearance and the phagocytosis activity of macrophage in mice.

In the studies of (Gokani *et al.*, 2007) and (Aribi *et al.*, 2013) who reported that the administration of extraction of *Clerodendrum phlomidis*, *Premna integrifolia* roots and *Argania spinosa* respectively in mice increased the phagocytic index at different concentrations.

Phagocytes as macrophage and neutrophils mediate their innate immunological response by releasing products that damage invading microorganisms. These products include proteins such as lysozyme, peroxidases, and elastase as well as reactive oxygen species such as superoxide, hydrogen peroxide, hypohalous acid, and hydroxyl radical (Rosen *et al.*, 1995). Many diseases are associated with the production of reactive oxidizing species that damage physiologically essential molecules. The classical view is that antioxidants scavenge these reactive oxidizing molecules and thus offer protection against disease (Guido *et al.*, 2014). Antioxidants are molecules able to inhibit the oxidation of other molecules, in another way they can protect other molecules from the reactive oxygen species effect. From this point, many phytochemicals from vegetables and fruits are used for their high antioxidant potential (Hasan *et al.*, 2010). In the second part of the study we evaluated the effect of the *Phoenix dactylifera* extract (AZ and HM varieties) on the glutathione reduced (GSH) values from the liver's homogenate.

Glutathione (GSH) is a major antioxidant that protects tissues from free radical injury and a vital component of host defences. In addition to protecting against free radical injury, it is important in the activation of lymphocytes, critical for the function of natural killer cells and lymphocyte-mediated cytotoxicity, and may have a role in the protection of neutrophils and macrophages against oxidative damage (Hong *et al.*, 1991). Glutathione reduced plays a central role in a multitude of biochemical processes, and disturbances in its homeostasis are implicated in the etiology and progression of a number of diseases. GSH is required for proper protein and DNA synthesis, cell cycle regulation, thermo tolerance, exocrine secretion, maintenance, and regulation of the thiol-redox status of the cell, protection against oxidative damage, detoxification of endogenous and exogenous reactive metals and electrophiles, biosynthesis of mercapturic acids (S-substituted N-acetyl-l-cysteines), and storage and transport of cysteine (Ballatori *et al.*, 2005). Reduced glutathione contains a thiol group which can function as a free radical scavenger. By reacting with reactive oxygen species such as free radicals or peroxides, GSH serves as an electron donor and is converted to its oxidized form, GSSG. GSSG can be reduced back to GSH by glutathione reductase in the presence of reduced nicotinamide adenine dinucleotide phosphate (NADPH) (Cao *et al.*, 2013).

Our results showed potential antioxidant effect of the *Phoenix dactylifera* extracts presented by a high significant decrease in the GSH values from liver in the groups treated by three doses of the *Phoenix dactylifera* extracts (AZ and HM varieties), with a higher effect at the dose of 50 mg/kg (AZ) and 150 mg/kg (HM) comparing to the control groups. This result agrees with the results of (Hasan *et al.,* 2010) and (Hasnaoui *et al.,* 2012) who reported respectively that fibre concentrates from Moroccan date flesh and various dates extract (*Phoenix dactylifera* L.) from Lybia have high antioxidant propriety. Also those of (Patel *et al.,* 1987) demonstrate in their study that the oral hypoglycemic agents, tolbutamide and glyburide, employed in the treatment of type II diabetics increase biliary glutathione release which appears as an increase in biliary GSH secretion by the liver.

These results suggest that the *Phoenix dactylifera* extracts have a potential antioxidant effect by stimulating the excretion of the GSH from the liver which will lead to eliminate the reactive oxygen species and protect the organism. This effect may be induced by the influence of the plant extracts on the enzymatic mechanisms of production of the GSH. As it is known that the synthesis and catabolism of GSH and its adducts occurs by a regulated series of enzymatic and plasma membrane transport steps that are collectively referred to as the g-glutamyl cycle GSH is synthesized in every cell of the body, but the liver is quantitatively the major site of synthesis. GSH is synthesized intracellularly from its precursor amino acids by the ATP requiring cytosolic enzymes g-glutamylcysteine synthetase and GSH synthetase (Ballatori *et al.,* 2005). After its synthesis, GSH is delivered to other intracellular compartments, including mitochondria and endoplasmic reticulum, and to the extracellular space (e.g., blood plasma and bile) for utilization by other cells and tissues (Ballatori *et al.,* 2005). This makes another possibility of the levels that the *Phoenix dactylifera* extracts enhance to influence the level of the GSH in liver. One of the proteins responsible of the GSH transport is the canalicular multispecific organic anion transporter (cMOAT). cMOAT a member of the ATP-binding cassette transporter family mediates the transport of a broad range of non-bile salt organic anions from liver into bile. cMOAT-deficient Wistar rats (TR−) are mutated in the gene encoding cMOAT, leading to defective hepatobiliary transport of a whole range of substrates, including bilirubin glucuronide. These mutants also have impaired hepatobiliary excretion of GSH (Paulusma *et al.,* 1999).

In this study, our results demonstrated that animals administered with *Phoenix dactylifera* extracts stimulated phagocytic activity and the release of GSH from the liver but this effect decreased with higher doses (100 mg/kg Azarza, 200mg/kg Homayra). These

results could be explained by the hormesis concept (Benmebarek, 2014). This hormesis (Greek word meaning "to excite ") concept has become progressively used in different fields, especially in environmental toxicology and biology when describing a dose-response continuum involving a low-dose stimulation/high-dose inhibition (Calabrese and Baldwin, 2001).

These results could be explained by the presence of two receptor subtypes affecting cell regulation, one with high and the other with low affinity for the agonist but with notably more capacity (Calabrese and Blain, 2005). This may lead to the biphasic dose response, with the high-affinity receptor activated at low concentrations, which stimulates DNA synthesis and cellular proliferation; and the low affinity/high-capacity receptor becoming dominant at higher concentrations decreasing the cell proliferative response (Benmebarek, 2014). This pharmacological mechanism may explain the biphasic dose response effect of *Phoenix dactylifera* extracts.

The next part of the study was to evaluate the effect of the *Phoenix dactylifera* extracts (AZ and HM varieties) on the hyperhomocysteinemia, inflammation and arthritis.
Hyperhomocysteinemia may promote the generation of reactive oxygen species (ROS) such as H_2O_2 and hydroxyl radicals via the auto-oxidation of sulfhydryl (-SH) group or by decreasing the intracellular levels of GSH that are involved in the elimination of free radicals (Bhandari *et al.,* 2008). An association of hyperhomocysteinemia with inflammation is supported by the fact that the level of circulating homocysteine can be effectively reduced by the administration of anti-inflammatory medications. Several anti-inflammatory compounds such as resveratrol, aspirin, salicylic acid and atorvastatin have all been shown to down-regulate the release of homocysteine from stimulated human peripheral blood mononuclear cells (Wu, 2007).

The results of the Hcy quantification showed that there was a significant decrease in the Hcy in the plasma of the treated group with the *Phoenix dactylifera* extracts (AZ and HM varieties) comparing to the other groups. These results are compatible with those of (Shaker *et al.,* 2013) who reported that avocado showed the best results among tomato and broccoli extracts in decreasing plasma Hcy. Also (Nolla *et al.,* 2009) reported that red wine polyphenolic extract (PE) administration in low quantities to mice is able to block the rise of plasma Hcy levels and has beneficial effects on biochemical markers of hepatic and endothelial dysfunction due to hyperhomocysteinemia. (Olas *et al.,* 2010) and (Malinowska *et al.,* 2012) showed that in the presence of the extract of *A. melanocarpa*, decreased

significantly the amount of thiols, including Hcy, in plasma from patients with invasive breast cancer and patients with benign breast diseases. Also (Esfahani *et al.,* 2011) reported that daily consumption of mixed fruits and vegetable supplements significantly increases serum levels of antioxidant and reduces homocysteine and markers of oxidative stress.

The potential effect of *Phoenix dactylifera* extracts (AZ and HM varieties) may be explained by the influence of the plant extract on the enzymatic mechanisms of the regulation of homocysteine concentration in the organism. It is clear that tissue concentration of Hcy is maintained at low levels by regulated production and efficient removal of this thiol (Stipanuk, 2004). In most tissues, Homocysteine itself is located at a branch-point of metabolic pathways: either it is irreversibly degraded via the transsulfuration pathway to cysteine or it is remethylated back to methionine exported out of the cell. The liver is the main organ of degradation of excess methionine and in maintaining homocysteine at adequate levels via a unique set of enzymes, including methionine-adenosyltransferase (MAT) I/III, cystathionine β-synthase (CBS), cystathionine γ-lyase (CTH), betaine homocysteine methyltransferase (BHMT), glycine N-methyltransferase (GNMT) (Blom and Smulders, 2011). Also another explanation may come from the previous activities and from the chemical composition of the plant extract. *Phoenix dactylifera* varieties which as demonstrated previously, had an antioxidant propriety, In addition to its containment of some active substances such as alkaloids, tannins, flavonoids, terpenes and sugars (Wu, 2007) and (Amiour, 2009), which could be responsible for the anti-inflammatory and analgesic effects. Also flavonoids have been shown to possess various biological properties related to antioxidant, anti-nociceptive, and anti-inflammatory mechanisms by targeting reactive oxygen species and prostaglandins which are involved in the late phase of acute inflammation and pain perception (Nagore *et al.,* 2010) and (Agnel *et al.*, 2012).

The anti-inflammatory and anti-arthritis activities were tested by the formalin induced paw edema test. It is well known that the inhibition of formalin–induced paw edema is one of the most suitable test procedures to screen anti-arthritic and anti-inflammatory agent as it closely resembles human arthritis. Injection of formalin subcutaneously into hind paw of rats produces localized inflammation and pain. Thus formalin induced arthritis is a model used for the evaluation of an agent with probable anti-proliferate activity (Hemamalini *et al.*, 2010).

The *Phoenix dactylifera* extracts (AZ and HM varieties) significantly inhibit the proliferative global edematous response compared to the positive and negative controls also the anti-inflammatory drug standard Diclofenac. These results are compatible with those of

(Mazumder *et al.,* 2012) who recorded that in the formaldehyde induced arthritis inflammation test, methanol extract of *Barleria lupulina* showed a significant inhibition of the edema formation during the experimental period of 10 days. Also a compatibility with those of (Dheeba *et al.,* 2012) and (Kumar *et al.,* 2014) who reported that *Alternanthera brasiliana* leaves extract and *Spirulin fusiformis* extract are able to suppress the edematous changes produced during formaldehyde-induced arthritis. Also a compatibility with those of (Kyei *et al.,* 2012) who reported that *Pistia stratiotes* compared to diclofenac and methotrexate has anti-arthritic and antipyretic effect in formalin-induced arthritis.

The injection of formaldehyde into animal paw produced localized inflammation (releasing of histamine, serotonin and kinin) and pain. Formaldehyde induced arthritis is biphasic in nature i.e. an untimely neurogenic element followed by a later tissue mediated response (Rodrigues *et al.,* 2012) and (Kumar *et al.,* 2013). The results obtained from this test may been explained by the influence of *Phoenix dactylifera* extracts (AZ and HM varieties) on the secretion of inflammatory mediators and to have an effect similar to non-steroid anti-inflammatory drugs (NSAIDs) in both phases of inflammation. The initial phase where histamine and serotonin are released (0-2 h) (Viswanatha *et al.,* 2011), followed by kinins like bradykinin, lasting 30-60 min (Hunskaar and Hole, 1987) and then a phase mediated by prostaglandins (>4 h) (Viswanatha *et al.,* 2011). Thus prostaglandins act relatively late in the development of inflammation. It is well established that NSAIDs prevent the inflammation by blocking the prostaglandin synthesis (Hunskaar and Hole, 1987).

It should be noted that the anti-inflammatory, anti-arthritis activities and other biological effects of many plants have been attributed to their triterpene or flavonoid contents. It has been also demonstrated that various flavonoids (such as rutin, quercetin, and luteolin), biflavonoids, and triterpenoids (such as ursolic acid) produced significant antinociceptive and/or anti-inflammatory activities (Çadirci *et al.,* 2012). *Phoenix dactylifera* is rich in phenolic compounds, such as phenolic acids and flavonoids, in addition to terpenoids (Al-farsi and Lee, 2008), (Benmeddour *et al.,* 2013) and (Delphin *et al.,* 2014).

The anti-cyclic citrullinated peptide (anti-CCP) antibody recognises arginine residues modified by peptidyl arginine deiminases (PAD). Anti-CCP belongs to the family of anti-filaggrin autoantibodies, accompanied by the anti-keratin antibody and the anti-perinuclear factor (Kastbom *et al.,* 2004). Anti-CCP antibodies have emerged as one of the most important biomarkers in RA, and their detection has been greatly facilitated by the development of the CCP test. Anti-CCP antibodies might have a key role in the

pathophysiology of RA (Serdaroflu *et al.,* 2008), as they are already present early in the disease course, are highly specific for the disease, can predict disease development, and can cause or enhance arthritis in experimental animal models (Van Venrooij *et al.,* 2011) In this part of the study our result showed that *Phoenix dactylifera* extracts (AZ and HM varieties) significantly decreased the CRP and Anti-CCP antibodies levels comparing to the control groups positive and negative. The possible explanation is that *Phoenix dactylifera* extracts enhanced also the inflammation development by inhibiting the filtration of inflammatory immune cells to the inflammation sit. It is known that inflammation is initiated as a vascular response followed by leukocytes filtration (Thomson *et al.,* 1999) which secrete the majority of the inflammation mediators like tumor necrosis factor-a (TNF-a), plasminogen activator inhibitor-1 (PAI-1), interleukin (IL)-1b, IL-6, IL-8, IL-10, IL-15, complement factors, and prostaglandin E2 (PGE2). These mediators stimulate the secretion of the acute-phase protein, C-reactive protein (CRP), by the liver (the presence of CRP in plasma is recognized as a marker for low-grade systemic inflammation) (Othman and Moghadasian, 2011). Like the other activities, the anti-inflammatory effect of *Phoenix dactylifera* extracts may returns to its chemical composition. Plant sterols have been shown to reduce plasma levels of CRP, IL-6, TNF-a, phospholipase A1, and fibrinogen (Bouic *et al.,* 1999), (Nashed *et al.,* 2005) and (Devaraj *et al.,* 2006). These effects may be mediated through beneficial alterations in the membrane composition, affecting membrane fluidity, sensitivity, and signalling pathways. All of these changes may subsequently alter the immune response by influencing the synthesis and secretion of eicosanoids, leukotrienes, and prostaglandins (Othman and Moghadasian, 2011).

These results are compatible with those of (Mikuls *et al.,* 2004) who reported that shorter disease duration predicts greater declines in anti-CCP antibody levels with treatment in RA. Also(Chen, Lin *et al.,* 2006), (Tsuji *et al.,* 2013), (Hetland *el al.* 2012) and (Gardette *et al.,* 2014) who reported that the treatment with Disease Modifying Anti-Rheumatic Drugs (DMARDs), Betamethasone, Adalimumab, Rituximab, Infliximab and Etanercept leads to greater decrease in the serum levels of anti-CCP, RF and CRP in rheumatoid arthritis.

The anti-inflammatory drugs such as indomethacin and diclofenac and anti-arthritis drugs act by the reduction of sensitization of pain receptors caused by prostaglandins at the inflammation site (Delphin *et al.,* 2014). The observed anti-inflammatory activities of these extracts may be attributed to the overall effects of the plant constituents or the compounds

having actions similar to anti-inflammatory drugs, it should be noted that the extracts have different compositions of several substances.

From the histological study of the mice's joint, it is evident that the inflammation was controlled by treatment with *Phoenix dactylifera* extracts, which confirmed that *Phoenix dactylifera,* possess anti-arthritic analgesic and anti-inflammatory activities. These results are compatible with those of (Mali *et al*., 2011) who reported in their histopathological studies of Freund's complete adjuvant induced arthritis, that treatment with *Phyllanthus amarus* extract (200 and 400 mg/kg), controlled the inflammation of the connective tissue in the tibiotarsal joint. These results agree also with those of (Aribi, 2015) who reported from the histological study of rat's inflamed joints, that the treatment using *Argania spinosa* seeds extract combating the inflammation caused either by formalin or formalin and methionine and it was quite similar to the action of Ibuprofen the reference anti-inflammatory drug.

The experiment evaluates the effect of formalin induced inflammation on the weight and food consumption of mice. The results show a significant decrease in the weight of mice in groups treated with the plant extracts (AZ and HM) and with the Diclofenac (D), when compared with the control groups (FF) and (N. Meanwhile we had obtained a significant decrease in the food consumption in groups treated with the plant extracts (AZ and HM) and with the Diclofenac (D), when it is compared with the control groups (FF) and (N).

This gain of weight and the loss of it may be defined by excess adipose mass and adipose tissue expansion and decrease. Adipose tissue is an important energy storage organ and has only been progressively recognized over the past two decades as a key endocrine organ with active metabolism (Wang *et al.*, 2014). As a possible explanation for the reduction in body weight of the groups treated by *Phoenix dactylifera* extracts and the anti-inflammatory drug, we suggest that there is in influence on the adipogenesis or the lipogenesis pathways. In the study of (Kwak *et al.*, 2012), they found that extract of *Aristolochia Manshuriensis Kom* (AMK) which is a traditional Korean medicinal herb used for the treatment of arthritis, rheumatism, hepatitis, and anti-obesity, inhibited adipocyte differentiation and reduced the body weight and the fat accumulation in HFD-induced obesity mice. It's anti-obesity mechanism involves the down-regulation of the major transcription factors of the adipogenesis pathway including peroxisome proliferation-activity receptor-c (PPAR-γ) and CCAAT/enhancer binding protein-a (C/EBP-α) by regulation of Akt and extracellular signal regulated kinases (ERK1/2) pathway, and resultant down regulation of lipid metabolizing enzymes, fatty acid synthase (FAS), lipopreotein lipase (LPL) and

adipocyte fatty acid binding protein 2 (aP2), which are involved in the transport, uptake and synthesis of lipids needed for the accumulation of lipid in adipocytes.

The other suggestion for this effect is coming from the anti-inflammatory and the antioxidant effect of *Phoenix dactylifera* extracts. In Indeed, several cell, animal and human studies provide strong evidence that dietary bioactive compounds act as anti-oxidant and anti-inflammatory agents to increase thermogenesis and energy expenditure while decreasing inflammation and oxidative stress, further supporting progress towards weight loss and/or decreased metabolic disorders (Wang *et al.,* 2014). In the review of (Serviddio *et al.,* 2013), the authors reported that some of antioxidants have a beneficial modulation of redox status and lipid metabolism.

The result obtained from this part of the study where compatible with the results of (Balasubramanian *et al.,* 2010) who reported that the treatment with the ethanolic extract of *Stereospermum suaveolens* and the standard drug indomethacin slightly reduced the body weight gain during the inflammation. But in another study of (Simplice *et al.,* 2011), the authors reported an increase in body weight during the treatment with indomethacin and the methanolic extract of *Hibiscus asper* leaves of carrageenan induced edema and complete Freund's adjuvant-induced arthritis in rats. Also and concerning the body weight, (Zerizer and Naimi, 2004) reported an increase in the weight of rats treated with 200 mg/kg of L-methionine for 21 days.

In the *in vitro* study we have achieved interesting and original results never done before with two different varieties of *Phoenix dactylifera* (AZ and HM) on breast cancer (MCF7) and hepatocarcinoma (HepG2) cells viability. Our research was followed by the investigation of the *Phoenix dactylifera* effect on gene expression of BAX and Bcl2 on breast cancer cells (MCF7). The quantities of RNA were decreased in all the groups treated with *Phoenix dactylifera* when it is compared to the control group; this is may be due to the potential inhibition of *Phoenix dactylifera* extracts on cell viability or the high dose used in this experimental study.

Liver cancer is one of the common malignant tumors with the fifth highest rate of morbidity. It is a leading cause of death in patients with malignant tumors, ranking second after lung cancer (Yue *et al.,* 2015). Its incidence has been increasing in developed countries, but 82% of cases (and deaths) are in developing countries and it becomes the leading cause of cancer-related deaths in the world. Liver cancer represents a highly lethal and aggressive malignant tumor because of its dormancy course, difficult diagnosis, early metastasis, strong invasion and poor prognosis (Chang *et al.,* 2011). In the other side, Breast cancer remains a

considerable health burden. It affects more than 1.3 million women worldwide annually and is responsible for about 14% of cancer-related deaths. Indeed, the incidences of breast cancer have increased over the past decades and a substantial rise is projected in the coming years. Breast cancer is a heterogeneous and polygenic disease which is influenced by epigenetic mechanisms that affect the transcriptomes, proteomes, and metabolomes (Pilane *et al.,* 2015).

The treatment with six different concentrations of *Phoenix dactylifera* (AZ and HM varieties) with the two extracts (acetone and methanol) showed a highly significant decrease of the HepG2 and MCF7 viability. Induction of apoptosis selectively in cancer cells is one of the prime objectives of cancer therapy. Recently medicinal plants have become the source of alternative therapy for cancer treatment due to their safety and efficacy where they simultaneously influence different phases of diseases through different mechanisms. Almost 90 out of 121 drugs prescribed to treat cancer are originated from plants (Shivaprakash *et al.,* 2015). This effect may be due to an effect on cellular apoptosis by influencing the expression of some major proteins involved in the apoptosis process like the Bcl-2 family of proteins. The Bcl-2 family of proteins is divided into two groups: suppressors of apoptosis (e.g., Bcl-2, Bcl-XL, and Mcl-1) and activators of apoptosis (e.g., Bax, Bok, Hrk, and Bad). Pathological increases in the amounts of one or more of the apoptosis suppressing proteins have been observed in several types of cancer, though, generally, the mechanisms that bring about their increased expression are poorly understood. The ratio Bcl-2/Bax might be a critical factor of a cell's threshold for apoptosis. Suppression of Bcl-2 has been demonstrated to promote apoptosis in response to a number of stimuli, including anticancer drugs (Tan *et al.,* 2009). Bax is a pro-apoptotic protein which resides in an inactive form in cytosol and after activation it gets translocated to mitochondria and plays an important role in mitochondria-mediated apoptosis. Activated Bax either in homo-oligomeric form or as complex with other proteins creates pores on the outer mitochondrial membrane, which leads to the leakage of ions, essential metabolites and cytochrome c from mitochondria to cytosol leading to cell death (Farahmandzad *et al.,* 2015). Another mechanism that may be influenced is the stimulation of the caspase cascade which leads to the cell death. The mechanisms of apoptosis predominantly involve two signalling pathways, namely mitochondrial (intrinsic) pathway and death receptor (extrinsic) pathway. The death receptor-mediated pathway engages Fas/FasL and other members of the tumor necrosis factor receptor family that activate caspase-8. The mitochondrial pathway, implicated in the function of a majority of anticancer drugs, utilizes cytochrome c (cyt. c), apoptotic protease activating factor 1 and caspase-9. The

activation of Bax and inhibition of Bcl-2, results in mitochondrial disruption and subsequent release of cyt C through the outer mitochondrial membrane into the cytosol. Inside the cytosol, cyt C associates with apoptotic protease activating factor 1 and activates caspase-9 which, in turn, triggers the activation of caspase-3 and/or caspase-7. The activated caspase-3 functions as the key executioner of apoptosis (Bhatia *et al.,* 2015).

In the study of (Lu *et al.,* 2011), the authors demonstrated a significant apoptotic effect of injectable seed extract of *Coix lacryma-job*i (SC) on HepG2 cells. The expression of caspase-8 was enhanced with rising SC concentration ($P<0.01$). Therefore, caspase-8 was one of the apoptosis inducing effects of SC in HepG2 cells. Caspase-8 is a downstream molecule in the cellular pathway of Fatty Acid Synthase (FAS) for transmitting death signals. Other study of (Bhatia *et al.,* 2015) on HepG2 cells, reported that HepG2 cells exposed to *Origanum dayi* and *Ochradenus baccatu* extracts for various time periods elevated the mRNA expression of BAX, CASP3, CASP7, CASP9 *and* exert apoptosis in HepG2 human cells. The study of (Blassan *et al*., 2016) demonstrated that *Rubus fairholmianus* root acetone extract has anti-proliferative activities against human breast cancer cells (MCF-7) via caspase 3/7 induced apoptosis. Also (Marrelli *et al.,* 2015) reported that *L. vulpinum* phenolic extract and *Origanum vulgare L. subsp. Viridulum,* and (Reisa *et al.,* 2016) who indicated that *Rosmarinus officinalis* L, inhibit the growth of the human MCF-7 breast cancer cell line and hepatic cancer HepG2, by inducing DNA damage, inhibiting cellular proliferation and leading to cell death by apoptosis.

Many studies reported the relation between the chemical composition of plant extracts, antioxidant and anti-proliferative and apoptotic effects on cancer cells. There has been cumulating evidence relating the reduced prevalence of chronic heart diseases, atherosclerosis and malignant cells development to the intake of polyphenols rich diet. Such a diet is represented by the Mediterranean diet where fruits, vegetables, olive oil and table olives are largely consumed. In addition, polyphenols were identified as valuable phytochemicals extensively discussed for their antioxidant and cancer preventive activities (Bouallagui *et al.,* 2011). Oxidative stress has been suggested to play an important role in the mechanisms of toxicity of a number of nanoparticles whether by the excessive generation of ROS or by depletion of cellular antioxidant capacity. (Javed *et al.,* 2012) reported in their study that, silica nanoparticles induced cytotoxicity and oxidative stress in human liver (HepG2) cells in a dose-dependent manner. Both the mRNA and protein expressions of cell cycle check point gene p53 and apoptotic genes (bax and caspase-3) were up-regulated while the anti-apototic gene bcl-2 was down-regulated in cells treated with silica nanoparticles. Moreover, co-

treatment of ROS scavenger vitamin C significantly attenuated the alteration of apoptotic markers along with the preservation of cell viability caused by silica nanoparticles. Also (Dikmen *et al.,* 2011) reported that (*Punica granatum* L.) which is a rich source of many phenolic compounds including flavonoids, anthocyanins, catechins, other complex phenolics, and hydrolysable tannins (punicalin, pedunculagin, punicalagin, gallagic, and ellagic acid esters of glucose) which are known to be potential antioxidant, exhibits anti-proliferative and apoptotic effects on MCF-7 breast cancer cells depending on concentration and incubation time. In another study of (Taing *et al.,* 2015) mentioned that the Mango cultivar "*Nam Doc Mai* "peel extract exhibited growth inhibitory effects by affecting the extracellular signal-regulated kinase (ERK) activity, and intracellular calcium (Ca^{2}) signalling in MCF-7 human breast cancer cells.

Another approach for belongs the anti-cancer therapeutic strategies, is to target some proteins of stress like Heat Shock Proteins 70 (Hsp70). This side also may give us a possible explanation for the mechanisms which are affected by *Phoenix dactylifera* (AZ and HM varieties).

In normal cells, under non stressed conditions, the expression of Hsp70 is usually very low. In contrast, high Hsp70 expression is a property of and essential for the survival of many cancers. The expression of Hsp70 is induced after many different stresses like after anti-cancer treatment. The protective function of Hsp70 is largely explained by its anti-apoptotic properties (Didelot *et al.,* 2007) and (Lanneau *et al.,* 2007). Hsp70 is a decisive negative regulator of the mitochondrial pathway of apoptosis that can block cell death at several levels: at a pre-mitochondrial stage by inhibiting stress inducing signalling, at the mitochondrial stage, by preventing mitochondrial membrane permeabilization through the blockage of Bax translocation; and finally, at the post‑mitochondrial level by interacting with apoptosis inducing factor (AIF) and apoptosis protease activating factor-1 (Apaf‑1) or by protecting essential nuclear proteins from caspase‑3 cleavage (Garrido *et al.,* 2006). (Joo *et al.,* 2005) found that the expressions of HSP70 and HSP27 play an important role in hepatocarcinogenesis, and especially HSP70 can contribute tumor progression by promoting hepatocarcinoma cell proliferation.

The last part of the study we had evaluated the cytotoxicity of the *Phoenix dactylifera* (AZ and HM varieties) on: normal human umbilical vein endothelial cells (HUVEC) and normal human hepatocytes **(**h NHEPS). The results obtained from this part of the study showed that the extracts of *Phoenix dactylifera* (AZ and HM varieties) have no toxicity on

normal cells and the influence of the two extracts on the normal cells viability could been explained by normal cells life time period. These results are compatible with those of (Lee *et al.,* 2015) who reported that *Nelumbo nucifera* leaf extracts have health-protecting effects as confirmed by their potent antioxidant effects on HUVEC cell line. Also (Atmaca and Bozkurt, 2015) who indicated that *Salvia triloba* extract inhibit the proliferation of prostate cancer cells but has no effect on human umbilical vein endothelial cells (HUVEC), and (Geisen *et al.,* 2015) who reported that *Fucus vesiculosus* extract inhibit the cell proliferation of Pancreatic Cancer Cells but has no effect on human umbilical vein endothelial cells (HUVEC) viability or proliferation. Also (Aghbali *et al.,* 2013), reported that grape seed extract (*Vitis vinifera*) has apoptotic potential by the inhibition of cell growth and viability in oral squamous cell carcinoma (KB cells) and without inducing damage to non-cancerous cell line HUVEC. In contrast to our results, in the study of (Ziaei *et al.,* 2015) the authors reported that the Treatment of HUVEC with teuclatriol from *salvia mirzayanii* showed adose-dependent decrease in cell viability.

However, our results were compatible with those of (Kuete, Sandjo *et al.,* 2013), (Kuete, Fankam *et al.,* 2013) and (Ikehara *et al.,* 2015) who reported that the treatment by cyanobacterial hepatotoxin microcystin-LR, the extarcts of *Echinopsgiganteus, Xylopiaaethiopica, Imperatacylindrica and Piper capense*, and other six Cameroonian medicinal plants had inhibitory effect on hepatocarcinoma cells but less effect on normal hepatocytes. Also (Schmidt *et al.,* 2005) reported that high concentrations of green tea extracts exhibit a low cytotoxicity in rat hepatocytes *in vitro*.

Conclusion and Perspectives

Conclusion and Perspectives

Our series of studies provide the evidence that Algerian dates fruit *Phoenix dactylifera* "Azarza and Homayra variety" are immunomodulatory agents and act immunostimulant activities in the reticuloendothelial system. The anti-inflammatory effect evaluated by the formalin test support the use of *Phoenix dactylifera* as analgesic drugs against pain, and inflammation, while the anti-arthritic study allow us to conclude the potential effect of this plant against chronic inflammation and edema. Considering the results obtained from the *in vitro* study we can conclude that the extracts used from *Phoenix dactylifera* "Azarza and Homayra varieties" have an anti-proliferative effect against two cancer cell lines (Breast cancer cells MCF7 and human hepatocarcinoma or liver cancer cell line HepG2) and no toxic effect on normal human cell lines (normal Human Umbilical Vein Endothelial Cells HUVEC and normal human hepatocytes h NHEPS).

Through the *in vivo* study of the anti-arthritic and anti-inflammatory effects of *Phoenix dactylifera* "Azarza and Homayra varieties", we are able to conclude that this plant possesses potential anti-arthritic and anti-inflammatory activities. On the basis of our findings and data from the literature we developed a model for describing the role of hyperhomocysteinemia in the alteration and aggravation of inflammation; this model involves formalin induced arthritis and methionine induced hyperhomocysteinemia in mice. In fact, our experiment consists of the administration of formalin and methionine in a high dose, its degradation product, homocysteine initiates an inflammatory process determined by the elevation of the plasma CRP, Anti-CCP antibodies and homocysteine levels, and confirmed by the increase of the inflammation process observed in paw thickness and histological study.

Based on the present results, our future work and perspectives can evaluate many topics:

- Purification of the bioactive molecules presented in the plant extracts.
- Biochemical analysis of our plant extracts.
- Evaluate the effect of *Phoenix dactylifera* extracts on gene expression and lymphocytes differentiation with much lower concentrations.
- The possible effect of Algerian date fruits *Phoenix dactylifera* "Azarza and Homayra varieties" on the action of pro-inflammatory cytokines such as IL-1, IL-6, TNFα and IL-17.
- Evaluation of *Phoenix dactylifera* "Azarza and Homayra varieties" on immune cells behavior like the balance between lymphocytes Th1/Th2 and Treg/Th17.
- Future studies need to characterize the role of *Phoenix dactylifera* "Azarza and Homayra varieties" on the regulation of some pro-apoptotic and anti-apoptotic proteins (Bax, Bcl2 and P53) and other proteins involved in the oxidative stress (Hsp27, HO1).
- Evaluate the effect of *Phoenix dactylifera* on other cancer types.
- Evaluation of *Phoenix dactylifera* "Azarza and Homayra varieties" on some enzymatic mechanisms involved in the pathogenesis of RA such as PAD and MMP.
- Use the *in ovo* technics to evaluate the effect of our plants on cardiovascular diseases.

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Appendices

Appendix I

I.1. PBS preparation

- ✓ Nacl: 0,8g
- ✓ Kcl: 0,2g
- ✓ Na2PO4: 1,15g
- ✓ KH2PO4: 0,2g
- ✓ Mgcl26H2O: 0,1g
- ✓ Cacl22H2O: 0,137g

QS: 1 liter of water

I.2. Treatment dose calculation

- ✓ Plant extracts given dose (100mg/kg)

0,1g ⟶ 1000g

X g Mouse ⟶ weight (g)

$$\textbf{Plant extract given dose} = \frac{\textbf{0,05g x Mouse weight (g)}}{\textbf{1000}}$$

I.3. Extraction table

Variety	Extraction solvent	variety FW (g)	extract weight (g)	% recovery	Extract used	Dilution 1:5 (W:V) Stock solution
A	A1	1,541	1,173	76,12	1 g	1g : 4 mL
	A2	3,031	2,48	81,82	1 g	1g : 4 mL
B	B1	1,537	1,208	78,59	1 g	1g : 4 mL
	B2	3,04	2,72	89,47	1 g	1g : 4 mL

A/B1 = Acetone

A/B2 = MeOH

The stock solution prepared with medium

I.4. Passage from T25 flask to T75 flask and from T75 to T75 flask

The passage of the cancer cells from a T25 flask to a T75 flask was made by a trypsinization protocol.

- ✓ First the medium was removed and the flask was washed with 2ml of DPBS (4ml for T75-T75).
- ✓ Then, the DPBS was removed and 1ml of the trypsin (1.5ml for T75-T75) was added to the cells and incubated in CO2 incubator for 5 min.
- ✓ After that, 3ml of the medium was added to the flask (4.5ml for T75-T75) and with a smooth pipetting the cells were separated and the mix was then transferred to a conic tube with 2ml of DPBS (3ml for T75-T75) used to wash the flask and then the solution was centrifuged for 5min/300g.
- ✓ After taking out the supernatant 4ml of the medium was added to the cells (by a smooth pipetting). At the end, 2 ml of the medium and cells solution was transferred to two T75 flasks filled with 7 ml of the medium each.

The calculation

The result after the cell counting was

	HepG2	MCF7
TOT	3.5 x 10^6 cells/1ml	2 x 10^6 cells/1ml
LIVE	1.9 x 10^6 cells/1ml	1.9 x 10^6 cells/1ml
DEAD	1.5 x 10^6 cells/1ml	7 x 10^4 cells/1ml
VIABILITY	56%	96%

	h NHEPS	HUVEC
TOT	4.6 x 10^6 cells/1ml	7.8 x 10^5 cells/1ml
LIVE	4.5 x 10^6 cells/1ml	6.3 x 10^5 cells/1ml
DEAD	9 x 10^4 cells/1ml	1.5 x 10^5 cells/1ml
VIABILITY	98%	81%

	LT CD4
TOT	1.1 x 10^5 cells/1ml
LIVE	6 x 10^4 cells/1ml
DEAD	5 x 10^4 cells/1ml
VIABILITY	52%

A cells/1ml ⟶ in 2ml of cell solution = B cells.

- For the MTT test we needed 5000 cells in 100µl for each well

B cells ⟶ 2000 µl of cells solution

5000 cells ⟶ X µl

$$X = \frac{5000 \times 2000}{B}$$

Meduim volum = 100 µl – X

EXP:

1.9 x 10^6 cells/1ml ⟶ in 2ml of cell solution = 3.8 x 10^6 cells.

3.8 x 10^6 cells ⟶ 2000 µl of cells solution

5000 cells ⟶ X

X= 2.63 µl

In each well for the MTT test: 2.63 µl of cell solution and 97.37 µl of medium = 100 µl (5000 cells/100µl).

- For gene expression we needed 60x10^3 cells in 500µl for each well.
- For the MTT test we needed 5000 cells in 100µl for each well.

I.5. Hepatocytes medium

- 500 ml of HBM Basal Medium.
- 10 ml BSA.

- 0.5 ml ascorbic acid.
- 0.5 ml Hydrocortisone.
- 0.5 ml human h EGF.
- 0.5 ml Transferrin.
- 0.5 ml insulin.

I.6. Lymphocytes medium

- 176 ml of RPMI 1640 red phenol free (SIGMA R 7509).
- 20 ml FBS.
- 2 ml Glutamine.
- 2 ml Antibiotics

I.7.Viability experiment

	CONCENTRATIONS												
	2µg/100µl		4µg/100µl		7.5µg/100µl		8µg/100µl		16µg/100µl		20µg/100µl		Stock solution used
	stk	med	stk	med	stk	med	stk	med	stk	med	stk	med	
	10 µl	90 µl	20 µl	80 µl	37,5 µl	62,5 µl	40 µl	60 µl	80 µl	20 µl	100 µl	0	
VOL Triplicate (X3) two cell lines (X2)	60 µl		120 µl		225 µl		240 µl		480 µl		600 µl		1725 µl

I.8.Gene expression experimen

	CONCENTRATIONS						
	4µg/100µl		7.5µg/100µl		16µg/100µl		Stock solution used
	STK	MED	STK	MED	STK	MED	
	100µl	400µl	187.5µl	312.5µl	400µl	100µl	
VOL Triplicate (X3)	300µl		526.5µl		1200µl		2062.5 µl

I.9. Protocol for Cryoconservation

The medium of the cryoconservation is made of: 50% meduim (used in the cell culture), 40% FBS and 10% DMSO.

- First the medium was removed and the flask was washed with 2ml of DPBS
- Then, the DPBS was removed and 1.5ml of the trypsin was added to the cells and incubated in CO2 incubator for 5 min.
- After that, 4.5ml of the medium was added to the flask and with a smooth pipetting the cells were separated and the mix was then transferred to a conic tube with 3ml of DPBS used to wash the flask and then the solution was centrifuged for 5min/300g.
- After removing the supernatant, the cells were re-suspended in the cryoconservation medium.
- The cells in 1 T75 flask were conserved in 3 cryotubes of 1ml. At the end of the experiment we had 4 T75 flasks of MCF7 cells and 2 T75 flasks of HepG2 cells = 18 cryotubes = 18 ml of cryoconservation medium.

Medium + DMSO = 50% + 10% = 9ml + 1.8 ml = 10.8 ml.

- The cells in each flask were re-suspended in 1.2 ml of FBS then400 µl of that solution was transferred in 1 cryotube with 600µl from the 10.8 ml of the medium + DMSO solution.

The primers used were from Eurofin MWG Operon in the genes expression experiment:

Beta Act Forward (Beta Act F): 5’ GCATGGGTCAGAAGG 3’

Beta Act Reverse (Beta Act R): 5’ AGGCGTACAGGGATA 3’

Bcl2 Forward (Bcl2 F): 5’ GCCTCATGAAATAAAGAT 3’

Bcl2 Reverse (Bcl2 R): 5’ TGGATGTACTTCATC 3’

BAX Forward (BAX F): 5’ GCTTCAGGGTTTCATC 3’

BAX Reverse (BAX R): 5’ CCTTGAGCACCAGTTT 3’

The 3 phases of PCR

	Temperature °C	Time	Cycle
Denaturation	95	30”	1
PCR	95	5”	40
	50	10”	
	72	12”	
	82	1’	
Melting	95		1
	45		
	95		

Table 14: Comparison between the effects of the two *Phoenix dactylifera* varieties extracts on RNA quantities ANEX

Samples	RNA (µg/µl)
A1.1 (4µg/100µl)	0,37 ±0,24
A1.2 (8µg/100µl)	0,40 ±0,16
A1.3 (16µg/100µl)	0,28 ±0,19
A2.1 (4µg/100µl)	0,282 ±0,16
A2.2 (8µg/100µl)	0,4 ±0,31
A2.3 (16µg/100µl)	0,34 ±0,40
B1.1 (4µg/100µl)	0,44 ±0,28
B1.2 (8µg/100µl)	0,575 ±0,1
B1.3 (16µg/100µl)	0,378 ±0,51
B2.1 (4µg/100µl)	0,224 ±0,20
B2.2 (8µg/100µl)	0,469 ±0,33
B2.3 (16µg/100µl)	0,148 ±0,05
CTR	2,657 ±0,62

Paper I

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Original Article

IMMUNOSTIMULATORY ACTIVITY OF PHOENIX DACTYLIFERA

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ABSTRACT

Objective: The aim of our study was to evaluate *in vivo* the immunostimulatory properties of *Phoenix dactylifera* "AZARZA variety".

Methods: The immunostimulant potential of the plant extract of Phoenix *dactylifera* on the phagocytic activity was measured by the carbon clearance rate test. The anti-oxidant activity was measured by spectrophotometric determination of glutathione from liver's homogenate.

Results: Our results obtained in this study shown that the phagocytic and the anti-oxidant activities was increased significantly in animals injected with *Phoenix dactylifera* "AZARZA" extract at doses (30,50 and 100mg/kg) P<0,05. The clearance rate of carbon was significantly faster at the concentration of 50 mg/kg when is compared to the two concentrations 30 and 100mg/kg (P= 0,004) and the release of the GSH from the liver was significantly higher at the concentration of 50 mg/kg when is compared to the two concentrations 30 and 100mg/kg (P= 0,003).

Conclusion: The *Phoenix dactylifera* extract revealed an immune-stimulatory effect on the reticuloendothelial system and anti-oxidant activity with higher effect by the administration of 50 mg/kg.

Keywords: Phoenix dactylifera, Immunostimulatory activity, Carbon clearance rate, Glutathione.

INTRODUCTION

The term immunostimulation comprises a prophylactic or therapeutic concept which aims at the stimulation of our non-specific immune system. This implies primarily the non-antigen dependent stimulation of the function and efficiency of granulocytes, macrophages, complement and natural killer cells. In contrast to immunity achieved by immunization or antibody injection, this type of immunity, arising from unspecific immunostimulation, is termed paramunity and the agents responsible are known as paramunity inducers. It is characteristic for these agents that they do not affect immunological memory cells [1]. Immunostimulation is also indicated to counteract immunosuppression and ineffectively working immune system, manifesting itself for example by a reduced resistance against infectious diseases, which may be the consequences of serious infections, physical and psychological stress, alcoholism, environmental damages such as pesticides, excessively applied chemotherapy, or long term treatment with immunosuppressive drugs [1].

Herbal drugs are known to possess Immunomodulatory properties and generally act by stimulating both specific as well as non-specific immunity. Immunomodulatory agents are used to either suppress or stimulate the immune responsiveness of an organism against the invading antigens [2].

Immunostimulatory therapy is now being recognised as an alternative to conventional chemotherapy for a variety of disease conditions, involving the impaired immuno-response of the host [3].

Glutathione (L-g-glutamyl-L-cysteinylglycine) is the principal non protein thiol involved in the antioxidant cellular defense. It is a tripeptide composed of cysteine, glutamic acid and glycine, and its active group is represented by the thiol (–SH) of cysteine residue. Glutathione is a ubiquitous molecule that is produced in all organs, especially in the liver [4].

Glutathione reduced (GSH) plays an important role in many biological processes such as intracellular reduction–oxidation metabolic cycles, transportation, protein synthesis, catabolism, and metabolism [5].

The *Phoenix dactylifera* is a monocotyledonous woody perennial belonging to the Arecaceae family, which comprises 200 genera and 3000 species. The beneficial health and nutrition values of date palm, for human and animal consumption, have been claimed for centuries [6].

Algeria is the sixth important countries in date world production. During 2007, 468000 metric tons were produced in Algeria. The Algerian dates represented about 7.28% of the total world production as reported by FAO in 2009 [6].

Fruits of the date palm (*Phoenix dactylifera* Fruits) are commonly consumed in many parts of the world especially the Arabian countries. Date fruit are used as nutrient while the pollen grains used in the treatment of infertility [7]. Traditional medicines are gaining importance and nowadays are being studied to find the scientific basis of their therapeutic actions. The use of herbal medicine has become increasingly popular worldwide especially in the Asian and African countries. The various parts of *Phoenix dactylifera* widely are used in traditional medicine for the treatment of various disorders which include memory disturbances, fever, and inflammation [8].

MATERIALS AND METHODS

Plant material

Collection

The jam was prepared from the date palm (*Phoenix dactylifera* AZARZA variety) which was collected from Ghardaïa (Algerian septentrional Sahara).

Preparation of the extract

The jam concentrations of 30, 50 and 100 mg/kg were diluted into 10 ml of Nacl (0,9%).

Animals

Adult male Mus Musculus mice (2-2.5 month old) were procured from central pharmacy Algeria. The animal experiments weighing (20–33 g) were used for determination of the phagocytic activity. The animals were kept in polyacrylic cages and maintained under standard housing conditions (room temperature 24-7 with 12:12 light: dark cycles). Food was provided in the form of dry pellets (SARL Production Locale, Bouzaréah. Algeria) and water adlibitum. The animal studies were conducted after obtaining clearance from Institutional

Animal Ethics Committee and the experiments were conducted in strict compliance according to ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Phagocytic activity

Phagocytic activity of reticuloendothelial systems (RES) was assayed by carbon clearance test. Phagocytic index was calculated as a rate of carbon elimination of reticuloendothelial systems by carbon clearance test determined by a reported method (Biozzi et al.,1955). Animals were divided into four groups, GI, GII, GIII and GIV. Group I (Control) was given by i.p injection 0.9% NaCl (0.5 ml/mouse.), groups II, III and IV were administered with different concentrations of the Phoenix dactylifera extract (30, 50 and 100 mg/kg) respectively.

After 48 h of i.p injection, Carbon ink suspension was injected via the tail vein to each mouse at a dose of 0.1 ml/10g, the mixture consisted of black carbon ink 3 ml, saline 4 ml and 3% gelatin solution 4 ml. Blood samples (≈14 drops or 25µl) were then withdrawn from the retro-orbital plexus at 5 and 15 minutes after injection of colloidal carbon ink via an heparin glass capillaries and lysed in 0.1% sodium carbonate solution (4ml). The optical density was measured spectrophotometrically at 676nm.

The phagocytic activity is expressed by the phagocytic index **K** which measures all the reticuloendothelial system function in the contact with the circulating blood and by corrected phagocytic index **α** which expresses this activity by unit of active weight organs: liver and spleen. The clearance rate is expressed as the half-life period of the carbon in the blood (t1/2, min) [9].These parameters are calculated using the following formulas:

$$K = \frac{\log OD\ 1 - \log OD\ 2}{t2 - t1}$$

$$t\frac{1}{2} = \frac{0.693}{K}$$

$$\alpha = \sqrt[3]{K} \times \frac{\text{Body weight of animal}}{\text{Liver wt} + \text{spleen wt}}$$

OD1 and OD2 are the optical densities at time t1 and t2, respectively.

Glutathione assay (GSH)

The animals were sacrificed and the liver and spleen dissected and weighted immediately in the wet state**.**

Preparation of the homogenate

The weight of 0,5g of the liver was homogenized in 2ml of TBS (Tris 50 mM, NaCl 150 mM, pH 7.4). Then the homogenates were centrifuged at 9000 g for15 min at 4°C after that the supernatant was used for determination of glutathione reduced (GSH).

Method

The glutathione reduced content in the liver was measured spectrophotometrically by using 5,5′-dithiobis-(2 nitrobenzoic acid) (DTNB) as a coloring reagent, following the method of Weckbekeret al.,1988 [10].

Statistical Analysis

Results were analyzed for differences between the groups across dietary treatments by one –way ANOVA test and Tukey's multiple comparison tests (SPSS version 9).The values of,

P<0,001, P< 0,01,P< 0,05 were considered to indicate the significant levels.

RESULTS

The present data showed that there is a significant difference in the means for the phagocytic index (K) between groups (NaCl, 30 mg, 50 mg and 100 mg) P= 0,003 and the group 50 mg has the Highest significantly difference from groups (NaCl, 30 mg and 100 mg) at P=0,002.This indicates that *Phoenix dactylifera* enhanced the phagocytic activity by stimulating the reticuloendothelial system (Figure 1).

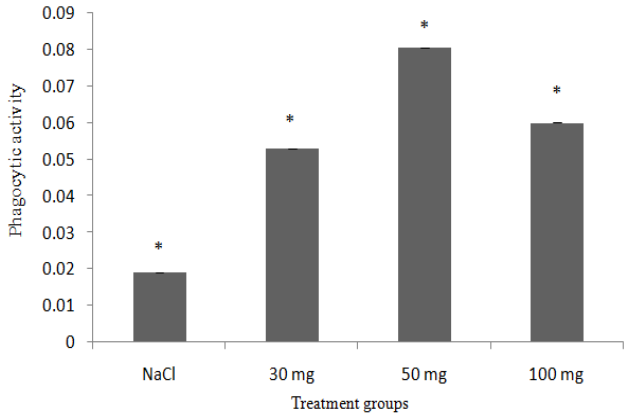

Fig. 1: It shows the effect of Phoenix dactylifera extract on phagocytic activity.

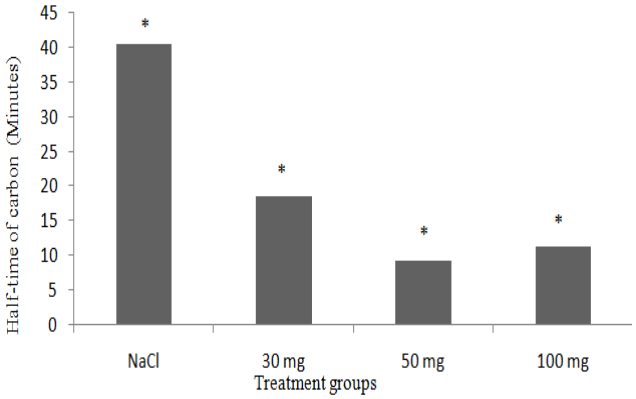

Fig. 2: It shows the effect of *Phoenix dactylifera* extract on half time t1/2 of carbon in blood.

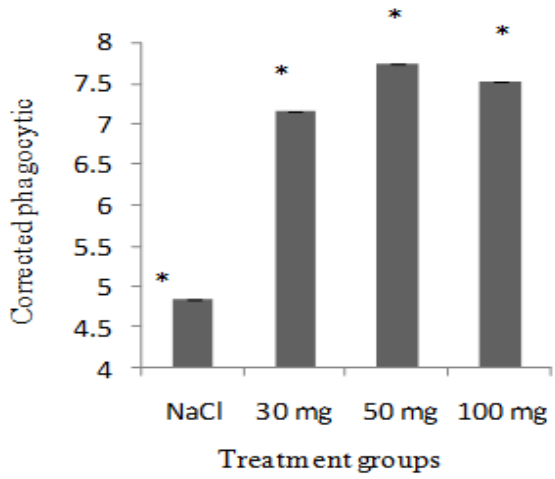

Fig. 3: It shows the Effect of *Phoenix dactylifera* extract on corrected phagocytic index α.

As shown in the figure 2, the half time of colloidal carbon was decreased significantly between groups P= 0,003 however at the concentration of 50mg/kg was faster when it is compared to the other groups P= 0,004.

The results of this study showed that there is a significant difference in the means for the corrected phagocytic index α between groups (NaCl, 30 mg, 50 mg and 100 mg) P= 0,004 and the corrected phagocytic index α was increased significantly in groups (30 mg, 50 mg and 100 mg) when it is compared to the control group (NaCl) P<0,05 but at the concentration of 50mg /kg the corrected phagocytic index α was higher than the other groups P= 0,006 (Figure 3).

The last part of this study showed that there is a significant difference in the means for the Glutathione values between groups (NaCl, 30 mg, 50 mg and 100 mg) P= 0,002 and the Glutathione values was decreased highly and significantly in groups (30 mg, 50 mg, and 100 mg) when it is compared to the control group (NaCl) P<0,05 however the glutathione reduced was lower than the other groups P= 0,003 (figure 4). This indicates that the extract liberates the glutathione particles from liver and affirms that *Phoenix dactylifera* enhanced the anti-oxidant activity.

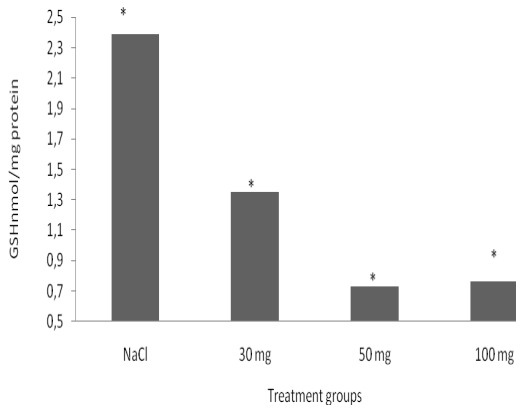

Fig. 4: It shows the effect of *Phoenix dactylifera* on Glutathione reduced values.

DISCUSSION

Today, the use (which we can say 'return to nature') of traditional herbal medicines, herbal health products, pharmaceuticals food supplement, cosmetics, etc. is increasing due to the growing recognition that natural products are safe, have either no or negotiable side effects[11].

Some of these plant products are believed to enhance the natural resistance of the body to infection, on the basis of their constituents like polysaccharides, lectins, saponins and flavonoids etc. Some of these stimulate both 'humoral and cell mediated immunity', while others activate only the cellular components of the immune system [12]. Immunostimulatory molecules intensify and modify the lymphocyte mediated immune response and its duration. Such molecules can, therefore, be potentially applied as adjuvants in vaccines and allergy preparations [13].

The activity was investigated by phagocytic carbon clearance by the phagocytic function of the reticuloendothelial system which is known to be important in the removal and destruction of pathogenic organisms from the tissues and blood [14].

Glutathione is a major antioxidant and a vital component of host defenses. In addition to protecting against free radical injury, it is important in the activation of lymphocytes, critical for the function of natural killer cells and lymphocyte-mediated cytotoxicity, and may have a role in the protection of neutrophils and macrophages against oxidative damage [15].

From ages dates are consumed by humans for its beneficial health and nutritional values [16].

In this study we observed that the animals administered with the extract of *Phoenix dactylifera* stimulates the phagocytic index at different concentration. So, this result agrees with those of Gokani *et al*. [17] and Aribi *et al* [18] who reported that the administration of extraction of *Clerodendrum phlomidis* and *Premna integrifolia* roots and *Argania spinosa* respectively in the mouse are increased the phagocytic index at different concentrations.

Treatment by the extract of *Phoenix dactylifera* enhanced the rate of carbon clearance from the blood when it is compared to the control group. Cells of the reticuloendothelial systems play important role in the clearance of particles from the blood stream. When colloidal carbon particles in the form of ink are injected directly into the systemic circulation, the rate of clearance of carbon from the blood by macrophage is increased during the treatment of rats by the methanolic extract of *Morus Alba* Linn (Mulberry) leaves. [19]. Also the jam reduces the glutathione particles from liver and affirms that Phoenix dactylifera enhanced the glutathione reduced concentration and anti-oxidant activity. This result agrees with those of Hasnaoui *et al* [20].

CONCLUSION

In vivo investigations showed that the jam of *Phoenix dactylifera* at concentration of 50mg/kg increased the phagocytic index, corrected phagocytic index α and decreased the half time of carbon and the concentration of the glutathione reduced. This Immunomodulatory effect of *Phoenix dactylifera* could be attributed to its interesting chemical composition. It is essentially characterized by the presence of unsaturated fatty acids, antioxidant compounds (Vitamin E-C family) and phenolic compounds [21].

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Paper II

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Anti-inflammatory effect of Algerian date fruit (Phoenix dactylifera)

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Anti-inflammatory effect of Algerian date fruit (*Phoenix dactylifera*)

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ABSTRACT

From ages, dates are consumed by humans for its beneficial health and nutritional values. The aim of this study was to evaluate anti-inflammatory effect of Algerian date fruit (*Phoenix dactylifera*). A quantity of 50 mg/kg of the *P. dactylifera* extract was analyzed *in vivo* using the formalin-induced paw edema test in mice. The inflammation level was detected by the measure of the edema size, also the dosage of C-reactive protein (CRP) and homocysteine levels in the blood. The results showed a highly significant decrease in the edema size and the level of the homocysteine in the blood ($P = .000$) and significant decrease in CRP values ($P < 0.05$) in the 50 mg/kg plant extract group compared to Control, Methionine 400 mg/kg and (Plant + Methionine) groups. The study suggests that the extract possesses enough potential to reduce inflammation by *in vivo* and directs the importance of further research and development of novel anti-inflammatory agents.

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KEYWORDS
Anti-inflammatory; formalin; paw edema; *Phoenix dactylifera*; CRP; homocysteine

1. Introduction

Inflammation plays an important role in various diseases, such as rheumatoid arthritis (RA), atherosclerosis and asthma, which all show a high prevalence globally (Mueller, Hobiger, & Jungbauer, 2010). Inflammation is the protective mechanism of the local microcirculation to tissue injury, which is caused by physical trauma, noxious stimuli by chemical agents, heat, antigen–antibody reaction and microbial effect. It is a body defense reaction in order to eliminate or limit the spread of injurious agents (Soni et al., 2014).

The clinically useful drugs against pain and inflammation exhibit many adverse effects; this leads to considerable interest in search of safer drug for these conditions (Prabhu, Nalini, Chidambaranathan, & Kisan, 2011).

C-reactive protein (CRP), a plasma protein synthesized by the liver, is a sensitive and dynamic systemic marker of inflammation. Its concentration in the circulation can increase during acute responses to serious infection or major tissue damage (The Emerging Risk Factors Collaboration et al., 2010). CRP is a pattern recognition molecule,

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binding to specific molecular configurations that are typically exposed during cell death or found on the surfaces of pathogens. Its rapid increase in synthesis within hours after tissue injury or infection suggests that it contributes to host defense and that it is part of the innate immune response (Black, Kushner, & Samols, 2004). In the inflamed tissues, CRP directly activates immune cells with the secretion of other inflammatory molecules, by initiating a vicious circle that maintains and increases the inflammatory state. Also, the inflammatory disorders which are characterized by high levels of CRP can develop a secondary immune cell activation, which may result in the increase of atherogenesis. Therefore, the chronic increased CRP serum levels in RA patients can directly induce an acceleration of atherosclerosis and its complications. Numerous prospective epidemiological studies showed that in healthy subjects, serum CRP predicts myocardial infarction mortality, stroke and arrhythmias, including sudden cardiac death in diabetes mellitus and hypertension (Dehghan et al., 2011). A meta-analysis of 14 prospective long-term studies (Montecucco & Mach, 2009) showed that after correction for age, smoking and other cardiovascular risk factors, CRP was strongly related to coronary heart disease. These studies show that CRP should be considered a direct pro-inflammatory factor in the pathogenesis of inflammatory diseases such as RA (Montecucco & Mach, 2009).

Homocysteine (Hcy), a sulfur-containing amino acid, is a metabolite of the essential amino acid l-methionine (Miller, 2003). An association of hyperhomocysteinemia (abnormally high blood levels of Hcy) with inflammation is supported by the fact that the level of circulating Hcy can be effectively reduced by the administration of anti-inflammatory medications. Several anti-inflammatory compounds such as resveratrol, aspirin, salicylic acid and atorvastatin have all been shown to downregulate the release of Hcy from stimulated human peripheral blood mononuclear cells (Wu, 2007). Because of this association, it is not surprising that varying degrees of hyperhomocysteinemia are detectable in all inflammatory diseases. In fact, detection of hyperhomocysteinemia has been reported in patients with well-known inflammatory diseases such as RA (Provenzano, Termini, Le Moli, & Rinaldi, 2003; Roubenoff et al., 1997; Wu, 2007), inflammatory bowel disease and also psoriasis (Wu, 2007).

Since ancient times, in various cultures worldwide, inflammatory disorders and related diseases have been treated with plants or plant-derived formulations (Mueller et al., 2010). Medicinal plant is defined as any substance with one or more of its organs containing properties that can be used for therapeutic purposes or which can be used as precursors for the synthesis of various drugs (Uma, Balasubramaniam, & Kumar, 2014).

Phoenix dactylifera (a monocotyledonous woody perennial), commonly known as date, is the only species of the genus *Phoenix* which is cultivated for its fruit. Dates are produced in 35 countries worldwide and cultivated on about 2.9 million acres of land. There are over 23.5 million numbers of trees and are about 450 cultivars of date palms found in Saudi Arabia (Bhat & Al-Daihan, 2012). Algeria is considered as one of the major date-producing countries. The annual production reaches up to 468,000 tons; most of it is consumed locally (Boukouada & Yousfi, 2009). Since ages, dates are consumed by humans for its beneficial health and nutritional values. Dates are very rich in vitamin A, B and C and dietary fibers. The antioxidants present in dates can also aid in lowering the risk of cancer and cardiovascular conditions and also improve immune system. Almost all the parts of this plant are used as traditional medicine for the treatment of inflammation, fever, paralysis, nervous disorders (Bhat & Al-Daihan, 2012).

The aim of this study was to evaluate anti-inflammatory effects of oral administration of the Algerian date fruit (*P. dactylifera*) to validate its traditional use on arthritis.

2. Methods

2.1. Plant material

2.1.1. Collection

The jam was prepared from the date palm (*P. dactylifera*) which was collected from Azarza, Ghardaïa (Algerian septentrional Sahara) and 50 mg was used (Kehili, Zerizer, & Kabouche, 2014).

2.2. Animals

Adult male Albinos mice (2–2.5 months old) weighing (20–33 g) were procured from central pharmacy in Constantine, Algeria and used for the *in vivo* experiment. The animals were kept in polyacrylic cages and maintained under standard housing conditions (room temperature 24–7 with 12:12 light:dark cycles) and water ad libitum. Food was provided from dry pellets (SARL Production Locale, Bouzaréah, Algeria). The animal studies were conducted after obtaining clearance from Institutional Animal Ethics Committee, and the experiments were conducted in strict compliance according to ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Animals were divided into four groups of five mice each: "F" (control group) was given flour balls orally and in three treated groups "P" was given the plant extract, "M" was given l-l-methionine 400 mg/kg (Boudebouz, 2013; Sakhri, 2014) and "MP" was given both plant extract and l-l-methionine. The administration was done orally by mixing the plant extract and the l-l-methionine of the treated groups into a flour balls.

2.2. Formalin test in mice

A volume of 100 µl of formalin (0.2%) was injected into the sub-plantar of the right hind paw and the edema size was measured by a digital caliper in the moment of the injection and after every 1 hour for 5 hours (Uma et al., 2014).

After the 5th hour, blood was withdrawn from the retro-orbital plexus for the hs-HS-CRP and Hcy dosage.

2.3. Statistical analysis

Results were analyzed for differences between the groups across dietary treatments by one-way ANOVA test and Tukey's multiple comparison tests (SPSS version 9). The values of $^{***}P < 0.001$, $^{**}P < 0.01$ and $^{*}P < 0.05$ were considered to indicate the significance levels.

3. Results

The results showed a highly significant decrease in the edema size ($P = .000$) in groups P (*P. dactylifera*) and MP with the best result in group P and a highly significant increase in the edema size ($P = .000$) in group M compared to the control group F (Figure 1).

As shown in Figure 2, there was a highly significant decrease in the edema size between groups ($P = .000$) during the 5 hours of the experiment. Also, a highly significant decrease in the P was observed (*P. dactylifera* and MP group's edema size $P = .000$) after 1 hour from induction of inflammation until it reached the lowest value in the 5th hour with a very close result between the two groups treated with (*P. dactylifera* T1: $P = 2.682 \pm 0.36$ mm, MP = 3.944 ± 0.34 mm) (T5: $P = 1.837 \pm 0.23$ mm, MP = 2.15 ± 0.052 mm) compared to the control (F). Also, a highly significant increase in the l-methionine (M) group edema size ($P = .000$) was observed from the 1st hour (T1 = 4.57 ± 0.51 mm, T5 = 6.767 ± 0.353 mm) compared to the control (F).

The last part of this study showed that there is a significant decrease in the means for the CRP values in group P (*P. dactylifera*) (0.452 ± 0.546 mg/l) and in group MP (0.84 ± 0.322 mg/l) ($P < .05$) when compared to the group treated by l-methionine M (1.76 ± 0.868 mg/l) and the control group F (1.31 ± 0.857 mg/l; Figure 3). Also, a highly significant decrease in the means for the Hcy values in group P (*P. dactylifera*) (7.122 ± 2.34 µmol/l) and group MP (6.124 ± 3.94 µmol/l) ($P = .000$) when compared to the group treated by l-methionine M (22.344 ± 3.27 µmol/l) and the control group F (7.85 ± 0.87 µmol/l) as shown in Figure 4.

4. Discussion

The present study establishes the anti-inflammatory activity of the Algerian date fruit (*P. dactylifera*) extract. Inflammation is associated with the pathophysiology of various

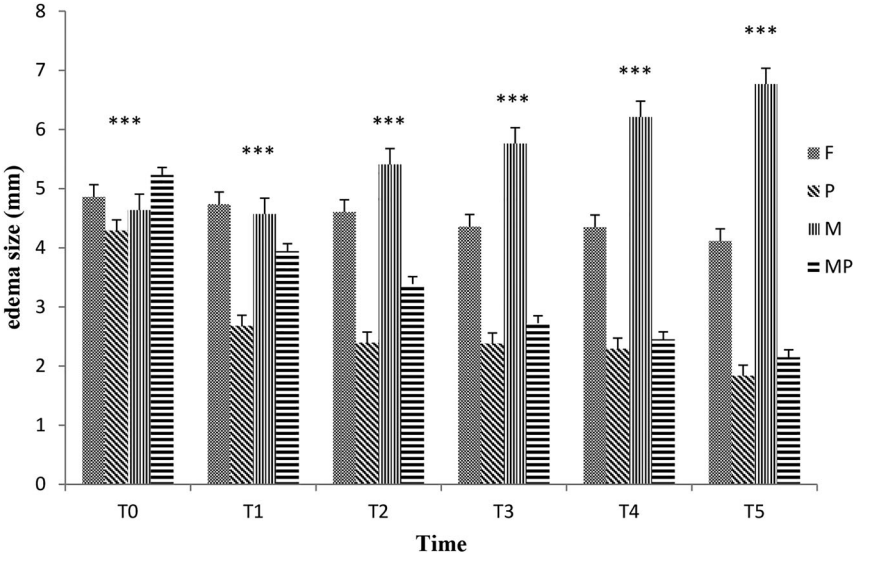

Figure 1. Comparison of paw edema size between different groups in each hour.

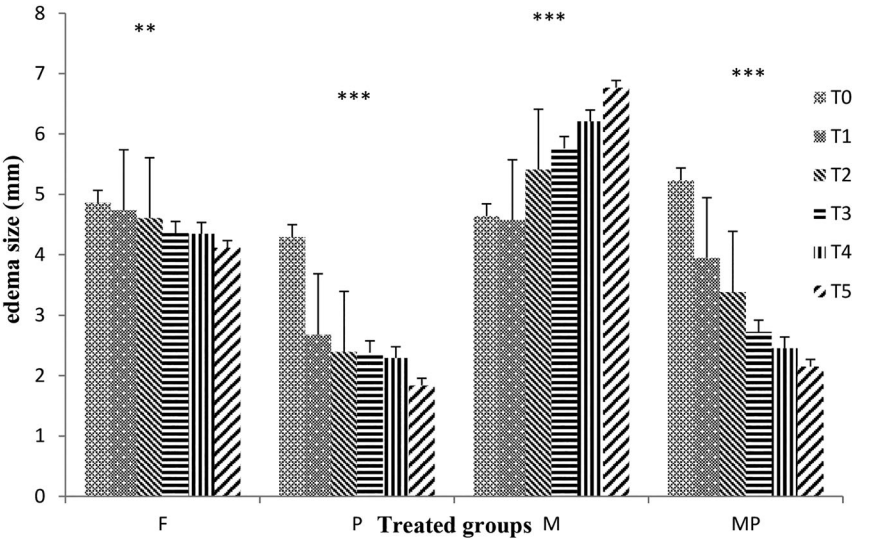

Figure 2. Comparison of paw edema size between different hours in each group.

clinical conditions such as arthritis, cancer and vascular diseases. Inflammatory reactions are not only the response of living tissues to injury and infection, but also are relevant to disease developments, such as asthma, multiple sclerosis and inflammatory diseases.

Inflammation is a pathophysiological response of living tissue to injuries that leads to the local accumulation of plasmatic fluid and blood cells. Although it is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases. However, studies have been continuing on inflammatory disease and the side effects of the currently available anti-inflammatory drugs pose a major problem during their clinical use. Therefore, development of newer and more anti-inflammatory drugs with lesser side effects is necessary (Vasudevan, Gunnam, & Parle, 2006).

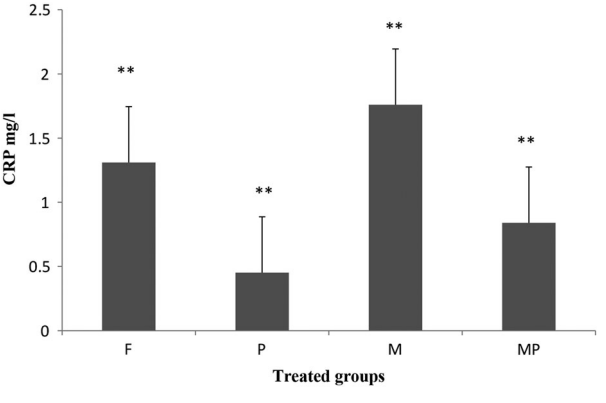

Figure 3. The effect of *P. dactylifera* extract on the CRP values.

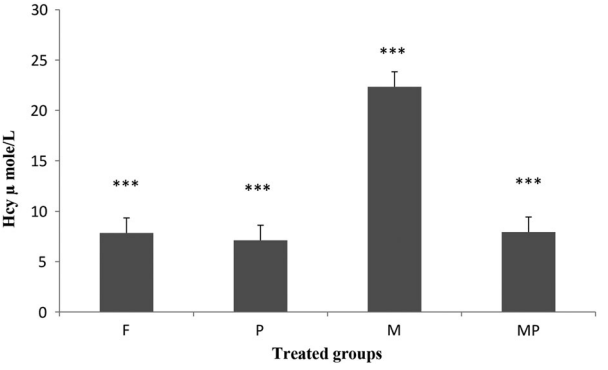

Figure 4. The effect of *P. dactylifera* extract on the Hcy values.

The formalin test provides a moderate and continuous pain because of tissue injury in the animal, which is a better approach to clinical conditions than more traditional tests of nociception (Khanavi, Davoodipoor, Sadati, Shams Ardekani, & Sharifzadeh, 2014). Formalin-induced inflammation involves three distinct phases based on the release of different inflammatory mediators, namely serotonin and histamine in the first phase (0–2 h), kinins like bradykinin in second phase (3 h) and prostaglandins in the third phase (>4 h) (Viswanatha et al., 2011).

By comparing the results of different groups treated with formalin induces edema over time, with the group F (control), the volume of edema began to decrease gradually over time as the immune system naturally degraded the rate of inflammation. However, in group P, the *P. dactylifera* had accelerated the anti-inflammatory activity and this is remarkable by lower volume of edema and the CRP values. *P. dactylifera*, which has antioxidant propriety according to Kehili et al. (2014) and the presence of some active substances such as alkaloids, tannins, flavonoids, terpenes and sugars (DaasAmiour, 2009; Hasnaoui, Elhoumaizi, Borchani, Attia, & Besbes, 2012) could be responsible for the anti-inflammatory and analgesic effects. Flavonoids have been shown to possess various biological properties related to antioxidant, anti-nociceptive and anti-inflammatory mechanisms by targeting reactive oxygen species and prostaglandins which are involved in the late phase of acute inflammation and pain perception (Agnel, John, & Shobana, 2012; Nagore, Ghosh, Patil, & Wahile, 2010).

While in group M, the inflammation increased the volume of edema gradually and the CRP values because the immune system became unable to fight this inflammation due to the presence of inflammation. This is even more remarkable than in Group F. In the last group MP, edema size and the CRP values decreased compared to group M, which confirms the potential effect of the *P. dactylifera* extract on the inflammation.

In this study, the result showed that *P. dactylifera* extracts significantly decreased the CRP levels compared to the control group. The possible explanation is that *P. dactylifera* extract enhanced also the inflammation development by inhibiting the filtration of immune cells to the inflammation site. It is known that inflammation is initiated as a vascular response followed by leukocytes filtration (Thomson et al., 1999) which secretes the majority of the inflammation mediators like tumor necrosis factor-a (TNF-a), plasminogen activator inhibitor-1 (PAI-1), interleukin (IL)-1b, IL-6, IL-8, IL-10, IL-15,

complement factors and prostaglandin E2 (PGE2). These mediators stimulate the secretion of the acute-phase protein, C-reactive protein (CRP), by the liver (the presence of CRP in plasma is recognized as a marker for low-grade systemic inflammation; Othman & Moghadasian, 2011). The anti-inflammatory effect of *P. dactylifera* extracts may return to its chemical composition. Plant sterols have been shown to reduce plasma levels of CRP, IL-6, TNF-a, phospholipase A1 and fibrinogen (Bouic et al., 1999; Devaraj, Autret, & Jialal, 2006; Nashed, Yeganeh, HayGlass, & Moghadasian, 2005). These effects may be mediated through beneficial alterations in the membrane composition, affecting membrane fluidity, sensitivity and signaling pathways. All of these changes may subsequently alter the immune response by influencing the synthesis and secretion of eicosanoids, leukotrienes and prostaglandins (Othman & Moghadasian, 2011).

Hyperhomocysteinemia has been known to exert its detrimental effects through induction of the acute and chronic inflammation pathway, such as endothelial dysfunction, leukocyte adhesion and oxidative stress (Wu, 2007). In this study, the use of 400 mg/kg of l-methioninein mice leads to a hyperhomocysteinemia according to Sakhri (2014) and Boudebouz (2013) who reported that the administration of 400 mg/kg of l-methioninein mice induces hyperhomocysteinemia.

The results of the Hcy quantification showed that there was a significant decrease in the Hcy in the plasma of the treated group with the *P. dactylifera* extracts (Azarza variety) compared to the other groups. These results are compatible with those of Shaker, Manaa, Mubarak, and Abd-El Hady (2013). The authors in this study reported that avocado showed a better decrease in plasma Hcy compared to tomato and broccoli extracts. Also, Nolla et al. (2009) reported that red wine polyphenolic extract administration in low quantities to cystathionine-β-synthase (CBS)-deficient mice is able to block the rise of plasma Hcy levels and has beneficial effects on biochemical markers of hepatic and endothelial dysfunction due to hyperhomocysteinemia. Malinowska, Babicz, Olas, Stochmal, and Oleszek (2012) and Olas et al. (2010) showed that in the presence of the extract of *Aronia melanocarpa*, changes in the amount of thiols, including Hcy, in plasma from patients with invasive breast cancer and patients with benign breast diseases were significantly decreased. Also in Esfahani et al. (2011), the authors reported that daily consumption of mixed fruits and vegetable supplements significantly increases serum levels of antioxidant and reduces Hcy and markers of oxidative stress.

The potential effect of *P. dactylifera* extracts may be explained by the influence of the plant extract on the enzymatic mechanisms of the regulation of Hcy concentration in the organism. It is clear that tissue concentration of Hcy is maintained at low levels by regulated production and efficient removal of this thiol (Stipanuk, 2004). In most tissues, Hcy itself is located at a branch-point of metabolic pathways: either it is irreversibly degraded via the trans-sulphuration pathway to cysteine or it is remethylated back to l-methionine exported out of the cell. The liver is the main organ of degradation of excess l-methionine and in maintaining Hcy at adequate levels via an unique set of enzymes, including methionine-adenosyltransferase I/III, CBS, cystathionine γ-lyase, betaine homocysteine methyltransferase and glycine N-methyltransferase (Blom & Smulders, 2011). Also another explanation may come from the previous activities and from the chemical composition of the plant extract. *P. dactylifera*, which was demonstrated previously, has an antioxidant propriety. In addition to its containment of some active substances such as alkaloids, tannins, flavonoids, terpenes and sugars, which could be responsible for the

anti-inflammatory and analgesic effects (DaasAmiour, 2009; Wu, 2007). Also flavonoids have been shown to possess various biological properties related to antioxidant, anti-nociceptive and anti-inflammatory mechanisms by targeting reactive oxygen species and prostaglandins, which are involved in the late phase of acute inflammation and pain perception (Agnel et al., 2012; Nagore et al., 2010).

5. Conclusion

The *P. dactylifera* extract possesses anti-inflammatory activity, which may be due to the presence of flavonoids and other polyphenol compounds (Al-Farsi et al., 2007; Mansouri, Embarek, Kokkalou, & Kefalas, 2005) present in it which seems to support the use of this plant in traditional medicine.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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الملخص بالعربية

ملخص بالعربية

مقدمة

إن ظاهرة الالتهاب تعمل عموما على حماية الجسم ضد الجراثيم, بينما التنظيم غير الطبيعي لهذه الظاهرة قد يؤدي الى تهديم الخلايا أو اضطراب في الميتابولزم الخلوي الذي يؤدي عموما الى أمراض مزمنة. عادة ظاهرة الالتهاب تكون قصيرة المدة وذلك يعود إلى بعض الخلايا المناعية والوسائط الالتهابية المتميزة بنصف عمر حيوي قصير مع تأخير في إنتاج السيتوكينات المضادة للالتهاب, ويطلق على هذا النوع من الالتهاب "الالتهاب الحاد". ومن جهة أخرى يوجد نوع آخر من الالتهاب الذي يستمر لفترة طويلة يطلق عليه "الالتهاب المزمن".

والاتهاب المزمن هو الظاهرة التي تؤدي إلى حدوث أمراض مزمنة مثل تصلب الشرايين , أمراض الأوعية القلبية , أمراض المناعة الذاتية مثل التهاب المفاصل الروماتيزم , والسرطان (Park وآخرون , 2013) .

إن التهاب المفاصل عبارة عن مرض مناعي ذاتي يصيب عدد من المفاصل في الجسم, يتميز بواسطة تهديم المفصل تدريجيا في منطقة غضروف التمفصل المتسبب بواسطة الخلايا الالتهابية , الخلايا الضامة الفتية المنشطة في الغشاء المصلي والخلايا الغضروفية الكهلة (Cojocaru و أخرون, 2013) . إن التهاب المفاصل باعتباره مرض مناعي ذاتي فإنه يتميز بواسطة غياب التسامح المناعي المؤمن بواسطة الخلايا اللمفاوية المنظمة Treg, هذا التسامح المناعي يحمي مولدات الضد الذاتية من تفاعل الجهاز المناعي.

تقترح بعض الدراسات أنه في حالة التهاب المفاصل فأن الخلايا اللمفاوية المنظمة ليست لها القدرة على توقيف افرازات معظم السيتوكينات المنبهة لالتهاب مثل (TNFα و IFNγ).

لوحظ في حالة التهاب المفاصل ارتفاع في الحمض الأميني الهوموسستيين , هذا الأخير عبارة عن حمض أميني بسيط يتشكل من خلال ميتابولزم الحمض الأميني مثيونين , وقد سجل ارتفاع غير طبيعي في الهوموسستيين البلازمي عند المرضى الذين يعانون من فرط في الهوموسستيين الحاد . أشير كذلك أن ارتفاع الهوموسستيين متواجدا في عديد من الأمراض منها التشوه الولادي, مرض الزهايمر, الاختلال الهرموني, القصور الكلوي والسرطان (Shaker وآخرون ,2013) . ومرتبطا مع أمراض الأوعية القلبية (Wald وآخرون , 2002).

من جهة أخرى فإن السيتوكينات المنبهة للالتهابات والخلايا المسؤولة , فهي مهمة في تشكيل بيئة صالحة لتكوين الأورام السرطانية, حيث لوحظ في بعض أنواع السرطانات فإن الالتهاب يكون موجود قبل تكوين الورم الخبيث, وبالعكس في

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أنواع اخرى من السرطانات فإن التغييرات الانكوجينية المولدة للورم تحفز وسط التهابي مما يسمح بتطور الأورام , يدمر المناعة المكتسبة , يغير من الاستجابة للهرمونات وعوامل العلاج الكيميائي (Mantovani وآخرون , 2008).

في الحالات الطبيعية , جسم الإنسان يتميز بميكانيزم طبيعي للمقاومة تشكيل وتطور الخلايا السرطانية والذي يطلق عليه بالموت المبرمج . الأخير هو عملية تنظيمية للاستقرار الذاتي للجسم والذي يؤدي الى تثبيط نمو الأورام السرطانية وهو يعتمد على سيطرة احد البروتينات وهي (Bcl2 , BAX) حيث نجد Bcl2 يحمي الخلايا التالفة من الموت المبرمج ويسمح بتطور السرطان , ومن جهة أخرى فإن BAX يسمح بالموت الخلوي وذلك بواسطة تثبيط نسخ بروتين BCL2 (Yu وآخرون , 2015) .

إن معرفة آليات الالتهابات , سمح بتعديل استراتيجيات العلاج للمرضى الذين يعانون من الأمراض الالتهابية مثل التهاب المفاصل الروماتويدي. في الواقع, نتائج العديد من الدراسات تشير إلى أن التدخل العلاجي بما يسمى بالأدوية المعدلة للمرض و المضادة للروماتيزم (DMARDs) والأدوية غير الستيروئيدية المضادة للالتهابات, يمكن أن يوقف أو يؤخر من تطور الالتهابات و بالتالي توقيف أو تأخير هذا المرض.كما أنه لوحظ بأن هذه الأدوية الصناعية باهظة الثمن لها تأثيرات سلبية على تغيير الترتيب الجيني, الميتابولزم الخلوي و أثار جانبية أخرى.

لهذا السبب نحتاج إلى مركبات طبيعية التي تحمينا من هذه التأثيرات الجانبية والتي تعتبر أدوية صحية وفعالة بدون أي تأثيرات (Rahmani وآخرون , 2014).

كانت النباتات مصدرا دائما للدواء والاستعمال الطبي المباشر للإنسان حيث أن جميع أنظمة الطب التقليدي لها أهميتها في الطب الشعبي والعلاجات المنزلية, ولهذا السبب فإن بعض الأدوية قد تم دراستها ومراجعتها من من خلال ممارسات رجال الطب المدربين والمتمرسين.

و في الطب الحديث قد ظهر عددا كبيرا من الأدوية التي كانت مذكورة في المخطوطات القديمة مثل القرآن والإنجيل من بينها النخيل.

النخيل (*Phoenix dactylifera* L) هي واحدة من أقدم النباتات المزروعة في تاريخ البشرية .وقد تم استهلاكها كغذاء منذ 6000 سنة .ويمكن استخدامها للأجيال القادمة نظرا لقيمتها الغذائية, الصحية والقيمة الاقتصادية, بالإضافة إلى فوائدها الجمالية و البيئية.

ملخص بالعربية

يعتبرالنخيل من الأشجار المعمرة بذورها احادية الفلقة, تنتمي الى العائلة الفوفلية (الاسم العلمي:Arecaceae), و التي تضم200 جنسا و3000 نوع (Boukouwada و Yousfi. 2009), المعروف منها باسم التمر هو النوع الوحيد من الجنس *Phoenix* الذي يزرع لثماره (Shafi-Bhat و Al-Daihan. 2012). و يعتبر تمر النخيل مصدرا مهما للطاقة, الألياف و ومصدر طبيعي للمواد الكيميائية النباتية النشطة بيولوجيا مثل مركبات الفينول ومركبات الفلافونويد (Louaileche و أخرون, 2015).

الهدف من دراستنا هو تقييم التأثير البيولوجي للتمور الجزائرية على جهاز المناعة, زيادة الهوموسستئين في بلازما الدم, التهاب الحاد والتهاب المزمن أثناء مرض التهاب المفاصل الروماتويدي (داء الرثة), وعلى الخلايا السرطانية.

في هذه الدراسة, قمنا بتقييم التأثير الحيوي لنوعين من التمر الجزائري: ("أزرزا" و المزروعة في منطقة غرداية, "حميرة" و المزروعة في منطقة أدرار), وتمحور هذا التقييم حول:

- اختبار سلامة استعمال مستخلصات التمر و تحديد الجرعات القاتلة باستعمال اختبارات السمية لدى الفئران.

- تقييم تأثيرمستخلصات التمر على جهاز المناعة باستخدام فحص إزالة الكربون.

- تقييم تأثير مستخلصات التمر كمضاد للاكسدة باستخدام تقدير كمية الجلوتاثيون المختزل (GSH) في الكبد.

- تقييم تأثير مستخلصات التمر كمضاد للالتهابات الحادة الناجمة عن الفورمالين وزيادة الهوموسستيين في بلازما الدم.

- تقييم تأثير مستخلصات التمرعلى مرض التهاب المفاصل (الرثة) المنبه بحقن الفورمالين ومقارنته مع ديكلوفيناك الصوديوم.

- دراسة تأثير مستخلصات التمر على الالتهاب المفاصل (مرض الرثة) من خلال قياس CRP, و أجسام مضادة ذاتية المناعة في البلازما (Anti-CCP).

- تأكيد مفعول مستخلصات التمر على مرض التهاب المفاصل (الرثة) المنبه بحقن الفورمالين خلال الدراسة النسيجية.

- تقييم نشاط مكافحة التضاعف الخلوي لمستخلصات التمر على خلايا سرطان الكبد (HepG2) و خلايا سرطان الثدي (MCF7) باستخدام تقنيات الزراعة الخلوية.

- دراسة تأثير مستخلصات التمر على خلايا بشرية سليمة: الخلايا البطانية للأوعية الدموية HUVEC, خلايا كبدية h (NHEPS.

- اختبار تأثير مستخلصات التمر على بعض الجينات المرتبطة بالموت الخلوي المبرمج Bcl2, BAX.

الطرق و الوسائل المستعملة

المستخلصات النباتية

استخدم خلال هذه الدراسة نوعين من التمور الجزائرية : ("أزرزا Azarza" و المزروعة في منطقة غرداية, "حميرة Homayra و المزروعة في منطقة أدرار) محضرة بشكل تقليدي "الُرب" فيما يخص التجارب على الفئران. اما التجارب المخبرية الخاصة بالزراعة الخلوية فتم انجازها باستخدام مستخلص كحول الميثانول و الاسيتون لكلا النوعين.

الحيوانات

الفئران المستعملة خلال الدراسات داخل العضوية (*in vivo*) كانت من نوعAlbinosو تم الحصول عليها من معهد الصيدلة بقسنطينة. أما التجارب فأجريت على الحيوانات بعد اعطائها مدة للتأقلم مع ظروف العمل قبل كل تجربة.

الخلايا المستعملة في الزراعة الخلوية

تم الاستعانة في هذه الدراسة بأربعة انواع من الخلايا البشرية, اثنين منها خلايا سرطانية و هي خلايا سرطان الكبد (HepG2) و خلايا سرطان الثدي (MCF7) واثنين منها خلايا سليمة هي الخلايا البطانية للأوعية الدموية (HUVEC), خلايا كبدية (h NHEPS). تم الحصول على هذه الخلايا من مختبر التشريح المرضي بالمستشفى المدني بكالياري ايطاليا.

طريقة العمل

تأثير مستخلصات التمر على السمية لدى الفئران

قد أجريت هذه الدراسة وفقا المبدأ التوجيهي المقترح من قبل منظمة التعاون الاقتصادي والتنمية (Aribi 2015) ويستند هذا المبدأ التوجيهي حول إجراءات (Bruce, 1985) حيث تم استخدام جرعة 2000 ملغ / كغ من مستخلصات التمر لخمسة فئران ذكور بالغين و في صحة جيدة. تعطى جرعة من 2000 ملغ /كغ للفأر الأول, ويلاحظ هذا الفأر

ملخص بالعربية

لاكتشاف أي علامات سريرية (السلوكيات: العدوانية غير عادية, والنطق غير عادي, والأرق, والتخدير ونعاس) لمدة الساعة الأولى, ثم كل ساعة لمدة 3 ساعات و , وأخيرا و دوريا حتى 48 ساعة .في حالة عدم تأثير الجرعة على الفأر, يتم إعطاء أربعة حيوانات إضافية نفس الجرعة 2000 ملغ / كغ بالتتابع على فترة 48 ساعة .ثم توضع كل الحيوانات تحت المراقبة الدقيقة لمدة 14 يوما, وتعتبر الجرعة غير سامة إذا نجا ثلاثة فئران أو أكثر.

تأثير مستخلصات التمر على تحفيز النشاط المناعي لدى الفئران

لدراسة هذا التأثير استعملت تجربة معدل إزالة الكربون أين اتبعت الطريقة (Biozzi واخرون,1953) ' قسمت مجموعة تحتوي على28 فأرا الى أربعة مجموعات و عوملت كما هو موضح في الجدول 1 و 2.

جدول رقم 1. يوضح نشاط الخلايا البلعمية عند الفئران المعالجة بواسطة مستخلص التمر ازرزا

المجموعة التجريبية	المعاملة	عدد الفئران	الجرعة
م1	**Nacl 0,9%**	5	0,5 مل لكل فأر
م2	***مستخلص*التمر**	5	30 ملغ/كغ
م3	***مستخلص*التمر**	5	50 ملغ/كغ
م4	***مستخلص*التمر**	5	100 ملغ/كغ

جدول رقم 2. يوضح نشاط الخلايا البلعمية عند الفئران المعالجة بواسطة مستخلص التمر الحميرة

المجموعة التجريبية	المعاملة	عدد الفئران	الجرعة
م1	**Nacl 0,9%**	5	0,5 مل لكل فأر
م2	***مستخلص*التمر**	5	30 ملغ/كغ
م3	***مستخلص*التمر**	5	50 ملغ/كغ
م4	***مستخلص*التمر**	5	100 ملغ/كغ

بعد 48 ساعة من معاملة الفئران وإعطائها الجرعات اللازمة تحقن جرعة تقدر ب 0,1 مل لكل 10 غ من الكربون عبر وريد الذيل و تؤخذ بعد ذلك عينتان من الدم عند 5 دقائق و عند 15 دقيقة من كل فأر. يضاف محلول كربونات الصوديوم وذلك لتحليل كريات الدم وتقاس الكثافة الضوئية لكل عينة. كما يؤخذ و يقاس وزن كل من الكبد والطحال.

يعبر عن النشاط المناعي للخلايا البلعمية بواسطة الثابت K و الثابت α ونصف العمر الحيوي t كما هو موضح في المعادلات الآتية:

$$K = \frac{\log OD1 - \log OD2}{t2 - t1}$$

$$\alpha = \frac{\sqrt[3]{K \times \text{وزن الحيوان}}}{\text{وزن الكبد + وزن الطحال}}$$

$$t1/2 = 0,693/K$$

1OD : الكثافة الضوئية عند زمن1

2OD :الكثافة الضوئية عند زمن 2

تأثير مستخلصات التمر كمضادات للأكسدة

تم قياس النشاط المضاد للأكسدة عن طريق تحديد الطيفي لكمية الجلوتاثيون المختزل في الكبد أين اتبعت طريقة (Weckbeker وآخرون 1988) في ذلك. في نهاية تجربة النشاط المناعي تم تخدير الحيوانات و تشريحها لنزع الكبد والطحال. بعد ذلك تم استخدام مسحوق الكبد المتجانس لمعايرة كمية الجلوتاثيون المختزل طيفيا باستخدام كاشف التلوين DTNB حسب المعادلة التالية:

$$\boldsymbol{GSH}\ (\mathbf{nmol}\frac{\mathbf{GSH}}{\mathbf{mg}}\mathbf{protein}) = \frac{\boldsymbol{DO} \times \mathbf{1} \times \mathbf{1.525}}{\mathbf{13100} \times \mathbf{0.8} \times \mathbf{0.5} \times \mathbf{mg\ protein}}$$

تم تحديد تركيز البروتينات بطريقة (Bradford 1976). عن طريق مقارنة إلى خط المعايرة BSA.

تأثير مستخلصات التمر كمضادات للالتهاب

تم تقييم النشاط المضادة للالتهابات لمستخلصات التمر في الفئران باستخدام اختبار مفعول حقن الفورمالين و الذي يسبب ظهور انتفاخ في قدم الفئران خلال 5ساعات (Uma وآخرون2014). وقد تم قياس مستوى الالتهاب بقياس حجم انتفاخ قدم الفأر, وأيضا مخبريا عن طريق قياس مستويات البروتين - C التفاعلي والهوموسستين في الدم. حيث خلال هذه التجربة قسمت مجموعة الفئران الى خمسة مجموعات و عوملت كما هو موضح في الجدول 3.

تم تغذية الفأران بمستخلص التمر و الحمض الأميني المثيونين بعد مزجها بعجينة الفرينة و تقديمها شفويا.

تم حقن 100ميكرولترمن الفورمالين تركيزه (0.2%) في قدم كل فأر وقياس حجم التورم باستخدام قدم قنوية رقمية خلال عملية الحقن وبعد كل ساعة لمدة 5 ساعات. بعد الساعة الخامسة يتم سحب الدم من الحيوانات لمعايرة مستويات البروتين – C التفاعلي والهوموسستيين.

جدول رقم 3. تاثير مستخلص التمر الحميرة على الالتهاب الحاد و المحفز بواسطة الفورمول

المجموعة التجريبية	المعاملة	حقن الفورمول	عدد الجرذان	الجرعة
م **N**	فرينة	---	5	0,1غ لكل فأر
م**F**	فرينة	+++	5	0,1غ لكل فأر
م**P**	فرينة/ *مستخلص* التمر **أزرزا**	+++	5	50مغ/كغ
م**HM**	فرينة/ *مستخلص* التمر **حميرة**	+++	5	150مغ/كغ
م**M**	**ميثيونين**/فرينة	+++	5	400مغ/كغ
م **MHM ,MP**	**ميثيونين**/فرينة /*مستخلص* التمر	+++	5	50, 150/400 مغ/كغ

تأثير مستخلصات التمر على التهاب المفاصل (الرثة)المحفز بواسطة الفورمول

تم دراسة التهاب المفاصل التجريبي في الفئران وفقا للأساليب التي وصفها (Mazumder'2012). حيث قسمت الفئران إلى أربع مجموعات :مجموعة الشاهد متغذية فقط بالفرينة (N), مجموعة محقونة ب 0.05 مل من الفورمالين (FF) (2%), المجموعة (P) متغذية بمستخلصات التمر وتمت تغذية المجموعة الأخيرة (D) بجرعة 10 ملغم / كغم من عقار مضاد للالتهابات ديكلوفيناك الصوديوم (50mg) (جدول رقم 4).

تم قياس وزن الجسم كل يوم من فترة التجربة. بعد ذلك تم قياس حجم التورم في قدم الفئران باستخدام قدم قنوية رقمية كل يوم لمدة 10 أيام. في نهاية الدراسة, تم تصويم الفئران , وجمع عينات الدم لتحقيق بعض الفحوصات المخبرية محددة لالتهاب المفاصل لنا مثل الكشف عن أجسام مضادة ذاتية ANTI-CCP وبروتين C التفاعلي.

جدول رقم 4. تاثير مستخلصات التمر الحميرة و ازرزا على الالتهاب المفاصل و المحفز بواسطة الفورمول

المجموعة التجريبية	المعاملة	حقن الفورمول	عدد الجرذان	الجرعة
م **N**	فرينة	---	5	0,1غ لكل فأر
م**FF**	فرينة	100 µl+++	5	0,1 غ لكل فأر
م**AZ**	فرينة/ *مستخلص* التمر **أزرزا**	100 µl+++	5	50مغ/كغ
م**HM**	فرينة/ *مستخلص* التمر **حميرة**	100 µl+++	5	150مغ/كغ
م**D**	ديكلوفيناك الصوديوم/فرينة	100 µl+++	5	10مغ/كغ

تحضير قطاعات نسيجية لمفاصل الفئران

بعد تشريح الفئران, تم اخد العينات و التي وضعت في قارورات صغيرة و نظيفة بها الفورمول المخفف10 ٪ . بعد ذلك تم وضع العينات في حمض الفورميك (2%) لمدة 48 ساعة لغرض تليينها.

بعد ذلك تم نزع الماء باستخدام كحول الإيثانول (50٪, 70٪, و 96٪), مدة كل خطوة لحوالي 30 دقيقة (3 × 30 دقيقة = 1سا30د). بعد هذه الفترة وضعت العينات في البيتانول لمدة أسبوع كامل. بعد ذلك, تم غمرها مرتين في حل زيلين Xylene, لمدة 10 دقيقة في كل مرة.

بعد ذلك تم طمر العينات في شمع البرافين لمدة أربعة ساعات مع تغيير البرافين كل ساعتين و في الأخير تم قطع العينات و تلوينها بصبغة الهيماتوكسلين ايزين

دراسة تأثير مستخلصات التمر على الزراعة الخلوية

تمت هذه الدراسة باستخدام أربعة انواع من خلايا الانسان, اثنين منها خلايا سرطانية و هي خلايا سرطان الكبد (HepG2) و خلايا سرطان الثدي (MCF7) واثنين منها خلايا سليمة هي الخلايا البطانية للأوعية الدموية(HUVEC),

خلايا كبدية (h NHEPS). تمت معاملة كل الخلايا بواسطة مستخلصات التمر لمدة 24 ساعة في الزراعة الخلوية بعد ذلك تم الكشف عن هذا التأثير باستخدام اختبار MTT.

أول مرحلة من هذا الاختبار هي معرفة عدد الخلايا الموجودة في مستوعب واحد وذلك بتلوين الخلايا بواسطة أزرق الميثيلين بعد ذلك تم حساب عدد الخلايا, وانطلاقا من العدد المتحصل عليه تم تقسيم الخلايا بمقداره 5000 خلية في 100 µl وتركت في الحاضنة مدة 24 ساعة. في اليوم التالي و بعد سحب كل الوسط الحيوي تم اضافة مستخلصات التمر بستة تراكيز مختلفة للخلايا و اعادتها للحاضنة مرة أخرى لمدة 24 ساعة (جدول رقم 5). في اليوم الأخير تم سحب المستخلصات بالكامل وإضافة محلول MTT 100µl(0.65 مغ/مل) ثم أرجعت الخلايا للحاضنة مدة ساعتين, ليتم التخلص بعدها من محلول MTT و اضافة 100µl من DMSO ليتم بعدها قراءة الكثافة الضوئية لكل عينة و مقارنتها بالشواهد.

تم حساب النسب المئوية لتكاثر الخلايا بالمعادلة التالية:

$$\text{الانقسام الخلوي } \% = \frac{\mathbf{At - Ab}}{\mathbf{Ac - Ab}} \times \mathbf{100}$$

بحيث:

At= كمية الامتصاص الضوئي للعينات المعالجة بمستخلصات التمر.

Ab= كمية الامتصاص الضوئي الخاص بالوسط الحيوي خال من الخلايا.

Ac= كمية الامتصاص الضوئي الخاص بالوسط الحيوي المضاف اليه الخلايا غير المعاملة.

جدول رقم 5. يوضح تاثير المستخلصات التمر على الخلايا السرطانية و الطبيعية

الرمز	التركيز	المعاملة	الرمز	التركيز	المعاملة
A1.1	**2µg/100µl**	اسيتون أزرزا	**B1.1**	**2µg/100µl**	اسيتون حميرة
A1.2	**4µg/100µl**	اسيتون أزرزا	**B1.2**	**4µg/100µl**	اسيتون حميرة
A1.3	**7,5µg/100µl**	اسيتون أزرزا	**B1.3**	**7,5µg/100µl**	اسيتون حميرة
A1.4	**8µg/100µl**	اسيتون أزرزا	**B1.4**	**8µg/100µl**	اسيتون حميرة
A1.5	**16µg/100µl**	اسيتون أزرزا	**B1.5**	**16µg/100µl**	اسيتون حميرة
A1.6	**20µg/100µl**	اسيتون أزرزا	**B1.6**	**20µg/100µl**	اسيتون حميرة
A2.1	**2µg/100µl**	ميثانول أزرزا	**B2.1**	**2µg/100µl**	ميثانول حميرة
A2.2	**4µg/100µl**	ميثانول أزرزا	**B2.2**	**4µg/100µl**	ميثانول حميرة
A2.3	**7,5µg/100µl**	ميثانول أزرزا	**B2.3**	**7,5µg/100µl**	ميثانول حميرة
A2.4	**8µg/100µl**	ميثانول أزرزا	**B2.4**	**8µg/100µl**	ميثانول حميرة
A2.5	**16µg/100µl**	ميثانول أزرزا	**B2.5**	**16µg/100µl**	ميثانول حميرة
A2.6	**20µg/100µl**	ميثانول أزرزا	**B2.6**	**20µg/100µl**	ميثانول حميرة
CTR+	**100µl**	وسط حيوي + خلايا			
CTR-	**100µl**	وسط حيوي			

ملخص بالعربية

دراسة تأثير مستخلصات التمر على الجينات المرتبطة بالموت الخلوي المبرمج في الخلايا السرطانية للثدي

لدراسة تأثير مستخلصات التمر على الجينات المرتبطة الموت الخلوي المبرمج في **الخلايا السرطانية للثدي**, تم زراعة خلايا سرطان الثدي بالإضافة الى مستخلصات التمر لمدة 24 ساعة (جدول رقم 6). ليتم بعد ذلك استخدامها لاستخراج الحمض النووي الريبي الرسول mRNA و الذي بدوره استعمل في عملية النسخ العكسية (Reverse Transcription) للحمض النووي المكمل cDNA. كخطوة اخيرة تم استخدام للحمض النووي المكمل cDNA في الكشف عن التعبير الجيني لبعض الجينات المرتبطة الموت الخلوي المبرمج مثل BAX و Bcl2 و ذلك بالاعتماد على RT-PCR protocol (Patel وآخرون 2009).

جدول رقم6. معاملة الخلايا في اختبار دراسة تأثير مستخلصات التمر على التعبير الجيني لخلايا سرطان الثدي

الرمز	التركيز	المعاملة	الرمز	التركيز	المعاملة
A1.1	**4µg/100µl**	اسيتون أزرزا	**B1.1**	**4µg/100µl**	اسيتون حميرة
A1.2	**8µg/100µl**	اسيتون أزرزا	**B1.2**	**8µg/100µl**	اسيتون حميرة
A1.3	**16µg/100µl**	اسيتون أزرزا	**B1.3**	**16µg/100µl**	اسيتون حميرة
A2.1	**4µg/100µl**	ميثانول أزرزا	**B2.1**	**4µg/100µl**	ميثانول حميرة
A2.2	**8µg/100µl**	ميثانول أزرزا	**B2.2**	**8µg/100µl**	ميثانول حميرة
A2.3	**16µg/100µl**	ميثانول أزرزا	**B2.3**	**16µg/100µl**	ميثانول حميرة
CTR	**500µl**	وسط حيوي + خلايا			

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النتائج

بينت النتائج الأولية لتجربة السمية على الفئران مستخلصات التمر الجزائري و المستخدمة في هذه الدراسة ليس لها أي تأثير سام بجرعة 2000مغ/كغ من كل مستخلص الفئران المعالجة والموضوعة تحت الملاحظة لمدة 14 يوم.

أشارت نتائج تأثير مستخلصات التمر الجزائري من نوع أزرزاعلى تحفيز النشاط المناعي للخلايا البلعمية إلى ارتفاع النشاط البلعمي بقيمة معتبرة بالنسبة للحيوانات المعاملة بجرعة 50ملغ/كغ مقارنة بالشاهد و الجرعات الأخرى, كما أن نصف العمر الحيوي لجزيئات الكربون في الدم انخفض بقيمة معتبرة كما هو موضح في الشكل (12,13 و 14) كما أظهرت نتائج معايرة كمية الجلوتاثيون المختزل في الكبد انخفاضه لدى الفئران المعالجة بجرعة 50ملغ/كغ مقارن بالشاهد و الجرعات الأخرى كما هو موضح في الشكل (15). أما فيما يخص نتائج تأثير مستخلصات التمر الجزائري من نوع حميرة على تحفيز النشاط المناعي للخلايا البلعمية فأظهرت ارتفاعا في النشاط البلعمي بقيمة معتبرة بالنسبة للحيوانات المعاملة بجرعة 150ملغ/كغ مقارن بالشاهد و الجرعات الأخرى, كما أن نصف العمر الحيوي لجزيئات الكربون في الدم انخفض بقيمة معتبرة كما هو موضح في الشكل (16,17 و 18) كما أظهرت نتائج معايرة كمية الجلوتاثيون المختزل في الكبد انخفاضه لدى الفئران المعالجة بجرعة 150ملغ/كغ مقارنة بالشاهد و الجرعات الأخرى كما هو موضح في الشكل (19).

وعلاوة على ذلك, تبين ان مستخلصات التمر الجزائري لها تأثير على الالتهابات المرتبطة بفرط الهوموسستيين في الدم والتي تمثلت في انخفاض ملحوظ في حجم التورم بعد 5 ساعات من حقن الفورمالين الشكل (20,21 و24, 25) وانخفاض ملحوظ في قيم في الشكل CRP (22 و 26) و الهوموسستين (HCY) في الشكل (23 و 27) في الفئران التي عولجت ب 50 ملغ/كغ لنوع أزرزا و 150 ملغ/كغ لنوع حميرة مقارنة مع الشواهد, كما لوحظ أن حقن المثيونين 400 ملغ /كغ تسببت في تفاقم التهاب القدم زيادة كبيرة في البروتين سي التفاعلي CRP وزيادة كبيرة في الهوموسستيين. وقد أجريت دراسة أخرى على تأثير مستخلصات التمر على الالتهاب المزمن في إحداث التهاب المفاصل بواسطة تنبيه الفورمالين. حيث أظهرت النتائج المتحصلة عليها انخفاض واضح في حجم انتفاخ قدم الفئران التي عولجت ب 50 ملغ/كغ لنوع أزرزا في الشكل (28 و 29) و 150 ملغ/كغ لنوع حميرة في الشكل (32 و 33) مقارنة مع الشواهد و كمية الأجسام المضادة Anti-CCP في الشكل (30 و 34). وذلك بعد انتهاء مدة الدراسة والمقدرة ب 10 أيام.

وعلاوة على ذلك, في هذه الأطروحة, أجرينا دراسة في المختبر لتقييم تأثير مستخلصات التمر الجزائري (المستخلص المثيلي والأسيتون) على نمو الخلايا السرطانية (سرطان الكبد HepG2, سرطان الثدي MCF7) والخلايا السليمة

(الخلايا البطانية للأوعية الدموية HUVEC, خلايا الكبد h NHEPS) .وأظهرت النتائج تناسبا طرديا بين التراكيز المستخدمة و نسبة انخفاض انقسام و تكاثر الخلايا السرطانية مقارنة بالشواهد. كما أظهرت النتائج أن العلاج بستة تراكيز مختلفة ليس لديها أي سمية على الخلايا السليمة مقارنة بالشواهد والموضحةفي الأشكال (من 40 الى47).

المناقشة

ان المقاومة الطبيعية للجسم ضد العدوى يمكن أن تتعزز من خلال استخدام الأدوية العشبية, وعلى نطاق واسع تستخدم عدة مستحضرات عشبية التي يمكن أن تعزز وضع نظام المناعة .هناك تصاعدا في الاستخدام السريري للأدوية الأصلية, حيث أنها خالية من الآثار الجانبية الخطيرة (Chandua وآخرون 2011), وقد أظهرت بعض النباتات الطبية والمنتجات النباتية تعديل الاستجابات المناعية الفطرية وكذلك الخلطية من خلال التفاعل مع تتالي معدلات المناعة وبالتالي تغيير انقسام الخلايا المناعية, البلعمية, نشاط الخلايا السامة , التعبير عن السيتوكينات, المسقبلات الخلوية و الأجسام المضادة (Maji و أخرون 2013).

وكان الجزء الأول من الدراسة هو تقييم تأثير مستخلصات التمر الجزائري على تحفيز النشاط المناعي.والمحفزات المناعية هي مواد يمكنها أن تحفز الاستجابة المناعية سواء الفطرية أو المكتسبة . وأطلقت العديد من المحفزات المناعية الاصطناعية من قبل شركات الأدوية ولكن لوحظ أن لها العديد من الآثار الجانبية (Talmale, وآخرون 2014).في الجانب الآخر يعتقد أن بعض المنتجات النباتية تعزز المقاومة الطبيعية للجسم ضد العدوى, على أساس ما تحتويه من مواد مثل السكريات, lectins لكتينات, الصابونين saponins وفلافونيداتflavonoids وما إلى ذلك. بعض من هذه المواد تحفز على حد سواء "المناعة الخلطية و الخلوية, في حين أن مواد أخرى تنشط فقط مكونات المناعة الخلوية (Jones Compton, 1985), ان الجزيئات المحفزة للاستجابة المناعية تكثف وتعدل الاستجابة المناعية اللمفاويات ومدتها,و بالتالي يمكن استخدامهاكمواد مساعدة في اللقاحات ومستحضرات الحساسية (Ranta وآخرون 2012).

لتقييم هذا الأثر تم إجراء اختبار تصفية الكربون لتقييم تأثير مستخلصات التمر على البطانة الشبكية (RES) , وهو نظام يتكون من الخلايا البلعمية. بحيث خلال حقن جزيئات الكربون في شكل الحبر, تقوم البالعات الكبيرة والخلايا البلعمية الأخرى بإزالة الكربون من الدم عن طريق ظاهرة البلعمة.

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وقد أظهرت النتائج أن جرعات مستخلصات التمر المستخدمة في المجموعات المعالجة كان لها تأثير على الجهاز المناعي عن طريق تحفيز الخلايا البلعمية للتخلص من الكربون في الدم في فترة زمنية قصيرة, وكانت لتراكيز 50 مغ/كغ (أزرزا AZARZ) و150 مغ/كغ (حميرة HOMAYRA) أفضل النتائج مقارنة بالشواهد.

وتشير الزيادة في معدل إزالة الكربون من الدورة الدموية الى زيادة نشاط الخلايا البلعمية وكفاءة نظام الخلايا الحبيبية في إزالة الجسيمات الغريبة. هذه النتائج متوافقة مع نتائج (Bharani وآخرون ,2010) التي ذكرت أن خلايا بطانة الجهاز الشبكي تلعب دورا هاما في إزالة الجسيمات الغريبة من الدورة الدموية. و أنه عندما يتم حقن جزيئات الكربون في شكل حبر مباشرة في الدورة الدموية, يزداد معدل إزالة الكربون من الدم عن طريق البلعمة بعد علاج الفئران بالمستخلص المثيلي لأوراق التوتLinn *Morus Alba*. كما وجدنا أن هذه النتائج متوافقة مع نتائج (Patel و آخرون 2010) و(Tang و آخرون 2012) التي سجلت على التوالي أن كلا من الجرعة المنخفضة (100ملغ /كغ),و الجرعة العالية (500 مغ/كغ) من *Aegle marmelos* تحفز جهاز المناعة, وأن *Tenebrio molitor Linnaeus* لها تأثير متناسب طرديا مع الجرعة المستخدمة وتزيد من تحفيز القدرة على إزالة حبيبات الكربون ونشاط البلعمة في الفئران.

ان الخلايا البلعمية مثل البالعات الكبيرة macrophage و الخلايا الحبيبية متعادلة الوسط neutrophils تسيطر على الاستجابة المناعية الفطرية عن طريق الإفراج عن المنتجات التي تضر الكائنات الدقيقة .وتشمل هذه المنتجات البروتينات مثل الليزوزيم lysozyme , والإيلاستاز elastase فضلا عن أنواع الاكسجين التفاعلية oxygen reactive species (Rosen وآخرون, 1995).

من وجهة النظر الكلاسيكية المواد المضادة للأكسدة تكنس هذه الجزيئات المؤكسدة , وبالتالي توفر حماية للجسم ضد الأمراض (Guido و آخرون, 2014). مضادات الأكسدة هي جزيئات قادرة على منع أكسدة جزيئات أخرى في الجسم, أي بطريقة أخرى يمكنها أن تحمي الجزيئات الأخرى في الجسم من تأثير أنواع الاكسجين التفاعلية .من هذه النقطة, يتم استخدام العديد من المواد الكيميائية النباتية من الخضار والفواكه لارتفاع احتمالات احتوائها على مضادات للأكسدة (Hasan وآخرون,2010). في الجزء الثاني من هذه الدراسة تم تقييم تأثير مستخلصات التمر (أزرزا وحميرة) على قيم الجلوتاثيون المختزل من الكبد. الجلوتاثيون المختزل هو أحد مضادات الأكسدة الرئيسية التي تحمي الأنسجة من الإصابة بالجذور الحرة و هو عنصرا حيويا في الدفاعات الخلوية .بالإضافة إلى حماية ضد الإصابة بالجذور الحرة, فهو يلعب دورا مهما في تنشيط الخلايا اللمفاوية, الخلايا القاتلة الطبيعية (natural killer cells) والسمية الخلوية بتدخل الخلايا اللمفاوية

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السامة, كما يمكن أن يكون له دور في حماية البالعات الكبيرة macrophage و الخلايا الحبيبية متعادلة الوسط neutrophils ضد الضرر التأكسدي (Hong وآخرون ,1991).

أظهرت النتائج من هذا الجزء من الدراسة تأثير محتمل مستخلصات التمر (أزرزا وحميرة) كمضادات الأكسدة والذي تمثل في انخفاض كبير لقيم الجلوتاثيون المختزل من الكبد في المجموعات المعالجة بثلاث جرعات من مستخلصات التمر , مع وجود تأثير أكبر للجرعة 50ملغ /كغ لنوع أزرزا و 150 مغ / كغ لنوع حميرة مقارنة مع المجموعات المراقبة.

هذه النتائج تتفق مع نتائج (Hasnaoui وآخرون ,2012) و نتائج (Hasan وآخرون, 2010)التي ذكرت على التوالي أن تركيز الألياف من ثمار التمر المغربي ومستخلصات مختلفة لتمر من ليبيا يحتوي عل نسبة عالية من مضادات الأكسدة. أيضا (Patel وآخرون ,1987) أظهروا في دراستهم أن العوامل التي تزيد من نسبة سكر في الدم عن طريق الفم, تولبوتاميد tolbutamide وغليبوريد glyburide, المستخدمة في علاج مرضى السكري من النوع الثاني يزيد من افراز الجلوتاثيون الصفراوي الذي يظهر على زيادة في إفراز الجلوتاثيون المختزل في الصفراوية من الكبد. وتشير هذه النتائج إلى أن مستخلصات التمرلها تأثير كمضادات للأكسدة من خلال تحفيز إفراز الجلوتاثيون من الكبد مما يؤدي إلى القضاء على أنواع الاكسجين التفاعلية وحماية الجسم. وقد يكون ذلك نتيجة التأثير على آليات الأنزيمية لإنتاج و افراز الجلوتاثيون المختزل.

في هذه الدراسة, أظهرت نتائجنا أن الحيوانات التي عولجت بمستخلصات التمركان لها نشاط بلعمة وافراز الجلوتاثيون المختزل من الكبد عند جرعات 50 مغ/كغ لنوع أزرزا و 150 مغ/كغ لنوع حميرة, ولكن انخفض هذا التأثير مع الجرعات الكبيرة 100 مغ /كغ لنوع أزرزا, و200 مغ/كغ لنوع حميرة ويمكن تفسير هذه النتائج وفقا لمفهوم hormesis (Benmebarek ,2014).أصبح استخدام مفهوم hormesis (كلمة يونانية تعني "لإثارة") في مختلف المجالات, وخاصة في علم السموم البيئية والأحياء وصف سلسلة متصلة بالاستجابة للجرعة التي تنطوي على تنشيط التحفيز بجرعة منخفضة وتثبيطه بجرعة عالية (Calabrese وآخرون, 2001).

وارتكز الجزء التالي من هذه الدراسة إلى تقييم تأثير مستخلصات التمر على فرط الهوموسسيين في الدم, والتهاب المفاصل.

فرط الهوموسستئين في الدم تؤدي الى تحفيز تكوين الجذور الحرة (ROS) مثل H2O2 أو من خلال خفض مستويات الجلوتاثيون المختزل التي تشارك في القضاء على الجذور الحرة (Bhandari وآخرون ,2008). ويربط زيادة الهوموسستيين البلازمي في الدم مع الالتهاب, في الحقيقة أن مستوى الهوموسستيين يمكن أن ينخفض بشكل فعال باستخدام

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الأدوية المضادة للالتهابات. كلما قد أظهرت العديد من المركبات المضادة للالتهابات مثل ريسفيراترول resveratrol, الأسبرين aspirin, حمض الساليسيليك salicylic acid وأتورفاستاتين atorvastatin إلى تخفيض افراز الهوموسستتين من خلايا الدم المحيطية وحيدات النوى"peripheral blood mononuclear cells" (Wu, 2007).

وأظهرت النتائج المتحصلة عليها ان هناك انخفاضا كبيرا في الهوموسستيين عند مجموعة الفئران التي تغذت على مستخلصات التمرمقارنة مع المجموعات الأخرى .هذه النتائج تتوافق مع نتائج (Shaker وآخرون ,2013) التي ذكرت أن الأفوكادو أظهر أفضل النتائج من بين مستخلصات الطماطم والقرنبيط في خفض مستويات الهوموسستيين في البلازما. وذكرت أيضا (Nolla وآخرون, 2009) أن مستخلص النبيذ الأحمر Extract polyphenolic (PE) عند تقديمه بكميات منخفضة للفئران يؤدي الى انخفاض تركيزالهوموسستيين البلازمي.

ويمكن تفسير التأثير المحتمل لمستخلصات التمرمن خلال التأثير على آليات الأنزيمية التي تنظم تركيز الحمض الاميني الهوموسستيين HCY في الجسم مثل methionine-adenosyltransferase (MAT) I/III, cystathionine β lyase (CTH), betaine homocysteine methyltransferase(BHMT), glycine N-methyltransferase (GNMT) (Blom وآخرون 2011).

تم اختبار الأنشطة المضادة للالتهابات والمضادة للالتهاب المفاصل الروماتويدي (داء الرثة) بحقن الفورمالين و الذي يسبب تورم و التهاب قدم الفأر.ومن المعروف جيدا أن الالتهاب الناجم عن حقن الفورمالين هو واحد من الإجراءات الأكثر ملائمة للكشف عن مواد مضادة للالتهابات لأنه يشبه التهاب المفاصل (Hemamalini وآخرون 2010).

أظهرت نتائج هذه الدراسة أن بمستخلصات التمر أدت الى تثبيط انتفاخ التورم في قدم الفئران مقارنة مع مجموعة الشواهد و مجموعة الفئران المعالجة بمضاد الالتهاب ديكلوفيناك. هذه النتائج متوافقة مع نتائج (Mazumder و آخرون ,2012) حيث ذكر أن مستخلص الميثانول من *Barlerialupulina* أظهر إعاقة واضحة في تشكيل التورم خلال الفترة التجريبية لاختبار التهاب المفاصل المحفز بواسطة حقن الفورمالين والمقدرة ب 10 أيام.كما ظهر أيضا التوافق مع اعمال (Kyei وآخرون ,2012) حيث قام باستخدام نبتة *Pistia stratiotes* في معالجة التهاب المفاصل ومقارنته مع ديكلوفيناك Diclofenac وميثوتريكسات methotrexate فوجد ان هذه النبتة لها تأثير مضاد للالتهاب المفاصل وخافض للحرارة في التهاب المفاصل الناجم عن الفورمالين.

ملخص بالعربية

ان حقن الفورمالين في قدم الحيوان ينتج التهاب موضعي وآلام وذلك نتيجة افراز الهستامين السيروتونين وكاينين .النتائج التي تم الحصول عليها من هذا الاختبار يمكن تفسيرها باحتمالية تأثير مستخلصات التمرعلى إفراز الوسائط الالتهابية كما يمكن أن يكون لها تأثير مماثل لمضادات الالتهابات غير الستيروئيدية في كل مراحل الالتهاب . المرحلة الأولى حيث يتم إفراز الهستامين و السيروتونين (0-2 سا) (Viswanatha وآخرون ,2011), تليه الكاينين مثل البراديكينين, وتستمر ما بين 30-60 دقيقة (Hunskaar و آخرون ,1987), ثم مرحلة افراز البروستاجلاندين (سا4>) (Viswanatha وآخرون, 2011). وهكذا البروستاجلاندين يعمل في وقت متأخر نسبيا في تطوير الالتهاب. ومن الثابت أن مضادات الالتهاب غير الستيروئيدية NSAIDs تمنع الالتهابات من خلال منع تخليق البروستاجلاندين (Hunskaar وآخرون ,1987).

في هذا الجزء من الدراسة أظهرت النتائج أن مستخلصات التمرأدت الى انخفاض كبير في البروتين سي التفاعلي (CRP) والأجسام المضادة الذاتية Anti-CCPمقارنة مع مجموعات المراقبة الإيجابية والسلبية .ويمكن تفسير ذلك بأن مستخلصات التمر عززت أيضا تطوير الالتهاب عن طريق تثبيط انتقال الخلايا المناعية إلى موقع التهاب. هذه النتائج متوافقة مع نتائج (Mikuls و واخرون,2004) التي ذكرت أنه بعد علاج التهاب المفاصل الروماتويدي يحدث تراجعا كبير في مستويات الأجسام المضادة Anti-CCP. أيضا (Hetland وآخرون ,2012) (Tsuji وآخرون, 2013) التي أفادت بأن العلاج بمضادات للروماتيزم (بيتاميثازون betamethasone , أداليموماب Adalimumab وما الى ذلك يؤدي إلى انخفاض في مستويات الأجسام المضادة Anti-CCP , وبروتين سي التفاعليCRP في التهاب المفاصل الروماتويدي.

تحصلنا خلال هذه الدراسة على نتائج جيدة و فعالة لمستخلصات التمر توقيف التمايز الخلوي لهذه الخلايا السرطانية, خلايا سرطان الكبد (HepG2) و سرطان الثدي (MCF7). كما لم تظهر أي سمية على الخلايا السليمة الخلايا البطانية للأوعية الدموية (HUVEC) و خلايا كبدية (h NHEPS).

تتوافق هذه النتائج مع نتائج (Lu و آخرون, 2011) أن حقن مستخلص بذور *Coixlacryma-jobi* لديه قدرة كبيرة على تحفيز الموت المبرمج في خلايا سرطان الكبد (HepG2). كما تتوافق أيضا مع نتائج (Blassan وآخرون, 2016) حيث ذكر كتاب المقال أن مستخلص الأسيتون لجذور *Rubusfairholmianus* لديه قدرة كبيرة على ايقاف تكاثر و انقسام خلايا سرطان الثدي (MCF7). أما فيما يخص الخلايا السليمة فتوافقت نتائجنا مع نتائج (Atmaca و آخرون ,2015) و (Geisen و آخرون ,2015) أين ذكر و على التوالي أن مستخلصات *Salviatriloba*و *Fucus*

vesiculosus لديهم قدرة على ايقاف تكاثر و انقسام خلايا سرطان غدة البروستات و البنكرياس لكن دون أي أثر على خلايا البطانية للأوعية الدموية (HUVEC). في حين أن هذه النتائج لم تتوافق مع ما جاء في المقال المنشور ل (Ziaei و آخرون ,2015) حيث ذكر أن معاملة الخلايا البطانية للأوعية الدموية (HUVEC) بـteuclatriol من *salviamirzayanii* ادى الى انخفاض في ابقاء الخلايا البطانية للأوعية الدموية على قيد الحياة كلما زادت الجرعة المستخدمة من teuclatriol .

وفيما يتعلق النتائج على خلايا الكبد السليمة, توافق نتائجنا مع نتائج (Ikehara و آخرون, 2015) (Kueteو آخرون ,2013)التي ذكرت أن معاملة خلايا كبدية سليمة ب cyanobacterial hepatotoxin microcystin-LR و مستخلصات *Echinopsgiganteus, Xylopiaaethiopica, Imperatacylindrica* و *Piper capense* وغيرها من النباتات الطبية الكاميرونية لديه تأثير كابح بشكل خاص على خلايا سرطان الكبد ولكن أقل تأثير على خلايا الكبد الطبيعية. أيضا في مقال (Schmidt و آخرون, 2005) أن التراكيز المرتفعة لمستخلصات الشاي الأخضر تبدي سمية خلوية منخفضة في خلايا الكبد السليمة في فئران المختبر.

Abstract

Inflammation plays an important role in various diseases, such as rheumatoid arthritis, atherosclerosis and asthma, which all show a high prevalence globally and the absence of the immune tolerance which is insured by the lymphocyte Treg. This tolerance protects the self-antigens from the immune system's reaction. In addition, some chronic inflammatory diseases are recently found to be associated with an increased levels of hs-CRP and plasma homocysteine.

In the present study, we evaluated the *in vivo*, the effect of two varieties of *Phoenix dactylifera* (Azarza variety grown in Ghardaia and variety Homayra grown in Adrar) on the toxicity using the up and down test, the immunomodulatory activity of the extracts using the carbon clearance from the blood, antioxidant by the measurement of the GSH from liver's homogenate, anti-inflammatory activity and anti-arthritis by the formalin and L-methionine test in Albino mice. Also we have carried a study *in vitro* to establish the anti-proliferative effect of the methanolic and acetone extracts dates on liver cancer cell line (HepG2), breast cancer cell line (MCF7) and healthy cells, endothelial cells (HUVEC) and hepatocytes (hNHEPS). In addition to this work, we tested the effect of *Phoenix dactylifera* extracts on apoptotic genes (Bcl2 and BAX) and on the differentiation of human naïve lymphocyte T CD4+ into regulatory lymphocytes Treg.

The results showed that the extracts of *Phoenix dactylifera* have no toxic effect at a dose of 2000mg/kg, also we found that the extracts increased significantly the phagocytic activity of the reticuloendothelial system, and release of glutathione reduced (GSH) from the liver. Furthermore, the extracts of *Phoenix dactylifera* studied reveal correction on the inflammation associated with hyperhomocysteinemia presented by a significant decrease in the size of the edema induced by formalin injection and a significant decrease in the hs- CRP

values and homocysteine Hcy ($p \leq 0.05$) in mice treated compared to controls, where it was observed that the administration of L-methionine 400 mg/kg caused a worsening of inflammation presented by a significant increase in protein C-reactive (CRP) $p \leq 0,05$ and a significant increase in homocysteine (Hcy) $p \leq 0,05$. Our results demonstrate a significant inhibition of edema of mice paws treated with our extracts with a decrease in the Anti-CCP values.

The results indicate that treatment with six different concentrations (2 mg/100mL, 4 mg/100mL, 7.5 mg/100mL, 8 mg/100mL, 16 mg/100mL and 20 mg/100mL) inhibited the growth of tumor cells but had no toxicity to healthy cells.

Keywords:

Phoenix dactylifera, immunomodulatory activity, GSH glutathione, inflammation, cancer hyperhomocysteinemia, anti-arthritis activity.

Résumé

L'inflammation joue un rôle important dans diverses pathologies telles que l'arthrite rhumatoïde, l'athérosclérose et l'asthme, montrant ainsi une prévalence élevée à l'échelle mondiale, avec une majeure caractéristique reconnue par l'absence de la tolérance immunitaire qui est assurée par les lymphocytes Treg. Cette tolérance protège les antigènes du soi des réactions immunitaires. En outre, certaines maladies inflammatoires chroniques récemment décrites, peuvent être associées à une augmentation du taux de hs-CRP et de l'homocystéine plasmatique.

Dans la présente étude, *in vivo,* nous avons évalué l'effet de deux variétés de dattes *Phoenix dactylifera* Azarza et Homayra récoltées en Algérie à Ghardaia et Adrar sur la toxicité en utilisant la méthode « up and down », l'activité immunomodulatrice des extraits en utilisant le test de l'épuration sanguine d'une dose de carbone colloïdale ; ainsi une autre étude a été effectuée sur l'activité anti-inflammatoire aigüe et chronique induite par le formol et une forte dose de la L-méthionine.

In vitro, nous avons étudié l'effet antiprolifératif des extraits methanolique et acétonique des dattes *Phoenix dactylifera* sur les lignées cellulaires du foie (HepG2) et du sein (MCF7), sur l'expression des gènes apoptotiques (Bcl2 et BAX) et sur la différentiation des lymphocytes T CD4+ naïve en lymphocytes Treg.

Nos résultats soulignent que les extraits du *Phoenix dactylifera* ne présentent aucun effet toxique à la dose de 2000 mg/kg, et que ces extraits ont augmenté l'activité phagocytaire du système réticulo-endothélial ainsi que la libération du glutathion réduit GSH à partir du foie. Ces activités s'expriment d'une façon significative dans les groupes de souris traitées par

Phoenix dactylifera « Azarza » (50mg/kg) et « Homayra » (150mg/kg) par rapport aux autres groupes P≤0,05.

Par ailleurs, les extraits de *Phoenix dactylifera* étudiés ont révélé un effet sur l'inflammation associée à l'hyperhomocystéinémie présentée par une diminution significative de la taille de l'œdème induit par l'injection du formol et une diminution significative des valeurs de la hs-CRP et l'homocystéine (P≤0,05) chez les souris traitées par rapport aux témoins, où nous avons constaté que l'administration de L- méthionine à 400 mg / kg provoquent une aggravation de l'inflammation. Cette dernière se manifeste par une augmentation significative de la protéine hs- C réactive (CRP) P≤0,05 et de l'homocystéine (Hcy) P≤0,05. En outre, nos données révèlent une inhibition significative de l'œdème des pattes de souris traitées par les différents extraits utilisés ainsi qu'une diminution des taux des auto-anticorps Anti-CCP.

En conséquence, le traitement avec les six différentes concentrations (2 mg/100mL, 4 mg/100mL, 7.5 mg/100mL, 8 mg/100mL, 16 mg/100mL et 20 mg/100mL) a inhibé la croissance des cellules tumorales mais n'a eu aucune toxicité sur les cellules saines.

Mots clés : *Phoenix dactylifera,* activité immunomodulatrice, glutathion réduit, inflammation, hyperhomocystéinémie, activité anti-arthrite, cancer.

ملخص

ان الالتهاب يلعب دور هام في امراض مختلفة من بينها التهاب المفاصل' تصلب الشرايين' و الربو المنتشرة عالميا. و الذي يمتاز بخاصية أساسية هي غياب التسامح المناعي المتمثل في الخلايا اللمفاوية التائية المعدلة Treg. هذا التسامح المناعي هو المسؤول عن حماية الجسم من هجوم الج بالإضافة الى ذلك ' بعض الأمراض الالتهابية المزمنة توجد حديثا هاز المناعي الذاتي. مرتبطة مع ارتفاع hs-CRP و زيادة الهوموسستئين البلازمي .

في هذه الدراسة, قمنا بتقييم التأثير الحيوي لنوعين من التمر الجزائري: ("أزرزا Azarza" و المزروعة في منطقة غرداية, "حميرة Homayra و المزروعة في منطقة أدرار), على السمية و ذلك باستخدام طريقة (up and down test), والاستجابة المناعية باستخدام تنقية التخلص من جزيئات الكربون من الدم, قياس مضاد الاكسدة الجلوتاثيون المختزل من الكبد, والنشاط المضادة للالتهابات الحادة و المزمنة المحفزة بواسطة الفورمول و جرعة مرتفعة من الميثيونين. في هذه الرسالة قمنا بدراسة خارج العضوية لتأثير مستخلصات التمر(ميثانول و الاسيتون) على الخلايا السرطانية للكبد HepG2 و الثدي MCF7. كما قمنا بتقييم تأثير التمر الجزائري على التعبير الجيني لبعض جينات الموت الخلوي المبرمج (BAX و Bcl2) و تمايز الخلايا اللمفاوية التائية.

من النتائج المتحصلة عليها وجدنا على انه التمر الجزائري المستخدمة في هذه الدراسة ليس لها تأثير سلبي عند الجرعة 2000 ملغ/كغ' كذلك هذه المستخلصات نبهت الجهاز المناعي مع زيادة تحرير الجلوتاثيون المختزل من الكبد. كما تم معالجة الالتهابات المرتبطة بزيادة الهوموسستئين حيث ادى الى انخفاض ملحوظ في حجم الورم الناتج عن حقن الفورمول مع انخفاض قيم hs-CRP و الهوموسستئين البلازمي بقيم معتبرة عند مقارنتها بالشواهد.

حيث لوحظ أن حقن الميثيونين 400مغ/كغ تسببت في تفاقم التهاب القدم زيادة كبيرة في البروتين سي التفاعلي(hs-CRP) وزيادة كبيرة في الهوموسستئين. تحصلنا كذلك على انخفاض واضح في حجم انتفاخ قدم الفئران و كمية الأجسام المضادة Anti-CCP.

ومن النتائج المتحصلة عليها خلال هذه الاطروحة هي تثبيط في تكاثر الخلايا السرطانية مع عدم تثبيط الخلايا الطبيعية.

الكلمات المفتاحية

التمر *Phoenix dactylifera*, النشاط المناعي, الجلوتاثيونGSH, الالتهاب, فرط الهوموسستئين, مضاد التهاب المفاصل, السرطان.

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Title: Biological activities of *Phoenix dactylifera* and Treg in Rheumatoid arthritis induced by hyperhomocysteinemia and formalin and on tumoral process

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