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Biological activities of some medicinal plants on rheumatoid arthritis induced by hyperhomocysteinemia and formalin and on tumoral process

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CHAPTER III RESULTS AND DISCUSSION

III. Results and Discussion

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International publications

Aribi B., Zerizer S., and Kabouche Z., (2013). Immunomodulatory activity of *Argania spinosa* seeds. *International Journal of Pharmacy and Pharmaceutical Sciences,* 5(3) 488-491.

Aribi B., Zerizer S., Kabouche Z., Screpanti I. and Palermo R. (2015). Effect of Argania spinosa oil extract on proliferation and Notch1 and ERK1/2 signaling of T-cell acute lymphoblastic leukemia cell lines. *Food and Agricultural Immunology*, DOI: 10.1080/09540105.2015.1104654. 1-8.

AD: Autoimmune Diseases

AdoHcy: S-Adenosyl Homocysteine

AdoMet: S-Adenosyl Methionine

ALL: Acute Lymphoblastic Leukemia

AML: Acute Myelogenous Leukemia

ANOVA: Analysis of Variance

APC: Antigen Presenting Cells

ATP: Adenosine Tri-Phosphate

B-ALL: B-cell Acute Lymphoblastic Leukemia

BD: Behçet's Disease

BSA: Bovine Serum Albumin

C5a: Complement Component 5a

CAM: Cell Adhesion Molecules

CD11: Cluster Differentiation 11

CD18: Cluster Differentiation 18

cIMT: Carotid Intima-Media Thickness

CLL: Lymphocytic Leukemia

hs-CRP: High Sensibility C-Reactive Protein Test

CVD: Cardiovascular diseases

DMSO: Dimethyl Sulfoxid

DPPH: 1,1-Diphenyl-2-Picryl-Hydrazyl free radical

ERK: Signal-Regulated Kinase

ESR: Erythrocyte Sedimentation Rate

H.E: Hematoxylin Eosin

Hcy: Homocysteine

HHcy: Hyperhomocysteinemia

i.p: Intraperitoneal

IBD: Inflammatory Bowel Diseases

ICCAM: Intracellular Adhesion Molecules

ICN: Intracellular Notch1

IL-1: Interleukin 1

IL-4: Interleukin 4

IL-8: Interleukin 8

LD50: Medial Lethal Dose

LDL: Low Density Lipoproteins

mAb: Mouse Monoclonal Antibody

MCP-1: Monocyte Chemoattractant Protein-1

MeAP: Methionine aminopeptidase

Methyl-THF: N-5-Methyltetrahydrofolate

MHC: Major Histocompatibility Complex

MS: Methionine Synthase

MTHFR: Methylentetrahydrofolate Reductase

MTT: Microculture Tetrazolium

NADPH: Nicotinamide Adenine Dinucleotide Phosphate

NK: Natural Killer

OECD: Organisation for Economic Cooperation and Development

PAF: Platelets Activating Factor

RA: Rheumatoid Arthritis

SD: Standard Deviation

SDS-PAGE: Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis

SLE: Systemic Lupus Erythematosis

SPSS: Statistical Package for Social Science

T-ALL: T-cell Acute Lymphoblastic Leukemia

THF: Tetrahydrofolate

TNFα: Tumor Necrosis Factor

Tregs: Regulatory T cells

An immune reaction is a complex reaction to tissue injury and infection, characterized by the classic response of redness, heat, swelling, pain and loss of function and the immune system consists of cells and soluble factors that mediate the reaction in order to eliminate the immune stimulus and initiate the process of immunological memory. Diseases of immunity can occur due to inappropriate inflammation or when the normal immune response progresses to chronic inflammation, either because of a long-term inappropriate response to stimuli (allergies) or because the offending agent is not removed (autoimmunity) (Koelink et al., 2012).

It is well known that acute inflammation is part of the defense response, in some cases acute inflammation fails to resolve, this is the case seen in chronic inflammatory conditions which mediate a wide variety of diseases, including cardiovascular diseases, cancers, diabetes, arthritis, Alzheimer's disease, pulmonary diseases, and autoimmune diseases (Medzhitov, 2008).

Rheumatoid arthritis is a chronic, inflammatory autoimmune disorder where the immune system targets and attacks the joints. It is a disabling and painful inflammatory condition, which can lead to substantial loss of mobility due to pain and joint destruction. Rheumatoid arthritis is a systemic disease, often affecting extra-articular tissues throughout the body (M.Saleh and Zangor, 2009).The suggested progression mechanism of rheumatoid arthritis develops in three stages. The first stage is the swelling of the synovial lining, causing pain, warmth, stiffness, redness and swelling around the joints. Second is the rapid division and growth of cell, or pannus, which causes the synovium to thicken. In the third stage, the inflamed cell releases enzyme that may digest the bone and cartilage, often causing the joints to loses its shape and alignments, more pain and loss of movements (Sueki and Brechter, 2009).

Because of the association of inflammation with the elevation of circulating homocysteine, it is not surprising that varying degrees of hyperhomocysteinemia are detectable in all inflammatory diseases. In fact, detection of hyperhomocysteinemia has been reported not only in patients with well-known inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease, but also in psoriasis, a chronic inflammatory skin disease. Hyperhomocysteinemia has also been reported in patients with cardiovascular disease, with type 2 diabetes, with chronic kidney disease and cancer. All these clinical disorders were only being recognized as inflammatory diseases in recently years (Wu, 2008).

Furthermore, association between rheumatoid arthritis and hyerhomocysteinemia was reported by many authors, they found for example that older women with rheumatoid arthritis had poor vitamin B-6 status and elevated plasma homocysteine concentrations compared to healthy control women, despite similar dietary intakes of vitamin B-6 and protein. Individuals with rheumatoid arthritis may display altered metabolism of vitamin B-6 that results in poor status of this nutrient. Furthermore, the poor vitamin B-6 status may explain why individuals with rheumatoid arthritis have increased plasma homocysteine concentrations and an increased risk of cardiovascular diseases (Woolf, 2008).

Several mechanisms for Hyperhomocysteinemia induced inflammation have been proposed. These include endothelial dysfunction, increased proliferation of smooth muscle cells, enhanced coagulability and increased cholesterol synthesis in hepatocytes. Endothelial dysfunction is considered to be one of the important mechanisms contributing to inflammation. It has been proposed that hyperhomocysteinemia-caused endothelial injury may be due to oxidative stress, attenuation of NO-mediated vasodilatation, and disturbance in the antithrombotic activities of the endothelium. On injury, endothelial cells are capable of producing various cytokines and growth factors that participate in inflammatory reactions (Wang et al., 2002). In addition, homocysteine can induce oxidative stress which favors lipid peroxidation and induces production of inflammatory factors such as MCP-1, IL-8, and TNFα, thus accelerating atherosclerosis (Lin et al., 2009).

In another side of chronic inflammation consequences, Cancer is the second leading cause of death in the world. It is an uncontrolled growth and spread of cells that can affect almost any part of the body. T-cell acute lymphoblastic leukemia is a highly aggressive hematologic malignancy characterized by the malignant clonal expansion of immature T-cell progenitors. T cell transformation is a multi-step process in which different genetic alterations cooperate to alter the normal mechanisms that control cell growth, proliferation, survival, and differentiation during thymocyte development (Van Vlierberghe and Ferrando, 2012).

It is well established that cell proliferation and differentiation is well controlled by a wide range of proteins, in this context we find NOTCH proteins and ERK proteins. Signaling due NOTCH proteins family plays a key role in the normal development of many tissues and cell types, through diverse effects on differentiation, survival and proliferation that are highly dependent on signal strength and cellular context. Because perturbation in the regulation of

differentiation, survival and proliferation underlie malignant transformation, pathologic NOTCH signals potentially contribute to cancer development (Allenspach et al., 2002).

Furthermore, ERK1/2 is an important subfamily of mitogen-activated protein kinases that control a broad range of cellular activities and physiological processes. ERK1/2 can provide anti-apoptotic effects by down-regulating pro-apoptotic molecules via a decrease in their activity or a reduction of their protein expression by transcriptional repression. ERK1/2 can also promote cell survival by up-regulating anti-apoptotic molecules via enhancement of their activity or activation of their transcription (Lu and Xu, 2006).

The most commonly prescribed medication for inflammatory diseases treatment is anti-inflammatory drugs. Though the goal of these drugs has been to relieve pain and to decrease inflammation, to prevent destruction and to restore function, these drugs are known to produce various side effects including gastrointestinal disorders, immunodeficiency and humoral disturbances. Accordingly, reducing side effects should be considered while designing improved therapeutics for inflammatory diseases, besides enhancing medicinal effectiveness (Scott et al., 1998).

Throughout the ages, natural products have always been the mainstay of disease therapy, and are still considered to play an important role in modern medicine. Almost half of the drugs approved since 1994 are based on natural products. Along this line, plant derived compounds have great potential to be developed into anti-inflammatory, anti-arthritic, immunomodulatory and anticancer drugs because of their multiple mechanisms and low side effects (Anh Thuy Do, 2012).

This study investigated the mode of action of three Algerian plants namely *Argania spinosa, Citrullus colocynthis, Boswellia serrata* with the olive oil. *Argania spinosa* belongs to the family *Sapotaceae, Citrullus colocynthis* to *Cucurbitaceae* while *Boswellia serrata* belongs to *Burseraceae*. Many medicinal uses of the various parts of plants from these three families have been reported in traditional medicines. These have reportedly been used in the treatment of arthritis, inflammation, cancer and cardiovascular diseases (El Monfalouti et al., 2010; Marzouk et al., 2011; Ammon, 2006).

In this thesis we have attempted to focus these objectives:

- Testing the safety of use of *Argania spinosa, Citrullus colocynthis* and *Boswellia serrata* and determining the lethal doses using toxicity tests in animals.
- Evaluation of immunomodulatory effect of the crude extract of *Argania spinosa* seeds using carbon clearance assay.
- Assessment of the immunomodulatory activities of the oils extracted from *Argania spinosa, Citrullus colocynthis* and *Boswellia serrata* in comparison with olive oil in order to determine the most effective as immunomodulant agent.
- Evaluation of the analgesic effect of *Argania spinosa, Citrullus colocynthis* and *Boswellia serrata* oil extracts using acetic acid induced pain to confirm their action as anti-nociceptive agents.
- Evaluation of the anti-inflammatory activity of *Argania spinosa, Citrullus colocynthis* and *Boswellia serrata* oil extracts in acute model of inflammation by xylene induced oedema comparing to standard drugs.
- Development of a novel animal model of chronic inflammation consisting in combination between the classical model of arthritis which is formalin induced arthritis and the model of hyperhomocysteinemia which is methionine induced hyperhomocysteinemia.
- Assessment of the antiarthritic and anti-inflammatory effects of *Argania spinosa* seeds in two animal models of inflammation, formalin induced arthritis and formalin/methionine induced inflammation.
- Examination of the effect of *Argania spinosa* on inflammation through the measurement of plasma hs-CRP and homocysteine.
- Confirmation of the action of hyperhomocysteinemia and plant extract on joint inflammation by histological study.
- Evaluation of the antiproliferative activity of *Argania spinosa, Citrullus colocynthis* and *Boswellia serrata* oils using cell culture of human lymphoblastic leukemia cell lines.
- Test of the effect of the oils in some proliferation-related proteins (ERK1/2 and NOTCH1).

Chapter I

Literature Review

I.1. Inflammation

Inflammation is a usual protective response of living mammalian tissues to injury. It is the body‘s response to inactivate or raze the invading organism, to eliminate the irritant and to set the stage for repair (Sosa et al., 2002; Ferrero-Miliani et al., 2007; Bhitre et al., 2008; Highleyman, 2011).

Inflammation is a series of synchronized mechanism consisting of precise vascular, humoral and cellular events that is characterized by the extravasations of fluids, plasma and inflammatory leukocytes to the inflammation site. An array of chemical mediators such as histamine, serotonin, leukotrienes, prostaglandins and oxygen derived free radicals (O2-, OH-, ONOO-) are produced by inflammatory and phagocytic cells primarily in the sequences which contribute to the initiation of inflammation (Safayhi and Sailer, 1997; Tripathy and Grammas, 2009). Inflammation response occurs in two phases, these are acute and chronic phases.

I.1.1. Acute Inflammation

Acute inflammation lasts from few minutes to hours or one to two days. The basic signs of acute inflammation were redness, heat, swelling and pain (Jain and Bari, 2010). The process involved in acute inflammation can be explained in two main respects as vascular and cellular processes.

The vascular process takes place in the microvasculature and it becomes evident in 15-30 minutes after a stimulus of inflammation. It is primarily mediated by chemicals such as serotonin and histamine released from mast cells. It is characterized by local vasodilatations of capillaries resulting in increased blood flow to the inflamed site hence the redness and heat followed by increased vascular permeability leading to transudation of fluids, plasma and proteins into the site of inflammation causing interstitial oedema (Nathan, 2002).

Numeral chemostatic agents including bacterial products possessing amino terminal N-formyl methionyl groups, C5a complement fragment and chemokines like IL-8 together with other mediators such as histamine, serotonin, leukotriene B_4 and platelets activating factor (PAF) induce intense polymorphonuclear leukocytes infiltration in a matter of 30-60 minutes (Asako et al., 1992).

Neutrophils are the first inflammation cells that are enlisted at the site of inflammation (Phillipson and Kubes, 2011), neutrophil interact with endothelium in post capillary venules and this process involves the sequential capture rolling along the endothelium, transmigration through the vessel wall and additional movement in extravascular tissue (Muller, 2003). These cascades of events are governed by cell adhesion molecules (CAM) which include selectin, integrin (CD11 and CD18) and intracellular adhesion molecules (ICCAM-1 and-2) (Palmblad and Lerner, 1992).

I.1.2. Chronic Inflammation

Chronic inflammation is marked by the infiltration of mononuclear cells (macrophages and lymphocytes), proliferation of fibroblasts, collagen fibers and formation of connective tissues which at the end of the day lead to the formation of granuloma. Tissues degeneration is an event involved in the chronic inflammation through the mediation activity of reactive oxygen, nitrogen species and protease released from infiltrated inflammatory cell (Winyard et al., 2000). These oxidants are potential mutagen, therefore causes permanent genomic alterations such as point mutations, deletion or rearrangement in case of repeated tissue damage and regeneration (Wiseman and Halliwell, 1996), for example some data indicate that p53 mutations occur in rheumatoid arthritis and inflammatory bowel diseases (IBD) at rates comparable to those in tumour (Yamanishi et al., 2002).

I.1.3. Resolution of Inflammation

The resolution of inflammation is a process in which all infiltrating inflammatory cells are removed from the site of inflammation and tissue homeostasis is restored. It is a complex active process in which several biochemical pathways are specifically regulated (Serhan, 2004).

Recent work has shown that during inflammation neutrophils can change their phenotype and start producing anti-inflammatory mediators to actively switch off acute inflammation and promote resolution. To date there are three types of molecules known to actively resolve inflammation: lipoxins, resolvins and protectins (Serhan, 2004). Lipoxins are derivates of arachidonic acid (Stajszczyk et al., 1993). Proinflammatory cytokines IL-1β and IL-4 promote the expression of lipoxins. The main action of lipoxins is to decrease neutrophils recruitment to the site of inflammation. Resolvins and protectins are recently

identified as anti-inflammatory molecules. They are very potent anti-inflammatory mediators; their mode of action includes decrease of production of pro-inflammatory molecules and down-regulation of recruitment of inflammatory cells to the site of inflammation (Serhan et al., 2007).

I.2. Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a common, chronic, systemic, autoimmune inflammatory disease that causes disability, chronic ill-health, and premature mortality. It targets synovial joints, in which there is a massive accumulation of blood-borne cells such as T cells and macrophages. Blood vessels are formed to support this new tissue and the whole mass is called a pannus. Progressive erosion to cartilage and bone leads to disability in patients (Leung, 2007).

I.2.1. Pathogenesis

The initiating events that lead to the disease still unknown, studies have demonstrated that joint damage is a result of chronic inflammation of the synovial membrane. The synovial membrane becomes hyperplastic with cellular infiltrates, which include macrophages, mast cells, CD4+ T cells, CD8+ T cells, B cells, plasma cells, natural killer (NK) cells and NKT cells. Synovitis is associated with extensive cellular infiltrates - pathogenesis of disease is thought to driven by antigen specific responses. This can be partly thought to be due to antigen presenting cells (APC) interacting with T cells through their MHC and T cell receptor (TCR) receptor respectively. This results not only in T cell activation, via co-stimulatory signals mediated through CD80/86 or CD28-B7 receptor (Van Boxel and Paget, 1975), but also in the activation of the APC. In response to activation, T cells and APC (macrophages or dendritic cells, or potentially B cells) produce pro-inflammatory cytokines, such as TNFα, IL-6, IL-12, IL-23 and IL-1 (Brennan et al., 1989; Burger et al., 2006; Nishimoto and Kishimoto, 2006). This cytokine environment can promote the differentiation of naïve T cells to alternative phenotypes such as T helper 1 and Th 17, an IL-6 dependent T cell phenotype that produces amongst other things IL-17. Cytokines are implicated at every stage of rheumatoid arthritis pathogenesis from maintaining a chronic inflammatory synovitis to promoting articular destruction, through the subsequent activation of osteoclasts, chondrocytes and synovial fibroblasts (Jimenez-Boj et al., 2005).

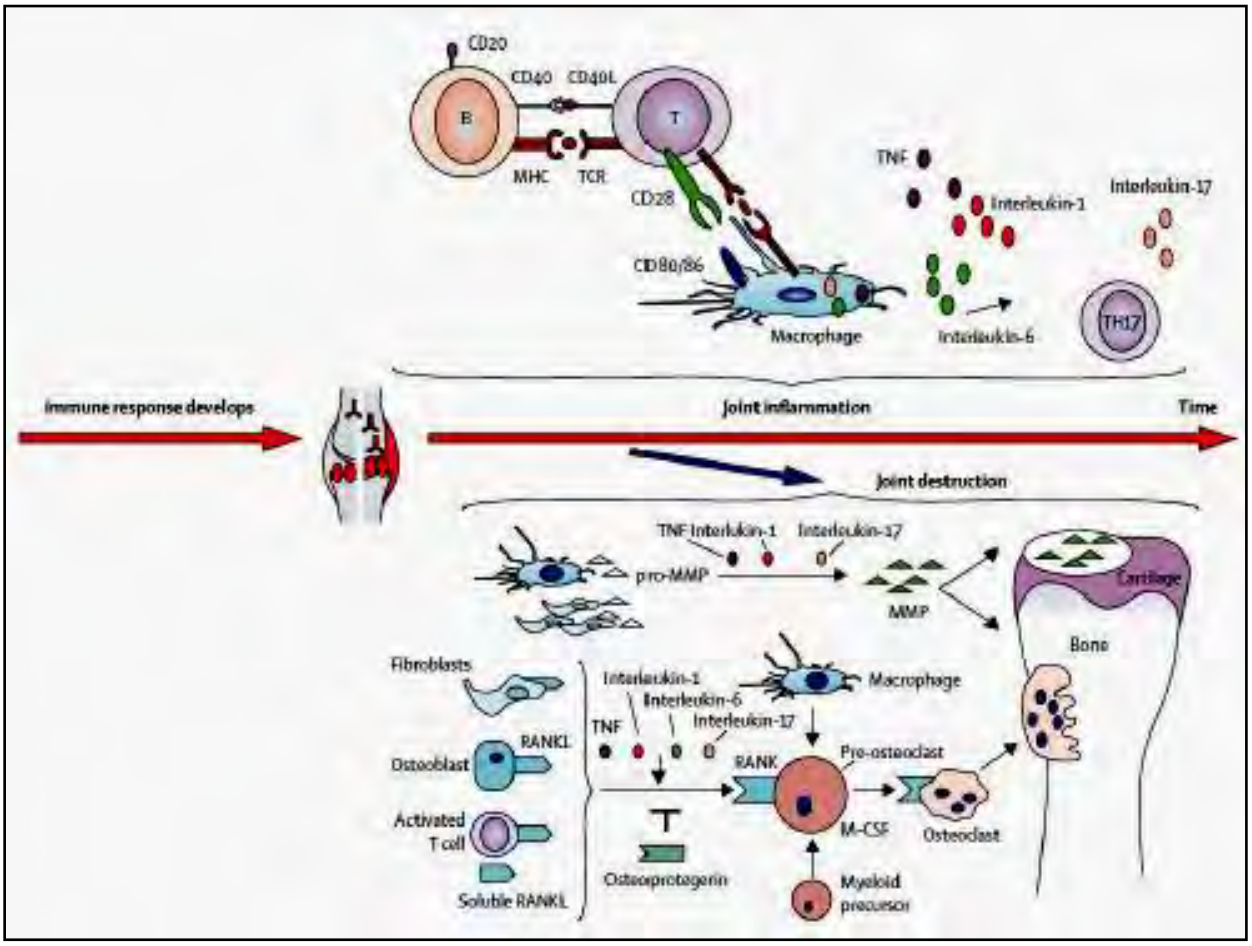

Figure 01. Immunological pathway of the arthritic joint, upper part show joint inflammation and lower part joint destruction (Klareskog et al., 2009).

I.2.2. Rheumatoid Arthritis and vascular risk

There is now extensive evidence based on observational cohort and case control studies that rheumatoid arthritis is strongly associated with cardiovascular morbidity and mortality (Wallberg-Jonsson et al., 1999; Farragher et al., 2008).

The results of a recent systematic review showed that rheumatoid arthritis is associated with a 60% increase in the risk of cardiovascular-related death (Meune et al., 2009). In particular, inflammation in rheumatoid arthritis (RA) is now considered as an independent risk factor for the development of atherosclerosis (Del Rincón et al., 2001).

Both RA and atherosclerosis are complex polygenic diseases with shared disease mechanism. There is increasing evidence that chronic inflammation and immune dysregulation contributes to accelerated atherogenesis and plays a role in all stages of atherosclerosis (Meune et al., 2009).

In a study to evaluate recent onset RA, patients were evaluated for sub-clinical atherosclerosis using ultrasonography to measure the carotid intima-media thickness (cIMT). The CRP level in these patients correlated with increased (cIMT) indicative of the presence of plaques in the vessel wall of the patients (Cuomo et al., 2004; Hannawi et al., 2007).

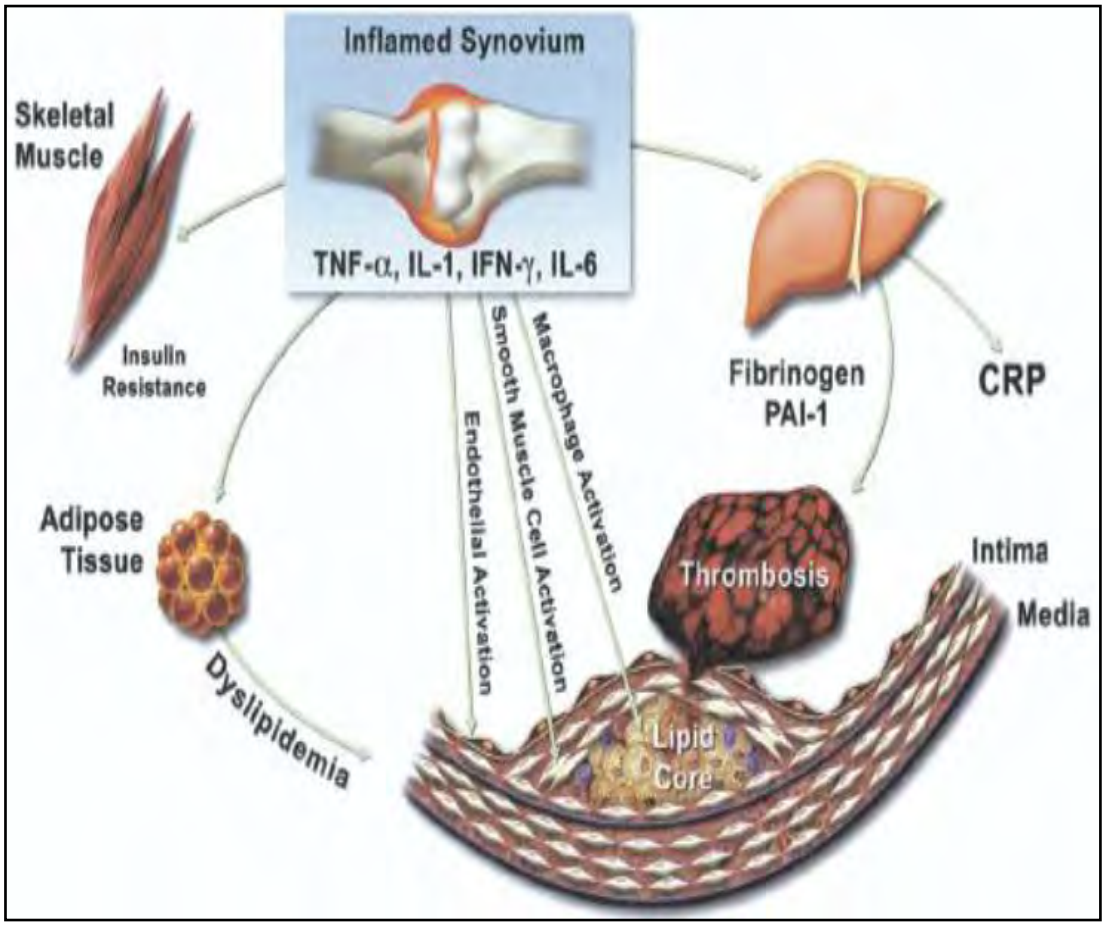

Figure 02. Inflammatory pathways by which mediators of synovitis including TNFα may alter biology and risk factor for atherosclerosis including insulin resistance, dyslipedimeia, fibrinogen, and increase the production by the liver of CRP (Libby, 2008).

I.3. Hyperhomocysteinemia

I.3.1. Historical overview

The arteriosclerotic and thrombotic complications of the severe inherited form of hyperhomocysteinemia (HHcy) was first described in 1960's based on postmortem findings of arteriosclerotic disease in patients with metabolic defects accompanied by high blood homocysteine levels. Elevated plasma homocysteine level is associated with the development of coronary artery disease and strongly predicts both cardiac mortality and restenosis after percutaneous transluminal coronary angioplasty. It is also an independent risk factor for cerebral vascular stenosis and ischemic stroke. Several lines of evidence suggest that this association represents a cause-and-effect relation and elevated Homocysteine (Hcy) levels are not simply a marker for another risk factor. A variety of conditions can lead to elevated homocysteine levels, but the relation between high Hcy levels and vascular disease is present regardless of the underlying metabolic cause. Moreover, vascular lesions, similar to those found in human, are known to develop in animal models of HHcy (Carey et al., 1968; McCully, 1969; Wilcken and Wilcken, 1976; Ueland and Refsum, 1989).

I.3.2. Homocysteine metabolism and causes of HHcy

Homocysteine (Hcy) is a thiol-containing non-protein forming amino acid that is formed from methionine, an essential amino acid found in large quantities in meat. The symmetrical disulfide of homocysteine is termed "homocystine," both names being chosen to indicate that each carbon chain of these compounds contained one -CH2- group more than those of cysteine and cystine, respectively. Normal human plasma contains total concentrations of homocysteine and its derivative disulfides close to 10 μmol/L (~2% as the thiol form, 98% is in the form of disulfides, from which 75% is bound to proteins, mainly albumin) (Yoo et al., 1998; Kittner et al., 1999; Eikelboom et al., 2000).

Metabolism of Hcy stands at the intersection of two pathways: remethylation to methionine, which requires folate and vitamin B12; and transsulfuration to cystathionine, which requires pyridoxal-5'-phosphate. In remethylation, Hcy acquires a methyl group from N-5-methyltetrahydrofolate or to form methionine. The reaction with N-5-methyltetrahydrofolate occurs in all tissues and is vitamin B12 dependent. A considerable proportion of methionine is then activated by ATP to form S-adenosylmethionine (AdoMet). AdoMet serves primarily as a universal methyl donor to a variety of acceptors. S-

adenosylhomocysteine, the by-product of these methylation reactions, is subsequently hydrolyzed, thus regenerating Hcy, which then becomes available to start a new cycle of methyl-group transfer. In the transsulfuration pathway, Hcy condenses with serine to form cystathionine in an irreversible reaction catalyzed by the B6–containing enzyme, cystathionine ß-synthase. Cystathionine is hydrolyzed by a second B6-containing enzyme, -cystathionase, to form cysteine and -ketobutyrate. Excess cysteine is oxidized to taurine or inorganic sulfates or is excreted in the urine. Thus, in addition to the synthesis of cysteine, this transsulfuration pathway effectively catabolizes excess Hcy, which is not required for methyl transfer (Fukagawa et al., 2000; Mudd et al., 2000).

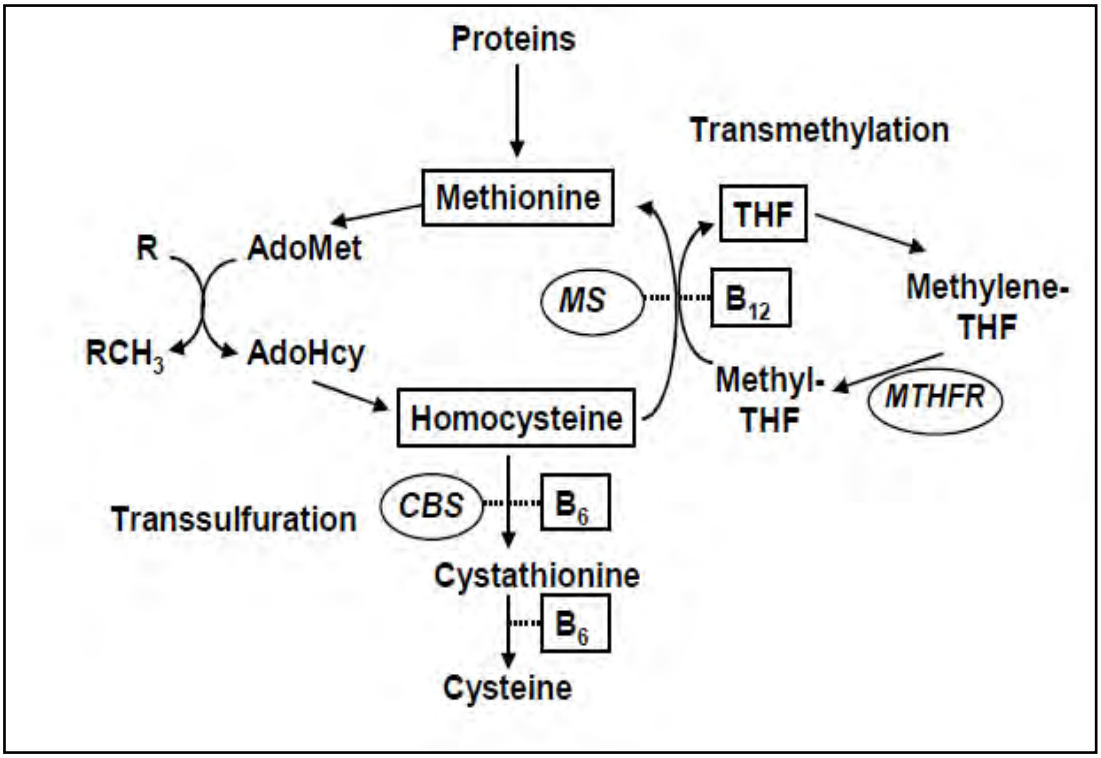

Figure 03. Simplified representation of homocysteine metabolism (Ungvari, 2001).

S-adenosylmethionine **(AdoMet);** Sadenosylhomocysteine **(AdoHcy);** tetrahydrofolate **(THF);** N-5-methyltetrahydrofolate **(Methyl-THF);** methylentetrahydrofolate reductase **(MTHFR);** methionine synthase **(MS)**

I.3.3. Homocysteine and immuno-inflammatory activation

The immuno-pathological processes supporting Autoimmune Diseases (AD) frequently produce a relevant inflammatory state that is representative of the disease activity. The most recent data seem to suggest a strict and bi-univocal relationship among immune activation, inflammation, and homocysteine (Hcy) levels. As a consequence, an explanatory mechanism contributing to the above mentioned high prevalence of HHcy in patients with AD may be provided. Moreover, a putative role for Hcy in the progression of the disease associated inflammatory damage may also be suggested (Lazzerini et al., 2007).

Several studies demonstrated that in the course of AD the degree of inflammation correlates with plasma concentration of Hcy. The most convincing data deal with rheumatoid arthritis (RA) patients, in which various authors reported a significant correlation between the level of Hcy and the expression of immuno-inflammatory markers. More in detail, a positive relationship was found between the concentration of Hcy and some bio-humoral parameters of inflammation, such as the circulating levels of soluble receptors for different cytokines (IL-2sRα, sTNF-R75) , adhesion molecules (sICAM-1), and C-reactive protein (CRP) (Wallberg-Jonsson et al. 2002; Schroecksnadel et al., 2003; Yxfeldt et al., 2003; Lopez-Olivo et al., 2006). An association exists also between HHcy and some clinical features of RA, such as accumulated disease activity, and higher radiological damage. Moreover, in 37 RA patients, Chiang et al. demonstrated that the increase in Hcy levels after methionine load correlated with erythrocyte sedimentation rate (ESR) and CRP levels, disability score, degree of pain, and number of painful and swollen joints (Chiang et al., 2003; Lopez-Olivo et al., 2006).

Among the other AD, a role for chronic inflammation in the pathogenesis of HHcy has been demonstrated in patients with Behçet's disease (BD), in which a significant and positive correlation between Hcy and CRP levels was found (Yesilova et al., 2005); additionally, Chung et al. reported higher concentrations of Hcy and CRP in systemic lupus erythematosis (SLE) patients with metabolic syndrome (Chung et al., 2007). The mechanisms involved in the development of HHcy as a consequence of a persistent immuno-inflammatory activation, although not completely clarified, are probably multiple and intriguing. However, the role of the depletion in vitamins implicated in the Hcy metabolism, such as folate, B12 and B6 seems particularly relevant (Schroecksnadel et al., 2003).

I.4. Tumorigenesis

Cancer become the second ranking cause of death worldwide led only by heart disease. from an immunologic perspective, cancer cells can be viewed as altered self cells that have escaped normal growth regulating mechanisms (kindt, 2007).

I.4.1. T Cell Lymphoblastic Leukemia

Leukemia is the unchecked proliferation of an abnormal clone of hematopoietic cells. Typically, leukemic cells respond poorly or inappropriately to regulatory signals, display aberrant patterns of differentiation, or even fail to differentiate. Furthermore, they sometimes suppress the growth of normal lymphoid and myeloid cells (kindt, 2007).

Leukemia can arise at any maturational stage of any one of the hematopoietic lineages. Lymphocytic leukemias display many characteristics of cells of the lymphoid lineage; another broad group, myelogenous leukemias, have attributes of members of the myeloid lineage. Aside from lineage, many leukemias can be classified as acute or chronic. Some examples are acute lymphoblastic leukemia (ALL), the most childhood leukemia; acute myelogenous leukemia (AML), found more often in adults than in children; and chronic lymphocytic leukemia (CLL), which is rarely seen in children (kindt, 2007).

T-cell acute lymphoblastic leukemia (T-ALL) is a highly aggressive hematologic malignancy that represents 10% to 15% of pediatric and 25% of adult acute lymphoblastic leukemia cases. Compared to B-cell acute lymphoblastic leukemia (B-ALL), patients with T-ALL commonly present large tumor burdens at diagnosis and invariably poor outcomes even after intensified chemotherapy. The specific biological and molecular mechanisms that account for the aggressiveness and poor therapy response in T-ALL remain unclear and T-ALL cells induced immune tolerance is an important hypothesis. Some reports showed that T-ALL cells are derived from regulatory T cells (Tregs), which suppress the reaction of lymphocytes to tumor antigens and induce immune tolerance and malignant neoplasm progression (Luo, 2013).

I.4.2. Proliferation related proteins NOTCH 1 and ERK

I.4.2.1. NOTCH 1 proteins

T-cell acute lymphoblastic leukemia (T-ALL) is characterized by the malignant clonal expansion of immature T-cell progenitors. More than 50% of cases of T-ALL involve somatic activating mutations of NOTCH1, a potent regulator known to play an oncogenic role in many malignancies, affecting proliferation, invasion, chemoresistance, angiogenesis and cell fate determination (Screpanti et al., 2003).

In the NOTCH1 signaling pathway, NOTCH1 transmembrane receptors become activated when ligands bind to their extracellular domains. This ligand binding results in two consecutive proteolytic cleavage events that liberate the intracellular NOTCH1 (ICN), which enters the nucleus and interacts with the DNA-binding protein CSL (CBF1/RBP-J, Su(H), Lag-1) to regulate expression of downstream genes. In murine models, constitutive activation of NOTCH1 signalling induced T-ALL, demonstrating the key role of NOTCH1 in the pathogenesis of T-ALL (Zou et al., 2013).

I.4.2.2. ERK1/2 proteins

The extracellular signal-regulated kinase (ERK) family is MAP kinases which consist of cytoplasmic protein serine/ threonine kinases that participate in the transduction of signals from the surface to the interior of the cell. The MAP kinases group includes also the p38 kinase family, and the c-Jun N-terminal kinase. ERK1/2 are related protein-serine/threonine kinases that participate in the Ras-Raf-MEK-ERK signal transduction cascade. This cascade participates in the regulation of a large variety of processes including cell adhesion, cell cycle progression, cell migration, cell survival, differentiation, metabolism, proliferation, and transcription (Roskoski, 2012).

ERK1/2 can provide anti-apoptotic effects by downregulating pro-apoptotic molecules via a decrease in their activity or a reduction of their protein expression by transcriptional repression. ERK1/2 can also promote cell survival by upregulating anti-apoptotic molecules via enhancement of their activity or activation of their transcription (Lu and Xu, 2006).

I.5. Medicinal plants

Natural products are believed to be important source of new chemical substances which have potential therapeutic effects. Medicinal plants, one of the important sources, are extensively investigated both *in vitro* and *in vivo* to examine their potential activities (Jalaiah, 2014). It has been estimated that less than 1 – 10% of the large diversity of 250000 – 500000 plant species on the earth have been studied chemically and pharmacologically for their medicinal properties (Farnsworth and Soejarto, 1991; Verpoorte, 2000).

Numerous medicinal plants extracts and products continue to be useful in the prevention and control of a wide range of inflammatory disorders. For example, the extract of *Phoenix dactylifera* has been shown to possess immunostimulatory effect in reticuloendothelial system of treated mice (Kehili et al., 2014); *Stachys Mialhesi* extract appears to prevent endothelial alteration *in vivo* (Benmebarek et al., 2013); Aqueous extract of *Carica papaya* leaves exhibits anti-tumor activity and immunomodulatory effects (Otsuki et al., 2010); while *Aristolochia Bracteata* extract exerts antiarthritic activity in experimental animals (Chitme and Patel, 2009).

I.5.1. Argania spinosa

I.5.1.1. Description

Argan tree or *Argania spinosa* (L). Skeels, in arabic الأرغان, is a rustic species, xero-thermophilic, which belongs to the tropical family of Sapotaceae, hence it is the only Northern representative in the Mediterranean region (Algeria and Morocco) where its endemism is marked at this region (Kechebar et al., 2013).

The Argan tree is exploited essentially for its fruits. The endosperm seed of the fruit constitutes a good potential source of edible oil for human consumption and is endowed with important medicinal properties (Khallouki et al., 2005).

I.5.1.2. Botanical classification

Kingdom Plantae

Sub-division Angiospermae

Class Dicotylédonae

Sub-class Asteridae

Order Ericales

Family Sapotaceae

Genus *Argania*

Species *Argania spinosa*

I.5.1.3. Therapeutic properties

Argan oil is a good ally for protecting the skin from fungal growths and sunburn, as well as an antiseptic for minor wounds. It is also used to treat skin diseases such as neurodermatitis and psoriasis, due to its analgesic and antiinflammatory properties. It has been demonstrated that polyphenols and sterols extracted from virgin argan oil exert an antiproliferative and pro-apoptotic effect on prostate cancer cell lines (Bennani et al., 2007).

One of the most interesting nutritional properties of argan oil is its ability to reduce harmful cholesterol levels (LDL) (Drissi et al., 2004) and to treat high blood pressure (Berrougui et al., 2004).

I.5.2. Citrullus colocynthis

I.5.2.1. Description

Citrullus colocynthis, in arabic الحنظل belonging to the family of Cucurbitaceae and growing in arid areas, is endemic in the South of Tunisia. This medicinal plant popularly known as Handhal, Hdaj or Dellaa El-Wad, is widely used in Tunisian folk medicine for treating many diseases such as hypertension and inflammation diseases, including rheumatism and rheumatoid arthritis (Marzouk et al., 2011).

I.5.2.2. Botanical classification

Kingdom Plantae

Sub-Kingdom Tracheobiontae

Division Spermatophytae

Sub-Division Angiospermae

Class Dicotylédonae

Sub-Class Dialypétalae

Order Violales

Family Cucurbitacea

Genus *Citrullus*

Species *Citrullus Colocynthis*

I.5.2.3. Therapeutic properties

Some studies have demonstrated the medicinal effect of *Citrullus colocynthis* as hypoglycemic agent in alloxan induced diabetic rats (Sangameswaran et al., 2008).

Another study proves that flavonoids extracted from *Citrullus colocynthis* extract exhibited significant anti-oxidant effect in (DPPH) assay (Delazar et al., 2006).

Previous investigation demonstrated that *Citrullus colocynthis* has antitumor effect on human breast cell cancer (Tannin-Spitz et al., 2007).

I.5.3. Boswellia serrata

I.5.3.1. Description

The family of Burseraceae is represented in the plant kingdom with 17 genera and 600 species, widespread in all tropical regions. The species are often a predominant component of the vegetation in dry, lowland areas. Some species of the two most important genera of this family, *Commiphora* and *Boswellia*, produce resins that are of considerable commercial value as raw materials of balm, myrrh and frankincense (Vollesen, 1989).

Olibanum; gum olibanum (الؤبان)incense or frankincense, are the common names given to the oleo-gum resin that exudes from incisions in the bark of trees of *Boswellia* (Burseraceae) (Blaschek et al., 1998).

I.5.3.2. Botanical classification

Kingdom	Plantae
Division	Spermatophyta
Sub-division	Angiospermae
Class	Anacardiales
Order	Sapindales
Family	Burseraceae
Genus	*Boswellia*
Species	*Boswellia serrata*

I.5.3.3. Therapeutic properties

Boswellia resin and its individual components have shown various biological activities; including anti-inflammatory activity (Sharma et al., 1989); leukotriene biosynthesis- inhibitory activity (Ammon et al., 1991); and anti-tumor activity (Shao et al., 1998). Another investigation showed that the alcoholic extract of the oleogum resin and boswellic acids (a mixture of triterpenoids obtained from the oleogum resin *Boswellia serrata*), influenced both cellular and humoral immune responses in rats and mice (Sharma et al., 1988 and 1996).

Figure 04. Photography of *Argania spinosa* tree and its fruits -place: la maison forestière de Taouref Bouaam, Tindouf, Algeria- (Ould Safi, 2014).

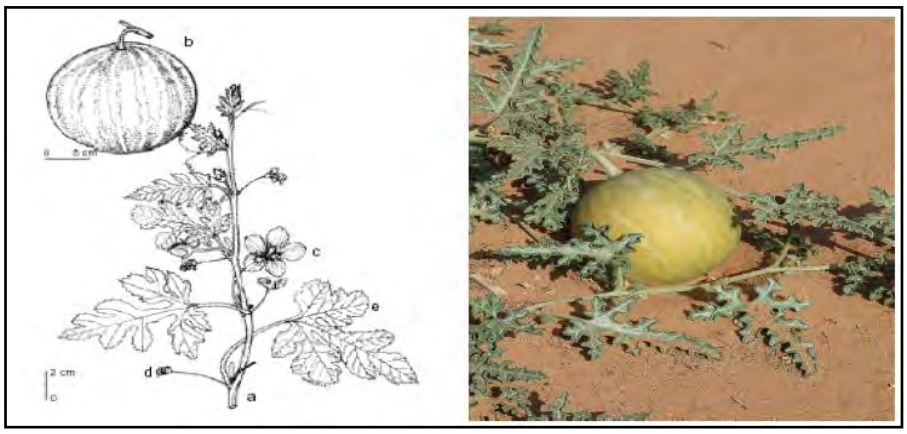

Figure 05. *Citrullus Colocynthis.*
a = branch ; b = fruit ; c = flower; d = tendril; e = leaf (Zoro et al., 2003).

Figure 06. *Boswellia serrata* (Aksamija, 2012).

Chapter II

Materials and Methods

II.1. Evaluation of the acute toxicity of the plants extracts

II.1.1. Used materials

II.1.1.1. Plant materials

II.1.1.1.a. Collection

Argania spinosa seeds were collected from Tindouf located in South west of Algeria, in December 2011.

Citrullus colocynthis seeds were collected from Tiguentourine (Ain Amenas) located in south of Algeria in November 2011.

Boswellia serrata gums were brought from Tiguentourine in December 2011.

II.1.1.1.b. Preparation

The crude extract of *Argania spinosa* seeds was obtained by cutting the fruits into pieces to obtain seeds, and then the seeds were subjected to size reduction to a coarse powder using a mechanical grinder. The powder (crude extract) was then used for the acute toxicity test.

The *Citrullus colocynthis* seeds oil was obtained by extraction with petroleum ether. 200g of the seeds (not grilled) were powdered and extracted in a soxhlet using petroleum ether. After evaporation of the solvent under reduced pressure, we obtained the respective oil.

The oil extracted from *Boswellia serrata* gum was obtained by hydrodistillation with 0.8 % yield.

II.1.1.2. Animals

Female Wistar rats weighing between 140-170g were used in this study. The animals were obtained from Central Pharmacy Institute of Constantine, Algeria. The animals were housed in polypropylene cages with soft wood shaving as bedding; they were maintained in the animal house under standard laboratory conditions of temperature and humidity with 12h light-dark cycle.

They were fed with normal commercial pellet diet (LA RATION, Bouzeriaa) and water *ad libitum*. The animals were acclimatized to laboratory conditions for one week prior to experiment.

II.1.1.3. Experimental procedure

- ✓ **Up and down method**

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 of 2000 mg/kg of the crude extract of *Argania spinosa* seeds was used in five female adult rats, the dose was given to the first rat, and this rat was observed for mortality and clinical signs (unusual aggressiveness, sedation, somnolence, twitch, tremor, catatonia, paralysis, convulsion, prostration and unusual locomotion) for the first hour, then hourly for 3 hours and, finally periodically until 48 h. If the animal survived, then four additional animals were given the same 2000 mg/kg dose. All of the experimental animals were maintained under close observation for 14 days, and the number of rats that died within the study period was noted. The LD_{50} was predicted to be above 2000 mg/kg if three or more rats survived.

The same procedure was used to evaluate the acute toxicity of *Boswellia serrata* and *Citrullus colocynthis* extracts.

II.2. Evaluation of the immunomodulatory activity of the medicinal plants by carbon clearance rate test

II.2.1. Evaluation of the immunomodulatory activity of *Argania spinosa* seeds

II.2.1.1. Used materials

II.2.1.1.a. Plant material

Crude extract of *Argania spinosa* seeds obtained by the method previously described in the up and down test was used in this study.

II.2.1.1.b. Animals

Adult male *Mus Musculus* mice (2.5- 3 month old) from central pharmacy Institute, Constantine, Algeria, weighing between 28 and 35g were used for determination of the phagocytic activity of *Argania spinosa* seeds.

The animals were obtained from Central Pharmacy Institute of Constantine, Algeria. The animals were housed in polypropylene cages with soft wood shaving as bedding; they were maintained in the animal house under standard laboratory conditions of temperature and humidity with 12h light-dark cycle. They were fed with normal commercial pellet diet (LA RATION, Bouzeriaa) and water *ad libitum.* The animals were acclimatized to laboratory conditions for ten days prior to experiments.

II.2.1.1.c. Experimental procedure

- ✓ **Macrophages phagocytosis using carbon clearance rate assay in mice**

The clearance rate of carbon was measured by the method of (Cheng et al., 2005).

Animals were divided into five groups, consisting of six mice in GI, GII, GIII, GIV and GV. Group I (control) was given 0,9% Nacl (0,5 ml/mouse i.p.), Groups II-III-VI and V were administered by intraperitoneal (i.p) injection with different concentrations of *Argania spinosa* crude extract (30, 50, 100 and 150 mg/kg/) respectively (table 01).

After 48h of i.p injection of the treatment, the mice were administered with carbon ink suspension at a dose of (0.1ml/10g) through the tail vein; the mixture consisted of black carbon ink 3ml, saline 4ml and 3% gelatine solution 4ml.

Table 01. Treatment of mice in carbon clearance rate test

Experimental groups	Treatment	Number of mice	Dose
GI	Nacl 0,9%	6	0,5 ml/mouse
GII	*Argania spinosa* crude extract	6	30mg/kg
GIII	*Argania spinosa* crude extract	6	50mg/kg
GIV	*Argania spinosa* crude extract	6	100mg/kg
GV	*Argania spinosa* crude extract	6	150mg/kg

✓ **Blood samples**

Blood samples were collected from retro-orbital plexus by using glass capillaries at an interval of 5 min and 15 min after the injection of ink solution.

Blood samples were added to 4ml of 0,1% sodium carbonate solution to lyse the erythrocytes and the absorbance of the samples was measured at 675 nm using a spectrophotometer.

After the last blood sampling the animals were sacrificed and the liver and spleen dissected and weighed immediately in the wet state.

✓ **Calculation**

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 ($t_{1/2}$, min). These are calculated by means of the following equations

$$K = \frac{\log OD1 - \log OD2}{t_2 - t_1}$$

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

$$t_{1/2} = 0{,}693/K$$

Where OD1 optical density at time 1 and OD2 optical density at time 2

II.2.2. Comparative screening of the immunomodulatory activities of the oils extracted from *Argania spinosa, Boswellia serrata*, *Citrullus colocynthis* and the virgin olive oil

II.2.2.1. Used materials

II.2.2.1.a. Plant materials

The oils extracted from *Argania spinosa* seeds, *Citrullus colocynthis* seeds, and *Boswellia serrata* gums and the commercial virgin olive oil were used in this study.

The oil extracted from *Argania spinosa* seeds was obtained by extraction with petroleum ether. 200g of the seeds (not grilled) were powdered and extracted in a soxhlet using petroleum ether. After evaporation of the solvent under reduced pressure, we obtained the respective oil.

The extraction of oils from *Citrullus colocynthis* and *Boswellia serrata* was precedently demonstrated in the first experiment.

II.2.2.1.b. Animals

Male and female Wistar rats weighing between 150-200g were used in this study.

The animals were obtained from Central Pharmacy Institute of Constantine, Algeria. The animals were housed in polypropylene cages with soft wood shaving as bedding; they were maintained in the animal house under standard laboratory conditions of temperature and humidity with 12h light-dark cycle. Females were non-pregnant and housed separately from males. They were fed with normal commercial pellet diet (LA RATION, Bouzeriaa) and water *ad libitum*. The animals were acclimatized to laboratory conditions for two weeks prior to experiment.

II.2.2.1.c. Experimental procedure

- ✓ **Macrophages phagocytosis using carbon clearance rate assay in rats**

The carbon clearance rate test was performed as described by (Hajra et al., 2011) with some modifications.

Animals were divided into 9 groups of 5 rats in each group.

Group I animals served as control and received flour (0,5mg/mouse per orally), group II-IX animals were treated with *Argania spinosa, Citrullus colocynthis, Boswellia serrata* and olive virgin oils with two different doses for each oil (table 02).

All the animals were treated as above from day 1 to day 3. On third day of treatment animals of the entire groups received an intravenous injection via tail vein (0,1ml/10g body weight) of Indian ink dispersion; the mixture consisted of black carbon ink 3ml, saline 4ml and 3% gelatine solution 4ml.

Table 02. Treatment of rats in carbon clearance rate test

Experimental groups	Treatment	Number of rats	Dose
GI	Flour	5	0,5mg/rat
GII	*Argania spinosa* oil	5	100mg/kg
GIII	*Argania spinosa* oil	5	200mg/kg
GIV	*Citrullus colocynthis* oil	5	100mg/kg
GV	*Citrullus colocynthis* oil	5	200mg/kg
GVI	*Boswellia serrata* oil	5	100mg/kg
GVII	*Boswellia serrata* oil	5	200mg/kg
GVIII	olive virgin oil	5	100mg/kg
GIX	olive virgin oil	5	200mg/kg

- ✓ **Blood samples**

Blood samples were collected from retro-orbital plexus by using glass capillaries at an interval of 5 min and 15 min after the injection of ink solution.

Blood samples were added to 4ml of 0,1% sodium carbonate solution to lyse the erythrocytes.

After the last blood sampling the animals were sacrificed and the liver and spleen dissected and weighed immediately in the wet state.

- ✓ **Calculation**

The phagocytic activity in this test was measured by phagocytic index K, corrected phagocytic index α and half time of carbon which are calculated with the same equations cited in the precedent experiment.

II.3. Evaluation of the analgesic activity of the plants extracts

II.3.1. Used materials

II.3.1.1. Plants materials

The following plants extracts was used in this study

The crude extract of *Argania spinosa* seeds which was obtained by the same method described in the up and down test.

The oil extracted from *Citrullus colocynthis* seeds was obtained by petroleum ether extraction.

The oil extracted from *Boswellia serrata* gum was obtained by hydrodistillation.

II.3.1.2. Animals

Male *Mus musculus* mice weighing between 18-22g were used in this study.

The animals were obtained from Central Pharmacy Institute of Constantine, Algeria. The animals were housed in plastic cages with saw dust bedding which was changed every day regularly, they were maintained in the animal house under standard laboratory conditions of temperature (25 ± 2°C) and humidity with 12h light-dark cycle.

Standard mouse diet (LA RATION, Bouzeriaa) and water were given in adequate *ad libitum.* The animals were acclimatized to laboratory conditions for five days prior to experiment.

II.3.1.3. Experimental procedure

- ✓ **Acetic acid induced pain test (writhing test)**

The writhing test in mice was carried out following the methods used by (Adzu et al., 2003; Ouachrif et al., 2012).

Animals were divided into control, standard and different test groups contain five mice in each (table 03). The control group received saline 0,9% (i.p.) and standard group was treated with DL-Lysine Acetylsalicylate (Aspegic) at a dose level of 100 mg/kg (i.p.).

Test samples (*Argania spinosa, Citrullus colocynthis, Boswellia serrata*) were administered orally 30 min before intraperitoneal administration of 0.6% acetic acid but DL-Lysine Acetylsalicylate (Aspegic) was administered intraperitonially 15 min before injection of acetic acid.

A period of 5 minutes was given to each animal to ensure bio-availability of acetic acid, following this period; the total numbers of writhes produced in these animals were counted for 30 min. For scoring purposes, a writhe is indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb.

Table 03. Treatment of mice in analgesic test

Experimental groups	Treatment	Number of mice	Dose
GI	0,9%Nacl	6	10ml/kg
GII	DL-Lysine Acetylsalicylate	6	100mg/kg
GIII	*Argania spinosa*	6	100mg/kg
GIV	*Argania spinosa*	6	200mg/kg
GV	*Citrullus colocynthis*	6	100mg/kg
GVI	*Citrullus colocynthis*	6	200mg/kg
GVII	*Boswellia serrata*	6	100mg/kg
GVIII	*Boswellia serrata*	6	200mg/kg

- ✓ **Calculation**

The data represent the total numbers of writhes observed during 30 min and are expressed as writhing numbers. The analgesic activity was expressed as percentage inhibition of writhing in mice according to the following formula

$$\text{Inhibition (\%)} = \frac{\text{Number of Writhes [Control]} - \text{Number of Writhes [Treatment]}}{\text{Number of Writhes [Control]}} \times 100$$

II.4. Evaluation of the acute anti-inflammatory activity of the plants extracts

II.4.1. Used materials

II.4.1.1. Plants materials

The anti-inflammatory activity was carried out using the crude extract of *Argania spinosa* seeds which was obtained by the same method described in the up and down test, the oil extracted from *Citrullus colocynthis* seeds and *Boswellia serrata* gum obtained by the same method described in the previous experiment.

II.4.1.2. Animals

Male *Mus musculus* mice weighing between 20-27g were used in this study.

The animals were obtained from Central Pharmacy Institute of Constantine, Algeria. The animals were housed in plastic cages with saw dust bedding which was changed every day regularly, they were maintained in the animal house under standard laboratory conditions of temperature (25 ± 2°C) and humidity with 12h light-dark cycle.

Standard mouse diet (LA RATION, Bouzeriaa) and water were given *ad libitum*. The animals were acclimatized to laboratory conditions for one week prior to experiment.

II.4.1.3. Experimental procedure

- ✓ **Xylene induced ear oedema test**

The anti-inflammatory effect of the plants extracts was evaluated by slightly modifying the method described by (Al Amin et al., 2012).

Eight groups of five mice each were used (table 04).

Argania spinosa crude extract at concentrations of 100 and 200mg/kg, *Citrullus colocynthis* oil extract at concentrations of 100 and 200mg/kg and *Boswellia serrata* at concentrations of 100 and 200mg/kg were administered orally one hour before xylene application.

Ibuprofen (100 mg/kg) was given as a reference anti-inflammatory drug and saline (10 ml/kg) given to control animals.

Ear oedema was induced by applying carefully a drop of xylene (0.03 ml) to the anterior and posterior surfaces of the right ear. The left ear remained untreated and considered as control.

One hour after xylene application, the animals were killed and both ears were cut off and weighed.

Table 04. Treatment of mice in xylene induced ear oedema

Experimental groups	Treatment	Number of mice	Dose
GI	0,9%Nacl	5	10ml/kg
GII	Ibuprofen	5	100mg/kg
GIII	*Argania spinosa*	5	100mg/kg
GIV	*Argania spinosa*	5	200mg/kg
GV	*Citrullus colocynthis*	5	100mg/kg
GVI	*Citrullus colocynthis*	5	200mg/kg
GVII	*Boswellia serrata*	5	100mg/kg
GVIII	*Boswellia serrata*	5	200mg/kg

- ✓ **Calculation**

Oedema size was quantified as the weight difference between the two ears (the right treated and left untreated ears). The anti-inflammatory activity was evaluated as percent inhibition of oedema in the treated animals relatively to the control animals using the following formula

$$\text{Inhibition (\%)} = 100\,[1 - (Et/Ec)],$$

Where Et and Ec represent average oedema of the treated group and the control group, respectively.

II.5. Evaluation of anti-arthritic and chronic anti-inflammatory activities of *Argania spinosa* seeds

II.5.1. Used material

II.5.1.1. Plant material

In this study the crude extract of *Argania spinosa* seeds was used.

II.5.1.2. Animals

Male and female Wistar rats weighing between 220-300g were used in this study.

The animals were obtained from Central Pharmacy Institute of Constantine, Algeria. The animals were housed in polypropylene cages with soft wood shaving as bedding, they were maintained in the animal house under standard laboratory conditions of temperature (25 ± 2°C) and humidity with 12h light-dark cycle. Females were non-pregnant and housed separately from males. They were fed with normal commercial pellet diet (LA RATION, Bouzeriaa) and water *ad libitum.* The animals were acclimatized to laboratory conditions for one week prior to experiment.

II.5.1.3. Experimental procedure

- ✓ **Formalin induced arthritis**

Experimental arthritis was induced in rats according to the methods described by (Vasudevan et al., 2006; Divakar et al., 2010) with some minor modifications.

Five groups were employed in the present anti-arthritic study. Each group consists of five rats. The treatment of groups is mentioned in (table 05a)

Table 05a. Treatment of rats in formalin induced arthritis

Experimental groups	Treatment	Formalin injection	Number of rats	Dose
Group N normal group	Flour	---	5	0,5mg/rat
Group F untreated control	Flour	+++	5	0,5mg/rat
Group FI standard	Ibuprofen	+++	5	100mg/kg
Group FP treated	*Argania spinosa*	+++	5	100mg/kg
Group FPP treated	*Argania spinosa*	+++	5	200mg/kg

+++: injection ---: no injection

A sublantar injection of 0,1 ml of 2% formaldehyde was administered to the right paw on the first and third day of the experiment.

Crude extract of *Argania spinosa* (100 and 200 mg/Kg) or Ibuprofen as a standard (100mg/Kg) were administered orally once daily for 10 days.

- ✓ **Methionine induced hyperhomocysteinemia associated with formalin induced inflammation**

Five complementary groups were used for this study. Each group consists of five rats (table 05b).

Table 05b. Treatment of rats in formalin and methionine chronic arthritis

Experimental groups	Treatment	Formalin injection	Methionine injection	Number of rats	Dose
Group FM untreated control	Flour	+++	+++	5	0,5mg/rat
Group FMI Standard	Ibuprofen	+++	+++	5	100mg/kg
Group FMP treated	*Argania spinosa*	+++	+++	5	100mg/kg
Group FMPP treated	*Argania spinosa*	+++	+++	5	200mg/kg
Group M methionin	Flour	---	+++	5	0,5mg/kg

+++: injection ---: no injection

Formalin was administered to group FM, FMI, FMP and FMPP in the same way of the experience describing above (Formalin induced arthritis by (Vasudevan et al., 2006; Divakar et al., 2010) and the methionine hyperhomocysteinemia was induced in combination with formalin administration, the group M serve as methionine induced hyperhomocysteinemia model. The methionine was administered to rats by intraperitoneal injection at the dose of 400mg/kg body weight diluted in saline (Fukada et al., 2006).

- ✓ **Blood investigation**

At the end of the experiments, animals from the entire groups were fasted overnight before collection of blood samples. Each rat was anesthetised and blood was withdrawn from retro orbital plexus. The separated plasma was assayed for hs-C-reactive protein by an immunoturbidimetric method on a Cobas integra 400 plus analyser (Roche) and total homocysteine concentration was measured by IMMULITE analyser.

Blood analysis was performed in the laboratory IBN SINA, Constantine.

- ✓ **Calculation**

The paw thickness (mm) of each group was measured every day during the experience period (10 days) using a digital Vernier Caliper.

Oedema was calculated as follows

$$\Delta T = Td - To$$

Where Td is the right hind paw thickness (mm) on day "d"

To is hind paw thickness (mm) before subplantar injection of formalin

Percentage inhibition of arthritis was calculated by the following formula

$$\% \text{ Inhibition} = \frac{\text{Mean paw inflammation of control} - \text{mean paw inflammation of test}}{\text{Mean paw inflammation of control}} \times 100$$

At the end of the experiment, and after sacrifice of animals the inflamed paws (right) were cut off then immediately weighed.

- ✓ **Photography of rats paws**

At the end of the experiment, the inflamed paws of each group were photographed using a digital Photographer Nikon.

- ✓ **Histological section procedure**

At the end of the experiment (day 10), each rat was sacrified with chloroform and hind paws were cut off and fixed in 10% formalin for at list 24 hours, and then decalcified in 0,3% formic acid. The tissues were then embedded in paraffin and cut in 5 µm thick sections after alcohol dehydratation (details in appendix 01).

II.6. Evaluation of antiproliferative activity of plants extracts

II.6.1. Used material

II.6.1.1. Plant material

The antiproliferative activity was carried out using three different oils; the oil extracted from *Argania spinosa* seeds, from *Citrillus colocynthis* seeds and from *Boswellia serrata*. The preparation method of the oils was the same described in the precedent experiments.

II.6.1.2. Cell culture

Three acute lymphoblastic leukemia cell lines (JURKAT, MOLT3 and DND41) were used in the antiproliferative tests. Four non transformed cell lines (murines thymocytes: pre T 2017 and M31, Human Keratinocytes: Hacat and Human embryonic kidney cells: HEK 293) were used to confirm the non toxicity of plants extracts on normal cells.

Cell lines were obtained from Laboratory of Molecular pathology, University La Sapienza, Rome. Cell lines were free from any kind of bacterial and fungal contamination.

JURKAT, MOLT3, DND41, pre T 2017 cell lines were cultured in complete RPMI supplemented with 10% fetal bovine serum, 2% antibiotic (penicillin/streptomycin) and 1% Glutamine. M31, HEK 293 and HaCat cells were maintained in Dulbecco's modified Eagle's medium (Gibco) containing 10% fetal bovine serum, 2% antibiotic (penicillin/streptomycin) and 1% Glutamine.

II.6.1.3. Antibodies

Mouse monoclonal antibody (mAb) against phospho-ERK (P-ERK) (Cell Signaling), Rabbit mAb against ERK (ERK) (Cell Signaling), Mouse mAb against NOTCH1 (N1), Goat anti-mouse IgG secondary antibody and Mouse anti-rabbit IgG secondary antibody from Santa Cruz Biotecnology were used.

II.6.2. Experimental procedure

II.6.2.1. Trypan blue exclusion assay

Trypan Blue is a blue acid dye that has two azo chromophores group. Trypan blue will not enter into the cell wall of cells grown in culture. Trypan Blue is an essential dye, use in estimating the number of viable cells present in a population (Tripathy and Pradhan, 2013).

To assess cell growth, $1x10^6$ cells were incubated for 48h with 100µg/ml of the oils extracts (*Argania spinosa* oil, *Citrullus colocynthis* oil or *Boswellia serrata* oil), diluted in DMSO (dilution 1:10) or with DMSO alone.

In detail, $1x10^6$ cells suspended in 1 ml of medium was treated with 1,1µl of test samples diluted 1:10 in DMSO and Control cells were exposed to the same volume of DMSO. The comparison of the number of viable cells incubated in the presence of DMSO versus the counterpart incubated with medium alone revealed that the concentration of DMSO used in the assay (1,1µl/ml) did not influence the cell viability.

After incubation, trypan blue dye exclusion assay was performed by mixing 20 µl of cell suspension with 20 µl of 0.4% trypan blue dye (Sigma Aldrich) before injecting into a hemocytometer and cell counting. The numbers of cells that exclude the dye (viable cells) were counted.

II.6.2.2. Microculture Tetrazolium (MTT) assay

The extent of the cell proliferation and cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. Cell proliferation and viability were determined by MTT (cell growth determination Kit, Sigma Aldrich).

This Colorimetric assay is based on the capacity of Mitochondria succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3- (4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into an insoluble, colored formazan product which is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells (Patel et al., 2009).

Cell proliferation was assessed with Cell Growth Determination Kit MTT Based (Sigma Aldrich) as previously described by (Kumar et al., 2014).

In brief: cells were seeded at $8x10^4$ cells/ml and incubated with 100µg/ml Argan oil or vehicle alone for 72h. After that, the culture medium was changed, and the MTT solution (stock solution: 5 mg/ml) was added in an amount equal to 10% of the culture volume and incubated for 4 hr. The viable cell number was directly proportional to the production of formazan, which was solubilized with isopropanol and measured spectrophotometrically at OD560 with GloMax-Multi Microplate Multimode Reader (PROMEGA). The experiments were performed in triplicate.

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

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

Where,

At= Absorbance value of test compound (Argan oil)

Ab= Absorbance value of blank (Medium alone)

Ac=Absorbance value of control (DMSO)

II.6.2.3. Immunoblotting

Total protein extracts preparation was described elsewhere by (Palermo et al., 2012).

In brief: cell pellets were resuspended in lysis buffer (Tris 20 mM pH 7.5, NaCl 150 mM, NaF 1mM, Triton X-100 1%, Sodium-Orthovanadate 1 mM, PMSF 1mM, Sodium Butyrate 10mM) plus protease inhibitors and incubated for 20 min on ice. After a centrifugation at 13000 rpm for 20 min, supernatants were boiled for 5 min in Laemmli sample buffer 1X (Biorad). Concentration of Protein was determined by Bradford assay (BioRad). 30 µg of protein was separated on 10% SDS-PAGE and transferred to nitrocellulose membranes (Perkin Elmer).

After the transfer, the blots were incubated for two hours at room temperature with the following primary antibodies: mouse mAb against phospho-ERK (P-ERK) (Cell Signaling) (diluted 1:1000 in BSA 5%); rabbit mAb against ERK (ERK) (Cell Signaling) (diluted 1:1000 in BSA 5%); mouse mAb against NOTCH1 (N1) (diluted 1:1000 in Milk 3%); and then incubated overnight at 4°C with a goat anti-mouse IgG secondary antibody or a mouse anti-rabbit IgG secondary antibody (both diluited 1:3000 in Milk 3%); The membranes were visualized by chemiluminescence detection (Western Bright ECL + Western Bright peroxide).

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 and Discussion

III.1. Up and down test

In the acute toxicity study represented in our study by up and down method, our crude extract of *Argania spinosa* at a dose of 2000 mg/kg caused neither visible signs of toxicity nor mortality. A total of five female rats were treated orally with the same extract at the same dose and observed for 14 days. All five rats survived until the end of the observation period.

The same observation was found for the test extracts *Citrullus colocynthis* and *Boswellia serrata.*

So we can draw a conclusion that the minimum lethal dose of the plant extracts tested in our study *(Argania spinosa, Citrullus colocynthis* and *Boswellia serrata)* is more than 2000 mg/kg.

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, usually rats are treated at specific doses for a defined period of time. At this period, the rats are controlled (Almança et al., 2011).

In the present toxicity test, the up and down method was used. This method consumed a fewer animals than conventional method. Mortality, signs of toxicity and abnormalities were observed during the experimental period.

Since the dose 2000mg/kg failed to produce any clinical signs of toxicities such as convulsion, hyperactivity and sedation, the estimated LD_{50} of the entire test extracts is more than 2000mg/kg, so our extracts obtained from *Argania spinosa, Citrullus colocynthis* and *Boswellia serrata* are toxically safe by oral administration in rats.

III.2. Evaluation of the immunomodulatory activity of medicinal plants extracts by carbon clearance rate test

III.2.1. Immunomodulatory activity of *Argania spinosa* seeds

The immunomodulatory activity of the crude extract of *Argania spinosa* seeds was evaluated by carbon clearance rate test in mice.

As shown in the figure 07, there is a highly significantly difference in the means for the phagocytic index (K) between groups (GI, GII, GIII, GIV and GV) P= 0,000 and the group V is highly significantly different from groups (GI, GII, GIII and GIV) at P=0,000.

This indicates that *Argania spinosa* crude extract enhanced the phagocytic activity by stimulating the reticuloendothelial system in a dose dependant manner.

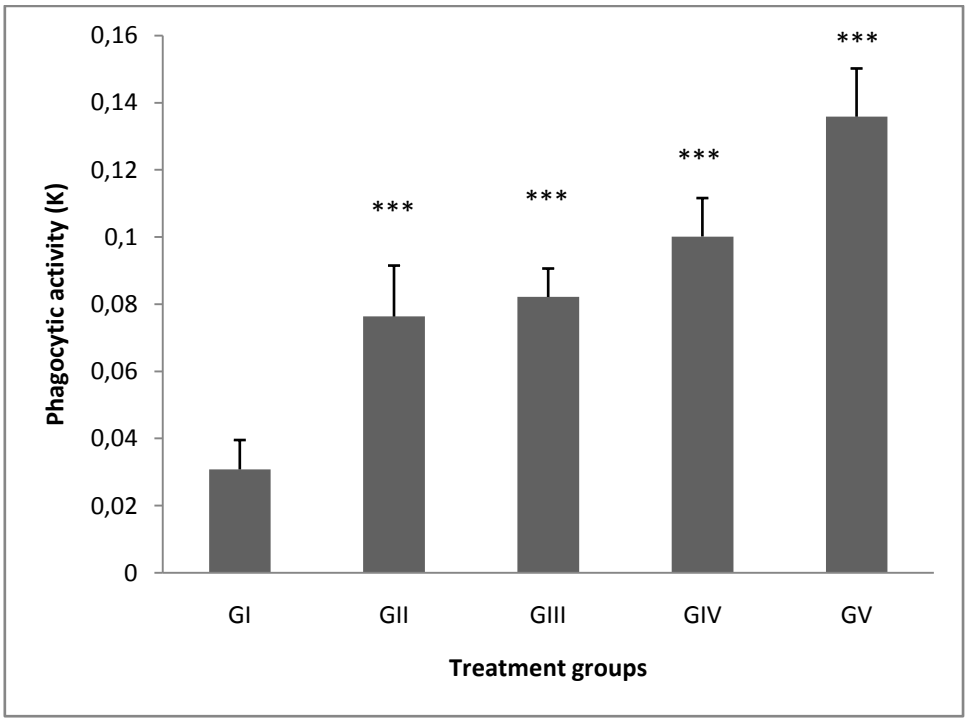

Figure 07. Effect of *Argania spinosa* crude extract on phagocytic activity in mice. Results are shown as mean ± SD (n=6) and significant difference from the control group is shown as *p<0,05, **p<0,01, ***p<0,001

GI: Control group received saline; **GII:** group received *Argania spinosa* crude extract at dose 30mg/kg; **GIII:** group received *Argania spinosa* crude extract at dose 50mg/kg; **GIV:** group received *Argania spinosa* crude extract at dose 100mg/kg; **GV:** group received *Argania spinosa* crude extract at dose 150mg/kg.

As shown also in Figure 08, the half-time of colloidal carbon was highly significantly faster at 48h, after the administration of *Argania spinosa* crude extract between groups (P= 0,000) and the clearance rate was decreased highly and significantly in groups (GII,GIII,GIV and V) when it is compared to the control group (GI) P=0,000. This indicates that the extract reduces the elimination time of carbon particles from blood and affirms that *Argania spinosa* crude extract enhanced the phagocytic activity.

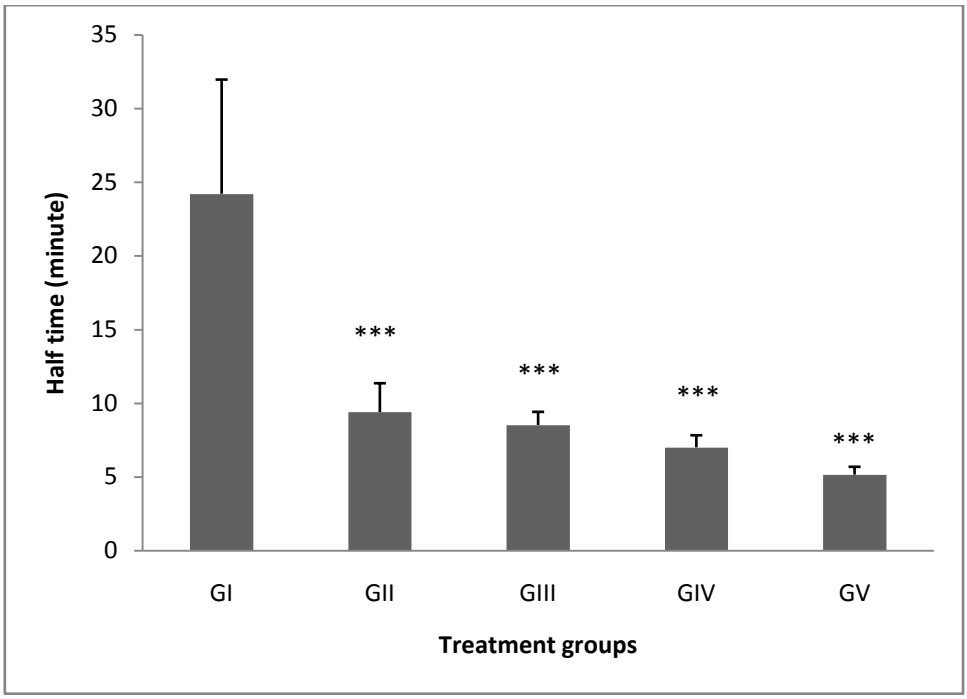

Figure 08. Effect of *Argania spinosa* crude extract on half time $t_{1/2}$ of carbon in blood. Results are shown as mean ± SD (n=6) and significant difference from the control group is shown as *p<0,05, **p<0,01, ***p<0,001

GI: Control group received saline; **GII:** group received *Argania spinosa* crude extract at dose 30mg/kg; **GIII:** group received *Argania spinosa* crude extract at dose 50mg/kg; **GIV:** group received *Argania spinosa* crude extract at dose 100mg/kg; **GV:** group received *Argania spinosa* crude extract at dose 150mg/kg.

Figure 09 confirm the phagocytic activity of *Argania spinosa* seeds including the carbon clearance from the animals blood versus the weight of their active organs (spleen and liver), the figure show a significant activity in dose manner.

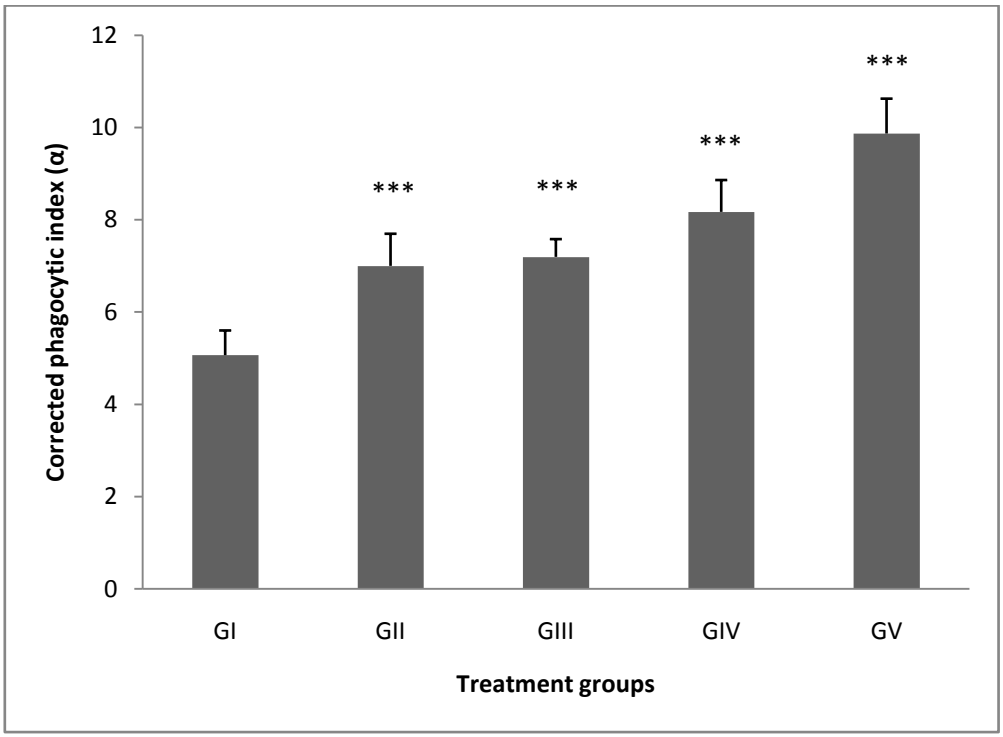

Figure 09. Effect of crude extract of *Argania spinosa* seeds on corrected phagocytic activity (α). Results are shown as mean ± SD (n=6) and significant difference from the control group is shown as *p<0,05, **p<0,01, ***p<0,001

GI: Control group received saline; **GII:** group received *Argania spinosa* crude extract at dose 30mg/kg; **GIII:** group received *Argania spinosa* crude extract at dose 50mg/kg; **GIV:** group received *Argania spinosa* crude extract at dose 100mg/kg; **GV:** group received *Argania spinosa* crude extract at dose 150mg/kg.

II.1.2. Comparative screening of the immunomodulatory activities of the oils extracted from *Argania spinosa, Citrullus colocynthis, Boswellia serrata* and the virgin olive oil

As it shown in figure 10, all the groups treated by both concentrations (100 and 200mg/kg) of the different plants oils presented an increase of the phagocytic activity in dose manner comparing to the control group (saline) indicating that there was increase in the clearance of colloidal carbon from the blood after administration of these drugs. However, this activity is different from one oil to another (groups treated with *Boswellia serrata* and olive oil was found to be more effective than the groups treated with *Argania spinosa* and *Citrullus colocynthis* (highly significant increase of phagocytic index in groups BS and OO comparing to the control p=0,000).

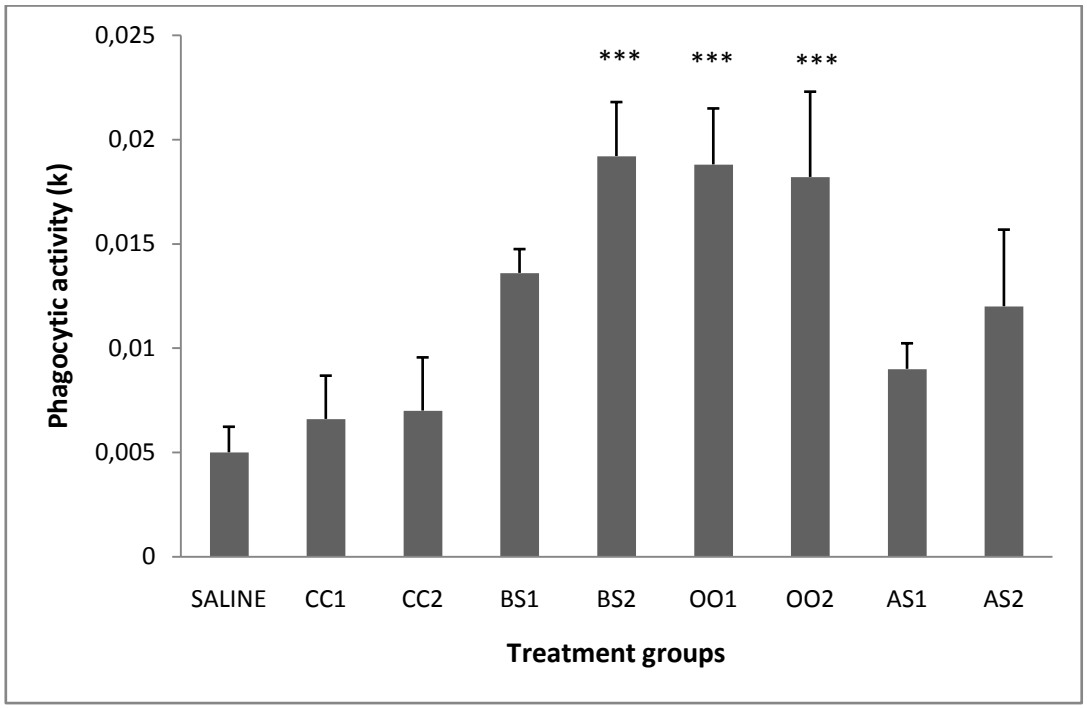

Figure 10. Effect of *Citrullus colocynthis, Boswellia serrata,* olive oil and *Argania spinosa* on phagocytic activity. Results are shown as mean ± SD (n=5) and significant difference from the control group is shown as *p<0,05, **p<0,01, ***p<0,001

Saline: Control group received flour at 0,5mg/rat; **CC1:** group received *Citrullus colocynthis* extract at dose 100mg/kg; **CC2:** group received *Citrullus colocynthis* extract at dose 200mg/kg; **BS1:** group received *Boswellia serrata* extract at dose 100mg/kg; **BS2:** group received *Boswellia serrata* extract at dose 200mg/kg; **OO1:** group received olive oil at dose 100mg/kg; **OO2:** group received olive oil at dose 200mg/kg; **AS1:** group received *Argania spinosa* crude extract at dose 100mg/kg; **AS2:** group received *Argania spinosa* crude extract at dose 200mg/kg.

As seen in Figure 11, the half time of elimination of carbon particles from the blood in the treated groups is lower than that in normal control it is obviously improved in groups treated by *Boswellia serrata* and olive oil (high significantly in groups BS and OO $P < 0.001$).

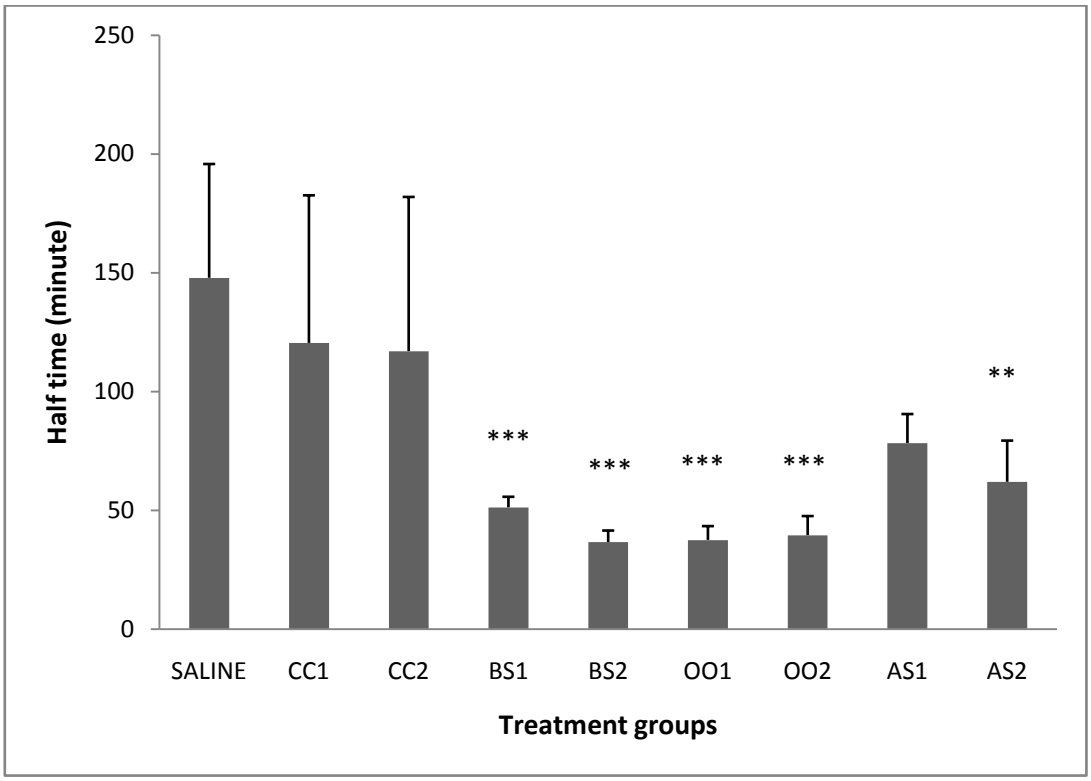

Figure 11. Effect of *Argania spinosa, Boswellia serrata*, *Citrullus colocynthis* and olive oil on half time of carbon in blood. Results are shown as mean ± SD (n=5) and significant difference from the control group is shown as *p<0,05, **p<0,01, ***p<0,001

Saline: Control group received flour at 0,5mg/rat; **CC1:** group received *Citrullus colocynthis* extract at dose 100mg/kg; **CC2:** group received *Citrullus colocynthis* extract at dose 200mg/kg; **BS1:** group received *Boswellia serrata* extract at dose 100mg/kg; **BS2:** group received *Boswellia serrata* extract at dose 200mg/kg; **OO1:** group received olive oil at dose 100mg/kg; **OO2:** group received olive oil at dose 200mg/kg; **AS1:** group received *Argania spinosa* crude extract at dose 100mg/kg; **AS2:** group received *Argania spinosa* crude extract at dose 200mg/kg.

Figure 12, which mentioned the corrected phagocytic index (phagocytic activity including spleen and liver weights) confirm the enhancement of the phagocytic function exerted by the different treatments comparing to the control group.

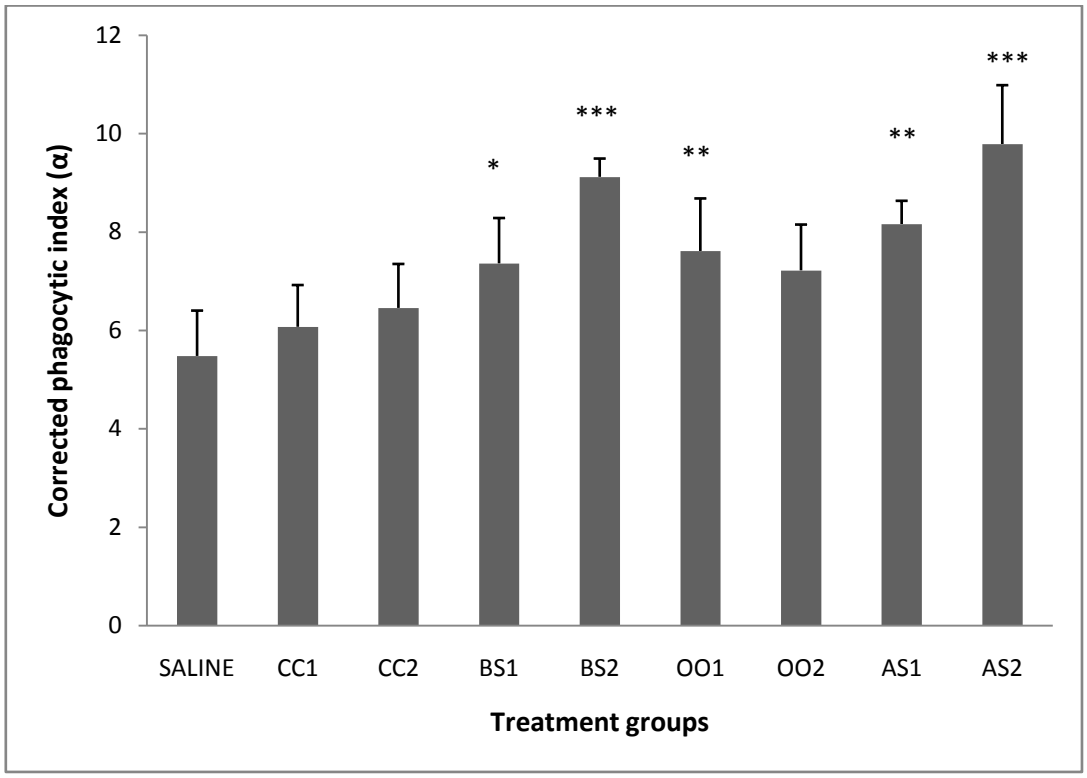

Figure 12. Effect of *Argania spinosa, Boswellia serrata*, *Citrullus colocynthis* and olive oil on corrected phagocytic index α. Results are shown as mean ± SD (n=5) and significant difference from the control group is shown as *p<0,05, **p<0,01, ***p<0,001

Saline: Control group received flour at 0,5mg/rat; **CC1:** group received *Citrullus colocynthis* extract at dose 100mg/kg; **CC2:** group received *Citrullus colocynthis* extract at dose 200mg/kg; **BS1:** group received *Boswellia serrata* extract at dose 100mg/kg; **BS2:** group received *Boswellia serrata* extract at dose 200mg/kg; **OO1:** group received olive oil at dose 100mg/kg; **OO2:** group received olive oil at dose 200mg/kg; **AS1:** group received *Argania spinosa* crude extract at dose 100mg/kg; **AS2:** group received *Argania spinosa* crude extract at dose 200mg/kg.

The first experimental approach in the scope of this thesis was to evaluate the immunomodulatory activity of the crude extract of *Argania spinosa* seeds then the immunomodulatory effect of four oils extracted from: *Argania spinosa, Citrullus colocynthis, Boswellia serrata* and olive as comparative study using *in vivo* carbon clearance assays in mice and rats.

Immunomodulation is a procedure which can alter the immune system of an organism by interfering with its functions; if it results in an enhancement of immune reactions, it is named as an immunostimulative drug which primarily implies stimulation of specific and non specific system that is, granulocytes, macrophages, complement, certain T lymphocytes and different effectors substances (Tilwari et al., 2011).

There are a number of natural agents from medicinal plants which are used for the enhancing of the body's response to disease. In recent time a large number of drugs extracted from the plants are coming in to the marked by proper clinical trials. When taking any of these agents take proper advice on dose and length of treatment (Singh et al., 2011).

The carbon clearance test was done to evaluate the effect of drugs on the reticuloendothelial system. The reticuloendothelial system is a diffuse system consisting of phagocytic cells. In fact, the role of phagocytic cells is primarily the removal of microorganisms and foreign bodies, but also the elimination of dead or injured cells. Phagocytic defects are associated with varied pathological conditions in humans. In view of the pivotal role played by the phagocytic cells and particularly macrophages in coordinating the processing and presentation of antigen to B-cells, our plants extracts were evaluated for its effect on macrophage phagocytic activity. 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 governed by an exponential equation (Gokhale et al., 2003).

It is known that plants are rich in a variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosides and saponins. Many biological activities including modulation of the immune system have been assigned to these compounds in plants (Banso and Adeyemo, 2006).

Argania spinosa is a plant characterised by the presence of triterpenoids, sterols and saponins (El Monfalouti et al., 2010), these compounds might be involved in the modulation of the immune system caused by *Argania spinosa* extract, while *Citrullus colocynthis* presented phytosterols, saponins, tanins and alcaloids as secondary metabolites (Uma and Sekar, 2014) and the water extract from the fruit of *Citrullus colocynthis* were found to possess immunostimulant activity (Bendjeddou et al., 2003).

Boswellia serrata in its turn present as main active constituents terpenoids and are composed of boswellic acids which may exhibit immunomodulatory properties (Kumar et al., 2011). In the same context, olive oil is rich of polyphenols, sterols and tocopherols, these compounds have been presented as responsible for the pharmacological effects of the oil including immunomodulatory effect (Garcia-Gonzalez et al., 2008).

Both studies showed remarkable augmentation in the phagocytic index after extracts treatment due to the ability of these compounds to increase the activity of the reticuloendothelial system.

Results of the first study using carbon clearance assay in mice clearly indicate that the crude extract of *Argania spinosa* activates the process of phagocytosis and this activation is increased when increase the dose.

In the second part of the study using carbon clearance assay in rats we observed that the rats administered with the different oils stimulate the phagocytic index at two different concentrations (100 and 200mg/kg).

Treatment by the different extracts used in these studies enhanced the rate of carbon clearance from the blood when it is compared to the control group. This reflects the enhancement of the phagocytic activity of phagocytes and non specific immunity, which includes opsonisation of the foreign particulate matter with antibodies and complement C3b, leading to a more rapid clearance of foreign particulate matter from the blood (Singh et al., 2012).

Another mechanism by which the extracts used in these studies may show an immunostimulant effect is the fact to influence the role of neutrophils, digestive enzyme in phagocytic vesicle, and the synthetic processes in the cytoplasm. In treated animal, hypergranulation of white blood cells is the evidence of it. The secretory material appeared in the cytoplasm is to meet the necessity of the cell to phagocytose and digest the antigen, stimulation of pagocytosis was influenced by the macrophages, these secret a number of cytokines such as IL-1, IL-2 (Rastogi et al., 2008).

Considering the results of the present studies, when all extracts exhibited the stimulatory effect on the reticuloendothelial system depending on dose, the compounds of this herbal extracts may influence the function of enzyme system that is involved in the immune response. The metabolic activation of phagocytes during phagocytosis causes the activation of NADPH oxidase: a complex enzymatic system that catalyses NADPH oxidation to produce superoxide radical and other reactive products of oxygen. An alternative pathway in macrophages and granulocytes leads to the production of hypochlorous acid that has a potent antimicrobial activity (Raińska-Giezek et al., 2011).

It is well evident, that the results of both studies using *in vivo* immunomodulatory activity showed a stimulation of the phagocytic index at different concentrations. Accordingly, these results agrees with those of (Shuklaa et al., 2009) and (Benmebarek et al., 2013) who reported that the oral administration of ethanolic extraction of *Caesalpinia bonducella* and *Stachys mialhesi* respectively in the mouse increased the phagocytic index at different concentrations.

Numerous other studies have shown the immunostimulant effect of plants extracts, (Gaoa et al., 2004) have reported that the extract of Curcumin from *Curcuma longa* inhibited the IL-2 induced proliferation of spleen cells completely at concentrations of 25mmol/L. The study of (Subhadip et al., 2012) confirmed that the methanolic extract of *Swietenia mahagoni* seeds has therapeutic potential and could be served as an effective immunomodulatory candidate without any side effects. Another study of Algerian *Phoenix dactylifera* recorded by (Kehili et al., 2014) revealed its immunostimulatory effect on the reticuloendothelial system with higher effect by the administration of 50 mg/kg.

III.3. Analgesic activity by acetic acid induced writhing

The effects of *Argania spinosa, Citrullus colocynthis and Boswellia serrata* extracts on acetic acid induced writhing in mice were summarized in figure 13 and figure 14.Oral administration of the extracts at doses of 100 and 200mg/kg significantly decreased the number of writhings and stretchings when compared to the control (14±0,89 vs 8,83±0,75, 7,83±0,16 vs 7,33±1,21, 5,16±1,16 vs 5,66±1,86, 4,66±0,81, respectively). This activity was dose-related.

The reference drug Aspegic at the dose of 200mg/kg significantly decreased the number of writhings when compared to the control (7,5±1,37 vs 14±0,89) and the percentage of inhibition was 46,06. The results indicated that *Boswellia serrata* at both doses 100 and 200mg/kg and *Citrullus colocynthis* at dose 200mg/kg showed more effective analgesic effect comparing to reference drug, while *Argania spinosa* extract at both doses (100 and 200mg/kg) showed an analgesic activity which could be comparable to the standard Aspegic at the dose of 100mg/kg.

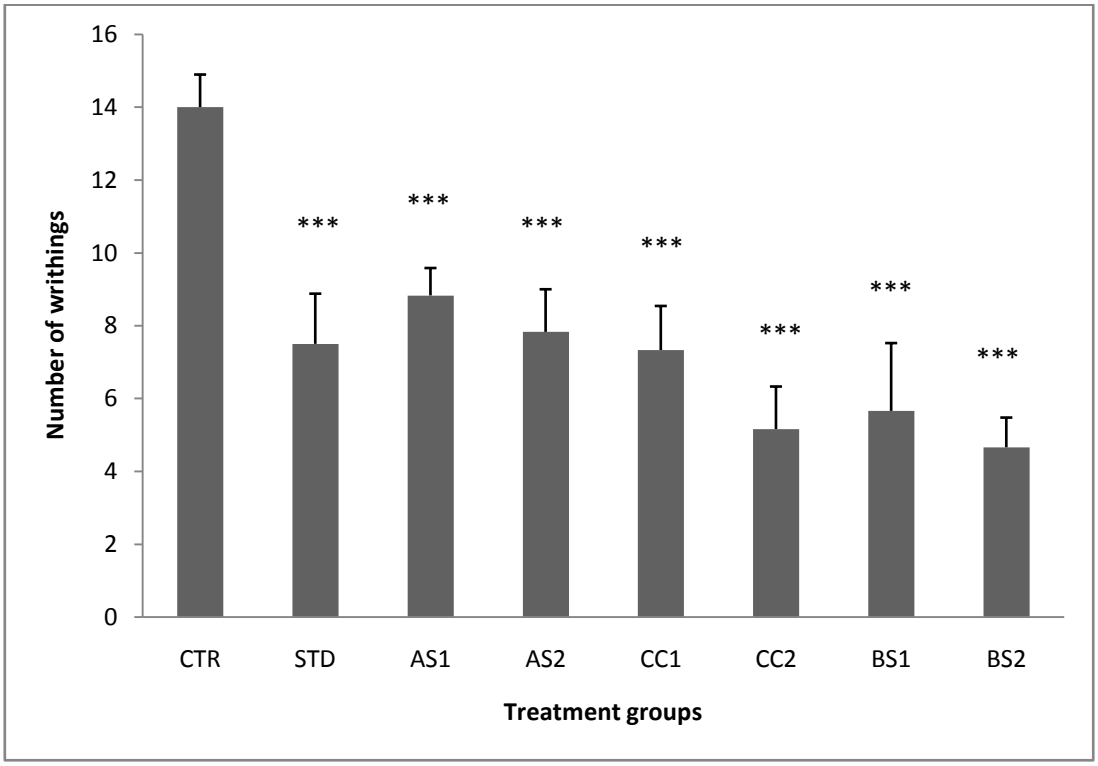

Figure 13. Effect of *Argania spinosa, Citrullus colocynthis and Boswellia serrata* on the writhing number in acetic acid induced pain in mice. Results are shown as mean ± SD (n=6) and significant difference from the control group is shown as *p<0,05, **p<0,01, ***p<0,001

CTR: Control group received saline; **STD:** reference group received DL-Lysine Acetylsalicylate at dose 100mg/kg; **AS1:** group received *Argania spinosa* crude extract at dose 100mg/kg; **AS2:** group received *Argania spinosa* crude extract at dose 200mg/kg **CC1:** group received *Citrullus colocynthis* extract at dose 100mg/kg; **CC2:** group received *Citrullus colocynthis* extract at dose 200mg/kg; **BS1:** group received *Boswellia serrata* extract at dose 100mg/kg; **BS2:** group received *Boswellia serrata* extract at dose 200mg/kg.

Figure 13 showed the numbers of the abdominal writhing episodes evoked by intraperitoneal injection of acetic acid in mice as well as the anti-nociceptive effect of *Argania spinosa, Citrullus colocynthis and Boswellia serrata* comparing to control group. It can be seen that treatment with the different plant extracts could dose-dependently reduce the number of writhing of mice in comparison with that of control animals; while the standard drug Aspegic which showed quite similar analgesic effect in this nociceptive model.

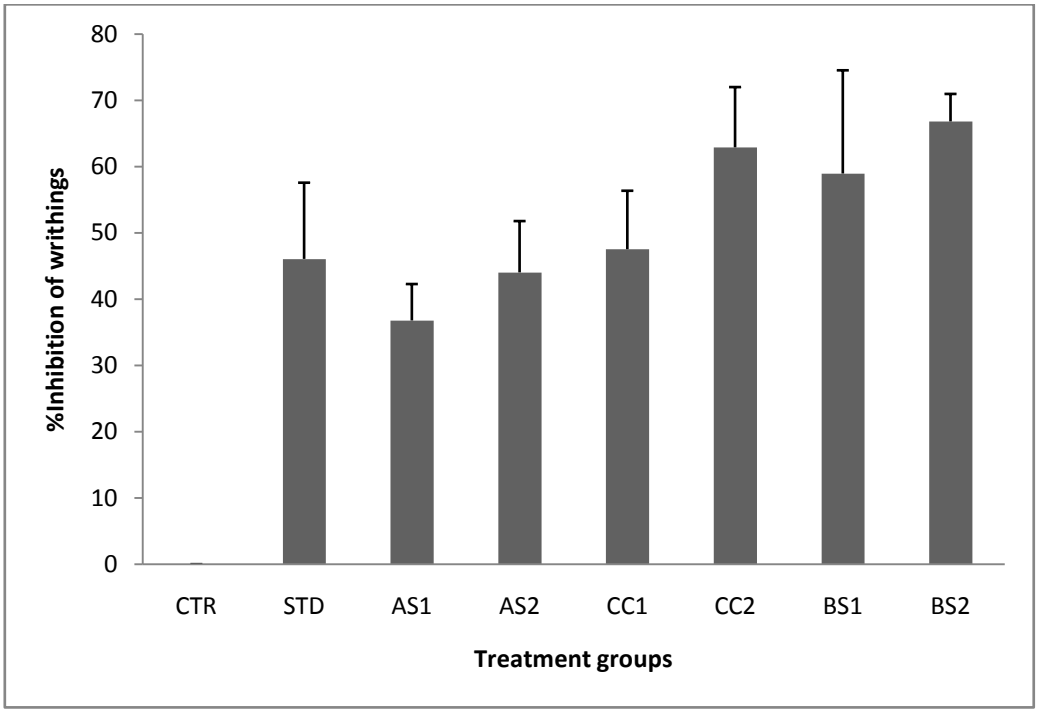

Figure 14. Effect of *Argania spinosa, Citrullus colocynthis and Boswellia serrata* on the percentage of inhibition of oedema in acetic acid induced pain in mice. Results are shown as mean ± SD (n=6) and significant difference from the control group is shown as *p<0,05, **p<0,01, ***p<0,001

CTR: Control group received saline; **STD:** reference group received DL-Lysine Acetylsalicylate at dose 100mg/kg; **AS1:** group received *Argania spinosa* crude extract at dose 100mg/kg; **AS2:** group received *Argania spinosa* crude extract at dose 200mg/kg **CC1:** group received *Citrullus colocynthis* extract at dose 100mg/kg; **CC2:** group received *Citrullus colocynthis* extract at dose 200mg/kg; **BS1:** group received *Boswellia serrata* extract at dose 100mg/kg; **BS2:** group received *Boswellia serrata* extract at dose 200mg/kg.

Acetic acid-induced writhing model represents pain sensation by triggering localized inflammatory response. Such pain stimulus leads to the release of free arachidonic acid from tissue phospholipid. The acetic acid induced writhing response is a sensitive procedure to evaluate peripherally acting analgesics. The response is thought to be mediated by peritoneal mast cells, acid sensing ion channels and the prostaglandin pathways (Shaikh et al., 2012).

In fact, acetic acid causes pain and localized inflammation by the action of prostaglandins production (mainly, prostacyclines and prostaglandin-E (PG-E)) which have been reported to stimulate the Aγ-fibres that cause a sensation of sharp well localized pain. There are various peripherally acting analgesic drugs such as ibuprofen, aspirin, diclofenac sodium and indomethacin that have been reported to inhibit acid induced writhing by inhibition of prostaglandin synthesis (Khadem et al., 2012).

Therefore the result of the plants extracts in acetic acid-induced writhing method suggests that the reduction of pain presented by reduction of number of writhing might be occurred due to the presence of analgesic properties in the entire extracts via inhibition of prostaglandin synthesis.

The research group of (Deraedt et al., 1980) described the quantification of prostaglandins by radioimmunoassay in the peritoneal exudates of rats, obtained after intraperitoneal injection of acetic acid. They found high levels of prostaglandins PGE2α and PGF2α during the first 30 min after acetic acid injection. Nevertheless, it was found that the intraperitoneal administration of acetic acid induces the liberation not only of prostaglandins, but also of the sympathetic nervous system mediators (Hokanson, 1978; Duarte et al., 1988).

The results of the present study confirm previous data of literature by demonstrating that Aspegic cause significant inhibition of acetic acid-induced pain. Furthermore, the oil extract of *Boswellia serrata* at both doses 100 and 200mg/kg and *Citrullus colocynthis* at dose 200mg/kg were more potent and efficacious than Aspegic. Thus, the result of the present study demonstrates that *Argania spinosa* extract possesses, at least partly, the same bioavailability comparing to Aspegic when administered to mice in the acetic acid test.

Recently, (Ribeiro et al., 2000) have demonstrated that the nociceptive activity of acetic acid may be due to the release of cytokines, such as TNF α, IL-1 and IL-8, by resident peritoneal macrophages and mast cells. So, the previous findings and these results presented here might indicate that the antinociceptive action of the entire extracts used in the acetic acid-induced writhing test could be due to inhibition of the release of TNF α, IL-1 and IL-8 by resident peritoneal cells.

The results of our study agrees with other studies; the group of Hosseinzadeh demonstrated that saffron stigma and petal aqueous and ethanolic maceration extracts have antinociceptive effect in acetic acid induced pain in mice, The analgesic effect of these extracts may be due to their content of flavonoids, tannins, alkaloids and saponins (Hosseinzadeh and Younesi, 2001).

III.4. Anti-inflammatory activity in acute inflammation by xylene induced ear oedema

The plant extracts exhibited a significant inhibition on ear edema formation. the oedema size was high significantly decreased in the treated groups comparing to the control group (Figure 15), the extracts caused 52.61% ($P<0.001$) and 50.25% ($P<0.001$) at the doses of 100 and 200 mg/kg body weight respectively in groups treated with *Argania spinosa* in comparison with the standard drug Ibuprofen where the inhibition was 56.48% ($P<0.001$) at the dose of 100 mg/kg body weight (Figure 16).

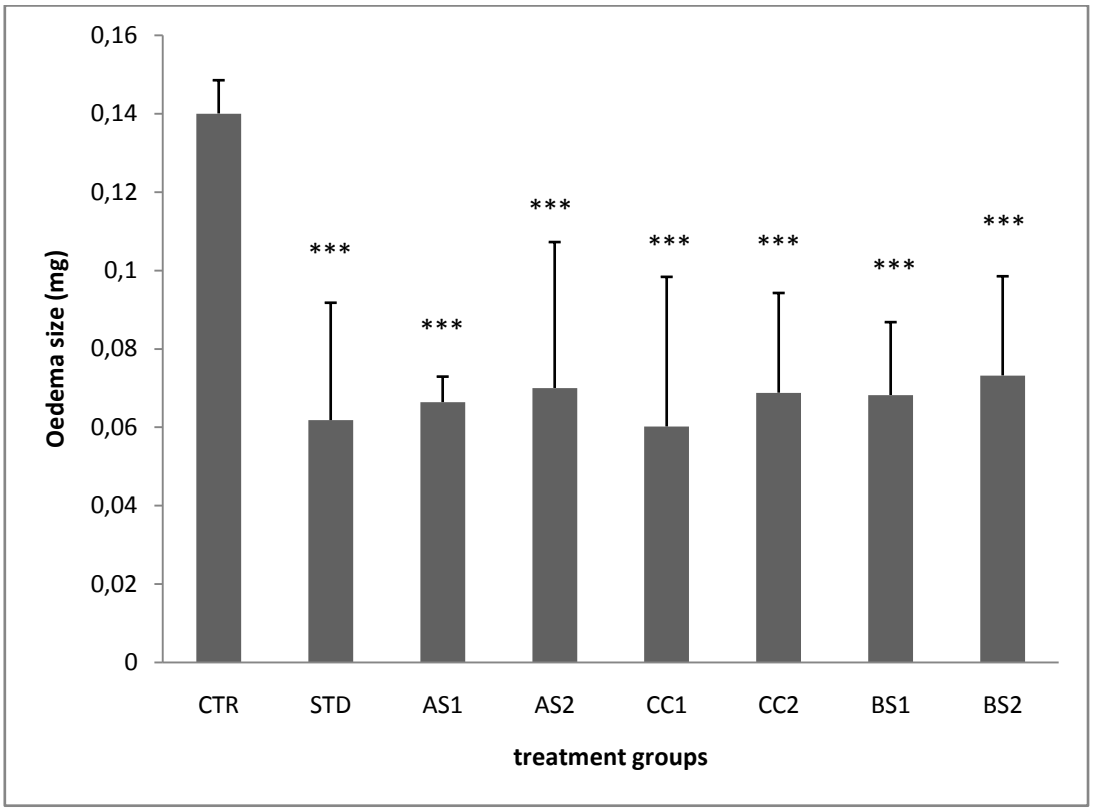

Figure 15. Effect of *Argania spinosa, Boswellia serrata*, *Citrullus colocynthis* on oedema induced by xylene in mice. Results are shown as mean ± SD (n=5) and significant difference from the control group is shown as *p<0,05, **p<0,01, ***p<0,001

CTR: Control group received saline; **STD:** reference group received Ibuprofen at dose 100mg/kg; **AS1:** group received *Argania spinosa* crude extract at dose 100mg/kg; **AS2:** group received *Argania spinosa* crude extract at dose 200mg/kg **CC1:** group received *Citrullus colocynthis* extract at dose 100mg/kg; **CC2:** group received *Citrullus colocynthis* extract at dose 200mg/kg; **BS1:** group received *Boswellia serrata* extract at dose 100mg/kg; **BS2:** group received *Boswellia serrata* extract at dose 200mg/kg.

Also, *Citrullus colocynthis* and *Boswellia serrata* extracts at doses 100, 200 mg/kg administered 30 min before the injection of xylene inhibited the formation of edema by 57.09, 51.10, 51.15 and 47.24%, respectively, 3 h after injection of the inflammatory stimulus. This result is quite similar to the one observed for Ibuprofen at 100 mg/kg, which inhibited the oedema by 56.48%. All results were statistically significant (Figure 15, $p < 0.001$). Moreover, there was no dose response correlation for the tested concentrations in the ear oedema test.

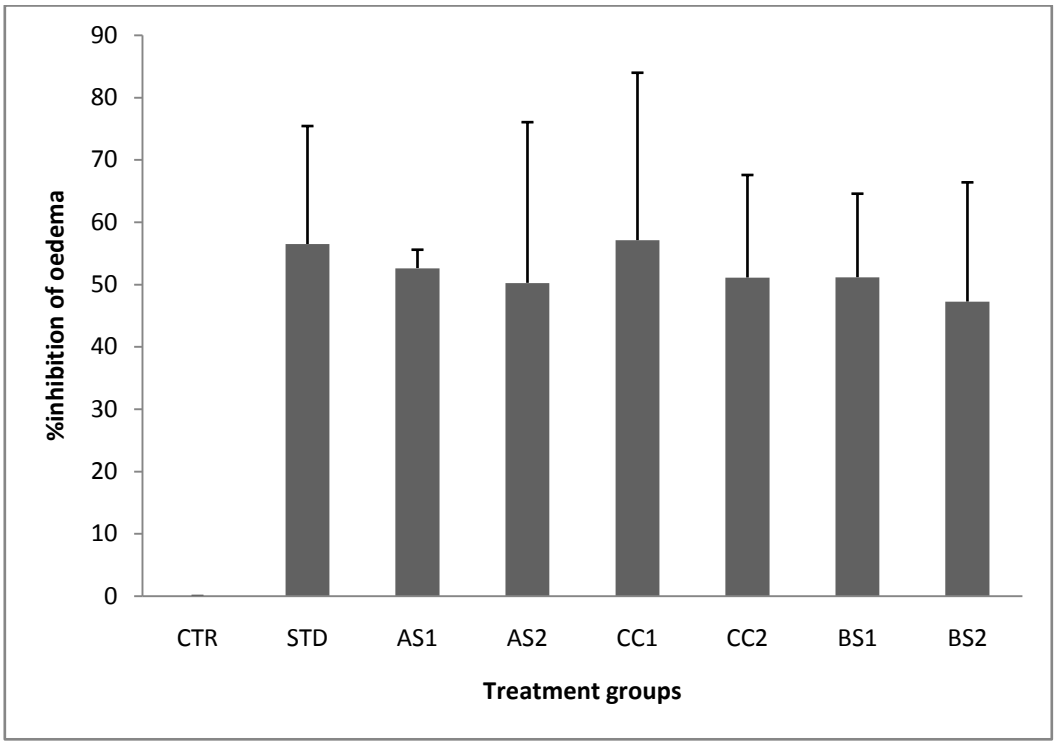

Figure 16. Effect of *Argania spinosa, Boswellia serrata*, *Citrullus colocynthis* on the percentage inhibition of oedema induced by xylene in mice. Results are shown as mean ± SD (n=5) and significant difference from the control group is shown as *p<0,05, **p<0,01, ***p<0,001

CTR: Control group received saline; **STD:** reference group received Ibuprofen at dose 100mg/kg; **AS1:** group received *Argania spinosa* crude extract at dose 100mg/kg; **AS2:** group received *Argania spinosa* crude extract at dose 200mg/kg **CC1:** group received *Citrullus colocynthis* extract at dose 100mg/kg; **CC2:** group received *Citrullus colocynthis* extract at dose 200mg/kg; **BS1:** group received *Boswellia serrata* extract at dose 100mg/kg; **BS2:** group received *Boswellia serrata* extract at dose 200mg/kg.

Xylene-induced ear oedema is an acute inflammation model which may involve inflammatory mediators such as histamine, serotonin and bradykinin. These mediators induce ear oedema and an increase of the ear thickness by promoting vasodilation and increasing vascular permeability (Birari et al., 2009).

In the present investigation, the plant extracts used significantly inhibited the xylene-induced increases in ear weight but not in a dose related manner. This inhibition capacity of the plant extracts can be regarded as the evidence of anti-inflammatory efficacy through reducing vasodilatation and so that improving oedematous condition.

The effectiveness of our plant extracts in this model probably provides the active principles present in the extracts which interfere with the action of the inflammatory mediators -previously cited- to produce its anti-inflammatory effect.

The preliminary phytochemical analysis of the plant extracts showed the presence of reducing sugars, alkaloids, flavonoids, tannins, steroids, gums and glycosides (ElManfalouti et al., 2010; Kumar et al., 2011; Uma and Sekar, 2014). The previous scientific studies have been reported that alkaloids, flavonoids and tannins are known to inhibit prostaglandin synthetase that is responsible for its antinociceptive and anti-inflammatory effects (Khadem et al., 2012). Therefore this anti-inflammatory effect of the extracts may be due to the presence of flavonoids, tannins, and alkaloids either singly or in combination in the entire extracts used.

The results of the anti-inflammatory activity of our plants extracts corroborate several other studies which indicate the key role of plants extract as anti-inflammatory drugs, in this context we can mentioned the recent studies of Khadem and Hosseinzadeh confirming the anti-inflammatory activity of the saffron extracts and the extract of *Typhonium trilobatum* in xylene induced ear oedema in mice (Khadem et al., 2012; Hosseinzadeh and Younesi, 2001).

III.5. Anti-arthritic and anti-inflammatory activities in chronic inflammation induced by hyperhomocysteinemia and formalin using *in vivo* induced arthritis

Signs of arthritis appeared after one day and reached its maximum level on three day (after 3 hours of the second injection of formalin). Symptoms include redness, swelling and deformity in hind paws and these symptoms were prominent in non treated arthritic groups (group F and group FM). No such symptoms appeared in case of normal group (N) and methionine group (M). Symptoms were less in case of treated groups (group FP, group FPP, group FMP and group FMPP), also the standard drug (Ibuprofen) show moderate arthritis as compared with the arthritic groups. The group received the formalin and methionine (FM) was the most affected with much more symptoms of arthritis.

Arthritic signs were reduced day after day during the experiment period until the 10^{th} day as seen in Figure 17, when the group received *Argania spinosa* at the dose 200mg/kg (FPP) at the end of the experiment was quite identical comparing to the normal (N) or the group received Ibuprofen (FI).

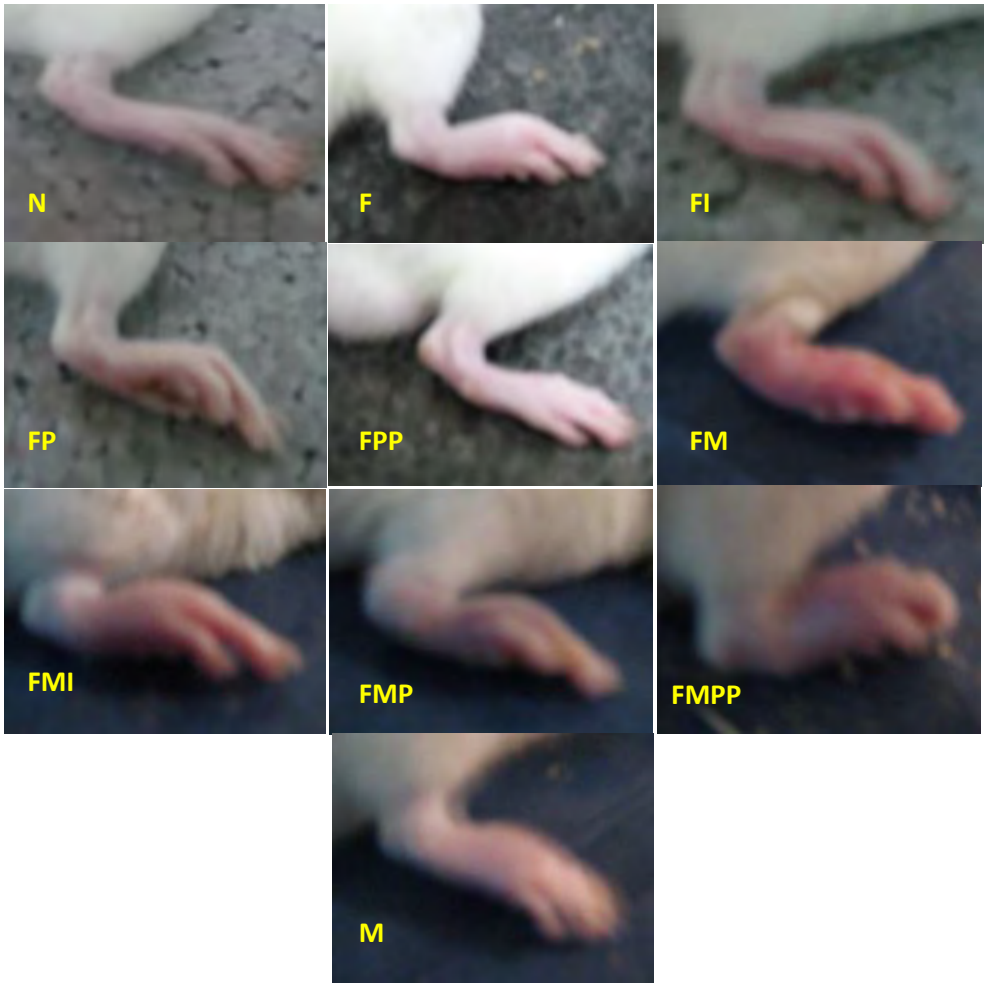

Figure 17. Photographic representation of the right (oedematous) hand paw of rats in formalin induced arthritis and on formalin/methionine induced inflammation (after 10 days of treatment)

N: normal group; **F:** formalin inflammation group; **FI:** formalin inflammation treated Ibuprofen group; **FP:** formalin inflammation treated *Argania spinosa* 100 mg/kg group, **FPP:** formalin inflammation treated *Argania spinosa* 200 mg/kg group; **FM:** formalin/methionine inflammation group; **FMI:** formalin/methionine inflammation treated Ibuprofen group; **FMP:** formalin/methionine inflammation treated *Argania spinosa* 100 mg/kg group; **FMPP:** formalin/methionine inflammation treated *Argania spinosa* 200 mg/kg group; **M:** methionine inflammation group.

As seen in figure 18 and 19 representing the size of the inflamed paw in day 1, 4, 7 and 10 during the experiment period which showed that the formalin injection caused increase of the paw diameter in all groups received formalin, on day 4 so after the second injection of formalin there was modest increase in paw diameter for all groups received formalin, but on day 7 and 10 we obtained a decrease in the paw size for all the groups receiving formalin or formalin and methionine and this diminution of paw size was most important in groups treated with *Argania spinosa* and Ibuprofen comparing to the normal. Group received formalin and methionine without treatment was the group with the largest diameter of inflamed paw at the end of the experiment.

The table 06 summarized the effect of *Argania spinosa* administred orally at the doses 100 and 200 mg/kg and Ibuprofen administred at the dose 100mg/kg during an experiment period of 10 days on formalin induced arthritis (which concern group F, FI, FP and FPP) and on formalin/methionine induced inflammation (which concern group FM, FMI, FMP and FMPP). *Argania spinosa* treatment significantly suppressed the joint oedema when compared with control between day 1 and day 10 post formalin injection or formalin and methionine injection.

The result obtained from these models for each of the two doses of extract tested (100 and 200 mg/kg) show that paw sizes of the animals were significantly reduced as from the 1st day of test. Ibuprofen which is a reference anti-inflammatory drug showed a lower inhibitory effect after formalin administration comparing to the effect of both doses.

In formalin/methionine induced inflammation, it was found that the plant extract exhibited a significant inhibition on oedema formation in a dose-related manner. It caused 44,47% ($P<0,01$) and 46,89% ($P<0,01$) at the doses of 100 and 200 mg/kg body weight respectively in comparison with the standard drug Ibuprofen where the inhibition was 37,56% ($P<0.001$) at the dose of 100 mg/kg body weight.

The maximum inhibition (45.26%) in the end of the experiment was recorded by the crude extract of *Argania spinosa* at 200mg/kg.

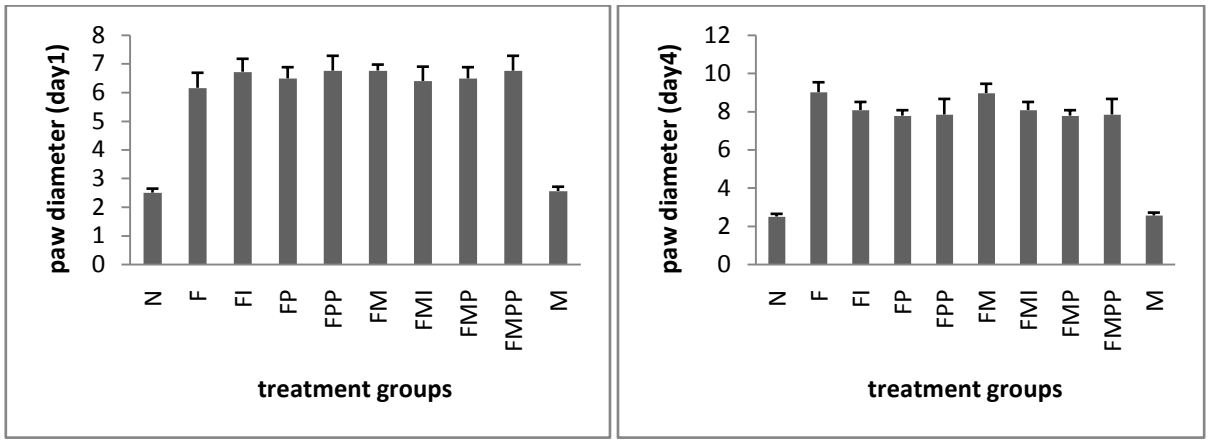

Figure 18. Effect of *Argania spinosa, Boswellia serrata*, *Citrullus colocynthis* in paw diameter on day 1 and day 4 of the experiment

N: normal group; **F:** formalin inflammation group; **FI:** formalin inflammation treated Ibuprofen group; **FP:** formalin inflammation treated *Argania spinosa* 100 mg/kg group, **FPP:** formalin inflammation treated *Argania spinosa* 200 mg/kg group; **FM:** formalin/methionine inflammation group; **FMI:** formalin/methionine inflammation treated Ibuprofen group; **FMP:** formalin/methionine inflammation treated *Argania spinosa* 100 mg/kg group; **FMPP:** formalin/methionine inflammation treated *Argania spinosa* 200 mg/kg group; **M:** methionine inflammation group.

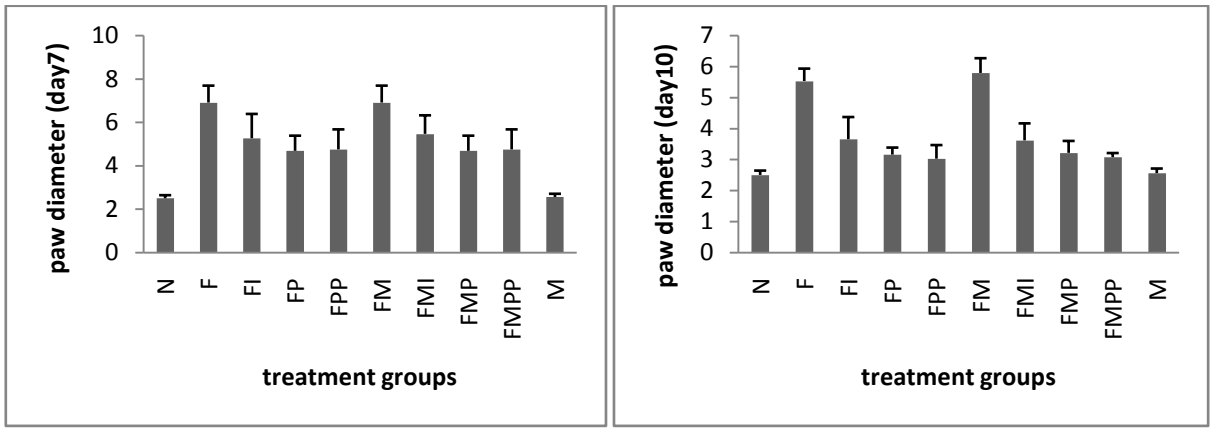

Figure 19. Effect of *Argania spinosa, Boswellia serrata*, *Citrullus colocynthis* in paw diameter on day 7 and day 10 of the experiment

N: normal group; **F:** formalin inflammation group; **FI:** formalin inflammation treated Ibuprofen group; **FP:** formalin inflammation treated *Argania spinosa* 100 mg/kg group, **FPP:** formalin inflammation treated *Argania spinosa* 200 mg/kg group; **FM:** formalin/methionine inflammation group; **FMI:** formalin/methionine inflammation treated Ibuprofen group; **FMP:** formalin/methionine inflammation treated *Argania spinosa* 100 mg/kg group; **FMPP:** formalin/methionine inflammation treated *Argania spinosa* 200 mg/kg group; **M:** methionine inflammation group.

Table 06 . Effect of the oral administration of *Argania spinosa* crude extract on paw diameter, ΔT and on percentage inhibition of arthritis in formalin induced arthritis and on formalin/methionine induced inflammation. 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

Group	Average diameter day 1	Average diameter day 4	Average diameter day 7	Average diameter day 10	ΔT (mm)	Inh%
Group N	2.50 ± 0.14	2.50 ± 0.14	2.50 ± 0.14	2.50 ± 0.14	00	--
Group F	6.16 ± 0.52	9.01 ± 051	6.21 ± 0.54	5.15 ± 0.39	1.63	0.00
Group FI	6.72 ± 0.45	8.07 ± 0.42	5.26 ± 1.11	3.65 ± 0.71**	0.61	33.93
Group FP	6.48 ± 0.39	7.78 ± 0.28	4.50 ± 0.44	3.15 ± 0.22**	0.45	42.87
Group FPP	6.75 ± 0.51	7.84 ± 0.81	4.61 ± 0.71	3.02 ± 0.43**	0.31	45.26
Group FM	6.76 ± 0.20	9.22 ± 0.81	7.32 ± 0.61	5.78 ± 0.47	2.77	0.00
Group FMI	6.40 ± 0.49	8.94 ± 0.37	5.71 ± 0.64	3.61 ± 0.54**	1.06	37.56
Group FMP	6.48 ± 0.39	8.21 ± 0.42	5.42 ± 0.48	3.21 ± 0.38**	0.77	44.47
Group FMPP	6.76 ± 0.52	7.93 ± 0.66	4.75 ± 0.91	3.07 ± 0.13**	0.57	46.89
Group M	2.56 ± 0.14	2.56 ± 0.14	2.56 ± 0.14	2.56 ± 0.14	00	--

N: normal group; **F:** formalin inflammation group; **FI:** formalin inflammation treated Ibuprofen group; **FP:** formalin inflammation treated *Argania spinosa* 100 mg/kg group, **FPP:** formalin inflammation treated *Argania spinosa* 200 mg/kg group; **FM:** formalin/methionine inflammation group; **FMI:** formalin/methionine inflammation treated Ibuprofen group; **FMP:** formalin/methionine inflammation treated *Argania spinosa* 100 mg/kg group; **FMPP:** formalin/methionine inflammation treated *Argania spinosa* 200 mg/kg group; **M:** methionine inflammation group.

The changes in the paws weight at the end of the experience (figure 20) have also been used to assess the course of the disease and the response to the therapy of anti-inflammatory drugs., The group (FM) received formalin subplantar injection (0,2%) and methionine intraperitoneal administration (400mg/kg) present the high significant increase in the arthritic paws weight comparing to the other groups.

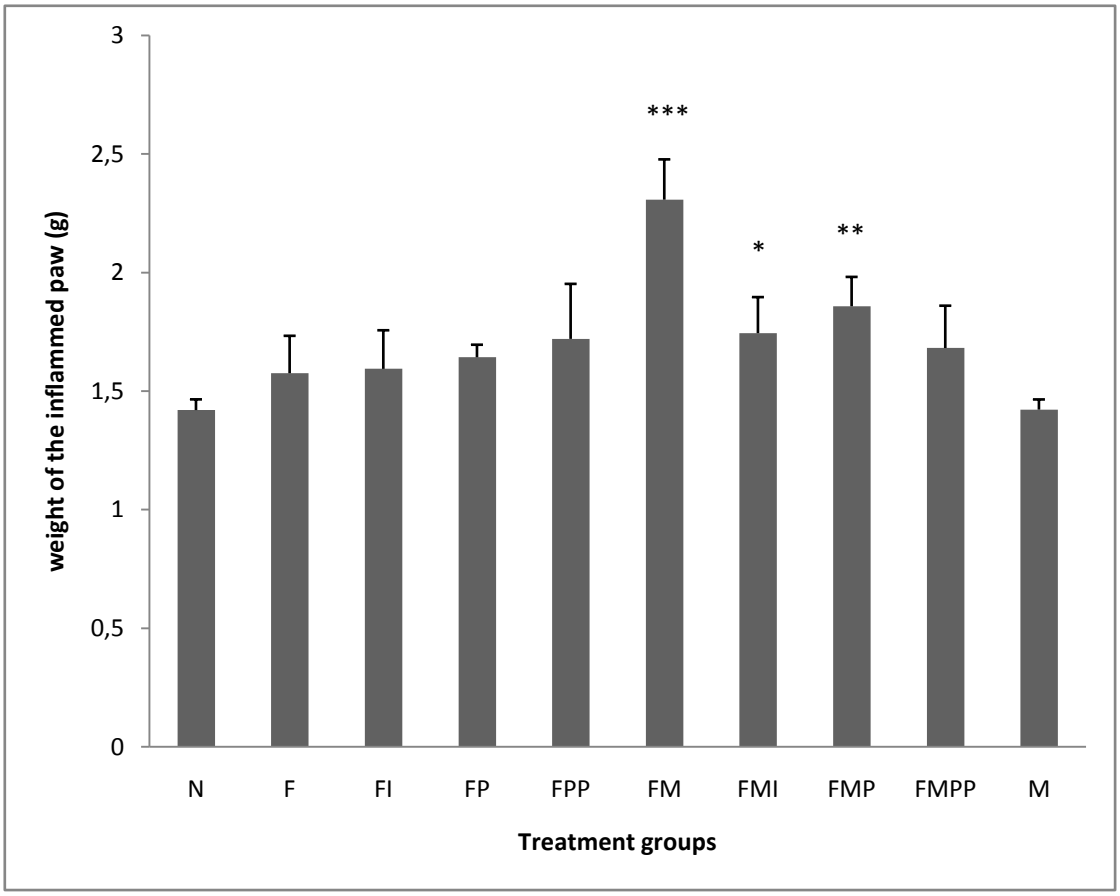

Figure 20. Effect of the oral administration of *Argania spinosa* crude extract in the weight of the right (inflamed) paw at the end of the experiments formalin induced arthritis and formalin/methionine induced inflammation. Results are shown as mean ± SD (n=5) and significant difference from the control group is shown as *p<0,05, **p<0,01, ***p<0,001

N: normal group; **F:** formalin inflammation group; **FI:** formalin inflammation treated Ibuprofen group; **FP:** formalin inflammation treated *Argania spinosa* 100 mg/kg group, **FPP:** formalin inflammation treated *Argania spinosa* 200 mg/kg group; **FM:** formalin/methionine inflammation group; **FMI:** formalin/methionine inflammation treated Ibuprofen group; **FMP:** formalin/methionine inflammation treated *Argania spinosa* 100 mg/kg group; **FMPP:** formalin/methionine inflammation treated *Argania spinosa* 200 mg/kg group; **M:** methionine inflammation group.

At the end of the experimental period, as the incidence and severity of arthritis decreased, the inflamed paws from the groups treated by *Argania spinosa* and reference drug either in groups received formalin injection alone (FP, FPP and FPI) or received formalin and methionine injection (FMP, FMPP and FMI) are quite comparable to the normal group (N), so except for the group received formalin and methionine the weight of animals inflamed paws at the end of the experiment of the entire treated groups was quite comparable to normal

animals due to the decrease in the severity of arthritis. Group received single administration of methionine at dose 400mg/kg did not show increased in paws weight.

In the blood investigation, and concerning the change caused by formalin injection, formalin and methionine injection and plant extract administration in some inflammatory parameters. Firstly, change of plasma CRP level was measured and the results are shown in figure 21 comparing to the normal group, the group received formalin subplantar injection (F) present an increase in hs-CRP level while groups received formalin in combination of Ibuprofen or *Argania spinosa* treatment showed a decrease in hs-CRP level. While the group received formalin subplantar injection in association with methionine administration showed a highly significant increase in plasma hs-CRP comparing to normal (N) and arthritic (F) groups.

The results also showed that treatment by crude extract of *Argania spinosa* seeds at two doses (100 and 200mg/kg) or by Ibuprofen decrease the level of hs-CRP in the blood and the action of the plant at the dose of 200mg/kg was more effective than the dose 100mg/kg and its action was similar to standard drug at dose 100mg/kg. The group received methionine alone presented also a high significant increase in hs-CRP comparing to normal group.

Homocysteine blood level was also measured as inflammation parameter in the study of the anti-arthritic effect of *Argania spinosa* seeds (figure 22).

A high dose (400mg/kg) of methionine administration for 10 days was sufficient to induce hyperhomocysteinemia in rats notable by high significant increase of plasma homocysteine compared to the normal group (Group M: HCY = 17,82±1,85 vs Group N: HCY=10,26±1,07) ; *Argania spinosa* seeds crude extract supplementation to the rats injected with the high-methionine dose prevented an elevation of Homocysteine levels in the blood.

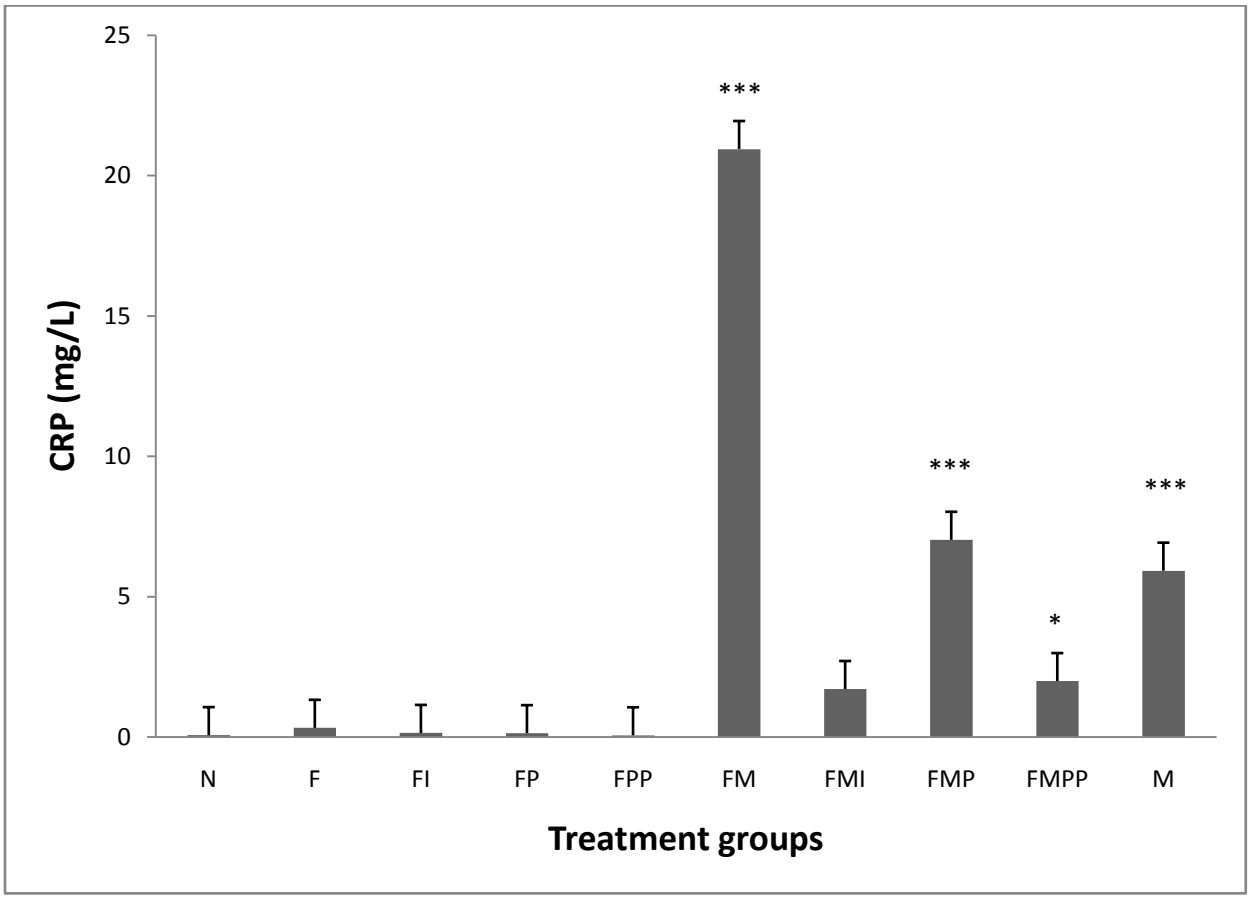

Figure 21. Effect of the treatment by *Argania spinosa* seeds on inflammatory marker (CRP blood level) during formalin induced arthritis and formalin/methionine induced arthritis experiments. Results are shown as mean ± SD (n=5) and significant difference from the control group is shown as *p<0,05, **p<0,01, ***p<0,001

N: normal group; **F:** formalin inflammation group; **FI:** formalin inflammation treated Ibuprofen group; **FP:** formalin inflammation treated *Argania spinosa* 100 mg/kg group, **FPP:** formalin inflammation treated *Argania spinosa* 200 mg/kg group; **FM:** formalin/methionine inflammation group; **FMI:** formalin/methionine inflammation treated Ibuprofen group; **FMP:** formalin/methionine inflammation treated *Argania spinosa* 100 mg/kg group; **FMPP:** formalin/methionine inflammation treated *Argania spinosa* 200 mg/kg group; **M:** methionine inflammation group.

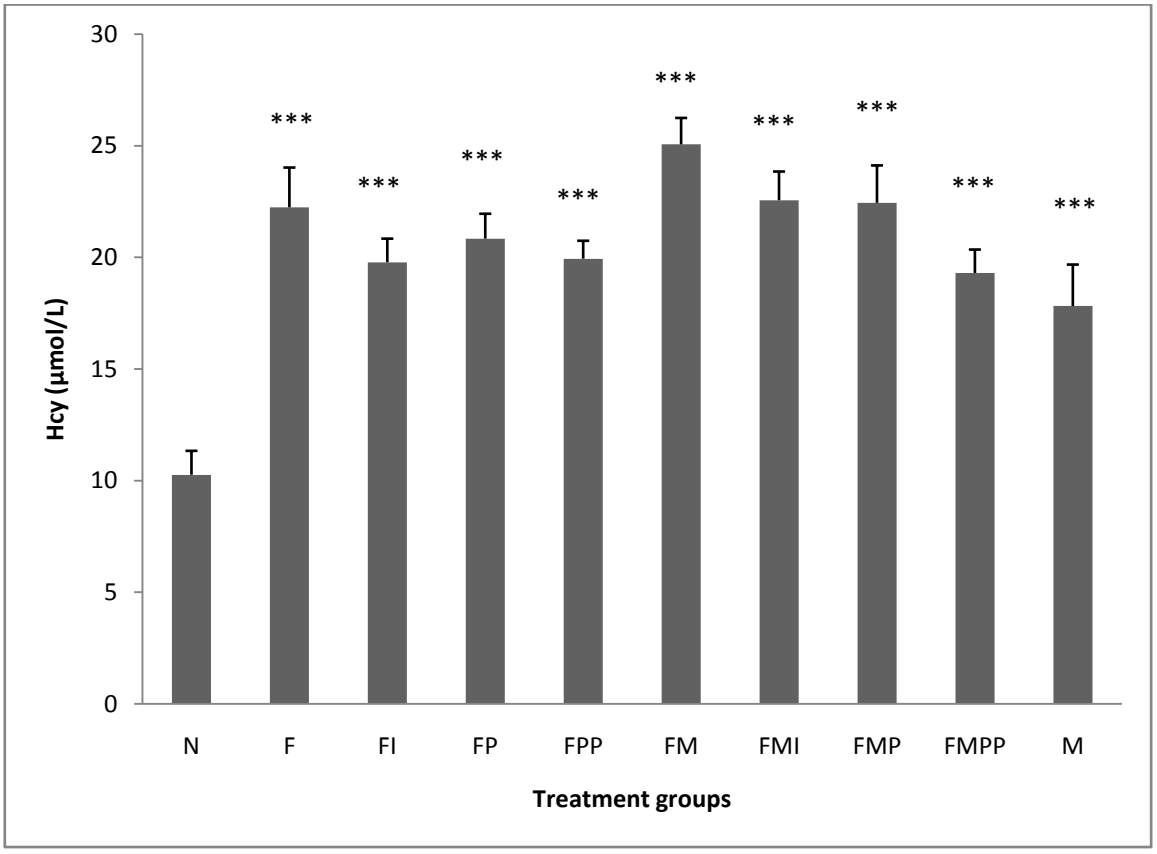

Figure 22. Effect of the treatment by *Argania spinosa* seeds on Homocysteine (Hcy) blood level during formalin induced arthritis and formalin/methionine induced arthritis experiments. Results are shown as mean ± SD (n=5) and significant difference from the control group is shown as *p<0,05, **p<0,01, ***p<0,001

N: normal group; **F:** formalin inflammation group; **FI:** formalin inflammation treated Ibuprofen group; **FP:** formalin inflammation treated *Argania spinosa* 100 mg/kg group, **FPP:** formalin inflammation treated *Argania spinosa* 200 mg/kg group; **FM:** formalin/methionine inflammation group; **FMI:** formalin/methionine inflammation treated Ibuprofen group; **FMP:** formalin/methionine inflammation treated *Argania spinosa* 100 mg/kg group; **FMPP:** formalin/methionine inflammation treated *Argania spinosa* 200 mg/kg group; **M:** methionine inflammation group.

Histology of the rat joint of arthritic rats belonging to arthritic groups (group F received subplantar injection of formalin and group FM received subplantar injection of formalin + intraperitoneal injection of 400mg/kg of methionine) showed necrotic and degenerative changes while in case of normal group no such signs are observed in histology analysis (figures 24 and 28) respectively.

In normal group, there was a normal vascular supply and intact cells including chondroblasts, also the synovium was not disrupted. In contrast, rats treated with *Argania spinosa* at both doses 100mg/kg (FP, FPP, FMP and FMPP) and standard groups (FI and FMI) in the both inflammatory models showed moderate destruction and less degenerative changes as compared to arthritic groups (Figures 26, 27, 30 and 31).

Histological assessment of the inflamed paws from group (F) received subplantar injection of formalin and group (FM) received subplantar injection of formalin and intraperitoneal injection of 400mg/kg of methionine showed only foci of mild chronic inflammatory change characterized by fibrosis, damaged articular cartilage with scattered lymphocytes. Tissue from the animals paws of the groups treated by *Argania spinosa* at both doses 100mg/kg (FP, FPP, FMP and FMPP) and standard groups (FI and FMI) in the both inflammatory models showed mild inflammatory changes dominated by lymphocytes with scattered plasma cells. The paws from untreated groups (formalin group F and formalin and methionine group FM) showed foci of intense inflammatory changes dominated by neutrophils, plasma cells, lymphocytes and focus of pus collection with necrotic debris.

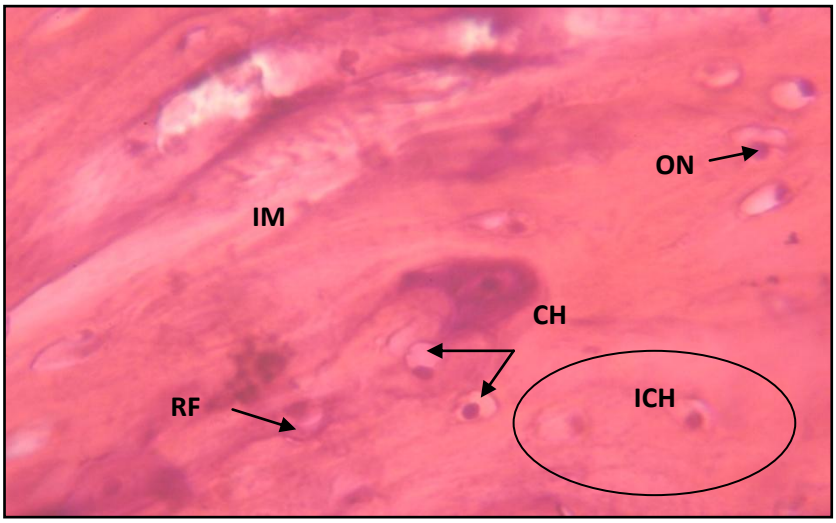

Figure 23. Histological section of rat normal joint 10 days oral flour application H.E staining (X400).

RF. Reticulin Fiber ON. Oval Nuclei

CH. Chondroplast IM. Intact Matrix

ICH. Intact Chondroblasts

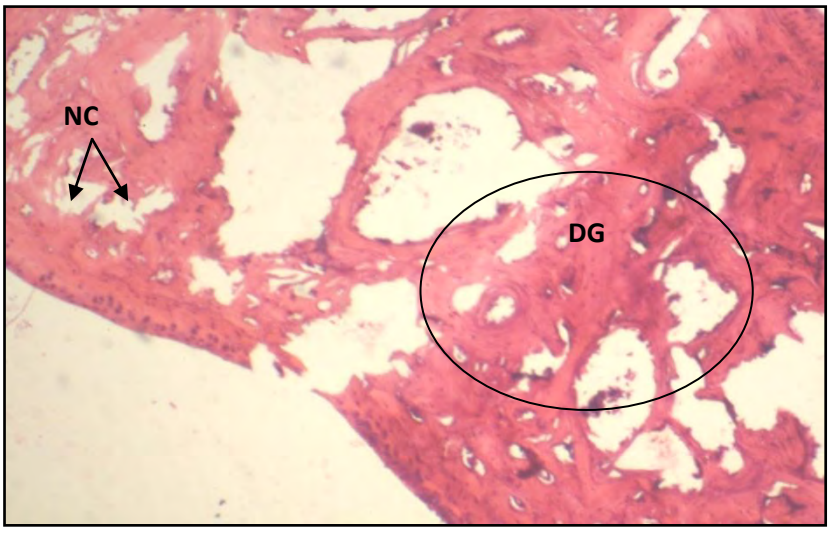

Figure 24. Histological section of rat joint 10 days flour + Formalin application H.E staining (X40).

DG. Degeneration of articular cartilage NC. Necrosis

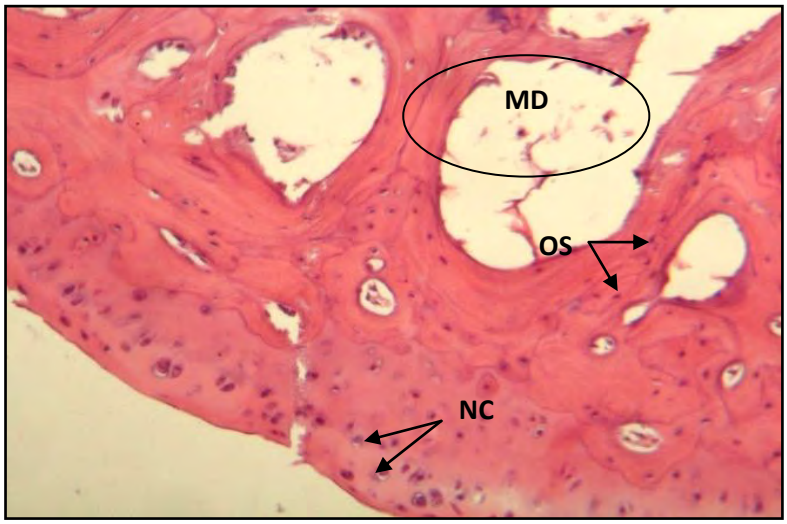

Figure 25. Histological section of rat joint 10 days oral Ibuprofen (100mg/kg) + formalin application H.E staining (X100).

MD. Moderate destruction NC. Normal chondroblasts

OS. Osteoblasts

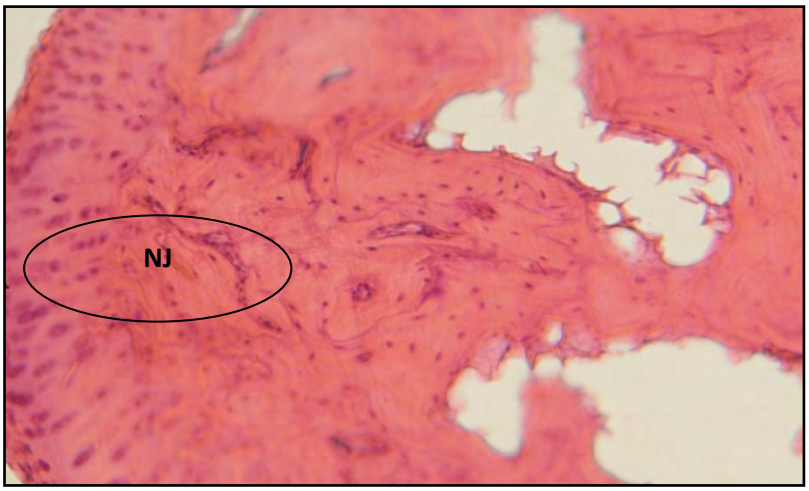

Figure 26. Histological section of rat joint 10 days oral plant extract (100mg/kg) + formalin application H.E staining (X100).

NJ. Normal joint with intact chondroblasts and osteoblasts

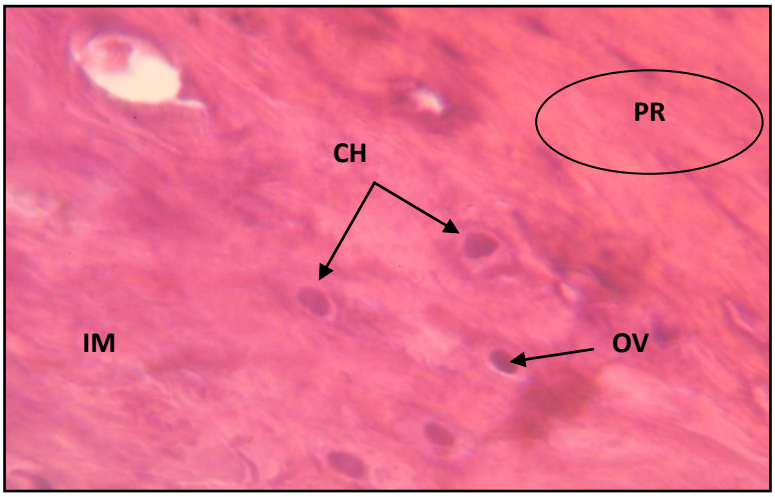

Figure 27. Histological section of rat joint 10 days oral plant extract (200mg/kg) + formalin application H.E staining (X400).

PR. Perichondrium ON. Oval Nuclei

CH. Chondroplast IM. Intact Matrix

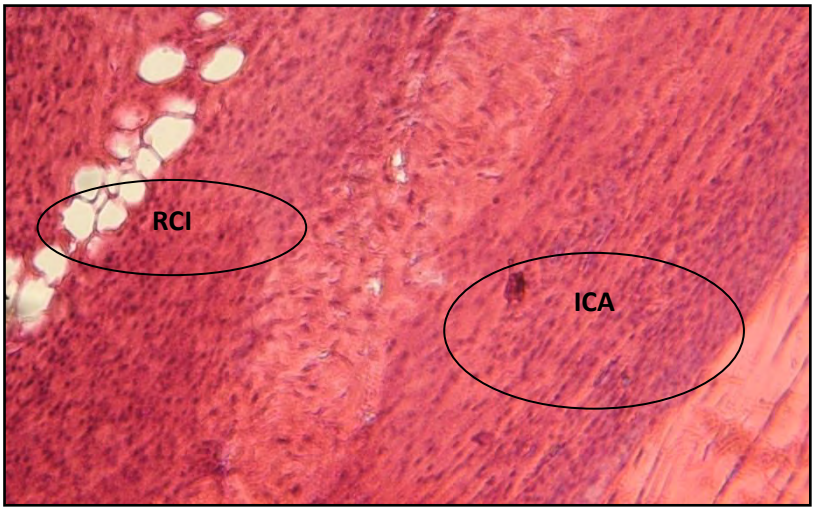

Figure 28. Histological section of rat joint 10 days methionine (400mg/kg) + formalin application H.E staining (X400).

ICA. Inflammatory cells accumulation RCI. Replacement of cartilage by inflammatory cells

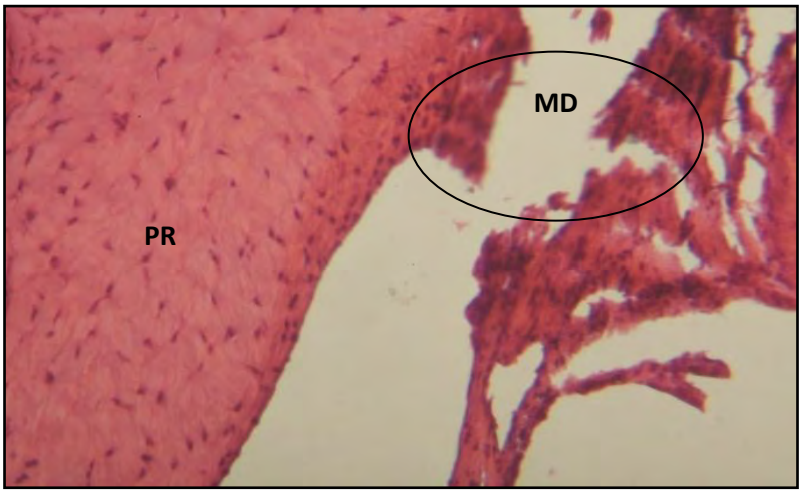

Figure 29. Histological section of rat joint 10 days oral Ibuprofen (100mg/kg) + methionine (400mg/kg) + formalin application H.E staining (X100).

PR. Perichondrium MD. Moderate degeneration

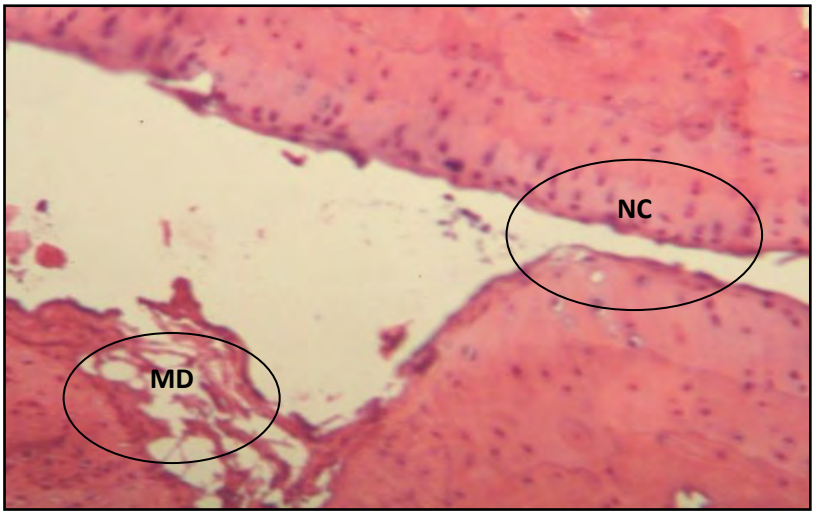

Figure 30. Histological section of rat joint 10 days oral plant extract (100mg/kg) + methionine (400mg/kg) + formalin application H.E staining (X100).

MD. Moderate degeneration NC. Normal cells

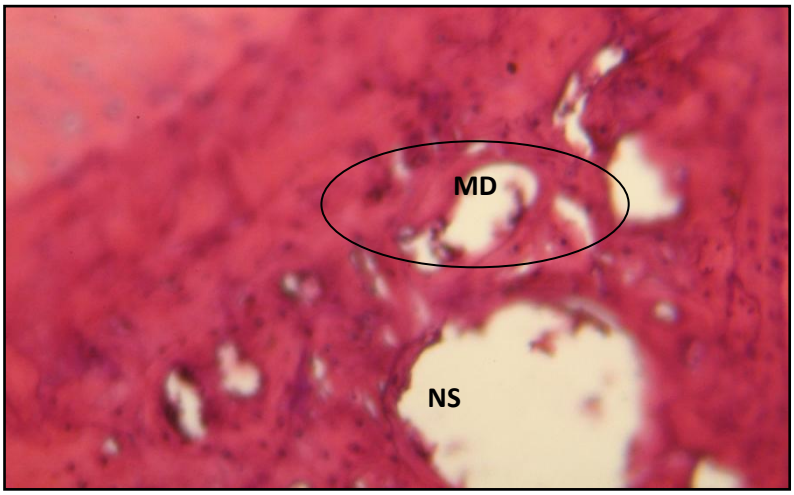

Figure 31. Histological section of rat joint 10 days oral plant extract (200mg/kg) + methionine (400mg/kg) + formalin application H.E staining (X100).

NS. Necrosis MD. Moderate degenaration

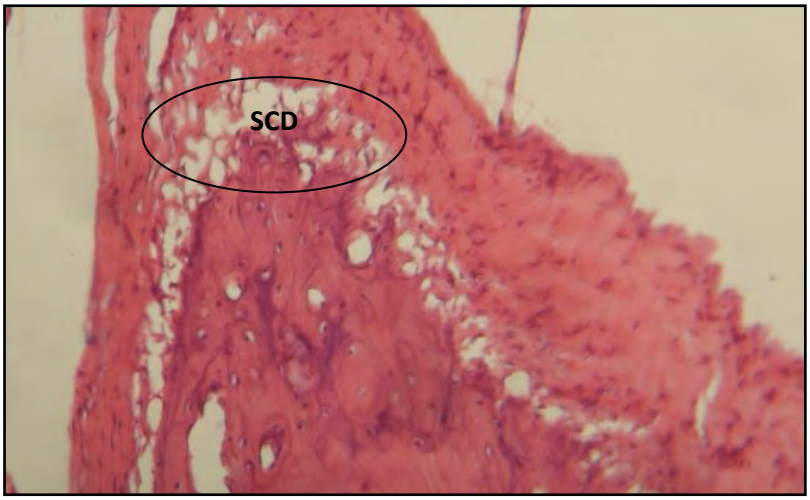

Figure 32. Histological section of rat joint 10 days oral flour + methionine (400mg/kg) application H.E staining (X100).

SCD. Superficial cartilage damage

Inflammation is the immune system's response to infection and injury and has been implicated in the pathogeneses of arthritis, cancer and stroke, as well as in neurodegenerative and cardiovascular disease (Littlefield et al., 2014).

The experimental models of inflammation are produced by different agents by releasing different types of inflammatory mediators. Each is known to elicit distinct mechanisms of action for producing inflammation by increased in vascular permeability, the infiltrations of leukocytes from the blood into the tissue or granuloma formation and tissue repair. Among the many methods used for screening of anti-inflammatory drugs, one of the most commonly employed techniques is based upon the ability of such agents to inhibit hind paw edema of the rat after the injection of a phlogistic (irritants) agent such as formaldehyde, carrageenan or xylene (Shaikh, 2011).

For evaluating the most effective and widely used model for inflammation formalin induced paw edema was used, this test is one of the most commonly used acute model for assessing anti-arthritic and anti-inflammatory potential of plant extract as it closely resembles human arthritis (Shastry et al., 2011).

Rheumatoid arthritis is a chronic inflammatory disease affecting about 1 % of the population in developed countries. The acute stage of arthritis is characterized by signs of hyperalgesia, lack of mobility and a pause in body weight gain; during the acute period, the hind and fore paw joint diameters increase. In chronic stages of the disease rats with arthritis are often relatively immobile due to the severity of paw swelling (Desai et al., 2012).

The development of arthritis in the rat paw after the injection of formalin is -as previously described- a biphasic event. The initial phase of the oedema is due to the release of histamine and serotonin and the edema is maintained during the plateau phase by kinin-like substance (Chauhan et al., 1998) and the second accelerating phase of edema formation may be due to the release of prostaglandin-like substances. Inhibition of edema observed in the formalin model may be due to the ability of bioactive substances present in *Argania spinosa* to inhibit these chemical mediators of inflammation. The results of the formalin induced arthritis ruled out a possible effect of the crude extract of *Argania spinosa* on formalin induced cell damage and accordingly, arthritic conditions (Firdous and Kineri, 2012).

Additionally, hyperhomocysteinemia, defined by elevated circulating blood levels of homocysteine, is associated with several pathologies including cardiovascular disease stroke (Refsum et al., 1998), neural tube defects in offspring (Van Der Put et al., 1997), certain cancers (Ueland et al., 2001), and rheumatoid arthritis (Wallberg-Jonsson et al., 2002).

Elevated homocysteine may be indicative of an underlying dysregulation of folate/Homocysteine metabolism. Several of the pathologies that are associated with folate/Homocysteine dysregulation have inflammatory components (Hammons et al., 2009).

The molecules and cellular mediators of inflammation such as tumor necrosis factor TNF α, IL-1, IL-6, intercellular cell adhesion molecule ICAM-1 and E-Selectin, which have roles in the inflammation of rheumatoid arthritis, can also play key roles in the development of atherosclerosis (Tiftikci et al., 2006).

The major molecular mechanisms of Hyperhomocysteinemia involve endothelial cell dysfunction (Austin et al., 2004), smooth muscle cell proliferation (Lee et al., 2002), and mononuclear cell activation (Silverman et al., 2002). Also Homocysteine may induce oxidative stress leading to impaired synthesis of nitric oxide (Upchurch et al., 1997; Romerio et al., 2004) and resulting in endothelial dysfunction (Stamler et al., 1993; Zhang et al., 2000). In addition, homocysteine-induced oxidative stress favors lipid peroxidation (Ferreti et al., 2004) and induces production of inflammatory factors such as monocyte chemoattractant protein-1, interleukin-8 (Mathiesen et al., 2001; Zeng et al., 2003), and tumor necrosis factor α (Hofmann et al., 2001; Bai et al., 2007), thus accelerating atherosclerosis.

Although results from *in vitro* studies suggest that Homocysteine, at pathophysiological concentrations, stimulates chemokine expression in vascular cells (Li et al., 2006), it is unknown whether hyperhomocystinemia can accelerate arthritis, leading to enhanced inflammatory response *in vivo*. On the basis of the pathogenic role of homocysteine in atherogenesis and arthritis, the objective of our study was to investigate whether hyperhomocysteinemia may accelerate arthritis *in vivo* using the combination between the subplantar injection of formalin which cause arthritis and intraperitoneal administration of high dose of methionine which lead to hyperhomocysteinemia.

In other-side C-reactive protein (CRP), a major systemic marker of inflammation, the plasma concentration of which is regulated by cytokines such as IL-6, have been used to predict the risk of cardiovascular events. Furthermore, it was recently demonstrated that CRP induces adhesion molecule expression in human endothelial cells, supporting the hypothesis of a direct role of CRP in promoting an inflammatory component in the atherosclerotic process (Bekpinar et al., 2003).

Our results showed that methionine induced hyperomocysteinemia in arthritic rats can accelerate and aggravate the pathogenesis of arthritis presented by a highly significant increase of CRP when comparing the formalin arthritic group (F) and the formalin/ methionine associated inflammation group (FM) and confirmed by histological study of the inflamed joints. Also, the results of this study mentioned the role of the treatment using *Argania spinosa* seeds extract in combating the inflammation caused either by formalin or formalin and methionine and this was quite similar to the action of Ibuprofen the reference anti-inflammatory drug.

Thus, the previous findings and these results presented here might indicate that the anti-inflammatory and antiarthritic activities of *Argania spinosa* could be due to the presence of a variety of antioxidant molecules such as sterols, polyphenols, and tocopherols (Cadi et al., 2013) which may have beneficial effects against inflammatory disorders including atherosclerosis and arthritis.

Our results confirm the study of the group of Schroecksnadel K. who suggested after a clinical study that immune activation could be involved in the development of hyperhomocysteinaemia because they found that the higher homocysteine concentrations and lower folate concentrations coincide with increased concentrations of immune activation markers in patients with RA (Schroecksnadel et al., 2003).

The results of the antiarthritic effect exerted by our crude extract of *Argania spinosa* agree with those of Kaithwas G. and his group who studied and confirmed the antiarthritic effect of *Ajuga bracteosa* in formalin induced arthritis (Kaithwas et al., 2012).

III.6. Antiproliferative activity

III.6.1. Effect of plants extracts on cell proliferation

III.6.1.1. Effect of Argan oil extracted from *Argania spinosa* seeds

As shown in Figures 33 and 34, Argan oil treatment strongly reduced the proliferation and the viability of all the three T-ALL cell lines in both tests MTT assay and Trypan blue assay.

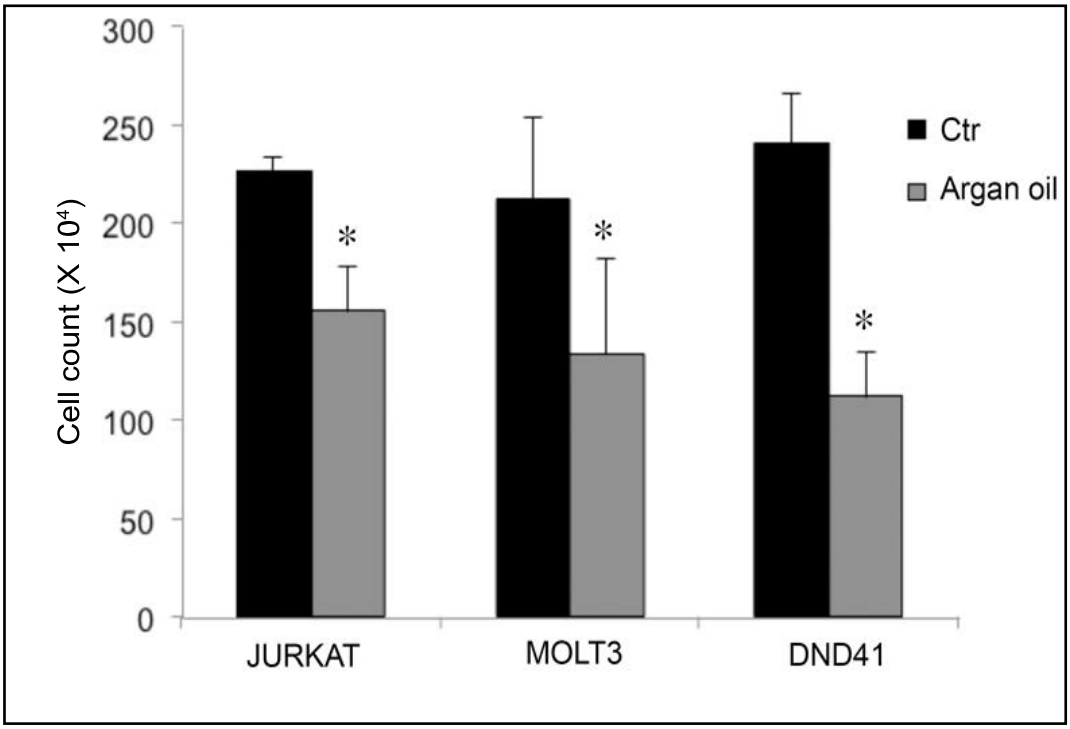

Figure 33. Effect of Argan oil on cell proliferation of JURKAT, MOLT3 and DND41 by trypan blue exclusion assay. Cell growth of JURKAT, MOLT3 and DND41 after 48h of treatment with 100 µg/ml of Argan oil (Argan oil) compared to the DMSO vehicle exposed cells (Ctr) as shown by the cell counts by using trypan blue staining. Results shown in the figures are expressed as the means average deviations of three separate experiments and bars indicate SD *P<0.05.

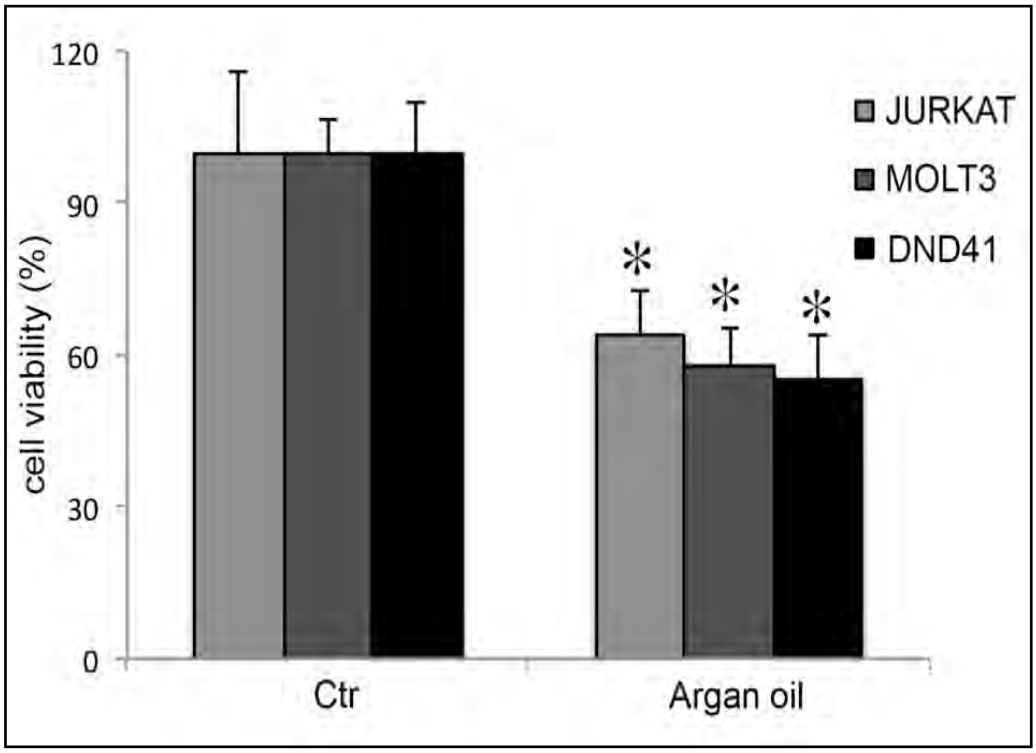

Figure 34. Effect of Argan oil cell viability of JURKAT, MOLT3 and DND41 in MTT assay. The Argan oil cytotoxicity was determined in JURKAT, MOLT3 and DND41 by MTT assay and calculated as percentage of inhibition of cell proliferation after 48h of treatment with 100 µg/ml of Argan oil (Argan oil) compared to the DMSO vehicle exposed cells (Ctr). Results shown in the figures are expressed as the means average deviations of three separate experiments and bars indicate SD *P<0.05.

On the other hand the Argan oil was evaluated in the growth and survival of four non tumor cell lines including murine preT 2017 and M31 immature T cell lines, human HEK 293 and HaCat-keratinocytes. The same treatment by Argan oil did not affect neither the growth of murine preT 2017 and M31 immature T cell lines nor of human HEK 293 and HaCat-keratinocytes (figure 35).

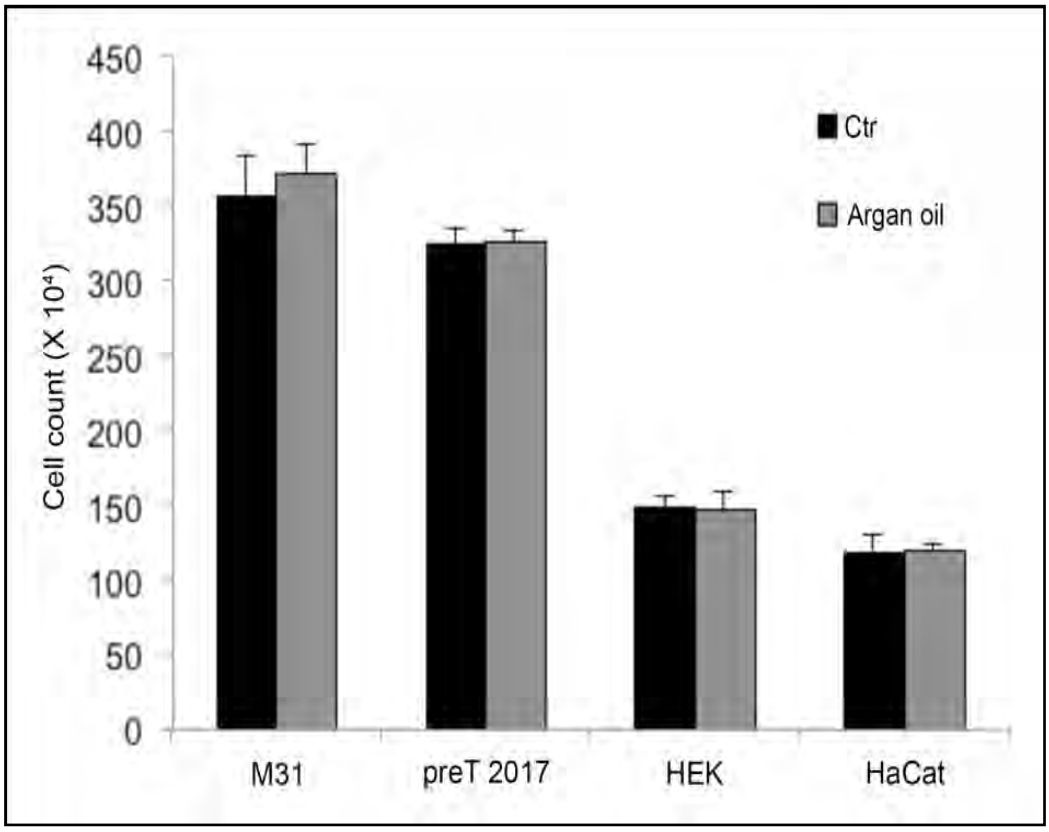

Figure 35. Effect of Argan oil on cell proliferation of M31, PreT, HEK and Hecat by trypan blue exclusion assay. The cell growth of M31, preT 2017, HEK and HaCat cell lines was not affected by 48h of exposure with 100 µg/ml of Argan oil (Argan oil) when compared with the DMSO treated counterpart cells (Ctr) and as shown by the cell counts by using trypan blue staining. Results shown in the figures are expressed as the means average deviations of three separate experiments.

III.6.1.2. Effect of the oil extracted from *Citrullus colocynthis*

The *in vitro* assay of Citrullus oil showed strong decrease in the proliferation and the viability of all the three T-ALL cell lines but didn't affect the proliferation of the normal cells in Trypan blue assay (Figure 36 and 37).

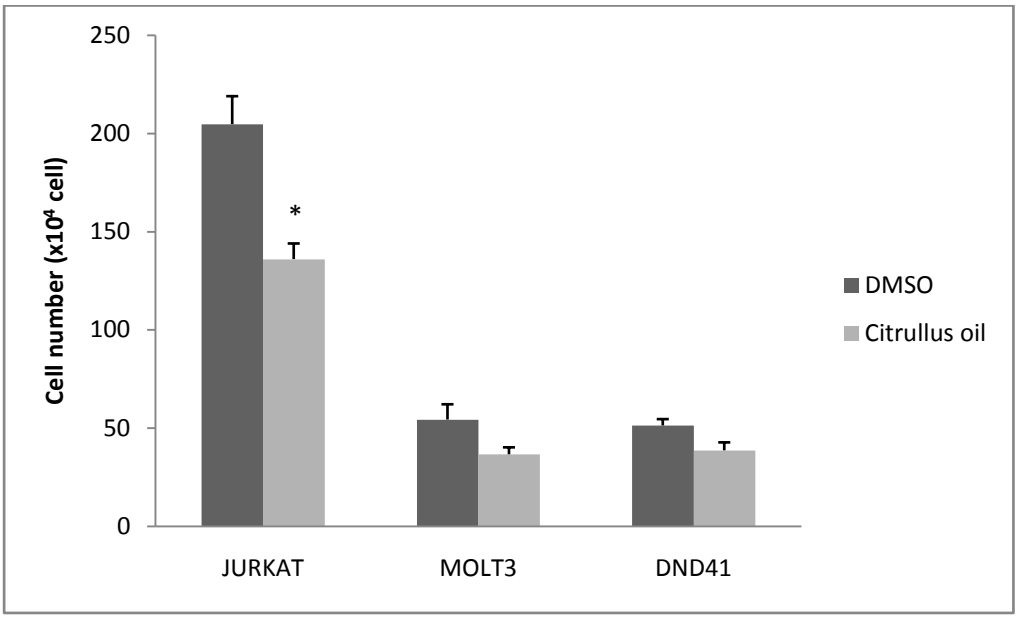

Figure 36. Effect of Citrullus oil on cell proliferation of JURKAT, MOLT3 and DND41 by trypan blue exclusion assay. Cell growth of JURKAT, MOLT3 and DND41 after 48h of treatment with 100 µg/ml of Citrullus oil compared to the DMSO vehicle exposed cells (Ctr) as shown by the cell counts by using trypan blue staining. Results shown in the figures are expressed as the means average deviations of three separate experiments and bars indicate SD *P<0.05.

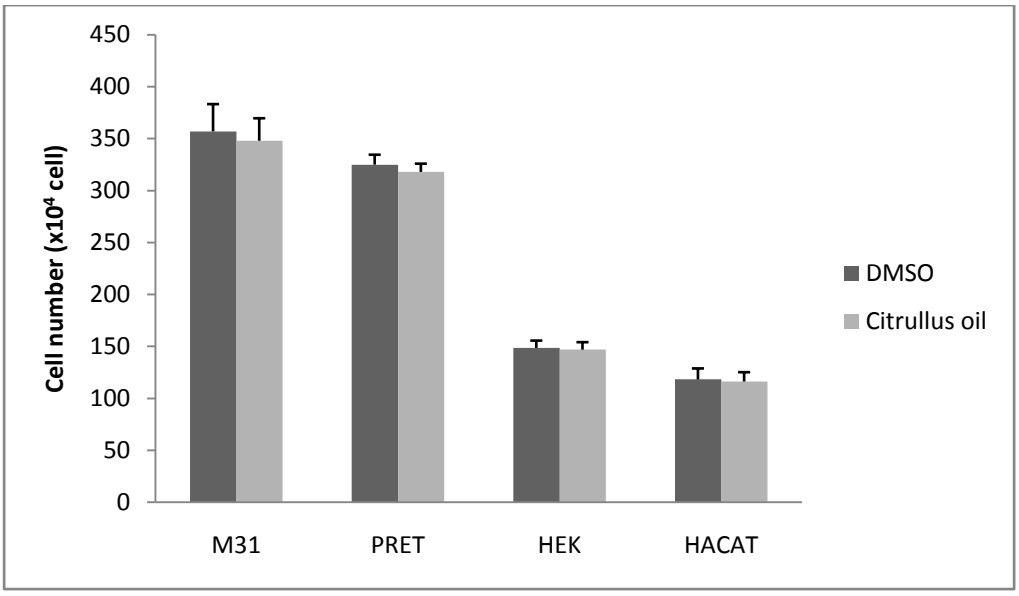

Figure 37. Effect of Citrullus oil on cell proliferation of M31, PreT, HEK and Hecat by trypan blue exclusion assay. The cell growth of M31, preT 2017, HEK and HaCat cell lines was not affected by 48h of exposure with 100 µg/ml of Argan oil (Argan oil) when compared with the DMSO treated counterpart cells (Ctr) and as shown by the cell counts by using trypan blue staining. Results shown in the figures are expressed as the means average deviations of three separate experiments.

III.6.1.3. Effect of the oil extracted from *Boswellia serrata*

The treatment using Boswellia oil decreased the proliferation of T-ALL cell lines and presented no effect in normal cell lines (figure 38 and 39).

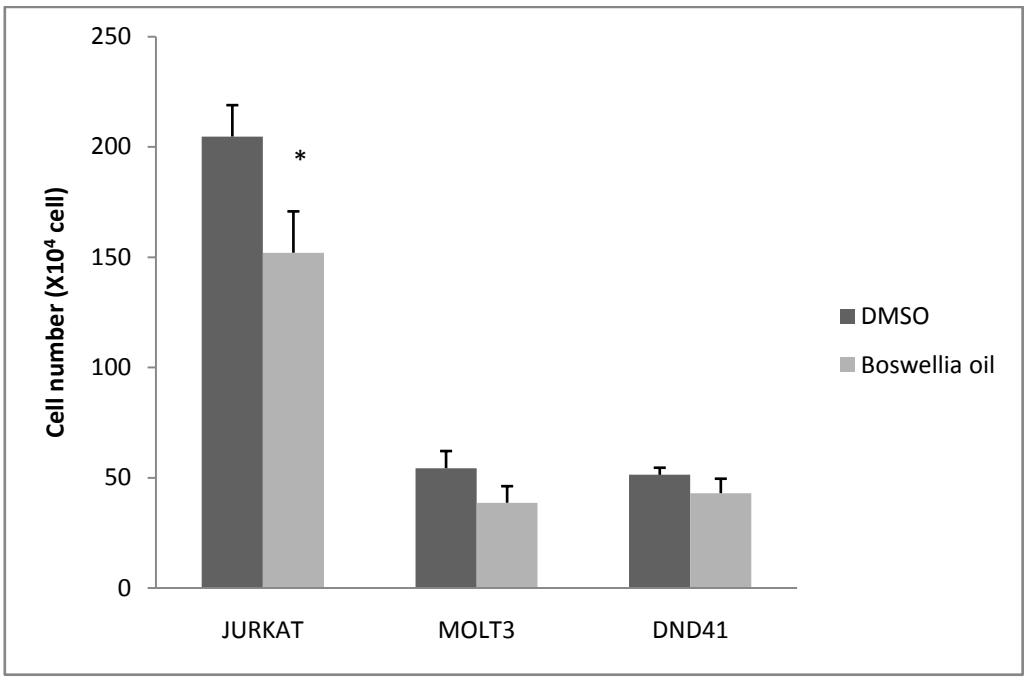

Figure 38. Effect of Boswellia oil on cell proliferation of JURKAT, MOLT3 and DND41 by trypan blue exclusion assay. Cell growth of JURKAT, MOLT3 and DND41 after 48h of treatment with 100 µg/ml of Boswellia oil compared to the DMSO vehicle exposed cells (Ctr) as shown by the cell counts by using trypan blue staining. Results shown in the figures are expressed as the means average deviations of three separate experiments and bars indicate SD *P<0.05.

All the data and the observations mentioned in the *in vitro* antiproliferative test concerning our oils revealed a specificity of action of Argan oil, *Citrullus* oil and *Boswellia* oil in malignant (tumor) cells and especially in T-ALL context.

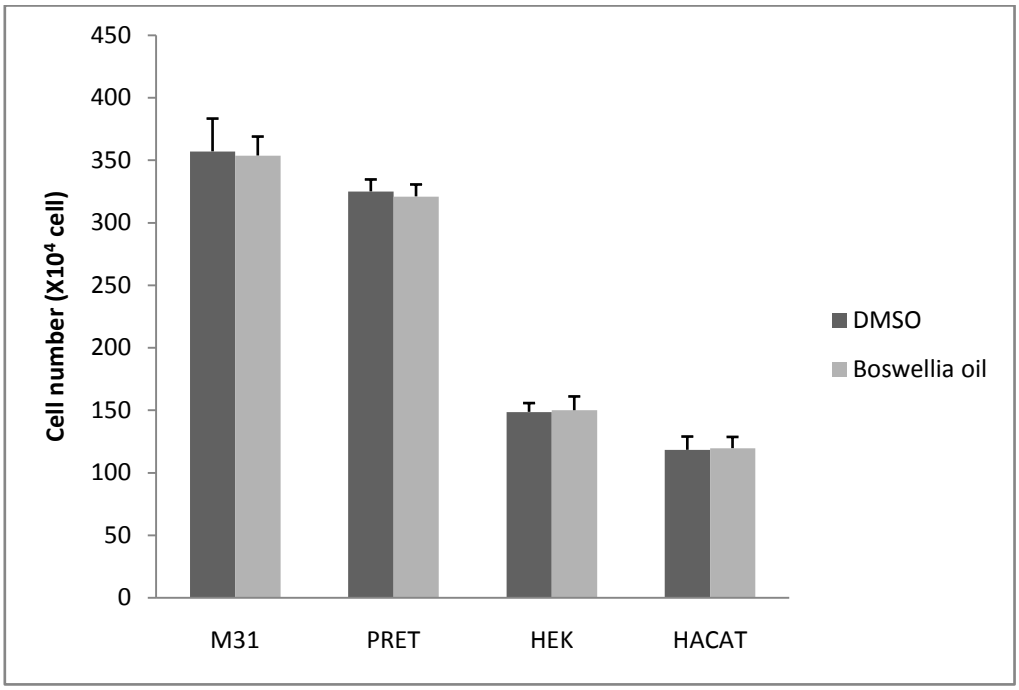

Figure 39. Effect of Boswellia oil on cell proliferation of M31, PreT, HEK and Hacat by trypan blue exclusion assay. The cell growth of M31, preT 2017, HEK and HaCat cell lines was not affected by 48h of exposure with 100 µg/ml of Boswellia when compared with the DMSO treated counterpart cells (Ctr) and as shown by the cell counts by using trypan blue staining. Results shown in the figures are expressed as the means average deviations of three separate experiments.

III.6.2. Effect of plants extracts on signaling proteins expression

III.6.1.2. Effect of the oil extracted from *Argania spinosa*

The anti-proliferative activity of Argan oil on T cell lymphoblastic leukemia cell lines was confirmed by testing the change in the expression/activity levels of the known proproliferative signaling NOTCH1 and ERK (Figure 40).

As shown in Figure 40, NOTCH and ERK signaling were differentially modulated by the Argan oil treatment in the three human T-ALL cell lines analysed.

The expression of the active intracellular domain of NOTCH1 (NOTCH1-IC) was strongly decreased by the Argan oil exposure both in MOLT3 and in DND41 while resulted increased in JURKAT T cells. On the other hand, the phosphorylation of ERK1/2 was impaired by Argan oil in JURKAT and MOLT3 while was not affected in DND41.

Interestingly, ERK1/2 expression was not modulated by the treatment thus suggesting that the decreased phosphorylation in JURKAT and MOLT3 is not done by a deregulated expression, but by the inhibition of the post-translational mechanism.

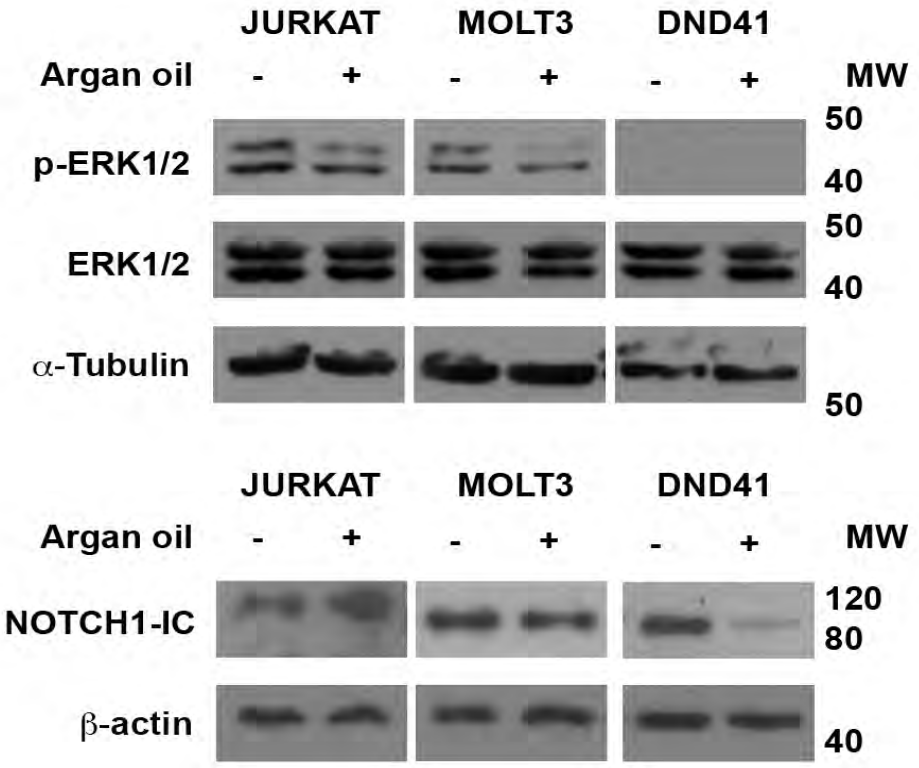

Figure 40. Effect of Argan oil in expression of proteins NOTCH1 and ERK1/2. Total cell extracts from JURKAT, MOLT3 and DND41 treated with DMSO (CTR) or with Argan oil (Argan) were subjected to western blot by using antibodies against NOTCH-1 (NOTCH1-IC), ERK1/2 (ERK1/2) and pospho-ERK1/2 (p-ERK1/2). The α- tubulin and β-actin expression were used as loading control.

\+ With oil treatment
\- Without oil treatment

III.6.2.2. Effect of the oil extracted from *Citrullus colocynthis*

The anti-proliferative activity of Citrullus oil on T cell lymphoblastic leukemia cell lines was tested in the expression of the proliferative signaling NOTCH1 and ERK, and as shown in Figure 41, NOTCH and ERK signaling were not modulated by the Citrullus oil treatment in the three human T-ALL cell lines.

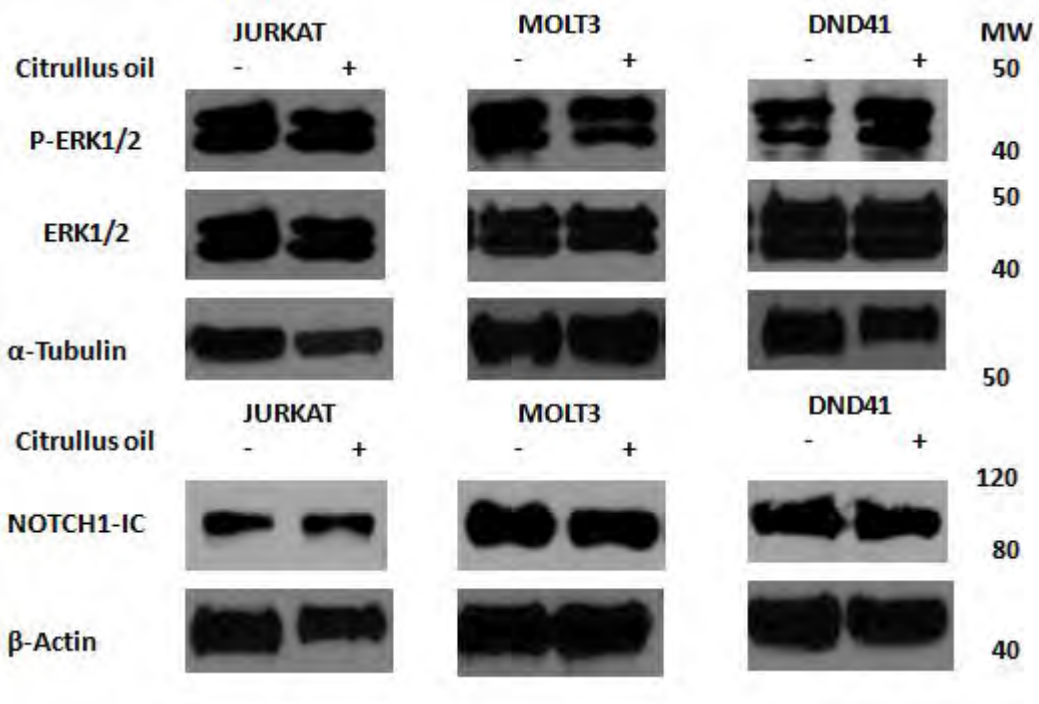

Figure 41. Effect of Citrullus oil in expression of proteins NOTCH1 and ERK1/2. Total cell extracts from JURKAT, MOLT3 and DND41 treated with DMSO (CTR) or with Citrullus oil were subjected to western blot by using antibodies against NOTCH-1 (NOTCH1-IC), ERK1/2 (ERK1/2) and pospho-ERK1/2 (p-ERK1/2). The α - tubulin and β -actin expression were used as loading control.

\+ With oil treatment
\- Without oil treatment

III.6.2.3. Effect of the oil extracted from *Boswellia serrata*

Boswellia oil was used to quantify the anti-proliferative activity in lymphoblastic leukemia in the molecular level using the estimation of the expression level of the proliferative signaling NOTCH1 and ERK, and as shown in Figure 42, there is no seen modulation in the expression of these proteins in the three human T-ALL cell lines.

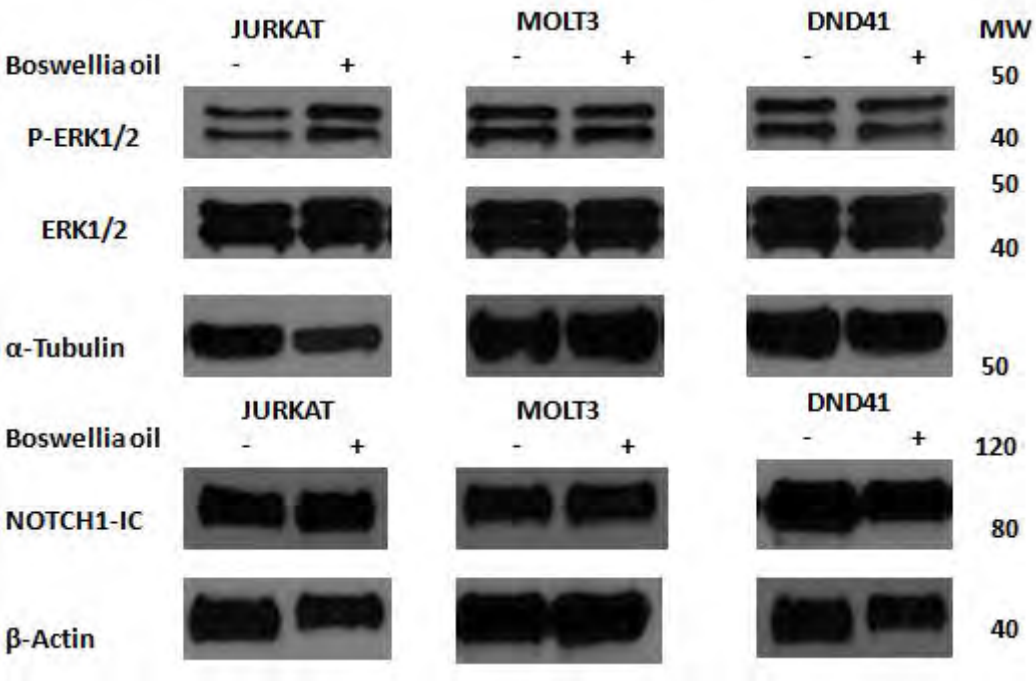

Figure 42. Effect of Boswellia oil in expression of proteins NOTCH1 and ERK1/2. Total cell extracts from JURKAT, MOLT3 and DND41 treated with DMSO (CTR) or with Boswellia oil were subjected to western blot by using antibodies against NOTCH-1 (NOTCH1-IC), ERK1/2 (ERK1/2) and pospho-ERK1/2 (p-ERK1/2). The α - tubulin and β -actin expression were used as loading control.

\+ With oil treatment
\- Without oil treatment

The expression of the active intracellular domain of NOTCH1 (NOTCH1-IC) was strongly decreased by the Argan oil exposure both in MOLT3 and in DND41 while resulted increased in JURKAT T cells. On the other hand, the phosphorylation of ERK1/2 was impaired by Argan oil in JURKAT and MOLT3 while was not affected in DND41. Interestingly, ERK1/2 expression was not modulated by the treatment thus suggesting that the decreased phosphorylation in JURKAT and MOLT3 is not done by a deregulated expression, but by the inhibition of the post-translational mechanism.

Currently applied radiation therapy and standard chemotherapeutic drugs using in cancer treatment kill some tumor cells through induction of apoptosis. Unfortunately, however, the majority of human cancers are resistant to these therapies. It is therefore urgent to look for novel natural compounds as candidate antitumor agents (Kazi et al., 2003).

Inhibition of proliferation has been a continuous effort in tumor treatment. Suppression of cell growth and induction of cell death are two major means to inhibit cancer growth (Huang et al., 2003).Natural antioxidants in vegetable food such as tea and olive oil are believed to reduce the risk of cancer (Paschka et al., 1998; Norrish et al., 1999).

A cell cycle arrest mediated by up-regulation of the p27 cell cycle regulatory protein may explain the observed physiological activity of the tocopherols, which is an important fraction present in Argan, Citrullus and Boswellia oils. Many epidemiologic studies using a γ-tocopherol-enriched diet have confirmed the beneficial effects of γ-tocopherol on prostate cancer prevention in humans (El Monfalouti et al., 2010). Consequently, these results have encouraged the study of the antiproliferative effects of the three oil extracts obtained from Algerian *Argania spinosa* seeds, *Citrullus colocynthis* seeds and *Boswellia serrata* found to inhibit cell growth and proliferation selectively in human tumor and transformed (JURKAT, MOLT3 and DND41), but not normal and non-transformed cells (M31, PreT, HEK and Hacat).

Our data indicated that Argan oil, Citrullus oil and Boswellia oil exerted an inhibitory effect on the proliferation of JURKAT, MOLT3 and DND41 cell lines, independently on their phenotypic or molecular differences.

The exploration of Argan oil, Citrullus oil and Boswellia oil on the proliferation of three T-ALL cell lines (JURKAT, MOLT3 and DND41), characterized by distinct immune-phenotype and molecular features. According to the characterization by markers expression proposed by Burger and colleagues, JURKAT resemble a mature T cell stage by expressing CD3, CD4 and TCR, while losing the CD1 antigen; MOLT3 have features of early cortical T stage characterized by CD1 and low CD3 co-receptor expression; and DND41 represent the transition stage from the cortical to the mature T-stage by expressing CD3 and TCR, while still bearing the CD1 antigen (Burger et al., 1999). In addition, although the three cell lines analyzed display the constitutive activation of NOTCH1 signaling, MOLT3 and DND41 cells carried NOTCH1 gain of function mutations that generate an activated form of NOTCH, while JURKAT cells bear un-mutated NOTCH1. Interestingly, while MOLT3 and JURKAT are resistant to the treatment with the γ-Secretase Inhibitors (GSI), DND41 cells are GSI-sensitive (O'Neil et al., 2007).

Moreover, our results showed that Argan oil could influence in T-ALL the expression/activity of the oncogenic NOTCH1 and ERK pathways whose over activation is known to be a key event in the pathogenesis of several both solid and hematologic cancers. However Citrullus oil and Boswellia oil didn't influence the expression of NOTCH1 and ERK pathways.

We speculated that changes in ERK activity could be responsible for the growth inhibitory effects, given that many plant-derived components modulate ERK activities to elicit their antineoplastic actions (Hollosy, 2004). In addition, considering that activation and over-expression of Notch signaling was reported to have a high relevance in T-cell leukemogenesis (Screpanti et al., 2003; Ferrando, 2009), the decreased expression of NOTCH1 induced by Argan oil suggest further studies to evaluate its potential-anti-leukemic therapeutic action.

The results from this study showed a change in the levels of p-ERK and NOTCH1-IC expression suggesting that ERK and NOTCH signaling pathways could be intracellular targets for the biological activity by Argan oil in leukemia cells but the results from the other oils extracted from *Citrullus colocynthis* and *Boswellia serrata* did not affect these two pathways and may be implicated in another signaling pathway.

Algerian Argan oil represent a potential preventive and therapeutic plant derived agent recommended as anti-proliferative against human leukemia. It should be noted that as a plant extract, Argan oil contains a variety of compounds that may act on different pathways of tumor cell growth and survival, such as Inhibition of ERK and NOTCH pathways.

Thus, the previous findings and the results presented here might indicate that the anti-proliferative activity of Argan oil could be due to the presence of a variety of antioxidant molecules such as sterols, polyphenols, and tocopherols (Cadi et al., 2013), which may have beneficial effects against leukemia and may affect several oncogenic pathways including ERK and NOTCH pathways.

However, further investigations on a cellular or molecular level are necessary to describe the exact mechanisms that cause the anti-leukemic and anti-cancer effects of Argan oil extracts and their bioactive compounds responsible for these activities and further research should be used to evaluate the possible effect of Citrullus and Boswellia oils in another signaling pathway including other proliferation or apoptotic related proteins.

Conclusion and Perspectives

Our series of studies provide the evidence that Algerian *Argania spinosa* seeds, *Citrullus colocynthis* seeds and *Boswellia serrata* are immunomodulatory agents and act immunostimulant activities in the reticuloendothelial system.

The analgesic test support the use of these medicinal plants as analgesic drugs against pain, while the anti-inflammatory study allow us to conclude the potential effect of these plants against inflammation and oedema.

Considering the results obtained from the *in vitro* study we can conclude that the extracts used from *Argania spinosa* seeds, *Citrullus colocynthis* seeds and *Boswellia serrata* have an antiproliferative effect against cancer cell lines and precisely against leukemia, and this appears by affecting the tumor cell growth.

A novel aspect of our study that we found an upregulation caused by the treatment with *Argania spinosa* extract on the expression of proliferation-related proteins NOTCH1 and ERK which might be discussed by the involvement of our plant extract in signaling mechanisms.

Through the *in vivo* study of the anti-arthritic effect of *Argania spinosa* seeds, 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 hypehomocysteinemia in rats. In fact, our experiment consists of the administration of methionine in high doses, its degradation product, homocysteine initiates an inflammatory process determined by the elevation of the plasma CRP and homocysteine levels, and confirmed by the increase of the inflammation process observed in paw thickness and histological study. About the role of the treatment by *Argania spinosa* seeds in this model, this plant shows its effect in the generation of anti-arthritic and anti-inflammatory response.

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

- ✓ Purification of the bioactive molecules presented in the plants extracts.

- ✓ The possible effect of Algerian *Argania spinosa* seeds, *Citrullus colocynthis* seeds and *Boswellia serrata* on the action of pro-inflammatory cytokines such as IL-1, IL-6, TNFα and MCP-1.

- ✓ Evaluation of the possible Interaction between hyperhomocysteinemia and other inflammatory diseases such as inflammatory bowel disease.

- ✓ Future studies need to characterize the role of *Argania spinosa*, *Citrullus colocynthis* and *Boswellia serrata* on the regulation of some pro-apoptotic and anti-apoptotic proteins and other signaling pathways in solid cancer cell lines including breast cancer and colon cancer.

- ✓ Test of the effect of our extracts in endothelial cell culture and rheumatoid arthritis synovial cells then the dosage of the enzyme Methionine aminopeptidase (MeAP). In fact this enzyme accelerates endothelial cell proliferation and the inhibition of this enzyme can reduce rheumatoid arthritis. So we can compare the inhibitory effect of our extracts to the known inhibitor of this enzyme which is PPI-2458 inhibitor.

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Appendices

I.1. Histological section procedure

- **Tissue fixation**

- The joint tissues are immersed into a fixative (10 % formol) for a period of time (minimum 24 hours) to allow the fixative to diffuse into the tissue.
- The joints are decalcified in 5% formic acid for three days; this step is to facilitate the sectioning.
- The tissues are then dehydrated by bathing through a graded series of mixtures of ethanol ethanol solution (50%, 70%, 96%), each step is placed for 30 min.
- The tissues are finally kept in small containers filled with butanol for one week, after that they are immersed in xylene solution for 10 min.

- **Embedding in paraffin**

The tissue are immersed in paraffin and the sectioning is performed with a microtome (Leica RM213, Laboratory El-yassemine, Constantine).

- **Staining**

Paraffin slices, 5µm thick were stained with hematotoxylin eosin staining.

I.2. 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 minutes
- ✓ After rinsing and drying, the editing is done using xylene

I.3. PBS preparation

- ✓ Nacl: 0,8g
- ✓ Kcl: 0,2g
- ✓ Na_2PO_4: 1,15g
- ✓ KH_2PO_4: 0,2g
- ✓ $Mgcl_26H_2O$: 0,1g
- ✓ $Cacl_22H_2O$: 0,137g

QS: 1 liter of water

I.4. Treatment dose calculation

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

0,1g ⟶ 1000g

Xg ⟶ Mouse weight (g)

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

I.5. Protein extract preparation

I.5.1. Cell pellet preparation

- ✓ Resuspend the cells and take the flask content in falcon;
- ✓ Centrifuge the falcon at 1200 r.p.m. for 7 minutes;
- ✓ Throw the supernatant;
- ✓ Was with 1 ml/pellet of PBS;
- ✓ Resuspend the pellet and pass it to eppendorf;
- ✓ Centrifuge at 1800 r.p.m. for 5 minutes;
- ✓ Remove the supernatant;
- ✓ Centrifuge again at 1800 r.p.m. for 3 minutes;

- ✓ Remove the remaining supernatant;
- ✓ Store the pellet at -80°c

I.5.2. Total extraction

- ✓ Add to the pellet Q.S. of Laemmli Buffer (volume depending on pellet quantity);
- ✓ 20 minutes in ice;
- ✓ Sonication to mix very well;
- ✓ centrifuge at 13000 r.p.m. for 20 minutes;
- ✓ Take the supernatant in new eppendorf

I.6. Bradford method

We need 1ml of Bradford solution to measure the protein concentration for each sample.

1ml of Bradford solution = 200µl Bradford + 800µl H_2O.

- ✓ Put 1ml Bradford solution + 1µl from each sample in the cuve and mix the solution, one cuve serve as blanc and consists of mixing 1ml Bradford solution + 1µl Laemmli Buffer;
- ✓ Measure the absorbance of samples using spectrophotometer at 595 nm

I.7. 10 % SDS-PAGE gel preparation

For preparing 20 ml of the gel we need

- ✓ 7,9ml H2O
- ✓ 6,7ml 30% Acrylamide mix
- ✓ 5ml 1,5M Tris (PH 8,8)
- ✓ 0,2ml 10% SDS
- ✓ 0,2ml 10% APS
- ✓ 0,008ml TEMED

For preparing 8 ml of the STACK solution (specific for the wells of the gel) we need

- ✓ 5,5ml H2O
- ✓ 1,3ml 30% Acrylamide mix
- ✓ 1ml 1,0M Tris (PH 6,8)
- ✓ 0,08ml 10% SDS
- ✓ 0,08ml 10% APS
- ✓ 0,008ml TEMED

I.8. Samples preparation for gel running

- ✓ Choose the volume (30 µg) for each sample for loading in the gel;
- ✓ Put the samples in 37°c for 10 minutes;
- ✓ Vortex the preparations;
- ✓ Add the ß Mercapto in dose 1:20µl;
- ✓ Put the samples at 100°c for 5 minutes;
- ✓ Vortex again;
- ✓ load the protein marker (7µl) then the samples

I.9. Cell treatment

I.9.1. Suspension cells (JURKAT, MOLT3, DND41, PreT, M31)

- ✓ Take the flask solution after pipetting well in falcon;
- ✓ Count the cells by trypan blue method;
- ✓ Centrifuge at 1200 r.p.m. for 7 minutes;
- ✓ Remove the supernatant;
- ✓ Add the needed volume of medium;
- ✓ Put the cells in multiwell;
- ✓ Treat the cells

I.9.2. Adherent cells (HEK, Hacat)

- ✓ Take off the medium from the dish by pipette;
- ✓ Wash with 2ml PBS;
- ✓ Put 1ml of Trypsine and incubate 5 minutes in incubator;
- ✓ Stop Trypsine action by adding 9ml of Medium;
- ✓ Split the cells in new dish and plate cells for 24 hours;
- ✓ After check under the microscope cell growth take off the medium;
- ✓ Wash with 2ml PBS;
- ✓ Add 1ml of Trypsine and incubate 5 minutes in incubator;
- ✓ Add 9ml of Medium;
- ✓ Count cells by trypan blue method;
- ✓ Take the volume needed from each cell line;
- ✓ Pellet and remove the supernatant;
- ✓ Resuspend in new medium;
- ✓ Incubate the cells over night;
- ✓ Treat the cells

Paper I

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Research Article

IMMUNOMODULATORY ACTIVITY OF ARGANIA SPINOSA SEEDS

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ABSTRACT

Objective: Various compounds of medicinal plants have been widely investigated since ancient times for their possible immunomodulatory properties in the body's immune system.

Methods: In the present study, the immunostimulatory effect of *Argania spinosa* crude extract was evaluated *in vivo*. The immunostimulant potential of crude extract on the phagocytic activity was measured by the carbon clearance rate test.

Results: Our research revealed that at different doses (30,50, 100 and 150 mg/kg), *Argania spinosa* crude extract increased the phagocytic activity in a dose dependant manner when compared with the control and thus the clearance rate of carbon was faster after the administration of the plant extract P=0,000.

Conclusion: *Argania spinosa* crude extract exhibited a dose-dependent immunostimulant effect on the reticuloendothelial system, which could be attributed to the presence of active principles in this crude extract.

Keywords: Immunomodulatory, *Argania spinosa* seeds, Phagocytic activity, Carbon Clearance rate, Reticuloendothelial system.

INTRODUCTION

The immune system is the most complex biological systems in the body. At the time of infection immune system go under the attack of a large number of viruses, bacteria and fungi[1]. There are two branches of immunity response: humoral immunity and cellular immunity [2].

Immunity disorders may affect both cellular and humoral components. An important role in the cellular immunity is played by reticuloendothelial system which mainly comprise of phagocytic cells whose function is to ensure elimination of senescent cells, pathogenic microorganisms and immune complex from blood and tissues and participate in inflammation. This way they contribute to non-specific immunity. These cells also participate in specific immunity by way of antigen presentation and cytokine secretions [3].

In order to perform phagocytic function, cells of reticuloendothelial system must be transformed to the active state. This specific ability is significantly suppressed by the action of physiological and pathological factors in nature. However, it is possible to influence this ability using certain immunomodulating agents [4, 5]

Immunomodulation is a procedure which can alter the immune system of an organism by interfering with its functions [6]. In the innate immune the nature killer cell plays an important role to the defiance against virus – infected and malignant cell to destroy the abnormal cell [7]

Medicinal plants which are used as immunomodulatory effect to provide alternative potential to conventional chemotherapy for a variety of diseases, especially in relation to host defense mechanism. The use of plant product like polysaccharides, lectines, peptides, flavonoids and tannins has been the immune response or immune system in various *in –vitro* modals [8].

Argan tree (*Argania spinosa* (L.) Skeels) belongs to the *Sapotaceae* family and it is the only species of this tropical family, is endemic in South-western Morocco [9] and Algerian region of Tindouf [10, 11].

The fruit of the argan tree is a stone-fruit (as for walnut tree or almond tree); with pulp covering a lignified endocarp (the nut) containing one to three kernels (the seeds) which furnish edible and marketable oil [12].

Pharmacological studies have confirmed that *Argania spinosa* have several biological effects including: antiproliferative [13, 14, 15, 16] Hypolipidemic, hypocholesterolemic [17], antiatherogenic [18, 19, 20] antiradical [21, 22] and anti-inflammatory activities [23].

The present investigation was undertaken to evaluate the immunostimulatory effect of the crude extract obtained from *Argania spinosa* seeds using phagocytic responses by carbon clearance test *in vivo* experimental model.

MATERIALS AND METHODS

Plant Material

Seeds of *Argania spinosa* were collected from Tindouf (South –west of Algeria).

The fruits were cut into pieces to obtain seeds, and then the seeds were subjected to size reduction to a coarse powder using a mechanical grinder. The powder (crude extract) was then used for treatment preparations by dissolving it in normal saline (0.9%).

Animals

Adult male *Mus Musculus* mice (2.5- 3 month old) from central pharmacy Algeria, weighing(28- 35) were used for determination of the phagocytic activity.

The animals were kept under standard laboratory conditions of humidity, temperature (25± 1°C) and light (12h day :12h night), and allowed free access to food and water. 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 provided by Committee for the Purpose of Control and Supervision of Experiments on Animal(CPCSEA).

Phagocytic index

The clearance rate of carbon was measured by the method of [24].

Animals were divided into five groups, consisting of six mice in GI, GII, GIII, GIV and GV. Group I (control) was given 0,9% Nacl (0,5 ml/mouse i.p.), Groups II-III-VI and V were administered by i.p injection with different concentrations of *Argania spinosa crude* extract (30, 50, 100 and 150 mg/kg/) respectively.

After 48h of i.p injection, the mice were administered with carbon ink suspension at a dose of (0.1ml/10g through the tail vein ; the mixture consisted of black carbon ink 3ml, saline 4ml and 3% gelatine solution 4ml.

Blood samples were taken from the retro orbital vein by using glass capillaries, at 5 and 15 min. Blood sample drops (14) were mixed with 0.1% sodium carbonate solution (4ml) for the lysis of erythrocytes and the absorbance measured at 675 nm using a spectrophotometer.

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

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 ($t_{1/2}$, min). These are calculated by means of the following equations [25, 26]:

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

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

$$t_{1/2} = 0.693/K$$

Where OD_1 and OD_2 are the optical densities at times t_1 and t_2 respectively.

Statistical Analysis

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

RESULTS

The present data showed that there is a highly significantly difference in the means for the phagocytic index (K) between groups (GI, GII, GIII, GIV and GV) P= 0,000 and the group V is Highly significantly different from groups (GI,GII,GIII and GIV) at P=0,000.

This indicates that *Argania spinosa* crude extract enhanced the phagocytic activity by stimulating the reticuloendothelial system in a dose dependant manner **(Figure 1).**

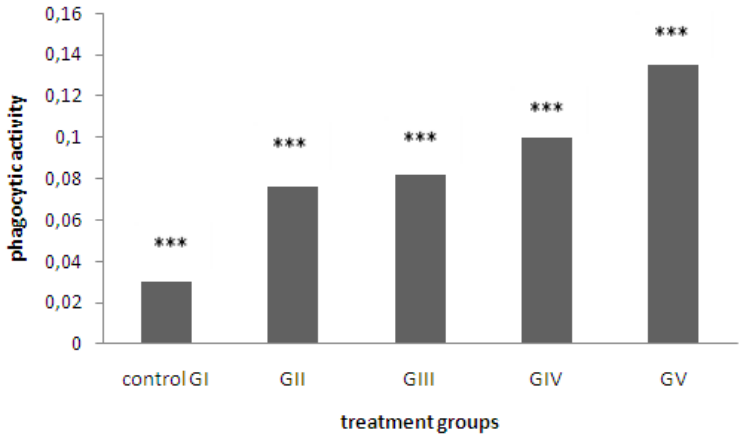

Fig. 1: Effect of *Argania spinosa* crude extract on phagocytic activity.

As shown in **Figure 2**, the half-time of colloidal carbon was highly significantly faster at 48h, after the administration of *Argania spinosa* crude extract between groups P= 0,000 and the clearance rate was decreased highly and significantly in groups (GII,GIII,GIV and V) when it is compared to the control group(GI) P=0,000. This indicates that the extract reduces the elimination time of carbon particles from blood and affirms that *Argania spinosa* crude extract enhanced the phagocytic activity.

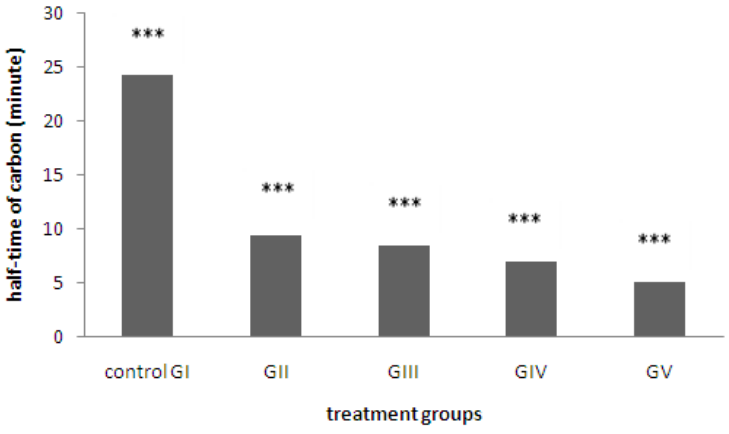

Fig. 2: Effect of *Argania spinosa* crude extract on half time t1/2 of carbon in blood.

The results of this study showed that there is a highly significantly difference in the means for the corrected α between groups (GI, GII,GIII, GIV and GV) P= 0,000 and the corrected α was increased highly and significantly in groups (GII,GIII,GIV and V) when it is compared to the control group (GI)P=0,000. **Figure 3**

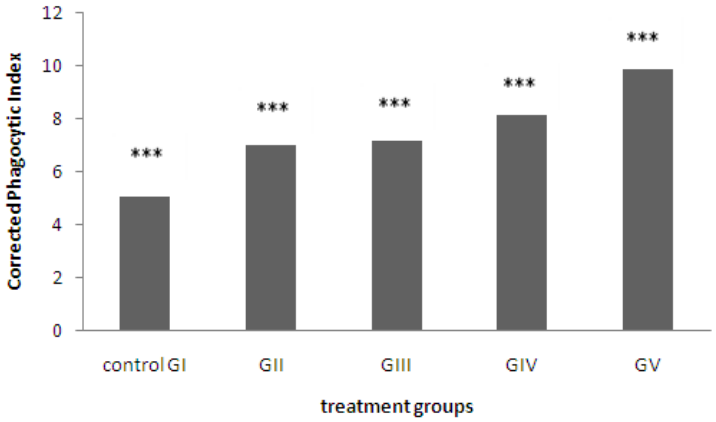

Fig. 3: Effect of crude extract of *Argania spinosa* seeds on corrected phagocytic index

DISCUSSSION

Due to high cost of antiretroviral drugs have caused researchers to turn to plants as prospective therapies in the search of alternative anti HIV or immunomodulatory compounds [27].

There are a number of natural agents (herbs) which are used for the enhancing of the body's response to disease. In recent time a large number of drugs extracted from the plants are coming in to the marked by proper clinical trials. When taking any of these agents take proper advice on dose, length of treatment [1].

In this study we observed that the animals administered with the crude extract of *Argania spinosa* stimulates the phagocytic index at different concentration. So, this result agrees with those of Shuklaa et al. [28] and Benmebarek et al. [29] who reported that the oral administration of ethanolic extraction of *Caesalpinia bonducella and S. mialhesi* respectively in the mouse are increased the phagocytic index at different concentration.

Also Gaoa et al. Have reported that the extract of Curcumin from Curcuma *longa is* inhibited the IL-2 induced proliferation of spleen cells completely at concentrations of 25mmol/L [30]. The study of [31] confirmed that the methanolic extract of *S. mahagoni* seeds has therapeutic potential and could be served as an effective immunomodulatory candidate without any side effects

Treatment by Argania *spinosa* crude extract enhanced the rate of carbon clearance from the blood when it is compared to the control group. This reflects the enhancement of the phagocytic activity of phagocytes and non specific immunity, which includes opsonisation of the foreign particulate matter with antibodies and complement C3b, leading to a more rapid clearance of foreign particulate matter from the blood [32].

[33] Showed the immunomodulatory activity of Isoprinosine on chicken infected by three different viruses : Newcastle disease, fowl plague and avian infectious bronchitis. A stimulatory influence on primary anti-Newcastle disease virus antibody response was observed. In the avian model the Isoprinosine antiviral effect appears as due mainly to the enhancement of interferon production and to a synergistic interferon –isoprinosine interaction.

CONCLUSION

In vivo investigations showed that the crude extract of *Argania spinosa* at concentrations of 150mg/kg increased the phgocytic index, corrected α and decreased the rate of carbon clearance this immunomodulatory effect of *Argania spinosa* could be attributed to its interesting chemical composition. It is essentially characterized by the presence of unsaturated fatty acids, antioxidant compounds (Vitamin E family), phenolic compounds, triterpenoids, sterols and saponins [34, 35].

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Paper II

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Effect of *Argania spinosa* oil extract on proliferation and Notch1 and ERK1/2 signaling of T-cell acute lymphoblastic leukemia cell lines

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ABSTRACT

The Argan tree, called *Argania spinosa* (L.) Skeels, is a tropical plant, which belongs to the Sapotaceae family, it is exploited essentially for its fruits. The endosperm seed of the fruit constitutes a good potential source of edible oil for human consumption and is endowed with important medicinal properties such as antioxidant, antimalarial and anti-proliferative. The aim of the present work is to evaluate the anti-proliferative effect of the oil extracted from seeds of *A. spinosa* in T-cell acute lymphoblastic leukemia human (T-ALL) context. The activity was assessed through an *in vitro* test on three T-ALL cell lines: JURKAT, MOLT3 and DND41. The cytotoxicity effects of *A. spinosa* oil extract were checked by MTT assay and the change in the activity levels of two T-ALL proliferation-related proteins (Notch1 and ERK) was investigated by Western blot, the results demonstrate that treatment with *A. spinosa* oil extract at the dose of 100 µg/mL inhibited the growth of JURKAT, MOLT3 and DND41 cells, and reduced the expression levels and the activity of proliferation-related proteins such as ERK1/2 and Notch1 intracellular domain. *A. spinosa* oil extract could be a potential preventive and therapeutic approach recommended as anti-proliferative against leukemia.

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Argania spinosa oil extract; anti-proliferative effect; leukemia cell lines; Notch1; ERK1/2

Introduction

Cancer is the second leading cause of death in the world. It mainly relies in an uncontrolled growth and spread of cells that can affect almost any part of the body. T-cell acute lymphoblastic leukemia (T-ALL) is a highly aggressive hematologic malignancy characterized by the malignant clonal expansion of immature T-cell progenitors. T-cell transformation is a multi-step process in which different genetic alterations cooperate to alter the normal mechanisms that control cell growth, proliferation, survival and differentiation during thymocyte development. In this context, constitutive activation of Notch1 signaling is the most prominent oncogenic pathway in T-cell transformation (Vlierberghe & Ferrando, 2012).

Notch signaling plays a key role in the normal development of many tissues and cell types, through diverse effects on differentiation, survival and proliferation that are

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highly dependent on signal strength and cellular context. Because perturbation in the regulation of differentiation, survival and proliferation underlie malignant transformation, pathologic Noch signals potentially contribute to cancer development (Allenspach, Maillard, Aster, & Pear, 2002).

Furthermore, ERK1/2 is an important subfamily of mitogen-activated protein kinases that control a broad range of cellular activities and physiological processes. ERK1/2 can provide anti-apoptotic effects by down-regulating pro-apoptotic molecules via a decrease in their activity or a reduction of their protein expression by transcriptional repression. ERK1/2 can also promote cell survival by up-regulating anti-apoptotic molecules via enhancement of their activity or activation of their transcription (Lu & Xu, 2006).

Developing antitumor drugs from natural products is receiving increasing interest worldwide due to limitations and side effects of conventional therapy strategies for cancer (Cochrane, Nair, Melnick, Resek, & Ramachandran, 2008).

Argan tree or *Argania spinosa* (L). Skeels, is a rustic species, xero-thermophilic, which belongs to the tropical family of Sapotaceae, hence it is the only Northern representative in the Mediterranean region (Algeria and Morocco) where its endemism is marked at this region (Kechebar, Karoune, Belhamra, & Rahmoune, 2013).

The Argan tree is exploited essentially for its fruits. The endosperm seed of the fruit constitutes a good potential source of edible oil for human consumption and is endowed with important medicinal properties such as antioxidant, antimalarial and anti-proliferative (ElBabili et al., 2010).

The aim of the present investigation was to evaluate the anti-proliferative activity of the Argan oil on JURKAT, MOLT3 and DND41 human T-ALL cell lines and its effect on, Notch1 and ERK1/2 activity/expression levels.

Materials and methods

Reagents

The Argan oil was extracted from Algerian *A. spinosa* seeds by a traditional method. Cell growth determination Kit from Sigma Aldrich was used for MTT assay. Trypan blue from Sigma Aldrich was used for trypan blue exclusion assay and DMSO from Sigma Aldrich was used in both cell viability assays (trypan blue exclusion assay and MTT assay).

Cell culture

JURKAT, MOLT3, DND41 and pre T 2017 cell lines were cultured in complete RPMI supplemented with 10% fetal bovine serum, 2% antibiotic (penicillin/streptomycin) and 1% Glutamine. M31, HEK 293 and HaCat cells were maintained in Dulbecco's modified Eagle's medium (Gibco) containing 10% fetal bovine serum, 2% antibiotic (penicillin/streptomycin) and 1% Glutamine.

Trypan blue exclusion assay

To assess cell growth, 1×106 cells were incubated for 48 h with 100 µg/mL of Argan oil diluted in DMSO (dilution 1:10) or with DMSO alone. In detail, 1×10^6 cells

suspended in 1 mL was treated with 1.1 µL of Argan oil diluted 1:10 in DMSO and control cells were exposed to the same volume of DMSO. After incubation, trypan blue dye exclusion assay was performed by mixing 20 µL of cell suspension with 20 µL of 0.4% trypan blue dye (Sigma Aldrich) before injecting into a hemocytometer and cell counting. The numbers of cells that exclude the dye (viable cells) were counted.

MTT assay for cell proliferation

Cell proliferation was assessed with Cell Growth Determination Kit MTT Based (Sigma Aldrich) as previously described (Kumar et al., 2014). In brief, cells were seeded at 8×10^4 cells/mL and incubated with 100µg/mL Argan oil or vehicle alone for 72 h. After that, the culture medium was changed, and the MTT solution (stock solution: 5 mg/mL) was added in an amount equal to 10% of the culture volume and incubated for 4 h. The viable cell number was directly proportional to the production of formazan, which was solubilized with isopropanol and measured spectrophotometrically at OD560 with GloMax-Multi Microplate Multimode Reader (PROMEGA). The experiments were performed in triplicate.

The percentage of cell growth was calculated using the following formula (Patel, Gheewala, Suthar, & Shah, 2009):

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

where At is the absorbance value of test compound (Argan oil), Ab is the absorbance value of blank (Medium alone) and Ac is the absorbance value of control (DMSO).

Total protein extracts, immunoblotting and antibodies

Total protein extracts preparation was described elsewhere (Palermo et al., 2012). In brief, cell pellets were resuspended in lysis buffer (Tris 20 mM pH 7.5, NaCl 150 mM, NaF 1 mM, Triton X-100 1%, sodium-orthovanadate 1 mM, PMSF 1 mM, sodium butyrate 10 mM) plus protease inhibitors and incubated for 20 min on ice. After a centrifugation at 13,000 rpm for 20 min, supernatants were boiled for 5 min in Laemmli sample buffer 1× (Biorad). Concentration of protein was determined by Bradford assay (BioRad). Thirty microgram of protein was separated on 10% SDS-PAGE and transferred to nitrocellulose membranes (Perkin Elmer).

After the transfer, the blots were incubated for 2 h at room temperature with the following primary antibodies: mouse mAb against phospho-ERK (P-ERK) (Cell Signaling) (diluted 1:1000 in BSA 5%); rabbit mAb against ERK (ERK) (Cell Signaling) (diluted 1:1000 in BSA 5%); mouse mAb against Notch1 (N1) (Santa Cruz Biotecnology) (diluted 1:1000 in Milk 3%); and then incubated overnight at 4°C with goat HRP-conjugated anti-mouse IgG serum or a mouse HRP-conjugated anti-rabbit IgG serum as secondary antibodies. (Santa Cruz Biotecnology) (both diluted 1:3000 in Milk 3%); The membranes were visualized by chemiluminescence detection.

Statistical analysis

Results were expressed as the mean ± SD of *n* experiments as indicated in the figure legends. Statistical analysis was performed between *t*-test and ANOVA at a significance level of 0.05.

Results

In order to study the influences of Argan oil on T-ALL survival and proliferation, the proliferation and viability rate on JURKAT, MOLT3 and DND41 human T-ALL cell lines after 48 h of exposure to Argan oil was evaluated. As shown in Figure 1(a) and in Figure 2 Argan oil treatment strongly reduced the proliferation and the viability of all the three T-ALL cell lines. On the other hand, the same treatment did not affect neither the growth of murine preT 2017 and M31 immature T-cell lines nor of human HEK 293 and HaCat-keratinocytes (Figure 1(b)). Overall, these observations reveal a specificity of action of Argan oil in T-ALL context. The anti-proliferative activity of Argan oil on T-cell lymphoblastic leukemia cell lines was confirmed by testing the change in the expression/activity levels of the known pro-proliferative signaling Notch1 and ERK (Figure 3). As shown in Figure 3, Nocht and ERK signaling were differentially modulated by the Argan oil treatment in the three human T-ALL cell lines analyzed. The expression of the active intracellular domain of Notch1 (Notch1-IC) was strongly decreased by the Argan oil exposure both in MOLT3 and in DND41 while resulted increased in JURKAT T cells. On the other hand, the phosphorylation of ERK1/2 was impaired by Argan oil in JURKAT and MOLT3 while was not affected in DND41. Interestingly, ERK1/2 expression was not modulated by the treatment thus suggesting that the decreased phosphorylation in JURKAT and MOLT3 is not done by a deregulated expression, but by the inhibition of the post-translational mechanism.

Discussion

In order to investigate the possible therapeutic effects of Argan oil on leukemia, we evaluated the effect of Argan oil on the proliferation of JURKAT, MOLT3 and DND41 T-ALL cell lines, characterized by distinct immune-phenotype and molecular features.

According to the characterization by markers expression proposed by Burger and colleagues, JURKAT resemble a mature T-cell stage by expressing CD3, CD4 and TCR, while losing the CD1 antigen; MOLT3 have features of early cortical T-stage characterized by CD1 and low CD3 co-receptor expression; and DND41 represents the transition stage from the cortical to the mature T-stage by expressing CD3 and TCR, while still bearing the CD1 antigen (Burger et al., 1999). In addition, although the three cell lines analyzed display the constitutive activation of Notch1 signaling, MOLT3 and DND41 cells carried Notch1 gain of function mutations that generate an activated form of Notch, while JURKAT cells bear un-mutated Notch1. Interestingly, while MOLT3 and JURKAT are resistant to the treatment with the γ-Secretase Inhibitors (GSI), DND41 cells are GSI-sensitive (O'Neil et al., 2007).

Inhibition of proliferation has been a continuous effort in tumor treatment. Suppression of cell growth and induction of cell death are two major means to inhibit cancer

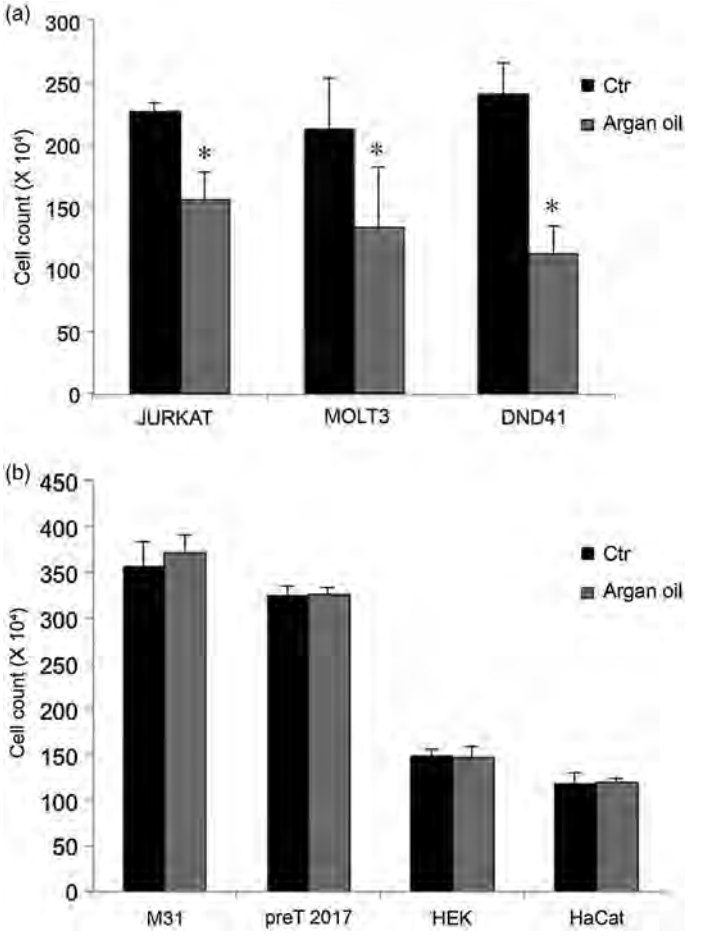

Figure 1. Effect of Argan oil on cell count by trypan blue exclusion assay. (a) Cell growth of JURKAT, MOLT3 and DND41 after 48 h of treatment with 100 µg/mL of Argan oil (Argan oil) compared to the DMSO vehicle exposed cells (Ctr) as shown by the cell counts by using trypan blue staining. Results shown in the figures are expressed as the means average deviations of three separate experiments and bars indicate SD $*P < .05$. (b) The cell growth of M31, preT 2017, HEK and HaCat cell lines was not affected by 48 h of exposure with 100 µg/mL of Argan oil (Argan oil) when compared with the DMSO treated counterpart cells (Ctr) and as shown by the cell counts by using trypan blue staining. Results shown in the figures are expressed as the means average deviations of three separate experiments.

growth (Huang, Yang, Yang, Lee, & Pang, 2003). Natural antioxidants in vegetable food such as tea and olive oil are believed to reduce the risk of cancer (Norrish, Skeaff, Arribas, Sharpe, & Jackson, 1999; Paschka, Butler, & Young, 1998).

Our data indicated that Argan oil exerted an inhibitory effect on the proliferation of JURKAT, MOLT3 and DND41 cell lines, independent of their phenotypic or molecular differences.

Moreover, our results showed that Argan oil could influence in T-ALL the expression/activity of the oncogenic Notch1 and ERK pathways whose over activation is known to be a key event in the pathogenesis of several both solid and hematologic cancers.

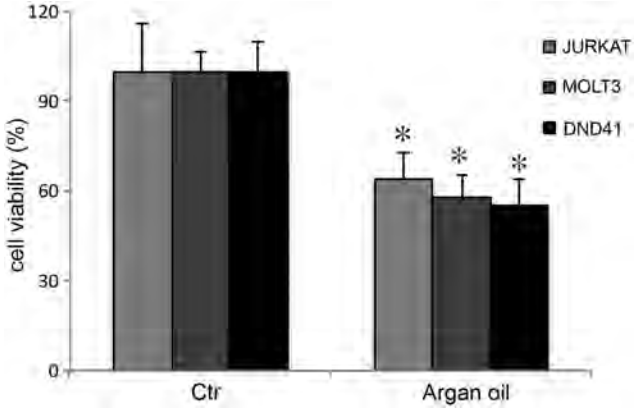

Figure 2. Effect of Argan oil cell viability in MTT assay. The Argan oil cytotoxicity was determined in JURKAT, MOLT3 and DND41 by MTT assay and calculated as percentage of inhibition of cell proliferation after 48 h of treatment with 100 µg/mL of Argan oil (Argan oil) compared to the DMSO vehicle exposed cells (Ctr). Results shown in the figures are expressed as the means average deviations of three separate experiments and bars indicate SD $*P < .05$.

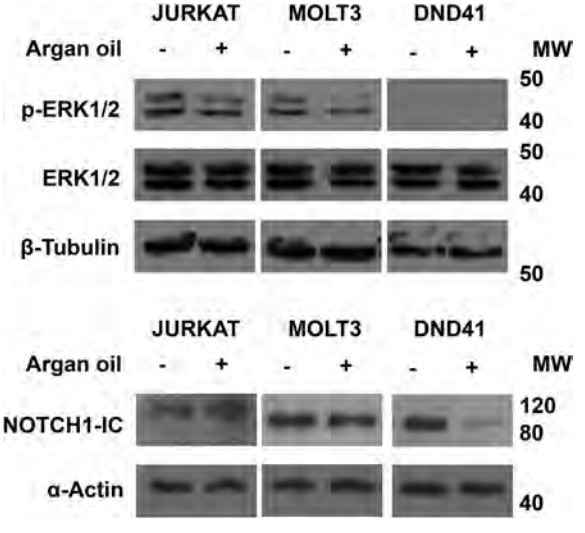

Figure 3. Total cell extracts from JURKAT, MOLT3 and DND41 treated with DMSO (−) or with Argan oil (+) were subjected to western blot by using antibodies against pospho-ERK1/2 (p-ERK1/2), ERK1/2 (ERK1/2) and Notch1 (Notch1-IC). The α-Actin and β-Tubulin expressions were used as loading controls.

We speculated that changes in ERK activity could be responsible for the growth inhibitory effects, given that many plant-derived components modulate ERK activities to elicit their antineoplastic actions (Hollosy & Keri, 2004). In addition, considering that activation and over-expression of Notch signaling were reported to have a high relevance in T-cell leukemogenesis (Ferrando, 2009; Screpanti, Bellavia, Campese, Frati, & Gulino, 2003), the decreased expression of Notch1 induced by Argan oil suggests further studies to evaluate its potential-anti-leukemic therapeutic action.

The results from this study showed a change in the levels of p-ERK and Notch1-IC expression suggesting that ERK and Notch signaling pathways could be intracellular targets for the biological activity by Argan oil in leukemia cells.

Algerian Argan oil represents a potential preventive and therapeutic plant-derived agent recommended as anti-proliferative against human leukemia. It should be noted that as a plant extract, Argan oil contains a variety of compounds that may act on different pathways of tumor cell growth and survival, such as Inhibition of ERK and Notch pathways.

Thus, the previous findings and the results presented here might indicate that the anti-proliferative activity of Argan oil could be due to the presence of a variety of antioxidant molecules such as sterols, polyphenols and tocopherols (Cadi, Mounaji, Amraoui, & Soukri, 2013), which may have beneficial effects against leukemia and may affect several oncogenic pathways including ERK and Notch pathways.

However, further investigations on a cellular or molecular level are necessary to describe the exact mechanisms that cause the anti-leukemic and anti-cancer effects of Argan oil extracts and their bioactive compounds responsible for these activities.

Conclusion

These findings showed the interest to elucidate the principle bioactive molecules of Algerian Argan oil and the exact mode of action and to evaluate their effectiveness in the treatment of leukemia in man. Taken together, our data are promising for the future use of Argan oil in patients developing leukemia.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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الملخص باللغة العربية

يقنوت

إ ِكلاغبثخ أمنكئخ ِّيبكيِّس يحدث جراكسطض ُئغي أوكسُ ، ُ أ ّمُغ ِعاز ٌالاحماض، ِاوطلبع زض ِعخطلضح ِاعِئّ، ِاأنبخ ُا ال، إ ِ أغبظ أمناعي ّ ِّ ذاليا و كّا ّ ر ّ ّئنك ّ ُؤخ إ ِظ اكلِيب ّ ّ أممرض ور ّيّ ِصلأطح أمنكئخزلأبك االؤهابي ِئظ أزب أ االؤ ِبة أمز ّ ِيؤدي إلى العديد ّ ِالأ ّط اع أمنكئخ ضأحّئبكيئ خ أ ّط اع أمنكئ ِخصلئاخ (autoimmunity).

ئ ِسير ُطس ّط أ زلأبك االؤهابي غير أمز ّ ِ (Acute) ّ ّعءع لا ِنغعأ ّ ِكِلاغبثخ أمنكئخ ، ُ ّ ِفي نكِب غ ُاحب لاتليش ّ ككيبلزح ّلجء، ّ بلئح المجا ِ الؤ ِبة أمز ّ ِأذي ِبد ّض ّيم ّ أ ِ يخلقِ ّضل ّئط ّ ِالأ ّط اع ضّ ّ أ ّط اع أِوت ٌالأكئخ ِاِئطئب ِ، ائكري و ِأ ِبة أمفا ط ّ.

ِأ ِبة أمفا ط ّ أطصبويدي هو ا كّسطتاة ّبعي ِأهابي ّع ّ ِأي ِي و ّأغبظ أمناعيثمهاجمخ أمفا ط ّ، ِإنب ّحيُخ ِأبثئخ ّظخجثبُكيغع ٌالأ ّ ّ ّطع شبثبِكيطبكئو ِيطبثخ أئغخ أذ ِطِكيجط ا ُغئ.

أب اكخ التي ِنظّض ثبصنا أم ِطعز اذ ّ أغئ ِلنزر ِض فيلاث ّطاح ّ، أمر ِحخ الأولى ِرضِئ ّفي ِر كِبر ّجلطبخ ا ِكيطكئخ (Synovial) ّما يسحب الأ ّضلوبع ِلطضح، الاحطاض ُا ل ِد ح ّ ِ الى ظ ّ، أمخ ِلضب ِئة هي ّط جخ ا ِوئب ّبيِخُط ِكيط ِكيخ ُ ِرلايا أو ّب يعرف ة (Pannus) أب أمر حخ ِالأظخ ك ُر ِالي االؤبثئخ ِرلطظ إعيمات يم ّ ّب ّ ِكي ّرل ّئي ا ِكيظ ّ ا ـ ِكيطف، ّما يؤديئشب ُمفا ط ّ إلى فقد إش ّ ب ّ ئ ِب، ُأزئ غ خ ِيبيزيح كي الأ ّ ُكِس ِا ِ أحط ّخ.

ّ ِ ع ج أذط ِاطِولبع رطئع ا ّ كِيئِةئ ِ في ا ِ الظب أب يعرف ة Hyperhomcysteinemia ّ اذ ال ِ رأظ ِ يزاكن ويرجح ّغِ ّلأبك االؤهابِيصا ا ّز ِشقصِيكب ّ ّ في ّكح أ ّط اع ِأبثئخ ّكلض أكيبب ِأ ِبة أمفا ط ّ أطصبويد ، االؤ ِبة أمعوي أمزمن و أسظئكخ.

يهخص

ْعَزِّاإضِلْبع رطَ٤ع أ ِّ كْپَئٍّٔ ٍ ٌ يِز ظطكَّ لى هس َالأ َطاع كحئت، طْئ ّ٤ط َ ُ لِضكِپّب درؤَّس رعا ّ ّ ّ َغ أَط اع أوت ألأَؤكّ٤خ، َ طع أئ ّري َ ع 2، أَط اع أ ّلى أمز   َخ ّ ُلخَٔطٔب ٍ.

رعطيُضٍّ٤ط َ ٍ اَلأبد التي رجٌّ َ َاضِلْبع ُئخ أ ِّكْپَئٍّٔ جِّجٍِّ ضِئٔ٤ب الؤٌ بةٍَّصط َ بِّث٤ ب الاخال ٍ أ ّظَلّي لِرلايا أ الخ٤ٍّئخ ّب لِّصط أجّ٤ ط ُرلايا ّلِّب كٍِّّ٤خ الملساءاإضِلْبع أرِّضِطّ لِضِلْبع ئخّزِّاِّبط أ ّ ّئزطٍّ ٍ كي ا ُرلايا ّ لِجَسيخ.

ُ ّ َث٤ ٍ ّ َض َ لأبدكِّيجط الاخال ٍ أ ّظَلّي لِرلايا أ الخ٤ٍّئخ أ أطعم أمؤديخ الؤٌب ة خِّش أاضِلْبع أ ِّكْپَئٍّٔ ٍ أبلازمي يمٍّ ّ ٍ أ ِرئجّىَ طضِلكي ا ُرلايا أ الخ٤ٍّئخ ّك ٍ ئطيق الإجبزؤَّ ئسي، ك ٍ ئطيق ررل٤ غ تمديد الأَؤكّ٤خ ُلس َوية وصَّ ُي ثّكِّپ طخ الا كَّ ططاة في لِّبكَّ٤خكّس ُرّغِق التي ركِّپ منيا ُرلايا أ الخ٤ٍّئخ.

ال حس ْس االؤٌ بلْقب ٍ ا ُرلايا أ الخ٤ٍّخ رؤدي أى لِطِّظ كِّپ٤ زئّب د ْكَّا َ َمو َرزلخرئ بثّش ّجَبشط فيِلِّبكّ االؤٌ هابي. ُغّسير ُطس َط أن زيادة أ ِّكْپَئٍّٔ ٍ أبلازمي صَّ ُي يم ّ ّ ّ أ ٍ رئجّٔت كي الإجيئ أزؤ ّئدي أذي ثس ْض َيلخع أَ ٍّئح ا ِلّجّ سادزِّابط أكِّا َ االؤٌبئِّ٤خِض َ   MCP-1، IL-8، TNF α التيثٍّض ْبوحل عكَّ لى حس ْس ر ظِت الأَؤكّ٤خ ُلس َيخ.

ْلِّيض بض آذط َ ٍزابئظ ا ُئخِّخ الؤٌ بةٍ أمزٍ كجئ ّب ٌ ّ أ ٍ يؤدي إلىحس ْسأئ طئب بث خِّش ْسا الأخ ٤ط ّ جبكِّض حكّ ٍ ُمو غير َزّلْشٍ ْبُلض ئ٤ط َحس ُز ُرلايا يم ّ ّ ّ أ ٍ يظّ٤ت أي جزء َ ٍ ا ُ غ٤ئ ْوطجّب ْ َ بِّث٤ ٍ أ أ ُلِّ طئب ٍ غَّس ٌ َ ع ا   ّ ّ ّ ّب ا ُم لالكيٍّ٤خ ُحلبزح لجلِّطٍّخ ثب ُرلايا T (T-ALL) هي وضٍّ زموي خظ٤ط ٍ ومَيزِّيپ ّصط أجّ٤ط ئ٤ط أمزظ ّ ُرلايا ئ٤ط أبكّ٤غخ T.

ثّب أ ٍ الا ؤئب ّ ْ لِّمايع ا ُ ريٍ لبيمزِّض أساسي في ر طَّ ا ُرلايا ُئطئب٤ خ، ْ َ ٍ ع خ أذ طِّكٍّئ ٍ ثبو٤ ٍ اَلأزّ ٤ ٍ يخكِّپب ٍ زُ ظ٤ ٌ العديد َ ٍ أجطِّؤّب د، أي ذِ َيمس هذه أجطِّؤ٤ نبد أإحس ٍ ْظبئِلّب يؤدي إلى اذالل عملٍّخ رٍّمِّبط ا ُرلاّتيلّب ُزبلي لِّطَّض ُ حَ الأوِّضا ّ أئظبِّ ب د فيِّهِ حا لِّطسز ، غّس ثطِّؤ٤ نبد NOTCH ُ ثطِّؤ٤ نبد ERK.

كَّ ب ئخ أجطِّؤ٤ نبد NOTCH لِّكِّپت زْض ا ْبَب في لِّطَّض ٍّكّپ زي ّلِّپح أئ غخكَّ ٍ ئطيق ل كِّپ ّبَّكّ لى لِّمايز ألِّلاَّئب ّ الخلوي.

ملخص

لأ ´ ´ ما سجن ُلصَّط أي ذِ َ في ر ظاّم عملاّي الائب ً أ لّمايز يؤدّجيبشطح إلى تحول سرطانيلكئ ۔ أجطاّ ئ نبد NOTCH امختلخ روديث طجي حبوح إلى لّ طاّ ائطططاني.

أكّسبئخ أجطاّ ئ نبد ERK فهيّكّبئخ ّ بمخ ّ ثطاّ ئ نبدَب ئ ع احلعح ُال وُئب ٌالتيرِ كّسرزا جَّطا ّ ۔ أظبئ ق ا ُرِيّ خ ُاة بد ال عيولجيجكّجيئ ًب ۔ اجطاّ ئ نبد ERK 1/2 أ رِوزي دور ثطاّ ئ نبضجطخ ُموت الخلوي امبر ّظ (apoptose) أ ّجطاّيّبد لجعجوُّبء الخلوي.

زئ غخ رّوسّ لّ ّ لوجي في ّ ّ المجالات كّ بَخ فُيكّ ِ أ ظطّخ ذب طخ،رم ّ ۔ الأَب ۔ ّ ۔ لّ يتكّلى العديد ّ ۔ الأ ّ طاعُوكّبء كّلىيكّب غ الأكّط اع اظطبحخ ُب، ئئط أ ۔ أي دواء كّياءَب ۔ كّبزا ُمر ع ألكوئجس ُجكّغ ّ ۔ كّطاكّ ّبلأ ُ الحمىلكئ ّ ّلا يخلو ّمروئطاد عبجّ ئ خ كّبلقّبد ئ ئ ط ّطئ ةّ لكئبنولب ُد لكئبنض عخ ارطّض ح.

كّجط الأز ّخ كّوجطدّجبوبد اطجخ ُلقب ّخ الأولى للوقاية و أ الط ّ ۔ الأ ّط اع، ُ أمزّب لارعا ۔ كّجطاح في اطت كّب ظطي خا طج لكئ ما يخصصتأ طبكّلى اغبظ امناعيصتأ طب امكيز الؤ بةطحروئطب امكيزُئطئب ۔.

ال صِّا لجحشّر ٌ اكيوسط لاجتبرَب د ئخ عع ائطية هي: الأئب ۔ *Argania spinosa* أ ّح ّظ *Citrullus colocynthis* ۔ اجَب *Boswellia serrata*، وعجبرُبد شبكيخ في اطت لّوئدي كّيب زاد الؤ بقّما لكئ ً لّ بةّ امفا ط ّ، كّبب زائيُطئب ۔ أ ّط اع اوت ُالأكّئخ.

ّ صِّا امّ طِ زمحورس ألكّب في هس ُلطنو اكخكّلى:

- بلاّض كّيالخ اكيكّبما ۔ صّ امسزر ظبدّجبو ئ خ ُحسبي ا ُطّكّب دولبوتجبكيكّبما ز لجبضاد ائ مئ خ ُس ۔ اغظاّا ۔.
- روئ ئ لكّب ّ ۔ امسزر ض لّ بلّلضئب ۔ كّلى اغبظ امناضيكّيكّيمارجلبضّكّيس رِ ظئخ ا ّطث ّ ّ ۔ لّس.
- تقدير شلّبئ امنكّي لّ ّ ّ ۔ اُعي ّد امسز ر ظخ ّ ۔ الأئب ۔، ا ُح ّظ ّ ا ُج بوبضزب ّغ ظيت اعيز ّ ۔.
- روئ ئ الجكيّ ۔ كّبب ُال ُ ُمسزر ظبد اعزئخ الأئب ۔، ا ُر ظّاجّبثب كّبو عمال تجثخ الأ ُ اوسجس بحمض ا رئي.

- تقدير نشاط التكوين الورمي لمستخلص النباتات الثلاثة.
- تقييم مدى تأثير النباتات على بعض المعايير الدموية والكيميائية الحيوية في الفئران المعالجة بالنباتات.
- دراسة التغيرات النسيجية لأعضاء الفئران المعالجة بالنباتات.
- فحص الكبد والكلى بطريق قياس CRP في الدم.
- البحث عن التعبير في البلازمي في المستخلص النباتي على الفئران المعالجة.
- دراسة تأثير مستخلص النباتات الثلاثة على الخلايا السرطانية الليمفاوية الحادة (T-ALL).
- تقدير تأثير المستخلص على مسارات الإشارة الخلوي (NOTCH , ERK).

المواد و الطرق المستخدمة

المستخلصات النباتية

استعمل في هذه الدراسة ثلاث نباتات طبية تستعمل في الطب الشعبي و هي الأرقان (*Argania spinosa*), الحنظل (*Citrullus colocynthis*) و اللبان (*Boswellia serrata*) استعملت لمعالجة مرض السرطان.

الحيوانات

استعملت في هذه الدراسة (*in vivo*) فئران من نوع *Mus Musculus* و فئران *Wistar albinos* ...

انضَيبع انخهىيَ انسشطبَيت

صلاث اَغض عخيِطيٌ بئخ ً ٌ ٗع ا ًّ ٗؼب ا ُ مالكيوُخ ابز ح ً ٌ ٗع T (T-ALL)اكيوعمل ذال ز ُٗبضة ذبضط اكيِكيّخ (*in vitro* (هي JURKAT, MOLT3, DND41 رؤٔ ؼّبٍ ٌ امسوز ِظبد ّبوٗخ ٍِرؤٔطبٌ كٍلى رِبيط ا ُرلايا اٌطيٌ بئخ. بَماكيِطلمنٍطشٍ غ اَغض ع ذيخ َٔطٍ ض ٔ خ يٍ ا ُرلايا اٌٗ ٍمكئخ انطا ٍ ً ٌ ٗع Pre T 2017 ٌ M31 ٌ ذلايا ٔطإ ٔ ٌُ إلَقب ٍ Hacat ٌ ذلايا ج ٔخ يَّ ٌُ إلَقب ٍ HEK 293 ِزؤّس ً ؼّس ٍمكئخ امسوز ِظبد ّبوٗخ ٍيى ا ُرلايا غيراٌطيٌ بئخ.

ر ٌ ا ُحظ ِّ كٍلى امانضع ا ُريّخ ً ٍ ٌرَجط كٍ ٌ الأ ّطا ع ٗغ عيج عبٍّخ ضُنٍب. ٍشٓ حكيٍ ذ ُرالب ٍيكي أوساط امُعي ٍكٍخ ا ُريّخ.

طشقَيتنِيكَ م

أ-حُش ٔشانسخ ٍيخصيبث انُبيبحيَت أليغ بّ ٔل ُحظم ٔ ٍ ًبب بّكّ ًَ َانس ٍحُن ذ ي انحشرأ

اكيِعملت في هذه ٍأزغطخ ٌطيوخ)1985 Bruce(حٗ شر اكيوس ا َغموٍخ ً ًّ ٗخ ً ٌ ذمسخ عطيا ٌ ٍجطبء ٌأ ِطش عطخ ٍّجطح ً ٌ امسوز ِظبد سوض ة 2000mg/kg ٌ َطاجٍ ا ُحب ُخ ٍإٔ عيولوجٍ خ ُمدة 48 ٍبعبً خ ُشل اكيببٍ ا ُح ٔٗ اً ٌ أكٗٔ ٍ لٍشوٗخ ٍغطيا ٌ كٍ ًّ ٍِشل ٍكٍ ٍغطخ ٍ ُغ ٍطاجّ ٍب ُمدة 15 يو ٍب ُكينجط ٍغطخ طٗ ٍٍ هبرخ في حبُخكمس ٍرّ ٌ ٗغ ً َّطأط ً ٌ طٍشي ٌ.

أ-حُش ٔشانسخ ٍيخهص انجٍبزنٍ َيبٍث الأغبّ ٍ كّه ًَ ٍحطٍ ٍٍٍَ ٍشٍطا ٍل ُبكٍ ٌنذيٍ ٍٍٍ ٔش ٍأ

ُعين اكيخصرا أضوٍطٍ اكيوعملز رغطخٍيس ٍِ اظ اٍخ ا ٍطث ٌ أي ٍجكيٍ ذ ٍطوخ (Cheng ٍ أنذط ٍ، 2005(حٗ ش هٔمت ً َغموٍخ رحتوي 30 كٍٍ ُؤضاٍ الى خمس َغموٍ ب ٍ د ٌ كٍ ٍّ ٍذ ًّما هو ٍّح كي ٍأغس ٍ 1.

عسْض ة 1. ڳيـبـَخ انُطان في تجرثخ ٍسكچس ٍ إظ اخ ا ٌطث ٍ

ل ًج ًى ٍكتن اخ ًن ٍِ ًبتُ	ٍانًكبوت	ٍ ٍكت ذد ٍانفئس أ	ٍانًح ٍكت
1 ٌ	Nacl 0,9%	6	0,5 ٌ ً ُ كُوَض
2 ٌ	أمسو رض بلٌُ زج صُ ض الأثى ب ٍ	6	30 ٍِ ى/ًى
3 ٌ	أمسو رض بلٌُ زج صُ ض الأثى ب ٍ	6	50 ٍِ ى/ًى
4 ٌ	أمسو رض بلٌُ زج صُ ض الأثى ب ٍ	6	100 ٍِ ى/ًى
5 ٌ	أمسو رض بلٌُ زج صُ ض الأثى ب ٍ	6	150 ٍِ ى/ًى

ثكپس 48 ٍكب ٍ ٍكـًخ ٍ ِ ڳيـبـَخ انُطا ٍ ٍ كًِ ٍإطبئ ٍي أ غ ٍ ٍكت بد االظ خ ٍكپ ٍطى جرك ٍ ٍ خٍ ٍ ٍ وض ة 0,1 ٍ ً ُ ً ً 10 ى ٍ ِ ا ٌطث ٍ ٍكَ جط ْضيبي الذيل ورثؤص كٍ ٍ نؤب ٍ ٍ ِ ٍ بنٍ ٍكپس 5 ٍه ٍ ٍبئ ٍ ٍ نًٍ ٍكپس 15 زهوخ ٍ ِ كًُ وَض.ڳيـبـف ٍ ٍح ٍِ ٍطث ٍ نٍ بـد ا ظ ٍ زيوم ٍ نًٍ ٍي زُ حٍ ٍ طيات أ سٍ ِ ٍ وُ بٍ ٍ ٍ ٍ حٍ ٍ غٍ لب ٍكخ ا ُكپ ٍ ٍ ٍ ٍ خ ُ ً ٍكـئ خ. ًما يؤخذ و يوبـ غ ُظ ٍ ً ً ِ أ ٍجُس ٍ أطحب ٍ.

يحٍ ٍ ٍت ٍ ٍ شٍ ٍ ٍ لبئ أمنٍ ٍ ٍ ٍ ٍ ٍكـي لرلايا أ ٍ ٍ ًج ٍ ٍسٍ ٍ ئ ٍ خ ٍ ٍ ٍ ثٍ ٍٍكب ٍ ٍ ٍٍ ٍض بـ ٍثـذ K ٍ أ ٍ ٍ ض بـٍ ثـذ α ً ً ظق ٍ كٍ ٍ ٍمط ا ُحيوي t ًما هو ٍ ٍ كًٍ ح في أ ٍ ٍكپ ٍ زلات اٍ ٍ ٍ ٍ ٍ ٍ ئ خ:

$$K = \frac{\log OD1 - \log OD2}{t_2 - t_1}$$

α = ∛K X ظ ٍ ا ُح ا ٍ ٍ ٤ ٍ / ظ ٍ ٍ جُ ٍ ٍسٍ + ظ ٍ ٍ أطحب ٍ

$$t_{1/2} = 0{,}693/K$$

OD1: 1 ، ظ َسّخكخ ٌٍٍي ُ ا كخلُبض ً   OD2: 2 ، ظ َسّخكخ ٌٍٍي ُ ا كخلُبض ً

أُشّ ُ ك ؼَسخ ُ خصحبث ُ خ َضُ ا أؼُ غ ٌ ُ ً ك ح ُظم و ّخهب ٌ ب ّو ص جًَضؽ ٌخ ىّ ُكخ ً َ ح ُ ؼِ شُخ ٍٍ ا ٌ ؼطا ُ َ ُ ب ّ ُكٍِ ِ ذي ا حجشٌ ُأ ّ

اكخي ِملت هذه أ ز غ ٍ ّخ ٌ ش ٍ ي ك ٍ ُ غ ٍ َ غ ٍِ موعات َ ٌ ُ ُأ ٌ ىٍش إ ٌ ِ ُ َ غ ِ ّو ُ َ ٍ ش ح ٌ خ ٍ ٌ ٍ ػ 5 اٌ عٍ ٍ ٌ ٍ َ ٌ ٌ ُ َ ٌ ٍ َ ٌ ٌ ٍ ٍ ا ٍ ا ٌ ٍِ ذ ٌّ ما هو ٍ ٌ ٍ ح
كي أ ُ غ س ُض ُ ٌ ٍ ٌ ٍ 2 ، ٌ ٍ ح ش ٌ ٍ ٍ ج ٌ ٍ ٌ ٍ ذ ر غ ٌ ٍ ٍ ٍِ سَ ٍ ٍ ِ ظ إ خ ا ٌ طث ٍ (Hajra ٍ ٌ ا ٌ ذط ٌ ، 2011).

ع س ُض ُ ٌ ٍ ٌ ٍ 2. ٌ ٍ ٍ ٌ خ أ غ ٍ ط ان في تج ٍ ٍ ٍ ٍ ٍ س ِ ظ إ خ ا ٌ طث ٍ

ٌ ٍ ٌ ٍ ٌ ٍ ٍ	ٌ ٍ ذد ٍ ٌ ٍ ش ٍ ٌ أ	ٌ ٍ ٍ ٍ ٍ ٌ ت	ٌ ٍ ٍ ٍ ٍ ٍ ٍ ٍ ٍ ٍ ٍ ٍ ٍ ٍ ٍ ٌ
0,5 ٌ ٍ ُ ٍ ٍ ٍ ٌ ط ش	5	ك ٍ ط ٍ خ	1 ٌ
100 ٌ ٍ ٍ / ٍ ٌ	5	الأ ٌ ٍ ب ٌ	2 ٌ
200 ٌ ٍ ٍ / ٍ ٌ	5	الأ ٌ ٍ ب ٌ	3 ٌ
100 ٌ ٍ ٍ / ٍ ٌ	5	أ ُ ح ظ ٌ	4 ٌ
200 ٌ ٍ ٍ / ٍ ٌ	5	أ ُ ح ظ ٌ	5 ٌ
100 ٌ ٍ ٍ / ٍ ٌ	5	أ ٍ ج ب ٌ	6 ٌ
200 ٌ ٍ ٍ / ٍ ٌ	5	أ ٍ ج ب ٌ	7 ٌ
100 ٌ ٍ ٍ / ٍ ٌ	5	ظيت أ ُ عيز ٌ ٌ	8 ٌ
200 ٌ ٍ ٍ / ٍ ٌ	5	ظيت أ ُ عيز ٌ ٌ	9 ٌ

يـطى أغطكبد ُغظا ِ ُمدة ثلاث أيام يهو ثبيـطى جركغرض ة 0.1 /10ٍ ئ ِ ٍ أ ّطث ِيجط ُضيى الذيل ورئوص

كذوب ِ ٍ بللرحس 5 ِهدي ئثيس 10 دِوٍئن ٍ ِ كوَض. ً كئ خب يف ب ُطثٍ َبد ا ظزيو ّزُ خ ً َطيات أ سّ ْ

روبّضلب كخ ا ُكيئ خ ُ ّ كئ خ. ثدس زُيتشطيح اهطا ِ ٍغ ظ ِ ْللأكيبء .

يحئ تشلب ئ امنكي لرلايا أجيمئخ ثكل ضبثذ K ضبثذ α ّ ظق كيمط أ ُحيوي t َما هو كح في الجزلات

اوئخ

$$K = \frac{\log OD1 - \log OD2}{t_2 - t_1}$$

$$\alpha = \frac{\sqrt[3]{K} \quad X \quad ظ ِ أ ُح أ٤ ِ}{ظ ِ ّجس + ظ إطحب ِ}$$

$$t_{1/2} = 0,693/K$$

خ ش OD1 أكض ُلب كخ ا ُكيئ خ كس ظ ٍ ِ 1

OD2 ُ ضلب كخ ا ُكيئ خ كس ظ ٍ ِ 2

خث ش أس خ خصبث أبيب حث ألي غ ب ّل ح ظم وهب ب ّكه ً لأى أس خ ذد ب حَض ل خم

ال ٍِضلظيا اكيخ اكيو عمل ذ ئطوخ) Adzu ، اذظ ِ 2003 ، Ouachrif ، اذظ ِ 2012 (وس يصرنا ئ ط ض ٍ

أمسور ِظبدكليرج ٤ئ أ لى غ الأ ُنكي اكيوس اسرج ٤ميائي حم غ ا ُر ك ي ا ُغبظ ُل موي.

ر ْ اكيو س طل ماني ٍ غموعات ً ٍ غموكخ ز ّ خ ٍ ِ 5 لكطا ِ ْ ك ٍ ذ َما هو كح لكي ا ُغس ِ 3.

عسٍض ة 3. يٍجبَخ انطان في تجبسة الأ ُ ٔسيخ ححثش سٌ غ ا ُ ر يك

انتشكيز	ػذد انفئشاٌ	انًعبيهت	انًجًٕعبث انتجشيبيت
10 ً / ل ً	6	0,9% Nacl	1 ّ
100 ِ ً/ ل ً	6	Aspegic	2 ّ
100 ِ ً/ ل ً	6	الأٔساق	3 ّ
200 ِ ً/ ل ً	6	الأٔساق	4 ّ
100 ِ ً/ ل ً	6	أ ح ظب	5 ّ
200 ِ ً/ ل ً	6	أ ح ظب	6 ّ
100 ِ ً/ ل ً	6	أجب ء	7 ّ
200 ِ ً/ ل ً	6	أجب ء	8 ّ

ثؼذ سبػخ يٍ انطان ء تى حقٍ انفئشاٌ ثبنغ ش ٌٕ حًط ا ُ ن ا ُ ش ٕ 0,6% ثبنطشٌق ثحذ ا ُ غ ش (intraperitonial) ٔ ثؼذْب يٍت

ٔصٌ زيىنت طبنت ذالت ضون 30 دقيقت.

ر يتى حسبة ب انُسبت أنتثبط سحزي انكعب خ

$$\text{Inhibition (\%)} = \frac{Wc - Wt}{Wc} \times 100$$

حيث Wc ٔسٌ عسٕ ِ ص ٌ ٔ ظ ب د في انًجًٕعت انشب ْ ذ ح

Wt ٔسٌ عسٕ ِ ص ٌ ٔ ظ ب د في انًجًٕعت انًعب ن ج خ

أُثُش اٌَسخخٍصبث اٌُببحُت ألٍىغ بٌٍ حُظم وٍٍبب بٌٍّه ٍَاخمبة الأ ًٌ اٌَسخخٍذ ببٍٍض ٍِ

رعطي هذه أٌ غطٌخرٌ ٍٍىٌب ٌٍٍُىٍ ٍٍُ ٍِص َ امسور ِظبدٍ كّ اٌُ القّ بة ٍْلٌُّخ األ ش ٍِ امسخوٌص ثب لزيلٍ ٍِ كِـٍى غطٍ ٍِىٌطال غبضة

لثوٌيجبع ئطيّخ (Al Amin, 2012) حٌٍشٍ ش ٍِّض ماني ٍَغموعات ٍَ ٌٌّحُ ٌَ ٍِ 5ٍٍىٍىٍطا ٍٍٍٍُُّ ٍَ ب ٍّكّ ٍَ ٌ ِذ ٍَما هو ٍّكَّح

كي أٌ غس ٍْ 4 ٍَجٍكٍبكٍّخ ٍَ ٍِاكٌوس ٌ أٌ ٌعال ٍِ..

عسٍْض ة 4. ٍَكٍبيٌّخ ٌُىٍطان في تجرٌخ أٌبة األ ش ٍِ امسخوٌص ث بٍُعيٍِ

ٌاٌجٍكّت	ٍَكٍذفلٍىٍ ئش أ	ٌٍَكٍبوٍت	ٌ ٍَجٍَىٍكٍتٍاخٍشٍّ ٍِبَت
10ml/kg	5	0,9% Nacl	1ّ
100 ٍِغ/ ٍَغ	5	Ibuprofen	2ّ
100 ٍِغ/ ٍَغ	5	الأٌثب ٍِ	3ّ
200 ٍِغ/ ٍَغ	5	الأٌثب ٍِ	4ّ
100 ٍِغ/ ٍَغ	5	أ ح ظٍَ	5ّ
200 ٍِغ/ ٍَغ	5	أ ح ظٍَ	6ّ
100 ٍِغ/ ٍَغ	5	أٌجب ٍِ	7ّ
200 ٍِغ/ ٍَغ	5	أٌجب ٍِ	8ّ

ثٍكٍبس ٍَكٍبيٌّخ ٌُىٍطا ٍِ حئث أٌ غس ٍْ ٍّكّلاه ركٍّ غطٍح ٍَ الزيلٍجٍبٍشٍطح فيٍجٌٍطٍب حٌ سٌا ٍِ ئٍ خٌٍُ ٍْلبٍضٍعٍ ك األ ش ٍِ ٌٍٍّمنى

ٌٌزطٍئثٍحٍشٍكٍيٍجٍ األ ش ٍِ ٍ ٍِئ طٍشٍبٍسٍبٍيٍكٍبكٍ خٍ ٍَكٍغ الزيلٍ ٍُ وٍتٍّوز ٍَ أ ح ٍَٔ ٍِ ٍُ ع عٍَاأل ش ٍِٔ أٌذ ٍْصٍ ظ ٍِ ٍّ ٍٍّ

ٍُّ ما. ٍِيٍجط ٍَلٌطٍٍٍشٍٍٍٍى ٍُ أظ ٍِ ٍُ ٍّ ٍِ ٍَٔ بغ اٌُّ ٍُ بة. ٍُّروٌب غ ئٌٍحٍّخٍرح ي اٌُّ بقٍّبٍُ الخٌ أٌبٍّخ

Inhibition (%) = 100 [1 – (Et/Ec)]

Et: عذد انخهبٌب انًحًشة فً يجًىػت انؼالج

Ec: عذد انخهبٌب انًحًشة فً انًجًىػت انشبهذة

ِشبط انًسخخهص انًبئً انًضبد نالنخهبة

1) ِشبط انًسخخهص انًبئً انًضبد نالنخهبة انًسخحذد ببنفىسًبنٍٍ

انًسخهذهت (Tang، 2004) نهًجًىػبث انخً حى حقسٍى انفئشاٌ إنى 5 يجًىػبث كًب هى يىضح فً انجذول 5.

جذول 5. حىصٌغ انفئشاٌ فً حجشبت انفىسًبنٍٍ

انًجًىػت	انًؼبنجت	حقٍ انفىسًبنٍٍ	ػذد انفئشاٌ	انكًٍت
N	يحهىل	---	5	0,5 يم
F	يحهىل	+++	5	0,5 يم
FI	Ibuprofen	+++	5	100 يغ/كغ
FP	الأوساق	+++	5	100 يغ/كغ
FPP	الأوساق	+++	5	200 يغ/كغ

حقٍ 0,1 يم يٍ انفىسًبنٍٍ (2%) فً انقذو انخهفٍت فً انٍىو الأول ثى انثبنث ويخببؼت انىصٌ نًذة 10 أيام.

2) ؤُثُش اٌَسخخص٥ ال٘ىٍعزيبد َبيبث الأغبّ ٌّخه ٍ االخ٘بة اٌّسخحذد ب٤اسطت ٍاس٤ٍ ى ل وسفخحش ٌٍّض الهع٤ٍ٤ اسخحاِص٢ي ب٤اسطتحش ٍٍحشصٌ٤ؤرة ٌِ ٢ ان ُ٘ ُثٌُ٢

ال ٍّص ٍأزغ ٌ٘طخ ش ٌّ ذ ذمس َ٘غمىٍّت بٍ ٌ٘ىٍذإب٘خر ٌحٍؤَّسِح 5 ِطش ٍإَّب ذ ٍ٘اكيبخ ّ٘ ما هو َ٘كح لكي أغس ٍْ ضه٘6.

عسْض ه 6. كجبخ أغطى٘ان في تجوث الري بة أوسجس شثٌ٢ط٘خ للض ٍّ ضِكغ رطٌَّ٤ع أ ٌ٘ ٍّكيئ٤ٌ٤ ٍ أبلازمي

شٌ٢طخ عخ٘طخ ٌطلح ٍ اصّي٤ٌ٤ ٍ

ل َجى ٌّّّكن٘اخ ٌَخبُث	ل٘كيبوت	ح ق ل ى٢ٍ٢سيٍفي	ح ق ل ٌخ٘ ٍى	ٌّكذدي ل٘ج٘شرأ	ٌّكذدي اج٘كتٌ
FMٌ	لكط٘يخ	+++	+++	5	ش٘ط ٌ٘ ٌ ٌ ى٘ 0,5
FMIٌ	Ibuprofen	+++	+++	5	ى٘/ى٘ 100
FMPٌ	الأوث ب ٍ	+++	+++	5	ى٘/ى٘ 100
FMPPٌ	الأوث ب ٍ	+++	+++	5	ى٘/ى٘ 200
Mٌ	لكط٘يخ	---	+++	5	ش٘ط ٌ٘ ٌ ٌ ى٘ 0,5

رْ ح ٍ أغطى بثٌٍوعز٘يوض ة 0,1 ٌ ٌ ٌ للض ٍّ ٌّة ٍ) 2% كجٌطخ ٌّ وسّ مى لِطش في أ ٤ ٌّ الأول ثم أ٘ ٍّطش ٍ ٌب شُ

زُغٌطخ. ٌ سٌّسح اكيبخ 10 أياضّ ٌأزغطخ ٌىبثو خ ٌّ٘كإب٘خ عطخ ٌ٘ ٍ ٌكف٤ٌ٤ ٍ أمقدرة ة 400 mg/kg.

ال ٍأزغطوى٤ أ٘بثوزى٤ ٍّرِوص طٍض وُسّ الموج خ ٌّ ٌ ٌّكسوخرِوٍّصه٤ بكيبد وُسّ الموج يّوعّثبب كيك٘ما ٍ وُسّ

و يّ اال ٌّزط٤ٌخ. أ ٌّ االوبة كبوبغ ٌ ٍ أ الحخ أُي٤خ

$\Delta T = Td - To$

حيث Td كمك وسّ ىمنى في أ ٤ d

T0 كمك وسّ ىمنى قح رطحئن للّضبّئ ,

كسّ بيخ ازغظوئ رثوصكئ نبد , ٌلس ّوؤب غ ئخخ ثطوئ , CRP رطئع أ . ّكيوئئ , في ال ظب ّماريض اح ٤أ ,

رثوصكئ نبد , امفا ط ضئ اكيخ ائئ غخ.

دراسة خثش رأس خصب ث رليب حت أل غ بّ ل حظم وهب بّكه لضلع ل خه يت

1) خثشيه كه نخ بش ل خى ّنه لب انس ش طبت

ذ نلايا ّ كـ DND41 ، JURKAT MOLT3 في اومثلخ ، T-ALL ع , نلايا خ غطأ زغ ص ال ذ اكيوعمل

T-ALL ثكيطخ ّئئر ظبد حبوبد المّضح كي الأكبي لمدة 48 كيبكخ في املضع ا ئيكيس شي ر ريّ , ارلايا

ثكيطخ ظضم لفن الكيس شي ر حئ بتكوز رالب احخ)التي لار زّ بشبني ال ؤبضزبيشس بش.

خثشيه كه نخ بش ل خى ّنه ال ٌشغ انس ش طبت

اكيذ لك ئطوخ ظضم لفن ال , ازيكيثوب كبي ا رلايا غيرائطئ بئخ التي رضى كي ارلايا ائم كيئخ اثطا , ّ ,

ع Pre T 2017 ، M31 ، نلايا اعطرئئ ، القب ، Hecat ، نلايا جئئخ بّخ الإنب ، HEK 293.

2) خثشيه كه نبش و جُ شل حبطب الق سب و ل خى ّ NOTCH و ERK

اطليخ امسرعملة هي طريخ Western blot ، ازيكيخ ّ , ئطف)Palermo ، اذظ , 2012(حيث رتكيب ر ال ص

اجطئ نبد , كيئ نبد سوضكيخ سيد ترّ ع ب في كيخ ص لكيبكب غطح كجط حو ّبطث بئي ثم ّوب ائ شئخ

ذب طخ يم ّ , ّ ذال بر طحئن ّس اد اكيس الأولئخ ّح ERK ، NOTCH ص رطئن ّس اسلكيض ب ّخ التي ش ّ ّ

كيسا غر ّي , ابب بثع رب لي تقدير ئخ اجطئ نبد.

انًهخّج

عب ءد زأبيئظ اهوح ظ َكّئِب ¯ ، ظإنيكبدز اذ َ اكيكيّخ هي اّيبلي:

ثئ ّذ زأبيئظ الأولخ ُز غهطخ اثمخ كًِى اغطوا ِ أ ِ ّيئزر ِظبد ً َ ّثصوض الأّثب ثصوض ا ُح ّظ إجب ِ كّسّ ظ ضّ أي كّط اع زُئمم بجرّخ 2000 ّ يُاي ئ ¯ ، ً َ ّيئزرض بُاتيُس ِ اغطوا ِ كيهمب ُغخ ُاموضوكّخر حذ املاحظخ ّسح 14 يوم.

أشبض زُئيظهىرؤ ط ّرز ِق امسوز ِظبد ّجيبوئخ كّلى رلج ؤ عشلبئ امنكّي لرلايا اكٍجمئخ إليهوضل بعشلبئ اجعمي ثوئمخ كيّهطح ثب ُئحخ ُح ؤاأبد اكيبيّضأخ ثشيبسح ًّب أ ِ ُ ظق كيبمط ا ُحيوي ُغ عيبد ا ّطث ِ في لس ّا زُل غثوئمخ كيّهطح ًما هو ّ كّح في اش ّ َ) 8،7 ، 9(ثب ُ ئخ غهطث خصوؤ طشلبئ امنكّي لمسوز رض بلزُجص ضُ الأّثب ِ كّلى شلبئ امناعي، و ًما هو جّ ِ في اش ّ َ) 11،10 ، 12(ثب ُ ئخ ُ ز غهطخ ضّ خصوؤ طشلبئ امناعي ُمسوز ظبد ا ُ عيزؤ خصوض الأّثب ِ ا ُح ّظ إجب ِ ّصرا ظيت ا ُ عيز ِ. جش أ ظبط امسوز رض ا ُ عزي للبا ِ ّظيت ا ُ عيز ِ جطصوؤ طر لجؤ زي شب ُ ئ امناعي ثوئمخ س كيّهطح بض كيّثش بس.

أظ بهطكنخصوؤ ط امسوز ِظبد ّجيبوئخ بُّربد الأّثب ، ا ُح ّظ إجب ِ كّلى الأ ً اوسجسرثحم غ ا ُر َ ُس ِ اهنط زُال بكّبثوئمخ كيّهطح زُوض اد أبعمخك ِ حم غ ا ُروَبض خُشبلنط اشبس حًب ذّ ةًخ ئموزن بًضثخ ّغ اهنط ِ كيمبج ّثب ُس اء امرجعي)Aspegic( )اش ّ ض ة 13(.

كئما يخص اكوس آجيبوبد كيبب زاد الزُيبة ا ُ يضيبكيئزنبؤ األش ِ لكيبسخ امسوز ِظبد ّجيبوئخ كّلى ا ُمحفز ثكيطخ ب ُ عي يًكيب ِ زأبيئظ اهوح ظ َكّئِب أ ظبطد ا زُلبع كّ ّي في اسزئبء األش ِ ثكيطخ اكوس آ س َ امسوز ِظبد ّجيبوئخ.

زأبيئظ اهوح ظ َكّئِب ّ ِ ر غهطخ ثيزئ ط امسوز رض ُلب جّصوض الأّثب ِ كّلى لُ بة امفا ط َ اوسجسس ثلّض ضًّا لُ بة امفا ط َ اوسجسس ثكيطخ لّض ِ ّضك غ رط ؤ ع ا ِّ كيئزئ ، ابلازمي عب ءد كّلى ا ّح أزبلي

- اكيبخشلك ُض ٍ أسهذ أبثبسوٌلبَذب اذي رمتبضزٌ ٌثئ ٍ امجموعاتلشبشس ح كلمزخ تثب ُسأء ا ٌكبز لإلو بخة)Ibuprofen(ٌ اكيبخشب ُمسوز ِض أ ٌبتيأل ضوشب بثئ ٌذ لكيبمئخ أخزخ إلى جا ٌت ُأسأء المركيبي في رلبع كي ي االلوُ بخة)عس ٌ ِضة 6 (.
- كيبخ اُطشإ ثغطكخ 400 ٌتُ/ٌت ٍ طمى ئٌ ٌئ كلبإلخ إلى كيبؤبشلك ُض ٍ أظ بطلول كبشثئمخ كيخطح ُ. ٌكيئٌتئ ٍ االظمي، اوضلب عشوئمخعس كيخطح ُمؤشر CRP.
- رزئغ ٌا رلبع ٌ ٌ ٌ ٍ أ .ٌكيئٌتئ ٍ االظمي ووٌش االلوُ بخة CRP في ٌغموكخ لكلطا ٍ زامصيخ كـلى جُخ الأوثب ٍ ٌهٌ ٌ 21 ٌ 22(.
- أظ بطد وبطكبد أ ٌئئغخ فيلب ط ٌ أغطا ٍ ا ٌكـبخشلك ُض ٍ لشلك ُض ٌ ٌ ٌ اصوئئكيب رآ ٌ ٌ رلزذ ٌغ ط كيما هس ٌالأكـط اع أ ٌئئغخ ثئط أكـحخ في امجموعات امعا ُخج ٌط ٌت كيب االلوُ بخة كلمب ُغخ ثكيطخ ٌتزرض جخ الأوثب ٍ 100 ٌ 200 ٌت/ٌت.

لبزأبيئظ أوح ظ ٌكـئب ٌ ُطبالجدبضط اكيكجلكج ٌذ بيلي:

- لكيبمئخ امسوز ِظبد ٌجبوئخ ُجبد الأوثب ٍ، ا ُح ظلأجب ٍكـلى املضع ا ُرئجئطئبمئخ ٌ ٍ ٌع ا ٌ ئميا ا ُمالكيئخ حلبزح T كيهخ كيغ ٌ ا رلبعشوئمخ كيجطحزلصبط ا ُرلايالئطئبمئخ شب ُئجخ كيئ ٌبد اكيبخ شب ُمسوز ِظبضخ ٌثشبسح ٌهٌ ٌض ٌ 33 ,36 ,38(.
- ٌض ٌ امسوز ِظبئثئ ٌذ ٌصئ ُسطورئطب كـبِ ا ُرلايا غيرائطئبمئخ ٌهٌ ٌض ٌ 35 ,37 ,39(.
- طوؤئط امسوز ِظبد ٌجبوئخ كـلى اجطؤئ ٌبد اجطولخ شبلائب ٌ الخلويئ ٌذرل ٌم امسوز ِض ا ُعيتيأل ضوشب ٍ طوؤئط ٌ اجطؤئ ٌبد NOTCH ٌ ERK، لب ٌتزرظبد ا ُح ظلأجب ٍك ِرظط لكيبكح بفيئبئ ٌصئ ٍ اجطؤئ ٍ.

لبيقشت

ريجطَلجبوبد ألطخ سَرطضا ئبَب ُطت لجسيث ُظطا لاحزلَب ػَلى َطجًبدرظط لكيب ُبث ٤ٌغخ ئب َخ ُظخح الاٍلجب ،ُالٍكجبزح َ ٍص َ ٍالكجب ُبدرغنتضاكخ ُتحديد سمغخ ٍص ٍلجبوثبب كيزيما ٍرو٤ ٤ٌ كيم٤خ ٍس ٍلجبوبد ثلكيطخ ح٤ا َئبر غبضةٍحسيبى أ غطفبد ا٥ َ َ ُالكوس ا ٍجٍشري)Almançaa ٍ آنظ ُ، 2011(.

أمسزو ِظبد ٍلجبو٤ خ ُ ٍ َ ٍشحصٍض الأٍث بثصٍضٍ ا ُح ٍظ َ ٍأٍجب ٍ ٍرظ بط أي ٍكطط اعرسمٍُس ٍ أغظٍا ٍ.
كيلمجٍ ٍئب ُغطخ 2000 ٍ ٍي/ىيٍ ٍماكيطي مجالا وكيب لاسكيما ٍ ٍصٍجبٍار ٍب د ٍ ٍطٍجبوب.

وٍضركيبكيسيدةرمتٍضٍاكخ لطزو٤ط ٍرلج٤زي أو ٍطمجٍتٍشٍب ٍى أمناعي و ٍطجبوبثب ٍئ ٍجخ ٍجبوبد ألط٤ خ ركيجط رغطٍثجٍكيس ٍر ظط٤خ ٍلس ٍ ٍ ٍ ٍأ ٍطث ٍ ٍأثططٍض ٍلض ٍكيبد لأٍبٍرو ٍكٍب ٍى ا ٍرلايا أٍلج٤ٍخ التي ٍو ٍث ٍىظ ٍاٍ ٍع ٍغ ٍبٍد أ ٍطث ٍ ٍ.
ٍ ٍلس ٍ)Gokhale ٍ آنظ ٍ، 2003(.

ٍشٍلب ٍى ٍرلج٤زي ٍغبظ أمناعي أذيث٤ز ٍزٍبئغٍب ٍلي ٍطثو ٍج ٍ ا ٍرلايا أٍلج٤ٍخ ٍح ٤ا ٍبد كيلمب ٍغخ التيٍشٍضٍب
رحلع زأكي٤ ٍ الخلوي)Opsonisation(ٍكٍ ٍى ٍطن ال ٍع ٍى ٍب ٍ أمكيبزح ٍالجزيء C3b ٍُمتمم ٍما يزيد في سرٍكغ ٍنكيٍمخ
ٍس كيلس)Singh ٍ آنظ ٍ، 2012(.

ٍ ٍي ٍعس ذال ٍصٍ ٍلطٍاكخ أ ٍ الأ ٍ ٍأٍمجسريثحم غ ا ٍر ٍ يحدثروظب د ٍأ زآءاد ي ظبط ا ٍح ٤ ٍ ٍكيس ٍ
ٍص ٍرو ظبد ا ٍرل ٍغ ٍثب ٍئ ٍخ ٍمجموعات كيلمب ٍغ ٍثب ٍمسزو ِظبد ٍلجبو٤ ٍخٍ آ ٍبولطٍط ٍثو ٍ حم غ ا ٍر ٍ يخ ٍرٍكيٍل ٍغبثخ
ٍرلبثئ٤خ ٍ ٍكيك٤خكيٍجٍضا ثبلأ ٍصا الأ ٍ ٍرٍيٍجت فيٍيٍك ٍغ تحرير حم غ الٍدا ٤س٤ي ٍ ٍ أ ٍل ٍكيل ٍجٍجٍا ٍضٍك ٍغ ٍى ز ٍ
أٍجٍطٍوٍب ٍثنٍدين ٍوث ٍكي ٍغ أكيأ ٍ ٍ ٍطٍسوٍطحٍكيلى أغبظ العصبي)Shaikh ٍ آنظ ٍ، 2012(.

ٍكيجط ٍمٍشٍطبلوٍخ الٍش ٍ أمسٍجٍوس ٍث ٍب ٍعيٍ ي ٍس ٍ ٍلٍظا ٍ ٍشٍوعب ٍ بما في ٍرو٤ ٤ أمرٍجٍبد كيلمبزح ٍالوٍ ٍبة
ٍظٍطٍو ٍلأب ٍ ٍأ ٍعيٍ ٍي ٍمر ٍت ٤ميائي بزيادة ٍى ٍز ٍثكي ٍغ أكيأ ٍ ٍالوٍبثئ٤خ ٍبٍوٍئٍزب ٤ ٍ، أٍى ٤ط ٍر ٍ ٤ٍ،أٍجطٍزيٍ ٤ ٍ، ٍص ٍ
أكيأ ٍ ٍر ٍ ٍ ٍ ٍى ٍوٍخٍكٍ ٍرٍكي٤ ٍغ الأوعيٍضٍوك ٍغ ٍبلٍبٍث٤خ ٍكٍلٍبٍئ٤خ)Birari ٍ آنظ ٍ، 2009(.

ٍبل ٍلكيب٤ٍخ ٍس ٍ أمسزو ِظبد ٍلجبو٤خ أمسٍوعملخ في ٍهٍذا ٍلجحش ٍ ٍلٍو ٍكي ٍو ٍ إلى اح زلٍب ٍكٍلى ٍطجًبد ٍشٍطٍخٍضٍ ٍ
أ ال ٍك ٍ ٍ ٍسٍيٍا ٍ ٍألأ ٍ ٍيٍساٍشاد لطزو٤ط ٍكٍ الوٍهابي.

إ ، رُ بـة أمفا ط َ أونجس سرشٍلَض ٍ ّ ثٍ، بلبَش ط أم مخ أمسزعملخ طكيخ رؤ٤ط أمسزر ظبد أطجي٤خ ظطونٌبث . ً غ رُ بـة أمفا ط ٌس ، الأثٍب ،)Shastry ُ آنظ ، 2011(. ح ٌ الَض ٍ يسي غبثخ لأخ جبكَض حٍ زرٌبخ يسج ٌ تحرير رٌ٤زب ٍ ٤ ٍ أئ ٤ط ٌ ٍ ٤ ٍ أ ّ ٌ ٤ ، أمخ ِض ب ٤خ رزسذ ٍ كذ بلفل ب ع ٌخ أطجٍٍ ب ث ٌس ي ،)Chauhan، 1998(.

كجطوول بـع رط ٤ ع أ . كيئ ٍ ، الظ ٌي أس أكيا ٌ ٌ أمسجخ الرُ بـة أمز ّش لخس يثا ظط اولإض ٍ كَلى رلى ٤ عزابطث كي غ أكيا ٌ الأمبث٤خض ٌ MCP-1، IL-8 ٌ TNF α(Tiftikci, 2006) جسُ أب إولبـع رط ٤ ع أ . كيئ ٍ ، الظمي ججب في زيادة حدة رُ بـة أمفا ط ٌ ، ٌ صٍا أمطن عب ءضلاكي ٍخرؤ٤ط أمسزر ض أ ٍباتي جُصٍ الأثٍب ، كِ الرُ بـة أونجس سرثح ٍ الَض ٌ ٍ غ لنٌ ٤٤ ، ث غ طكٍ خرٍض ة 400 ٌ ي/ ٌ ي رُ ي كٌ طوٌ بيظ شاد ةمخ كجطرظط زُ ضراولبـع رط ٤ ع أ . كيئ ٍ ، في زيادة حدة رُ بـة أمفا ط ٌ ، زُض أمسزر ض أ ٍباتي ٌمكي ز الرُ بـة ألخ ظلي، ٍهٌا كـِى الأرجح ضا عغ لاحزٍئ كـلى ٌطجٌبد ٌكيز ح ُأمئ س حٍض ٌ أئ ٤ض ٍ ٍأ ز ٌ ك ٤ ط ٍ.

كي الأ ٤ طرح ظِب ٌ ٍ ُطٍس اكيذبضط اكيكيٍتيكيكيما ٍ ئطوخ أمٍضع ا ُرًٌ ئطئب ٤خ أ ٍ أمسزر ظبد جُ بوة خ ُ ٌ ٌ ، الأثٍب ، ُح ظِلٌجب ٍ بُلكيسب ٤خكي ٌٍبز حُئطئب ، وبالتحديد كـس ا ٍ ٤ميا، هذه الكيسب ٤خ كمٍبز ح ٍئطئب ٍ كيجزب ٍ ضكيبد ٌ لأخ كـِى زيت الاثٍب ٍ ٍصٍ ٤ط ٍ اكيهزٍ ئطئب ٍ الذي وصّ ُيشب ٌ ئح خ ُح ظِلٌجب ٍ طلُا بٍ ٌ ت ُشبئب كي ٌبز ٍ أئطئب كيلبلخ إلى هيا أز ٍ ظيت الأثٍب ٍ إلى رؤ ٌ ٌ ئ زيّات ئ د أجطى ٤ نبد NOTCH 1 ٍ ERK1/2.

Abstract

Inflammation is an immune response that underlies a wide variety of physiological and pathological processes; there is growing evidence suggesting that inflammation plays a key role in the pathogenesis of rheumatoid arthritis, cardiovascular diseases and cancer. In addition, some chronic inflammatory diseases are recently found to be associated with an increased level of homocysteine (hyperhomocysteinemia). In fact, a link seems to connect homocysteine (Hcy) and the immuno-inflammatory activation, when inflammatory activation may contribute to Hcy increase, Hcy, in its turn, may act as a pro-inflammatory molecule cooperating to the injury. Natural products from medicinal plants play a crucial role in drug discovery; the investigation of medicinal plants products has resulted in wide number of compounds with promising biological activities.

In the present study we evaluated *in vivo* the effect of three Algerian medicinal plants namely *Argania spinosa, Citrullus colocynthis*, and *Boswellia serrata* harvested in Tindouf andTiguentourine respectively on the toxicity, immunostimulatory activity and pain. Another work was about a comparative screening of the immunomodulatory activity of the three oils which were compared with virgin olive oil.

The toxicity assessment was performed using up and down test in Albinos rats , the immunomodulatory activity was assessed in the reticulo-endothelial system using carbon clearance assay, the analgesic effect was studied by acetic acid induced writhing test in *Mus musculus* mice. The results obtained showed that the extracts of medicinal plants did not present any toxic effect with the administration of the dose 2000mg/kg; also increased significantly the phagocytic activity in dose dependent manner and have analgesic effect by reducing significantly the writhing in mice induced by acetic acid. Also we have obtained that the phagocytic activity was increased very highly significantly in the groups of rats treated with *Boswellia serrata* (200mg/kg) and olive oil (100mg/kg) $P \leq 0{,}001$.

Another study was carried on the anti-inflammatory activity of the three plants extracts which was assessed using an acute model of inflammation which is xylene ear oedema and other was carried with only *Argania spinosa* seeds on arthritis which was assessed in two chronic models of inflammation consisting of formalin induced arthritis and formalin induced arthritis associated with L- methionine induced hyperhomocysteinemia. The results obtained demonstrated that there was significant inhibition of ear oedema caused by the three extracts

treatment. Our research also revealed that administration of L- methionine at dose 400mg/kg caused aggravation of inflammation presented by very high significantly increase of C-reactive proteins (CRP) $P \leq 0,001$ and high significantly increase of homocysteine (Hcy)$P \leq 0,01$ in addition , necrosis, degeneration and inflammatory cells accumulation were observed in the articular cartilage.

But the treatment by crude extract of *Argania spinosa* seeds at doses of 100 and 200 mg/kg attenuate this effect reducing the paw thickness in both models of chronic arthritis and this also was confirmed by normal histological sections. Moreover, in the present thesis, we conducted a study *in vitro* to evaluate the anti-proliferative effect of the three oils on the T-cell acute lymphoblastic leukemia cell lines and on signaling proteins (NOTCH 1 and ERK1/2).The results revealed that treatment with the different oils at the dose of 100µg/ml inhibited the growth of tumor cells, but the *Argania spinosa* oil only reduced the expression levels of signaling proteins ERK1/2 and NOTCH1.

Keywords

Inflammation, rheumatoid arthritis, hyperhomocysteinemia, cancer, analgesic, anti-inflammatory, antiarthritic, antiproliferative, immunomodulatory activity, *Argania spinosa*, *Citrullus colocynthis*, *Boswellia serrata*.

Résumé

L'inflammation est une réponse immunitaire importante impliquée dans plusieurs processus physiologiques ou pathologiques ; diverses études confirment que l'inflammation joue un rôle clef dans la pathogenèse de plusieurs maladies inflammatoires tel que la polyarthrite rhumatoïde, les maladies cardiovasculaires et le cancer.

Des investigations récentes soulignent une relation entre certaines maladies inflammatoires chroniques et l'augmentation du taux d'homocystéine plasmatique (hyperhomocystéinémie). En fait, il semble y avoir une association entre l'hyperhomocystéinémie et l'activation immuno-inflammatoire ; quand l'activation inflammatoire contribue à l'élévation d'homocystéine plasmatique, l'homocystéine, à son tour, agit comme une molécule pro-inflammatoire en altérant les tissus cibles et aggravant ainsi l'inflammation.

Dans la présente étude, nous avons évalué *in vivo*, l'effet de trois plantes médicinales *Argania spinosa, Citrullus colocynthis* et *Boswellia serrata* récoltées en Algérie à Tindouf et Tiguentourine respectivement sur la toxicité en utilisant la méthode up and down, l'activité immunomodulatrice des extraits utilisant le test de l'épuration sanguine d'une dose de carbone colloïdale et l'activité antalgique qui a été évaluée par le test de la douleur induite par l'acide acétique chez des souris *Mus musculus*. En outre, une autre étude comparative a été effectuée sur l'activité immunostimulante des trois plantes indiquées ci-dessus et l'huile d'olive vierge.

Nos résultats montrent que les extraits des plantes ne présentent aucun effet toxique à la dose de 2000mg/kg ; en revanche à d'autres doses, nous avons constaté que les extraits ont augmenté l'activité phagocytaire du système réticulo-endothélial et ce d'une manière dose dépendante, cette activité phagocytaire s'exprime d'une façon hautement significative dans les groupes des rats traité par *Boswellia serrata* (200mg/kg) et l'huile d'olive vierge (100mg/kg) par rapport aux autres groupes $P \leq 0{,}001$. Par ailleurs, les extraits de plantes étudiés révèlent une activité antalgique présentée par une diminution significative du nombre de contorsions chez les souris *mus Musculus* induites par l'acide acétique par rapport aux témoins.Une autre étude a été effectuée sur l'activité anti-inflammatoire des extraits sur l'œdème de l'oreille induit par le xylène et d'autres ont été réalisées avec l'*Argania spinosa* seulement sur l'arthrite et a été évaluée dans deux modèles chroniques d'inflammation consistant à induire l'arthrite par le formol et l'arthrite induite par le formol associées à

l'hyperhomocysteinémie . Nos résultats démontrent une inhibition significative de l'œdème de l'oreille causée par le traitement des extraits. Notre recherche révèle également que l'administration de L- méthionine à 400 mg / kg a provoqué une aggravation de l'inflammation présentée par une augmentation fortement significative de protéine C réactive (CRP) P≤0,001 et une augmentation significative de l'homocysteine (Hcy) P≤0,01 ; en plus, une nécrose, une dégénérescence et une accumulation des cellules inflammatoires ont été observées au niveau du cartilage articulaire. Nous avons constaté que le traitement par l'extrait brut des grains d'*Argania spinosa* à des doses de 100 et 200 mg / kg a un effet atténuant l'inflammation et l'arthrite, ceci est confirmé par l'étude histologique.

En outre, dans la présente thèse, nous avons mené une étude *in vitro* pour évaluer l'effet antiprolifératif des huiles *d'Argania spinosa,* de *Citrullus colocynthis* et de *Boswellia serrata* sur les lignées cellulaires de la leucémie des cellules T aiguës lymphoblastiques et sur les protéines de signalisation (NOTCH 1 et ERK1/2). Les résultats révèlent que le traitement avec les différentes huiles à la dosede100 µg/ml a inhibé la croissance des cellules tumorales; en revanche, l'huile *d'Argania spinosa* réduit seulement les niveaux d'expression de protéines de signalisation ERK1 /2 et NOTCH1.

Mots clés

Inflammation, arthrite rhumatoïde, cancer, hyperhomocystéinémie, activité immunomodulatrice, *Argania spinosa*, *Citrullus colocynthis*, *Boswellia serrata,* huile olive vierge, protéines de signalisation ERK1 /2 et NOTCH1.

ملخص

[illegible]

[illegible]

[illegible] up and down [illegible] 2000 [illegible] (100 [illegible] 200) [illegible]

[illegible]

[illegible] CRP [illegible] 400 [illegible] 100-200 [illegible]

[illegible] 100 [illegible] NOTCH 1 [illegible] ERK1/2.

الكلمات المفتاحية

[illegible]

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Title: Biological activities of some medicinal plants on rheumatoid arthritis induced by hyperhomocysteinemia and formalin and on tumoral process

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Inflammation is an immune response that underlies a wide variety of physiological and pathological processes; there is growing evidence suggesting that inflammation plays a key role in the pathogenesis of rheumatoid arthritis, cardiovascular diseases and cancer. In addition, some chronic inflammatory diseases are recently found to be associated with an increased level of homocysteine (hyperhomocysteinemia). In fact, a link seems to connect homocysteine (Hcy) and the immuno-inflammatory activation, when inflammatory activation may contribute to Hcy increase, Hcy, in its turn, may act as a pro-inflammatory molecule cooperating to the injury. Natural products from medicinal plants play a crucial role in drug discovery; the investigation of medicinal plants products has resulted in wide number of compounds with promising biological activities.

In the present study we evaluated *in vivo* the effect of three Algerian medicinal plants namely *Argania spinosa, Citrullus colocynthis*, and *Boswellia serrata* harvested in Tindouf andTiguentourine respectively on the toxicity, immunostimulatory activity and pain. Another work was about a comparative screening of the immunomodulatory activity of the three oils which were compared with virgin olive oil.

The toxicity assessment was performed using up and down test in Albinos rats , the immunomodulatory activity was assessed in the reticulo-endothelial system using carbon clearance assay, the analgesic effect was studied by acetic acid induced writhing test in *Mus musculus* mice. The results obtained showed that the extracts of medicinal plants did not present any toxic effect with the administration of the dose 2000mg/kg; also increased significantly the phagocytic activity in dose dependent manner and have analgesic effect by reducing significantly the writhing in mice induced by acetic acid. Also we have obtained that the phagocytic activity was increased very highly significantly in the groups of rats treated with *Boswellia serrata* (200mg/kg) and olive oil (100mg/kg) $P \leq 0{,}001$.

Another study was carried on the anti-inflammatory activity of the three plants extracts which was assessed using an acute model of inflammation which is xylene ear oedema and other was carried with only *Argania spinosa* seeds on arthritis which was assessed in two chronic models of inflammation consisting of formalin induced arthritis and formalin induced arthritis associated with L- methionine induced hyperhomocysteinemia. The results obtained demonstrated that there was significant inhibition of ear oedema caused by the three extracts treatment. Our research also revealed that administration of L- methionine at dose 400mg/kg caused aggravation of inflammation presented by very high significantly increase of C-reactive proteins (CRP) $P \leq 0{,}001$ and high significantly increase of homocysteine (Hcy) $P \leq 0{,}01$, in addition , necrosis, degeneration and inflammatory cells accumulation were observed in the articular cartilage, but the treatment by crude extract of *Argania spinosa* seeds at doses of 100 and 200 mg/kg attenuate this effect reducing the paw thickness in both models of chronic arthritis and this also was confirmed by normal histological sections.

Moreover, in the present thesis, we conducted a study *in vitro* to evaluate the anti-proliferative effect of the three oils on the T-cell acute lymphoblastic leukemia cell lines and on signaling proteins (NOTCH 1 and ERK1/2).The results revealed that treatment with the different oils at the dose of 100µg/ml inhibited the growth of tumor cells, but the *Argania spinosa* oil only reduced the expression levels of signaling proteins ERK1/2 and NOTCH1.

Keywords

Inflammation, rheumatoid arthritis, hyperhomocysteinemia, cancer, analgesic, anti-inflammatory, antiarthritic, antiproliferative, immunomodulatory activity, *Argania spinosa*, *Citrullus colocynthis*, *Boswellia serrata*.

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