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Review

Thymoquinone in autoimmune diseases: Therapeutic potential and molecular mechanisms

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ABSTRACT

Autoimmune diseases (AUDs) are a multifactorial disease, among which rheumatoid arthritis, systemic lupus erythematosus and multiple sclerosis are more prevalent. Several anti-inflammatory, biologics, and AUD-modifying drugs are found effective against them, but their repeated use are associated with various adverse effects. In this review article, we have focused on the regulation of inflammatory molecules, molecular signaling pathways, immune cells, and epigenetics by natural product thymoquinone on AUDs. Studies indicate that thymoquinone can regulate inflammatory molecules including interferons, interleukins, tumor necrosis factor-α (TNF-α), oxidative stress, regulatory T cells, and various signaling pathways such as nuclear factor kappa beta (NF-κβ), janus kinase/signal transduction and activator of transcription (JAK-STAT), mitogen-activated protein kinase (MAPK) at the molecular level and epigenetic alteration. As these molecules and signaling pathways with defective immune function play an important role in AUD development, controlling these molecules and deregulated molecular mechanism is a significant feature of AUD therapeutics. Interestingly thymoquinone is reported to possess all these potential. This article reviewed the deregulated mechanism of AUDs, and the action of thymoquinone on inflammatory molecules, immune cells, signaling pathways, and epigenetic machinery. Thymoquinone can be regarded as a potential drug candidate for AUD treatment.

1. Introduction

Autoimmune diseases (AUDs) are a group of chronic diseases or conditions characterized by the defective immune system losing the ability to recognize self-antigens. In response to an unknown trigger, the immune system starts to produce auto-antibodies and inflammatory responses against itself [1]. There are more than 100 types of autoimmune diseases among them rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), multiple sclerosis (MS), and type 1 diabetes (T1D) are more prevalent [2]. But the severities of the diseases are organ specific. They may affect a single organ or different sites of a single organ or multiple organs that are linked with many systems [3]. It was estimated that approximately 7.6–9.4 % of the world people are affected by AUDs, and among them 80 % patients are women [4]. AUDs are among the predominant causes of the global death and also uprising concomitantly with allergy and cancer [5]. Unfortunately, women aged from 15 to 44 years are died of SLE alone, and thus SLE is regarded as one of the major 15 leading causes of the women death [6].

Common and conventional treatment strategy of AUDs include non-steroidal anti-inflammatory drugs (e.g., ibuprofen, methotrexate), biologics like anti- tumor necrosis factor-α (TNF-α) and corticosteroids [7,8]. However, their repeated use for a long time are associated with numerous unwanted effects such as cardiovascular complications, unresponsiveness, recurrent infections, economical loss, and others [9]. Consequently, interests are recently growing to the use of natural products having ailments against autoimmunity, and it is reported that more than 36 % of the American are using natural products as an option of complementary and alternative (CAM) therapies [9,10]. In this regard, thymoquinone (TQ), a phytochemical compound obtained from the black cumin seeds of a plant, *Nigella sativa*, has received attentions

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because of possessing anti-inflammatory, analgesic, anticancer, antioxidant, and antipyretic activities. It can inhibit pro-inflammatory cytokines, cyclooxygenase (COX) and lipooxygenase (LOX) enzymes, elastase, myeloperoxidase, reactive oxygen species (ROS), and epigenetic changes. Interestingly, it acts as an inhibitor of several signaling pathways such as nuclear factor kappa beta (NF-κβ), janus kinase/signal transduction and activator of transcription (JAK-STAT) and mitogen-activated protein kinase (MAPK), and thus it can improve the therapeutics of AUDs [11]. Up to date, many scientific studies have reported the benefits of TQ for treating AUD; nevertheless, the molecular mechanism is still obscure. Therefore, in this review, we have discussed the prominent therapeutic role of TQ that targets molecular signaling pathways, inflammatory molecules, pro-inflammatory transcription factors and inflammatory cells that are involved in AUDs.

2. Pathophysiology of autoimmune diseases

AUDs are developed due to the combination of genetic predisposition (HLA and MHC genes) [12], epigenetic alteration (DNA methylation, histone methylation, histone post-translational modification, microRNAs, acetylation-deacetylation, and ubiquitination) [13] and environmental factors (infection, UV radiation, toxin, drugs, chemicals, heavy metals, stress, smoking, hormones, and nutrition) [14].

Although the generation of autoimmunity is associated with the acquired or adaptive immune response, primarily the innate immune cells including natural killer (NK) cells, macrophages, dendritic cells (DCs) and polymorphonuclear granulocytes play major roles in alternating self-tolerance [15]. Cells of the innate immunity produce immune response against a particular antigen via pattern recognition receptors (PRRs) including Toll-like receptor (TLRs), which is considered as a major participant in AUDs [16]. Interaction between pathogen-associated molecular pattern (PAMPs) and PRRs on APCs in innate immunity results in the stimulation of cell surface marker molecules that are responsible for antigen presentation and generation of pro-inflammatory cytokines [17]. Unfortunately, certain human antigens have recognized theses TLRs, and as a result they are directly involved in developing AUDs through attacking the self-molecules and increasing the expression of inflammatory mediators [18]. Moreover, in the absence of T-cell support, TLRs also activates B-cell and it finally activates naïve T-cells that are key components for progression of AUDs [18].

Adaptive immune response is the master site of AUDs induction. The production of self-reactive T helper cells and pro-inflammatory cytokines is the hallmark of the pathogenesis of AUDs. These self-reactive T cells activate B cells and then these auto-reactive B-cells produce autoantibodies, which are the key factors in AUDs [19]. Initially, any risk factor predisposed to the AUD development including infections, pathogens, toxins and stress invade the body, and the cells of the innate immune system such as NK cells, macrophages, and DCs become immune to these factors. Therefore, macrophages release cytokines (IL-4, IL-5, IL-6, IL-10, IL-13, IL-23, TGF-β, and TNF-α) and activate B and T-helper cells (Th1, Th2 and Th17). B cells and T cells make a coordination that ultimately creates a balance between Th1 and Th2 and between Th17 and Treg cells [20]. Normally Th1 cells as well as TNF-α and interferon gamma (IFN-γ) protect body against intracellular pathogens. Th2 cells prevent infection by releasing cytokines including IL-4, IL-5, IL-10, and IL-13 and activating B-cells. However, deregulated Th17 cells along with IL-6, IL-23, TGF-β, TNF-α, IL-17A, IL-17 F, IL-21, and IL-22 play a major role on the pathophysiology of autoimmune diseases including rheumatoid arthritis, inflammatory bowel diseases, and systemic lupus erythematosus [21,22]. Unfortunately, this process lead to the development of chronic inflammation when the cells and tissues of infected organs are not repaired and these exacerbate the damage of that organ by recruiting more inflammatory and other cells and triggering inflammatory reaction to these site. Thus, above mentioned processes cause the death or malfunction of the infected organs by self-reactive immune responses leading to the expression of the AUDs symptoms (Fig. 1).

Abnormal miRNA expression and dysregulated immune responses are also considered for AUDs progression. For example, abnormal miRNA expressions can enhance the progress of SLE, psoriasis, systemic sclerosis, and dermatomyositis by breaking the maintenance of the immune cells (macrophages, DCs, B cell, and regulatory T cell) function and signaling pathways at the molecular level [23–25]. The dysregulated immune function, a fundamental mechanism of AUD, is manifested by the production of the excessive amounts of auto-antibodies by auto-reactive B cells, and the secretion of the uncontrolled levels of inflammatory cytokines such as TNF-α, IL-1, IL-2, IL-4, IL-5, IL-6, IL-10, IL-23, IFN-γ etc. by $CD4^+$ T-helper classes (Th1, Th2, Th17) and the faulty immune responses of FOXP3+ regulatory T cells and NLRP3 inflammosome [26–29]. These inflammatory cytokines subsequently stimulate endothelial cells, fibroblasts, other immune and inflammatory cells to release different types of cytokines, chemokines, ROS, nitric oxide (NO) and matrix metalloproteinases (MMP) from these cells that exacerbate the condition of AUD, specifically RA and ankylosing spondylitis (AS) [26]. It has been observed that the dysregulation of several molecular signaling pathways including NF-κβ, JAK–STAT, MAPK, Wnt/β-catenin, etc., also play detrimental roles in the pathogenesis of AUD due to the mutualistic effects of the above-mentioned molecules and vice-versa [3,29,30]. Additionally, several systemic AUDs like as RA, SLE, and psoriasis are developed due to interaction between auto-antibodies and many auto-antigens that are present in human body including intracellular matrix protein and cell surface molecules [31].

3. Therapeutic potential of thymoquinone

Historically, black cumin (*Nigella sativa*) has been used as a potential healer against various diseases including hypertension, asthma, diabetes, bronchitis, inflammation, and many others [32]. The main constituent of this plant is TQ that possesses these diverse pharmacological activities. TQ showed potential anti-cancer effects against various types of cancers and immunomodulatory activities by controlling inflammatory molecules (TNF-α, interleukins, and some growth factors) and NF-κβ signaling pathways involved in cancer development [33]. TQ showed an efficacy against RA and AUD by inhibiting COX, LOX, proinflammatory cytokines, elastase, myeloperoxidase, lipid peroxidation (LPO), and NO [11]. TQ has been reported to have beneficial roles in controlling allergic encephalitis, allergic asthma, allergic lung inflammation, experimental colitis, and RA by suppressing IL-6 signaling pathways [34]. TQ's role in controlling cardiovascular complications, bacterial infections against different types of bacterial strains, diabetes mellitus etc. are also well documented [34]. TQ also lowers blood cholesterol level and hepatic oxidative stress caused by the diet rich in cholesterol [35]. The gastroprotective effects of TQ are also found as TQ inhibited the proton pump (H+/K+-ATPase), acid secretion, neutrophil infiltration, and NO production [36]. Moreover, TQ improved stomach ulcer and ischemia/reperfusion (I/R)-induced gastric dysfunction in mice models [36]. Bone complications were also improved by TQ because of its involvement on bone formation, bone metabolism, bone healing, and osteogenesis [37]. TQ can improve brain impairment caused by sodium nitrate through the reduction of oxidative stress, pro-inflammatory cytokines, and apoptosis markers and the enhancement of glutathione levels [38]. The neuroprotective actions of TQ having anti-inflammatory and anti-oxidant activities have also been observed in different models of neurological disorders such as Alzheimer's disease, Parkinson's disease, autoimmune encephalomyelitis, stroke, schizophrenia, epilepsy, and neuropathic pain [39,40].

TQ improved various renal diseases whether it be diabetic nephropathy or renal ischemia-reperfusion in mice and *in vitro* models by altering inflammatory mediators (IL-6, IL-1, IL-18, IL-10, TNF-α, and NF-κβ), redox potential (MDA, GSH, CAT, GST, and SOD), and apoptotic

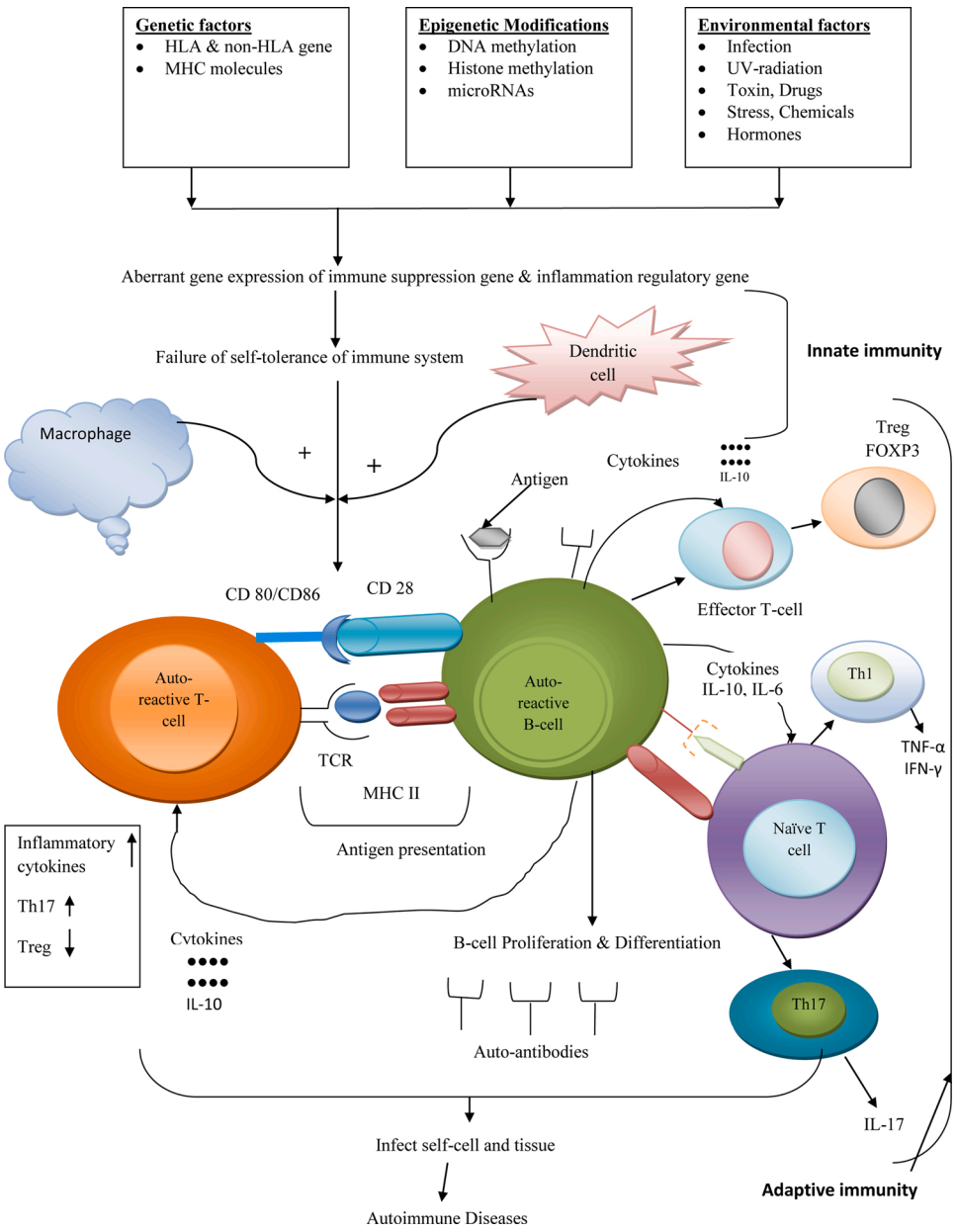

Fig. 1. Pathogenesis of autoimmune diseases. Factors affecting AUDs cause abnormal gene expression of immune suppression gene and inflammatory regulatory gene that ultimately disrupt immune tolerance. Interaction between auto-reactive B cell and T cell causes B cell proliferation and differentiation and produced autoantibodies. Antigen presenting cells such as macrophage and dendritic cell stimulated antigen presentation through interaction between T cell and B cell. Autoantibodies aberrantly affect cell and tissue of immune system and developed AUD.

states [41]. The anti-oxidant and anti-inflammatory properties of TQ corrected reproductive disorders such as spermatogenic and testicular steroidogenic abnormalities induced by lead exposure [42] and the low levels of serum testosterone caused by the exposure of cadmium [35]. It has been found that TQ alleviated liver fibrosis through the modulation of PI3K, Akt phosphorylation, CD14 and collagen-I expression, and TLR4 pathways in an in-vitro study done in a T-HSC/Cl-6 cell line [43]. Interestingly, TQ when formulated using nanotechnology for increasing its bioavailability and studied in animal models, it also showed protection against cancer, diabetes, hepatic injuries, microbes, inflammation, and CNS diseases [44]. Some pharmacological properties of TQ have been listed in Table 1.

4. Role of thymoquinone in autoimmune diseases

The development of AUD treatment is very challenging because of unclear scientific reports of their pathological mechanism and the involvement of multiple factors including genetic predisposition linked to the AUD progression. Moreover, treatment of AUDs with the use of chemical drugs cause different types of adverse effects on health which may cause the body prone to other diseases [5]. Natural compounds have been traditionally used to treat various diseases because of lower cost and belief of lower side effects. So, their use have been increasing for new drug discovery and development. Natural compounds, which are capable of modulating immune system through maintaining the balance of immunological cells (B cells and T cells) and cytokine (IFNs, ILs, TNF and TGF) productions and regulating different types of signaling pathways including NF-κβ, MAPKs, JAK-STAT, and PI3k/Akt can be used to treat AUDs [65,66]. Many experimental studies reported that natural compound which possess antioxidant activity are pharmacologically potent to treat AUDs [68]. Among them quercetin, kaempferol, caffeic acid, lutin, chlorogenic acid, genistein, ferulic acid, resveratrol, epicatechin, catechin, luteolin, galangin, curcumin, epigallocatechin-3-gallate (EGCG) along with TQ are most common [65]. They target different signaling pathway and control the expression of pro-inflammatory genes and regulate autoimmune response. Quercetin, resveratrol, genistein and EGCG targets NF-κβ signaling pathway. Quercetin, kaempferol, and resveratrol targets arachidonic acid

Table 1
Therapeutic potential of thymoquinone.

Therapeutic potential	Study model	TQ dosage	Studied parameters	Mechanism of action	Ref
Hepato-protective	Rats (Sodium fluoride induced hepatotoxicity and oxidative stress)	10 mg/kg	Liver function markers (ALT, AST, ALP, LDH, TB, ALB) LPO and tissue antioxidant enzymes (GSH, SOD, CAT, GST, GPx)	Reducing oxidative stress by elevating tissue antioxidant enzymes activity	[45]
	Rats (Carbon tetrachloride (CCl4)-induced hepatotoxicity)	5 mg/kg	ALT, GSSG, mRNA levels of GST, EPHX1, and NQO1	Increased transcription of GST, EPHX1, and NQO1	[46]
	Mice (Acetaminophen-induced hepatotoxicity)	0.5, 1.0, and 2 mg/kg	ALT, total nitrate/nitrite, lipid peroxide, GSH, ATP	Increased resistance to oxidative & nitrosative stress and improve mitochondrial energy production	[47]
Cardio-protective	Rats (Isoproterenol-induced myocardial infarction)	20 mg/kg	Oxidative stress markers (GSH, MDA, and NO), pro-inflammatory cytokines in heart (TNF-α, IL-1β, and IL-6) and cardiac markers (CK, LDH, ALT and AST)	Mitigating oxidative stress, elevating endogenous antioxidant enzymes, and maintaining structural integrity of heart tissue	[48]
	Mice (Doxorubicin-induced cardiotoxicity)	10 and 20 mg/kg	Cardiac markers (CK-MB, LDH, ALT and AST), antioxidant enzymes (GSH, SOD, CAT, GST, GPx), LPO And inflammatory cytokine (IL-2)	Elevating endogenous antioxidant enzymes and reducing inflammation	[49]
	Rats (Doxorubicin-induced cardiotoxicity)	10 mg/kg	Total oxidant status, total antioxidant status, HSP70, HSP90, GRP78, caspase-3, ANP and NT-proBNP	Increasing antioxidant effects and ameliorating cardiac markers	[50]
	Rats (Acrylamide-induced neurotoxicity)	2.5, 5.0 and 10 mg/kg	Gait score, MDA and GSH	Antioxidant activity	[51]
Neuro-protective	*In vitro* study (Amyloid β-induced neurotoxicity)	–	Aβ1-42	Inhibiting Aβ1-42 aggregation	[52]
	In vitro study (LPS-induced neuroinflammation)	2.5, 5.0 and 10 μM	Inflammatory mediators (TNF-α, IL-1β, IL-6, NO, PGE2)	Downregulation of NF-κβ and PI3k/Aktsignaling pathway	[53]
	In vitro study (HaCaT cells)	–	HO-1 expression	Activation of Nrf2 and phosphorylation of Akt and AMPKα	[54]
Anti-inflammatory	Mice (TPA-induced NF-κB and COX2 expression)	1 or 5 μmol/0.2 mL	NF-κBactivation and COX2 expression	Suppressing COX2 expression by down regulation of NF-κβ signaling pathway.	[55]
	Rats (Collagen induced arthritis)	5 mg/kg	Antioxidant markers (GSH, CAT, SOD, LPO, MPO, and NO) Inflammatory markers (TNF-α, IFN-γ, PGE2, IL-1β, IL-6) and rticularelastase	Enhancing antioxidant enzymes activities and maintaining the homeostasis in the cytokines imbalance	[56]
Antidiabetic	Rats (Streptozotocin –induced diabetes)	80 mg/kg	Glucose, total cholesterol, triglyceride, HbA1c, MDA, total antioxidant capacity, and Glut-2 mRNA expression.	Up-regulating Glut-2 and insulin receptor	[57]
	Rats (Streptozotocin + nicotinamide-induced diabetes)	10 mg/kg	Glucose, lipid profile, HbA1c	Not clear	[58]
	Rats (Streptozotocin + nicotinamide-induced diabetes)	80 mg/kg	Glucose, insulin, GSH, CAT, SOD, GPx, GST, LPO	Antioxidant activity	[59]
Anti-cancer	*In vitro*	5 μM	Expression of epithelial to mesenchymal transition gene(s)/protein(s)	Inhibition of TWIST1 promoter activity and Epigenetic modification	[60]
	Mouse (breast cancer model)	10 mg/kg	Expression of epithelial to mesenchymal transition gene(s)/protein(s)	Modification of epithelial to mesenchymal transition markers	[60]
	In vitro	25, 50 or 100 μM	Activities of Caspase, Bax and Bcl2 protein	Activation of caspase, regulation of Bax and Bcl2 protein expression, and release of cytochrome c	[61]
Autoimmune diseases	Rats (Collagen induced arthritis)	5 mg/kg	Antioxidant markers (GSH, CAT, SOD, LPO, MPO, and NO) Inflammatory markers (TNF-α, IFN-γ, PGE2, IL-1β, IL-6) and rticularelastase	Enhancing antioxidant enzymes activities and maintaining the homeostasis in the cytokines imbalance	[56]
	Rats (FLS and tat adjuvant-induced arthritis)	0–10 μM	TNF-α, PGE2, IL-1β,cyclooxygenase-2 and metalloproteinase-13	Downregulation of NF-κβ and MAPKssignaling pathway	[63]
	In vitro (IL-1β induced osteoarthritis chondrocytes)	0–15 μM	COX-2, PGE2, NO, iNOS, MMP1, MMP3, MMP13	Suppressing NF-κβ and MAPKs signaling pathway	[64]

dependent pathway. Quercetin, resveratrol, luteolin, and EGCG targets MAPKs pathway. Quercetin, resveratrol, and EGCG targets PI3k/Akt signaling pathway. And curcumin, quercetin, resveratrol, and EGCG targets epigenetic alteration pathway [65,69,70].

TQ could be an excellent therapeutic agent to treat AUDs due to its potent antioxidant, anti-inflammatory, and immunomodulatory activities [33,67]. TQ can control different type of inflammatory mediators and protect the immune system from AUDs via regulating cytokine production during inflammation, enhancing the activities of regulatory T cells, controlling the enzymes in arachidonic acid metabolism, regulating different signaling pathways, controlling epigenetic modifications and controlling oxidative stress (Figs. 2 and 3).

4.1. *Effect of thymoquinone on interferons*

IFNs are intercellular signaling molecules, secreted by the cells when infected by virus or foreign particles. They inhibit B-cell activation and stimulate T-cell and NK-cell activity [71]. IFN-α and IFN-β are secreted by macrophages and fibroblasts respectively, increase MHC class I molecule expression and activate NK-cells. IFN-γ, which is secreted by Th1 cell, CD8+ and NK-cells, activates macrophages, increase the expression of MHC class I and II molecules and enhance antigen presentation [72]. IFNs play an important role in AUD development including RA, SLE, Sjogren's syndrome, polymyositis and systemic sclerosis [73]. TQ plays an essential role to control AUDs by regulating the expression of IFNs. Aziz et al. [74] reported that TQ regulates the genetic expression of IFN-α and IFN-β via suppression of TBK1. This finding could be used to treat inflammation related AUDs such as RA, inflammatory bowel diseases (IBD), SLE, systemic sclerosis and chronic obstructive pulmonary disease. In another study, Umar et al. [56] found that TQ ameliorates collagen induced arthritis in Wistar rats by reducing IFN-γ with other pro-inflammatory cytokines. In an *in vitro* study, administration of TQ at low concentration during CD8 + T-cell activation enhanced the expression of CD62 L receptor of antigen presenting T-cell and CD8 + T cell produced huge amount of immunomodulatory cytokine IFN-γ. This finding might be helpful for T-cell therapy against AUD progression [75].

4.2. *Effect of thymoquinone on interleukins*

ILs are cytokines secreted by CD4+ cell, macrophage, monocytes and endothelial cells that are involved in immune cell proliferation, differentiation, maturation, activation and suppression. They have both inflammatory and anti-inflammatory activities [76]. In different immunological pathway, ILs collaborates in the pathogenesis of AUDs. IL-1 (IL-1α and IL-1β) participates in the development of RA, pemphigus, ermatomyositis, T1DM and encephalomyelitis. IL-2 also contributes to the autoimmune response of ulcerative bowel disease and the development of encephalomyelitis, MS, SLE and systemic sclerosis. Elevated level of IL-6 in serum was found in SLE and systemic sclerosis. In case of SLE, RA and encephalomyelitis development, IL-12 also plays key role whereas IL-15 contribute in Crohn's disease and RA development. IL-16, IL-17 and IL-18 participate in the pathogenesis of SLE, systemic sclerosis, and encephalomyelitis [77–79].

Various studies reported that TQ significantly reduce autoimmune arthritis score by decreasing IL-1β in experimental animal models [63, 80]. Badr et al. [81] found that in streptozotocin induced neonatal gestational diabetic rats pro-inflammatory cytokines IL-1β and IL-6 are much increased while IL-2 levels are decreased. The TQ administration during pregnancy and lactation period increased IL-2 level and improved autoimmune T1DM. In an *in vitro* study, TQ suppressed lipopolysaccharide induced pro-inflammatory cytokines, IL-5 and IL-13 by

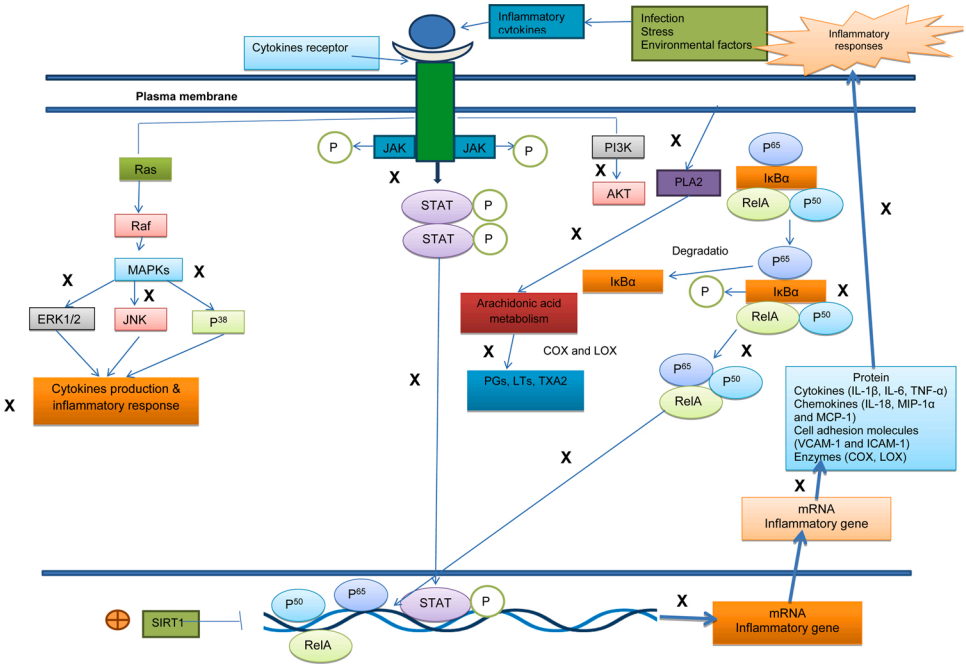

Fig. 2. Probable main mechanism action of thymoquinone against autoimmune disease. The factors of AUD stimulated inflammatory cytokines outside of the plasma membrane that activated JAK/STAT pathway. Pro-inflammatory cytokines and inflammatory response are also generated through MAPKs pathway. PGs, LTs, and TXA2 are produced via arachidonic acids metabolism. The end products of MAPKs, JAK/STAT, AAs metabolism, NF-κβ pathway affect the DNA of inflammatory cells in the nucleus. Via transcription produced mRNA of inflammatory gene and by translation outside of nucleus produced inflammatory proteins including cytokines, chemokines, cell adhesion molecules and enzymes. All of these proteins generated inflammatory response outside of the plasma membrane of the cell. TQ inhibits MAPKs, JAK/STAT, AAs metabolism and NF-κβ pathways and stimulated Treg cells and SIRT1. By these activities TQ ameliorates AUD. '+' *means activation and 'X 'means inhibition. COX: cyclooxygenase; LOX: lipooxygenase; APC: antigen presenting cell; Treg: Regulatory T cell, MAPKs: mitogen-activated protein kinases; PGs: prostaglandins; LTs: leukotrienes; ERK1/2: extracellular regulated kinases ½; IL: interleukin; INF: interferon; FoXP3: forkhead box P3; JAK: Janus kinase; STAT: signal transducer and activator of transcription; JNK: C-jun N-terminal kinase; TXA2: thromboxane A2; PI3K: phosphoinositide 3-kinase; AKT: protein kinase B; PLA2: phospolipase A2; IκBα: nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha; SITR1: silent mating-type information regulator 2 homology 1; MHC: major histocompability complex; TCR: T cell receptor.*

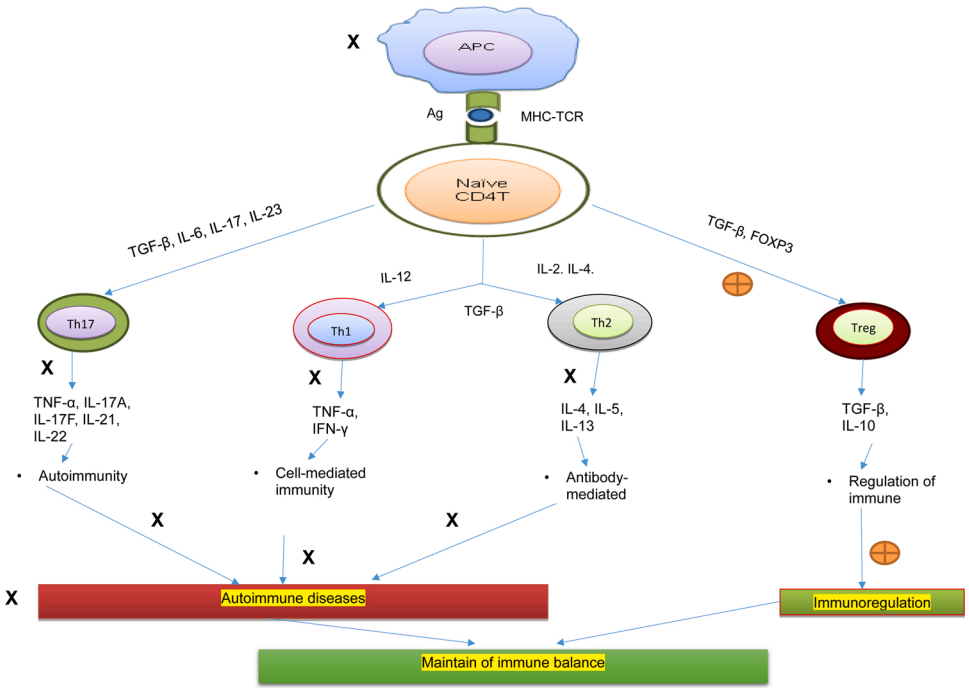

Fig. 3. Effect of TQ on naïve T cell differentiation in AUD. Interaction between APC and naïve T cell produced different types of helper T cells that produced ILs, TNF and Treg. All of these maintain immune balance.

blocking the expression of GATA transcription factor in RBL-2H3 cell [82]. In another study, TQ prevented allergen induced (bronchoalveolar lavage fluid) lung eosinophilic inflammation by reducing IL-4, IL-5 and IL-13 (Th2 cytokines) [83]. Umar et al. (2012) [56] found that TQ significantly decreased IL-1β and IL-6 and increased IL-10 in collagen induced arthritis in wistar rats. Cobourne et al. (2008) [84] found that TQ reduced neuro-inflammation by decreasing the expression of IL-2, IL-4, IL-6, IL-10 and IL-17a in LPS and IFN-γ activated BV-2 microglial cells. TQ treated experimental animal showed that TQ interfere with the LPS induced release of IL-10 and IL-12 by inhibiting DC maturation [85].

4.3. *Effect of thymoquinone on tumor necrosis factor-alpha*

TNF-α is a cytokine molecule generated by activated macrophages, NK-cells, and T-lymphocytes, which play a crucial role in the development of AUDs including SLE, RA, T1DM, Grave's disease, IBD, Crohn's disease, ankylosing spondylitis and MS [27,86]. Elevated level of TNF-α creates deleterious symptoms in AUD patients (Figs. 1 and 2). To minimize the deleterious effects of TNF-α, anti-TNF-α agents are used to block the activity of TNF-α. Anti TNF-α treatment ameliorates AUDs by suppressing or destroying auto-reactive T-cells that ultimately interfere with the pathogenesis of AUDs including RA, IBD, lupus and type 1 diabetes [87]. Although anti TNF-α therapy improves AUDs, but it possess some side effects worsening the patient condition [5]. To overcome this problem, active phytochemicals compounds are researched as an alternative therapy for treating TNF-α. TQ is such type of active compound inhibiting the LPS induced release of TNF-α by inhibiting DC maturation [85]. El-Mahmoudy et al. [88] found that TQ ameliorates autoimmune T1DM and also T2DM by reducing the key cytokine of AUDs macrophage derived TNF-α. Umar et al. [56] found that TQ ameliorates collagen induced arthritis in Wistar rats by reducing TNF-α with other pro-inflammatory cytokines. Vaillancourt et al. [63] and Parlar & Arslan [89] found that TQ reduces autoimmune arthritis score by decreasing TNF-α in experimental animal models. In BV 2 microglial cells, it has been observed that TQ inhibits the release of TNF-α by deactivating LPS-induced NF-κβ activation and P13 K/Akt phosphorylation [53] (Fig. 2).

4.4. *Effect of thymoquinone on NF-κβ signaling*

NF-κβ is a family of inducible transcription factors that are considered as a master regulator of innate and adaptive immune responses [90]. It regulates the expression of target genes, which contribute in inflammatory responses, including pro-inflammatory cytokines (IL-1, IL-2, IL-6, IL-8 and TNF-α), chemokines (IL-18, MIP-1α and MCP-1), cell adhesion molecules (VCAM-1 and ICAM-1) immune-receptor and proteins involved in antigen presentation [91]. Many AUDs including RA, SLE, IBD, T1DM and MS have been developed due to the deregulated activation of NF-κβ [92]. In AUDs, NF-κβ induces pro-inflammatory cytokines and chemokines, and activates auto-reactive T–cells by modulating DC functions, and finally establish inflammation in that infected organs [93]. In RA, dysfunctional activities of NF-κβ was observed in synovium of joints upon the enhancement of the NF-κβ DNA binding capability [94]. In SLE, deregulated activation of NF-κβ were developed in T-cell where p65 expression was decreased and c-Rel expression was found increased [95]. In MS, dysfunctional activities of NF-κβ was seen in MS brain tissue including microglia, astrocytes, oligodendrocytes and infiltrating macrophages where nuclear levels of c-Rel, p65, p50 and 1kβα were increased [96,97]. In T1DM, abnormal NF-κβ activation was observed in peripheral blood mononuclear cells where DNA binding of RelA, RelB, c-Rel and p50 in DCs are hampered and in monocytes, RelB and p65 are activated [98,99].

TQ has a therapeutic potential to cure AUDs by controlling NF-κβ signaling pathway [33]. Daily administration of TQ (1 mg/kg/day) in the animal model of MS ameliorates myelin basic protein induced experimental autoimmune encephalomyelitis through the inhibition of the activation of NF-κβ in the brain and spinal cord [100]. This finding suggests that TQ could be used in the treatment of MS. In another study, Wang et al. [64] found that TQ improve osteoarthristis by inhibiting IL-1β induced inflammation, and NF-κβ and MAPKs in human osteoarthritic chondrocytes. Sethi et al. [101] found that TQ exhibits anti-inflammatory properties through the suppression of the NF-κβ activation, IkBα kinase activation, phosphorylation and degradation of IKBα, nuclear translocation and phosphorylation of p65, and NF-κβ dependent reporter gene (TNFR1, TRAF2, TRADD, TAK1/TAB1, NIK

and IKK-β) expression. Vaillancourt et al. further [63] demonstrated that TQ showed protective effect against autoimmune RA through the suppression of LPS-induced phosphorylation of NF-κβ p65 along with p38 MAPK and extracellular regulated kinases 1/2 (ERK1/2). El Gazzar et al. [102] reported that TQ reduces pro-inflammatory responses generated by LPS-induced mast cells through increasing the number of repressive NF-κβ homodimer and decreasing the nuclear transactivation of NF-κβ and the production of TNF-α. In an *in vitro* and *in vivo* study, Hossen et al. found that TQ reduced inflammatory response by the downregulation of NF-κβ and AP-1. Due to the downregulation of NF-κβ, TQ strongly suppressed the expression of TNF-α, IL-6, IL-1β, COX-2, nitric oxide (NO) production, and NO synthase (iNOS) [103]. Therefore, the outcome of different experimental research convincingly support the involvement of TQ to ameliorate AUDs through the down regulation of NF-κβ signaling pathway (Fig. 2).

4.5. *Effect of thymoquinone on regulatory T cells*

Regulatory T cells, also known as Tregs, have an indispensable role to control the immunological responses against self and foreign antigens, and thus prevent numerous AUDs through suppressing and regulating other cells in autoimmune pathology [104]. Reduction of Treg activity increase the possibilities of AUD development including T1D [105], RA [106], SLE [107], autoimmune thyroid disease [108], and Sjogren's syndrome [109]. Treatment strategies of autoimmune patients along with Treg cells magically improved their deleterious symptoms [110, 111]. Treg cells performed their suppressive action through producing anti-inflammatory cytokines (TGF-β, IL-10 and IL-35) and inducing the apoptosis of infected cells and reducing the proliferation of effector T cells [112]. Among all Tregs, FoxP3+ (CD4+ CD25+ Tregs) performs main role to control auto-reactive T cells to prevent AUDs [113].

In a clinical experimental work, *N. sativa* oil ameliorated RA through increasing the expression of CD4+CD25+ Treg cell and decreasing the expression of CD8+ compared to the placebo or baseline group [114]. This novel finding supports the immunomodulatory effect of *N. sativa* oil in AUD patients. As the main bioactive compound of *N. sativa*, TQ might be involved in this anti-inflammatory activities through elevating the activity of FoxP3+ Treg cell in AUD patients (Fig. 3). In another study on gamma irradiation-induced rats, TQ ameliorated radiation-induced T cell exhaustion and apoptosis through increasing the percentage of CD4+ CD8+ cells by modulating Bcl-2, Bax, PD-1 and caspase-3 expression [115]. In STZ-induced gestational diabetic (GD) rats, TQ significantly ameliorated diabetic complication through increasing T-cell proliferation, rescuing thymus homing CD4+CD8+ and circulating T-cells [116].

4.6. *Effect of thymoquinone on mitogen-activated protein kinases pathway*

MAPKs constitute a group of serine/threonine kinases that regulate diverse range of cellular processes including proliferation and differentiation, gene expression, development, stress response, immune function, and cell death [117,118]. MAPKs consist of ERK and stress activated MAPKs, P38 MAP kinase and C-jun N-terminal kinases (JNKs) [119]. The MAPK signaling cascade in AUDs is activated in response to growth factor, environmental stress, and immunomodulatory cytokines such as TNF, IL-1, IL-1β, IL-12 and TLR ligands stimuli [120,121]. So, treatment of AUDs is associated with the suppression of MAPKs signaling pathway.

According to various studies, TQ downregulates MAPKs signaling pathway and performs immunomodulatory activity that might be associated with treatment of AUDs [122] (Fig. 2). Wang et. al. [64] demonstrated that TQ ameliorates osteoarthristis through the inhibition of IL-1β induced inflammation by downregulating NF-κβ and MAPKs in human osteoarthristic chondrocytes. In another study, TQ inhibited osteoclastogenesis through suppressing ERK/MAPKs signaling [123]. In that study, osteoclastogenesis was generated by RANKL in RAW-264.7 cells; whereas TQ significantly reduced ERK1/2, JNK and p38 phosphorylation in a concentration dependent manner and prevented osteoclastogenesis [123]. TQ also ameliorates neuro-inflammation through the downregulation of p38-MAPK in cisplatin-induced neurotoxicity in experimental rats [124]. In another study, TQ protect acrylamide-induced neurotoxicity in experimental rats through the modulation of MAPKs signaling pathway [125]. Vaillancourt et al. [63] found that TQ decreases arthritis scoring and prevents autoimmune RA through decreasing IL-1β, TNF-2, COX-2, PG-2, and metalloproteinase-13 and suppressing LPS-induced phosphorylation of p38 MAPK, ERK1/2 and NF-κβ.

4.7. *Effect of thymoquinone on JAK-STAT signaling pathway*

The JAK -STAT is an intracellular signaling pathway. It transmits signal to the nucleus from cell membrane receptor to produce growth factors and cytokines. These are involved in variety of cellular activities such as proliferation and differentiation, apoptosis, migration, and cell-cell communication of complex biological events including inflammation and immune response against disease condition [126–128]. Many cytokines and growth factors, which are involved in pathogenesis of inflammatory and autoimmune diseases, use JAK-STAT signaling pathway to transmit signals. So JAK-STAT pathway is directly involved in the pathogenesis of inflammatory and AUDs [30,129].

TQ can prevent AUD generation through the suppression of the activation of JAK-STAT signaling pathway (Fig. 2). Hu et al. [130] reported that TQ can block the stimulation of JAK2/STAT3 signaling pathway and inhibit cell proliferation. In an *in vitro* and *in vivo* study, TQ inhibited the activation and phosphorylation of STAT3 pathway and suppressed the expression of STAT3 dependent reporter genes such as survivin, cyclin D and VEGF, and thus TQ also prevent cell proliferation and induce apoptosis [131,132].

4.8. *Effect of thymoquinone on phosphatidylinositol 3-kinase/protein kinase B (PI3k/Akt) signaling pathway*

PI3k/Akt signaling pathway is an intracellular regulatory signal transduction pathway activated by toxic substances or cellular stimuli that regulate many cellular processes including proliferation, transcription, translation, metabolism, cell growth, survival and apoptosis [133]. Abnormal activation of PI3k/Akt signaling pathway is associated with the pathogenesis of many diseases including diabetes mellitus, cancer and different autoimmune diseases [133,134]. In autoimmune diseases, PI3k/Akt signaling pathway play an important role through the expression of different types of pro-inflammatory mediators that degrade Iκβ and activate NF-κβ signaling pathway [65]. There has been an experimental evidence that TQ deactivated PI3k/Akt signaling pathway via various mechanisms (Fig. 2). TQ induces apoptosis through blocking PI3k/Akt signaling pathway in DU-145 cell line [135]. In another study, TQ deactivated PI3k/Akt and NF-κβ signaling pathway and regulated various gene products such as P65, P-Akt, XIAP, VEGF and COX-2 [62]. Badr et al. [81] reported that TQ ameliorates diabetes through restoring cytokines (IL-1β, IL-6 and TNF-α), free radicals, blood sugar level, insulin and lipid profiles. TQ also restore the proliferation of lymphocyte through regulating PI3K/Akt signaling pathway. Xuam et al. [85] have reported that TQ possess anti-inflammatory properties through controlling the release of inflammatory cytokines and inhibiting DCs clustering, maturation and survival. In that study, DCs were activated through the induction of LPS, and activated DCs stimulate antigen presentation by expressing high level of CD11c, CD40, CD86, CD54 and MHC-II molecule which further stimulate the excess production of inflammatory cytokines (IL-1β, IL-12 and TNF-α). LPS activated DCs when treated with TQ were hampered for DCs clustering, maturation and survival and retarded the release of inflammatory cytokines through the down regulation of AKT and ERK1/2 signaling pathways. As TQ down

regulates PI3k/Akt signaling pathway, so it would be a potential therapeutic agent to treat AUDs.

4.9. Effect of thymoquinone on oxidative stress

Oxidative stress is an imbalance condition between the generation of ROS in cells and tissues and capability of the body to detoxify or counteract their adverse effects by antioxidants. ROS such as superoxide radicals ($O^{-}2$), hydroxyl radicals (OH^{-}), and hydrogen peroxide (H_2O_2) contribute to the generation of oxidative stress by altering enzymatic activity, leading DNA lesions, increasing cell permeability and finally damaging cells and tissues [136]. Oxidative stress accelerates the pathogenesis of AUDs by breaking down of self-tolerance of the immune system. An immunological activity against cell and tissue lead to generate oxidative stress and contribute to the pathogenesis of AUDs [137]. Antioxidant defense systems include both enzymatic and non-enzymatic processes that control ROS generation, compensate the effect of ROS by scavenging or reducing excess ROS levels, and maintain cellular redox homeostasis. Non-enzymatic antioxidants are dietary antioxidants including vitamins (vitamin C and vitamin E) and phytochemicals (polyphenols, beta-carotene, and flavonoids) presenting their antioxidant activity by neutralizing ROS and protecting cell membrane against lipid peroxidation [138]. Oxidative stress is also responsible for the post-translational modification of some protein antigen in autoimmune patients. This oxidative induced post-translational modification of protein antigen produces new epitopes leading to the production of auto-antibodies when immune system recognize them as non-self and stimulate B cells against it [137]. Anti-citrullined protein antibodies and anti-carbamylated protein antibodies are two examples of auto-antibodies generated in RA patients due to the oxidative post-translational modification [139].

TQ can scavenge oxidative free radicals including $O^{-}2$, OH^{-}, and H_2O_2, and is responsible for controlling oxidative stress [140]. Both environmental and epigenetic factors are responsible for the continuous generation of ROS in AUD patients. Due to oxidative damage of cells and tissues, an inflammatory response is generated. Inflammation is also considered as a physiological response of adaptive immune system which is stimulated by adverse conditions including drug and chemicals, toxin, stress, infection and tissue injuries [141]. TQ can normalize these adverse effect of inflammation and also prevent inflammation [67]. TQ is capable of scavenging 1,1-diphenyl-2-picrylhydrazyl (DPPH) and prevent lipid peroxidation (LPO) by reducing malondialdehyde (MDA) level in cell membrane [142,143].

Anti-oxidative enzymes including catalase (CAT), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), glutathione reductase and glutathione–S-transferase (GST) are the main enzymatic antioxidant system of cells and tissues. CAT, SOD and GSH-Px are primary antioxidant enzymes that are directly involved in the elimination of ROS (O^{-2}, OH-, H_2O_2). On the other hand, glutathione reductase and GST are secondary antioxidant enzymes that accelerate the activities of primary antioxidant enzymes function and causes the detoxification of ROS by reducing peroxide levels. There were many supporting *in vivo* experimental evidence that TQ elevated endogenous anti-oxidative enzymes such as SOD, CAT, GSH-Px, GST and glutathione reductase through protecting the tissue against oxidative damage. Sener et al. [144] reported that TQ protect kidney inflammation by increasing the anti-oxidant enzymes, SOD, CAT and GSH-Px against arsenic induced renal damage by targeting oxidative stress. Hamdy & Taha [145] found that TQ prevent STZ-induced oxidative stress in diabetic rats by increasing GST, CAT and GSH level and protect neuro-inflammation. Zafeer et al. [146] reported that TQ ameliorates hepatic inflammation through elevating the activity of SOD, CAT, and GSH against cadmium-induced liver toxicity in rats. In an experimental arthritis rat model, TQ shows anti-inflammatory activity by reducing oxidative stress, increasing the activity of SOD, CAT and GSH, and suppressing the expression of myeloperoxidase (MPO) and NO [56]. In EAE model rats, TQ significantly increased GSH level and improved autoimmune EAE through the down-regulation of NF-κβ signaling pathway [100]. The decreased level of CAT, SOD, GSH-Px and GST are restored by TQ in STZ-induced diabetic rats [59]. From the above findings we can suggest that TQ would be an appropriate natural compound to treat AUDs due to having strong anti-oxidant potential.

4.10. Effect of thymoquinone on cyclooxygenase and lipooxygenase

Cyclooxygenase (COX) and Lipooxygenase (LOX), two main enzymes involved in an arachidonic acid (AA) metabolism, are principle factors that participate in the inflammatory responses by the synthesis of prostaglandins (PGs) and leukotrienes (LTs) [147,148]. TQ shows its anti-inflammatory properties through the inhibition of both COX and LOX enzymes which result in the suppression of PGs and LTs [149] (Fig. 2). In human blood cells, TQ was found to inhibit LTs production in dose dependent manner through the downregulation of COX and LOX activity [150]. Mansour & Tornhamre [151] found that TQ inhibits LTs formation through suppressing 5-LOX and LT4 synthase activity in human blood cells. El Gazzar et al. [152] demonstrated that TQ attenuates an airway inflammation in a mouse model of allergic asthma through blocking 5-LOX expression and LTs synthesis. Al Wafai [153] found that TQ ameliorates pancreatic inflammation through suppressing COX-2 expression in the pancreatic tissue of STZ-induced diabetic rats. In another study, El Mezayen et al. [154] noticed that TQ shows anti-inflammatory effect against an allergic airway inflammation in lung in a mouse model. In this study, TQ significantly decreased lung eosinophilia, Th2 cytokines, and hyperplasia of goblet cell through the suppression of the expression of COX-2 and PGs synthesis. TQ also improved skin inflammation in an experimental mouse model through suppressing the expression of COX-2 protein by inhibiting NF-κβ signaling pathway activation and inducing the expression of cytoprotective enzymes [55].

4.11. Effect of thymoquinone on epigenetic modification

Epigenetic modification is characterized by the alteration of gene expression without modifying genome sequence. In AUDs, epigenetic modifications alter T regulatory cells and T helper cells function [155,156]. TQ ameliorates autoimmune diseases through activating or silencing gene expression without changes in the DNA sequences. In epigenetic mechanism, TQ act as a methylating and demethylating agent that can easily donate or accept a methyl group from cellular DNA [157,158]. TQ interfere with an epigenetic process targeting DNMT1 as TQ was found to decrees the methylation of DNA by binding to the catalytic site of DNMT1 and reactivate it through the percussion with Sp1-miR29b loop [159]. In other studies, it has been reported that by an epigenetic process TQ downregulates Twist1 and Zeb1 genes via methylation of their promoter region [60,160]. TQ suppressed the expression of UHRF1 gene and repaired wrong epigenetic code by the process of DNA demethylation [161]. TQ also suppressed the expression of epigenetic protein including HDAC1, 4, 9, KMT2A, B,C,D,E, DNMT1, 3A,3B, G9A and UHRF1 [162].

SIRT1 (silent mating-type information regulator 2 homology 1) is a NAD+-dependent deacetylase that eliminates acetyl groups from different proteins and regulates gene expression [163]. It has been reported that TQ is a strong activator of SIRT1 and control inflammatory gene expression upon the down regulation of NF-κβ signaling pathway [164]. Moreover, TQ activated SIRT1 expression in cardiomyocytes of experimental rats through the deacetylation of p53 gene and acted as an apoptosis inducer [164,165]. In another research work, the authors found that TQ ameliorates neuro-inflammation in BV-2 microglia by the activating SIRT1 and AMPK [166] (Fig. 2).

MicroRNAs are small non-coding RNAs that post-transcriptionally regulate gene expression. From previous studies, it has been demonstrated that the aberrant expression of microRNAs is associated with the pathogenesis of many autoimmune diseases [167]. Previous studies

documented the immunomodulatory effect of TQ on microRNAs. Luo et al. reported that in lithium-pilocarpine induced status epileptius (SE) rat model, the expression of inflammatory cytokines such as TNF-α, IL-6 and IL-1β are increased with the excessive elevation of microRNA-146a expression. However, a treatment of experimental rat with TQ lowers the expression of microRNA-146a and attenuates the expression of cytokines through the down regulation of NF-κβ signaling pathway. This finding strongly support the protective effect of TQ against micro-RNA created autoimmunity [168]. In another study, TQ increased the expression of microRNA-34a targeting TWIST1 and ZEB1 through the downregulation of EMT signaling pathway in MBC cell line BT-549 [169]. Overexpression of microRNA-206b-3p is associated with the generation of oxidative stress and necrosis of the liver tissue in mice model. After treatment of the experimental animals with TQ, it reduced oxidative stress and prevented tissue necrosis through the down-regulation of microRNA-200b-3p expression [170].

5. Limitations and future prospects

Scientists have been searching natural products or compounds, which could target the molecular mechanism of AUDs, for overcoming diverse health complications associated with the repeated use or withdrawal of conventional autoimmune therapeutics (ibuprofen, methotrexate, biologics, etc.). In addition to minimizing adverse effects, it is also a concerning factor to search for an easy, cost-effective natural source having anti-inflammatory, anti-oxidant, analgesic, and antipyretic properties, which are notable in TQ [11]. Though TQ possess water insolubility, photosensitivity, and poor bioavailability, it has been observed that the formulation of nano-TQ improved its bioavailability [44]. Thus it can be concluded that TQ could be a promising therapeutic option for treating AUDs, so significant research should be done on the isolation, formulation, and the design of clinical trials for developing TQ as a drug against AUD. Researchers can think about clinical studies on TQ now.

6. Conclusion

Natural products are considered nowadays as an option of complementary and alternative therapies among the most important therapeutics used to treat AUD. TQ, a promising natural component, can target inflammatory cytokines, oxidative agents, and molecular signaling pathways as well as can control inflammation, regulatory T cells and epigenetic alterations that are the most desirable characteristics for controlling AUD. Therefore, TQ could be a promising therapeutic option for treating AUDs, however, more insightful research should be done on the isolation, formulation, and the design of clinical trials for developing TQ as a drug against AUD.

Authors' contributions

MYA and ZA wrote the manuscript, ZM collected data and edited manuscript, MZ and MT helped in revision and rearranged the texts, figures and table; and MAK designed the work, supervised and finalized the manuscript. All of the authors reviewed final version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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References

[1] P. Zhang, Q. Lu, Genetic and epigenetic influences on the loss of tolerance in autoimmunity, Cell. Mol. Immunol. 15 (6) (2018) 575–585.
[2] I. Comerford, E.E. Kara, D.R. McKenzie, S.R. McColl, Advances in understanding the pathogenesis of autoimmune disorders: focus on chemokines and lymphocyte trafficking, Br. J. Haematol. 164 (3) (2014) 329–341.
[3] B. Bolon, Cellular and molecular mechanisms of autoimmune disease, Toxicol. Pathol. 40 (2) (2012) 216–229.
[4] F. Angum, T. Khan, J. Kaler, L. Siddiqui, A. Hussain, The prevalence of autoimmune disorders in women: a narrative review, Cureus 12 (5) (2020).
[5] P. Li, Y. Zheng, X. Chen, Drugs for autoimmune inflammatory diseases: from small molecule compounds to anti-TNF biologics, Front. Pharmacol. 8 (2017) 460.
[6] S. RR, in: Presented at ACR/ARHP Annual Meeting, Oct. 20-24, 2018; Chicago, 2018. Available in: https://www.healio.com/news/rheumatology/20181114/sle-leads-autoimmune-diseases-for-lost-life-years-among-young-women.
[7] S.H. Venkatesha, B. Astry, S.M. Nanjundaiah, H.R. Kim, R. Rajaiah, Y. Yang, L. Tong, H. Yu, B.M. Berman, K.D. Moudgil, Control of autoimmune arthritis by herbal extracts and their bioactive components, Asian J. Pharm. Sci. 11 (2) (2016) 301–307.
[8] C.M. Greco, C. Nakajima, S. Manzi, Updated review of complementary and alternative medicine treatments for systemic lupus erythematosus, Curr. Rheumatol. Rep. 15 (11) (2013) 1–9.
[9] S. Dudics, D. Langan, R.R. Meka, S.H. Venkatesha, B.M. Berman, C.-T. Che, K. D. Moudgil, Natural products for the treatment of autoimmune arthritis: their mechanisms of action, targeted delivery, and interplay with the host microbiome, Int. J. Mol. Sci. 19 (9) (2018) 2508.
[10] P.M. Barnes, B. Bloom, R.L. Nahin, Complementary and Alternative Medicine use Among Adults and Children, United States, 2007, 2008.
[11] A. Pannu, R.K. Goyal, S. Ojha, M. Nandave, Therapeutic Potential of Thymoquinone in Treatment of Rheumatoid Arthritis and Related Autoimmune Diseases, Bioactive Food As Dietary Interventions for Arthritis and Related Inflammatory Diseases, Elsevier, 2019, pp. 575–587.
[12] V. Taneja, C.S. David, Role of HLA class II genes in susceptibility/resistance to inflammatory arthritis: studies with humanized mice, Immunol. Rev. 233 (1) (2010) 62–78.
[13] M. Zouali, Epigenetics of Autoimmune Diseases, The Autoimmune Diseases, Elsevier, 2020, pp. 429–466.
[14] F.W. Miller, L. Alfredsson, K.H. Costenbader, D.L. Kamen, L.M. Nelson, J. M. Norris, A.J. De Roos, Epidemiology of environmental exposures and human autoimmune diseases: findings from a National Institute of Environmental Health Sciences Expert Panel Workshop, J. Autoimmun. 39 (4) (2012) 259–271.
[15] E. Toubi, Z. Vadasz, Innate immune-responses and their role in driving autoimmunity, Autoimmun. Rev. 18 (3) (2019) 306–311.
[16] S. Trivedi, E.L. Greidinger, Endosomal Toll-like receptors in autoimmunity: mechanisms for clinical diversity, Therapy 6 (3) (2009) 433.
[17] A.K. Shakya, K.S. Nandakumar, Applications of polymeric adjuvants in studying autoimmune responses and vaccination against infectious diseases, J. R. Soc. Interface 10 (79) (2013), 20120536.
[18] M.J. Shlomchik, Activating systemic autoimmunity: B's, T's, and tolls, Curr. Opin. Immunol. 21 (6) (2009) 626–633.
[19] J. Ermann, C.G. Fathman, Autoimmune diseases: genes, bugs and failed regulation, Nat. Immunol. 2 (9) (2001) 759–761.
[20] E. Bettelli, Y. Carrier, W. Gao, T. Korn, T.B. Strom, M. Oukka, H.L. Weiner, V. K. Kuchroo, Reciprocal developmental pathways for the generation of pathogenic effector TH 17 and regulatory T cells, Nature 441 (7090) (2006) 235–238.
[21] A. Hot, P. Miossec, Effects of interleukin (IL)-17A and IL-17F in human rheumatoid arthritis synoviocytes, Ann. Rheum. Dis. 70 (5) (2011) 727–732.
[22] S. Adami, A. Cavani, F. Rossi, G. Girolomoni, The role of interleukin-17A in psoriatic disease, BioDrugs 28 (6) (2014) 487–497.
[23] L. Duan, X. Rao, K.R. Sigdel, Regulation of Inflammation in Autoimmune Disease, Hindawi, 2019.
[24] C. Selmi, Q. Lu, M.C. Humble, Heritability versus the role of the environment in autoimmunity, J. Autoimmun. 39 (4) (2012) 249–252.
[25] X. Deng, Y. Su, H. Wu, R. Wu, P. Zhang, Y. Dai, T.M. Chan, M. Zhao, Q. Lu, The role of Micro RNA s in autoimmune diseases with skin involvement, Scand. J. Immunol. 81 (3) (2015) 153–165.
[26] G.C. Kaul A, M.K. Crow, Z. Touma, M.B. Urowitz, R. van Vollenhoven, G. Ruiz-Irastorza, G. Hughes, Systemic lupus erythematosus, Nat. Rev. Dis. Primers 2 (2016) 16039.
[27] J.S. Ellis, H. Braley-Mullen, Mechanisms by which B cells and regulatory T cells influence development of murine organ-specific autoimmune diseases, J. Clin. Med. 6 (2) (2017) 13.
[28] A. Mohr, M. Atif, R. Balderas, G. Gorochov, M. Miyara, The role of FOXP3+ regulatory T cells in human autoimmune and inflammatory diseases, Clin. Exp. Immunol. 197 (1) (2019) 24–35.
[29] H.-H. Shen, Y.-X. Yang, X. Meng, X.-Y. Luo, X.-M. Li, Z.-W. Shuai, D.-Q. Ye, H.-F. Pan, NLRP3: a promising therapeutic target for autoimmune diseases, Autoimmun. Rev. 17 (7) (2018) 694–702.
[30] S. Banerjee, A. Biehl, M. Gadina, S. Hasni, D.M. Schwartz, JAK–STAT signaling as a target for inflammatory and autoimmune diseases: current and future prospects, Drugs 77 (5) (2017) 521–546.
[31] A. Hansen, P.E. Lipsky, T. Dörner, B-cell lymphoproliferation in chronic inflammatory rheumatic diseases, Nat. Clin. Pract. Rheumatol. 3 (10) (2007) 561–569.

[32] M. Khader, P.M. Eckl, Thymoquinone: an emerging natural drug with a wide range of medical applications, Iran. J. Basic Med. Sci. 17 (12) (2014) 950.
[33] Z. Akter, F.R. Ahmed, M. Tania, M.A. Khan, Targeting inflammatory mediators: an anticancer mechanism of thymoquinone action, Curr. Med. Chem. (2020).
[34] S. Darakhshan, A.B. Pour, A.H. Colagar, S. Sisakhtnezhad, Thymoquinone and its therapeutic potentials, Pharmacol. Res. 95 (2015) 138–158.
[35] A. Fouad, I. Jresat, Thymoquinone therapy abrogates toxic effect of cadmium on rat testes, Andrologia 47 (4) (2015) 417–426.
[36] H. El-Abhar, D. Abdallah, S. Saleh, Gastroprotective activity of Nigella sativa oil and its constituent, thymoquinone, against gastric mucosal injury induced by ischaemia/reperfusion in rats, J. Ethnopharmacol. 84 (2-3) (2003) 251–258.
[37] P.K. Kirui, J. Cameron, H.A. Benghuzzi, M. Tucci, R. Patel, F. Adah, G. Russell, Effects of sustained delivery of thymoqiunone on bone healing of male rats, Biomed. Sci. Instrum. 40 (2004) 111–116.
[38] A.M. Hamdan, M.M. Al-Gayyar, M.E. Shams, U.S. Alshaman, K. Prabahar, A. Bagalagel, R. Diri, A.O. Noor, D. Almasri, Thymoquinone therapy remediates elevated brain tissue inflammatory mediators induced by chronic administration of food preservatives, Sci. Rep. 9 (1) (2019) 1–11.
[39] M. Jakaria, D.-Y. Cho, E. Haque, G. Karthivashan, I.-S. Kim, P. Ganesan, D.-K. Choi, Neuropharmacological potential and delivery prospects of thymoquinone for neurological disorders, Oxid. Med. Cell. Longev. 2018 (2018).
[40] M. Cascella, S. Bimonte, A. Barbieri, V. Del Vecchio, M.R. Muzio, A. Vitale, G. Benincasa, A.B. Ferriello, A. Azzariti, C. Arra, Dissecting the Potential Roles of Nigella sativa and Its Constituent Thymoquinone on the Prevention and on the Progression of Alzheimer's Disease, Front. Aging Neurosci. 10 (2018) 16.
[41] H. Shaterzadeh-Yazdi, M.-F. Noorbakhsh, S. Samarghandian, T. Farkhondeh, An overview on renoprotective effects of Thymoquinone, Kidney Dis. 4 (2) (2018) 74–82.
[42] A. Mabrouk, H. Ben Cheikh, Thymoquinone supplementation ameliorates lead-induced testis function impairment in adult rats, Toxicol. Ind. Health 32 (6) (2016) 1114–1121.
[43] T. Bai, L.-H. Lian, Y.-L. Wu, Y. Wan, J.-X. Nan, Thymoquinone attenuates liver fibrosis via PI3K and TLR4 signaling pathways in activated hepatic stellate cells, Int. Immunopharmacol. 15 (2) (2013) 275–281.
[44] A.H. El-Far, S.K. Al Jaouni, W. Li, S.A. Mousa, Protective roles of thymoquinone nanoformulations: potential nanonutraceuticals in human diseases, Nutrients 10 (10) (2018) 1369.
[45] W.M. Abdel-Wahab, Protective effect of thymoquinone on sodium fluoride-induced hepatotoxicity and oxidative stress in rats, J. Basic Appl. Zool. 66 (5) (2013) 263–270.
[46] W.M. El-Sayed, Upregulation of chemoprotective enzymes and glutathione by Nigella sativa (black seed) and thymoquinone in CCl4-intoxicated rats, Int. J. Toxicol. 30 (6) (2011) 707–714.
[47] M.N. Nagi, H.A. Almakki, M.M. Sayed-Ahmed, A.M. Al-Bekairi, Thymoquinone supplementation reverses acetaminophen-induced oxidative stress, nitric oxide production and energy decline in mice liver, Food Chem. Toxicol. 48 (8-9) (2010) 2361–2365.
[48] S. Ojha, S. Azimullah, R. Mohanraj, C. Sharma, J. Yasin, D.S. Arya, A. Adem, Thymoquinone protects against myocardial ischemic injury by mitigating oxidative stress and inflammation, Evid. Based Complement. Altern. Med. 2015 (2015).
[49] M.F. Alam, G. Khan, M.M. Safhi, S. Alshahrani, R. Siddiqui, S. Sivagurunathan Moni, T. Anwer, Thymoquinone ameliorates doxorubicin-induced cardiotoxicity in swiss albino mice by modulating oxidative damage and cellular inflammation, Cardiol. Res. Pract. 2018 (2018).
[50] D. Karabulut, E. Ozturk, E. Kaymak, A.T. Akin, B. Yakan, Thymoquinone attenuates doxorubicin-cardiotoxicity in rats, J. Biochem. Mol. Toxicol. (2020) e22618.
[51] S. Mehri, M. Shahi, B.M. Razavi, F.V. Hassani, H. Hosseinzadeh, Neuroprotective effect of thymoquinone in acrylamide-induced neurotoxicity in Wistar rats, Iran. J. Basic Med. Sci. 17 (12) (2014) 1007.
[52] A. Alhebshi, M. Gotoh, I. Suzuki, Thymoquinone protects cultured rat primary neurons against amyloid β-induced neurotoxicity, Biochem. Biophys. Res. Commun. 433 (4) (2013) 362–367.
[53] Y. Wang, H. Gao, W. Zhang, W. Zhang, L. Fang, Thymoquinone inhibits lipopolysaccharide-induced inflammatory mediators in BV2 microglial cells, Int. Immunopharmacol. 26 (1) (2015) 169–173.
[54] J. Kundu, D.-H. Kim, J.K. Kundu, K.-S. Chun, Thymoquinone induces heme oxygenase-1 expression in HaCaT cells via Nrf2/ARE activation: akt and AMPKα as upstream targets, Food Chem. Toxicol. 65 (2014) 18–26.
[55] J.K. Kundu, L. Liu, J.-W. Shin, Y.-J. Surh, Thymoquinone inhibits phorbol ester-induced activation of NF-κB and expression of COX-2, and induces expression of cytoprotective enzymes in mouse skin in vivo, Biochem. Biophys. Res. Commun. 438 (4) (2013) 721–727.
[56] S. Umar, J. Zargan, K. Umar, S. Ahmad, C.K. Katiyar, H.A. Khan, Modulation of the oxidative stress and inflammatory cytokine response by thymoquinone in the collagen induced arthritis in Wistar rats, Chem. Biol. Interact. 197 (1) (2012) 40–46.
[57] B. El-Aarag, W. Hussein, W. Ibrahim, M. Zahran, Thymoquinone improves anti-diabetic activity of metformin in streptozotocin-induced diabetic male rats, J. Diabetes Metab. 8 (12) (2017) 780.
[58] R. Rani, S. Dahiya, D. Dhingra, N. Dilbaghi, A. Kaushik, K.-H. Kim, S. Kumar, Antidiabetic activity enhancement in streptozotocin+ nicotinamide–induced diabetic rats through combinational polymeric nanoformulation, Int. J. Nanomed. 14 (2019) 4383.
[59] C. Sankaranarayanan, L. Pari, Thymoquinone ameliorates chemical induced oxidative stress and β-cell damage in experimental hyperglycemic rats, Chem. Biol. Interact. 190 (2-3) (2011) 148–154.
[60] M.A. Khan, M. Tania, C. Wei, Z. Mei, S. Fu, J. Cheng, J. Xu, J. Fu, Thymoquinone inhibits cancer metastasis by downregulating TWIST1 expression to reduce epithelial to mesenchymal transition, Oncotarget 6 (23) (2015) 19580.
[61] M.A. El-Mahdy, Q. Zhu, Q.E. Wang, G. Wani, A.A. Wani, Thymoquinone induces apoptosis through activation of caspase-8 and mitochondrial events in p53-null myeloblastic leukemia HL-60 cells, Int. J. Cancer 117 (3) (2005) 409–417.
[62] D. Xu, Y. Ma, B. Zhao, S. Li, Y. Zhang, S. Pan, Y. Wu, J. Wang, D. Wang, H. Pan, Thymoquinone induces G2/M arrest, inactivates PI3K/Akt and nuclear factor-κB pathways in human cholangiocarcinomas both in vitro and in vivo, Oncol. Rep. 31 (5) (2014) 2063–2070.
[63] F. Vaillancourt, P. Silva, Q. Shi, H. Fahmi, J.C. Fernandes, M. Benderdour, Elucidation of molecular mechanisms underlying the protective effects of thymoquinone against rheumatoid arthritis, J. Cell. Biochem. 112 (1) (2011) 107–117.
[64] D. Wang, J. Qiao, X. Zhao, T. Chen, D. Guan, Thymoquinone inhibits IL-1β-induced inflammation in human osteoarthritis chondrocytes by suppressing NF-κB and MAPKs signaling pathway, Inflammation 38 (6) (2015) 2235–2241.
[65] H. Khan, A. Sureda, T. Belwal, S. Çetinkaya, İ. Süntar, S. Tejada, H.P. Devkota, H. Ullah, M. Aschner, Polyphenols in the treatment of autoimmune diseases, Autoimmun. Rev. 18 (7) (2019) 647–657.
[66] J. Xu, J. Liu, G. Yue, M. Sun, J. Li, X. Xiu, Z. Gao, Therapeutic effect of the natural compounds baicalein and baicalin on autoimmune diseases, Mol. Med. Rep. 18 (1) (2018) 1149–1154.
[67] A. Khan, Antioxidant and Anti-inflammatory Action of Thymoquinone, Molecular and Therapeutic Actions of Thymoquinone, Springer, 2018, pp. 41–56.
[68] K.R. Rengasamy, H. Khan, S. Gowrishankar, R.J. Lagoa, F.M. Mahomoodally, Z. Khan, S. Suroowan, D. Tewari, G. Zengin, S.T. Hassan, The role of flavonoids in autoimmune diseases: therapeutic updates, Pharmacol. Ther. 194 (2019) 107–131.
[69] L. Chen, H. Teng, Z. Jia, M. Battino, A. Miron, Z. Yu, H. Cao, J. Xiao, Intracellular signaling pathways of inflammation modulated by dietary flavonoids: the most recent evidence, Crit. Rev. Food Sci. Nutr. 58 (17) (2018) 2908–2924.
[70] N. Yahfoufi, N. Alsadi, M. Jambi, C. Matar, The immunomodulatory and anti-inflammatory role of polyphenols, Nutrients 10 (11) (2018) 1618.
[71] C.M. Ahmed, H.M. Johnson, Interferons, eLS, 2017, pp. 1–6.
[72] T.J. Kindt, R.A. Goldsby, B.A. Osborne, J. Kuby, Kuby Immunology, Macmillan, 2007.
[73] L. Rönnblom, M.-L. Eloranta, The interferon signature in autoimmune diseases, Curr. Opin. Rheumatol. 25 (2) (2013) 248–253.
[74] N. Aziz, Y.-J. Son, J.Y. Cho, Thymoquinone suppresses IRF-3-mediated expression of type I interferons via suppression of TBK1, Int. J. Mol. Sci. 19 (5) (2018) 1355.
[75] M. Salem, F. Alenzi, W. Attia, Thymoquinone, the active ingredient of Nigella sativa seeds, enhances survival and activity of antigen-specific CD8-positive T cells in vitro, Br. J. Biomed. Sci. 68 (3) (2011) 131–137.
[76] A.K. Abbas, J.C. Aster, Robbins and Cotran Pathologic Basis of Disease, Elsevier/Saunders, 2015.
[77] M. Akdis, A. Aab, C. Altunbulakli, K. Azkur, R.A. Costa, R. Crameri, S. Duan, T. Eiwegger, A. Eljaszewicz, R. Ferstl, Interleukins (from IL-1 to IL-38), interferons, transforming growth factor β, and TNF-α: receptors, functions, and roles in diseases, J. Allergy Clin. Immunol. 138 (4) (2016) 984–1010.
[78] F. Caso, L. Costa, V. Nucera, G. Barilaro, I.F. Masala, R. Talotta, P. Caso, R. Scarpa, P. Sarzi-Puttini, F. Atzeni, From autoinflammation to autoimmunity: old and recent findings, Clin. Rheumatol. 37 (9) (2018) 2305–2321.
[79] K.D. Moudgil, D. Choubey, Cytokines in autoimmunity: role in induction, regulation, and treatment, J. Interferon Cytokine Res. 31 (10) (2011) 695–703.
[80] I. Tekeoglu, A. Dogan, L. Ediz, M. Budancamanak, A. Demirel, Effects of thymoquinone (volatile oil of black cumin) on rheumatoid arthritis in rat models, Phytother. Res. 21 (9) (2007) 895–897.
[81] G. Badr, M.H. Mahmoud, K. Farhat, H. Waly, O.Z. Al-Abdin, D.M. Rabah, Maternal supplementation of diabetic mice with thymoquinone protects their offspring from abnormal obesity and diabetes by modulating their lipid profile and free radical production and restoring lymphocyte proliferation via PI3K/AKT signaling, Lipids Health Dis. 12 (1) (2013) 37.
[82] M. El Gazzar, Thymoquinone suppressses in vitro production of IL-5 and IL-13 by mast cells in response to lipopolysaccharide stimulation, Inflamm. Res. 56 (8) (2007) 345–351.
[83] M. El Gazzar, R. El Mezayen, J.C. Marecki, M.R. Nicolls, A. Canastar, S.C. Dreskin, Anti-inflammatory effect of thymoquinone in a mouse model of allergic lung inflammation, Int. Immunopharmacol. 6 (7) (2006) 1135–1142.
[84] M.K. Cobourne-Duval, E. Taka, P. Mendonca, K.F. Soliman, Thymoquinone increases the expression of neuroprotective proteins while decreasing the expression of pro-inflammatory cytokines and the gene expression NFκB pathway signaling targets in LPS/IFNγ-activated BV-2 microglia cells, J. Neuroimmunol. 320 (2018) 87–97.
[85] N.T. Xuan, E. Shumilina, S.M. Qadri, F. Götz, F. Lang, Effect of thymoquinone on mouse dendritic cells, Cell. Physiol. Biochem. 25 (2-3) (2010) 307–314.
[86] J. Fragoso, G.A. Vargas, S.M. Jiménez, O.H. Reyes, J.B. Ramírez, Tumor necrosis factor alpha (TNF-α) in autoimmune diseases (AIDs): molecular biology and genetics, Gac. Med. Mex. 150 (4) (2014) 334–344.
[87] S. Kodama, M. Davis, D. Faustman, The therapeutic potential of tumor necrosis factor for autoimmune disease: a mechanistically based hypothesis, Cell. Mol. Life Sci. CMLS 62 (16) (2005) 1850–1862.

[88] A. El-Mahmoudy, Y. Shimizu, T. Shiina, H. Matsuyama, H. Nikami, T. Takewaki, Macrophage-derived cytokine and nitric oxide profiles in type I and type II diabetes mellitus: effect of thymoquinone, Acta Diabetol. 42 (1) (2005) 23–30.
[89] A. Parlar, S.O. Arslan, Thymoquinone exhibits anti-inflammatory, antioxidant, and immunomodulatory effects on allergic airway inflammation, Arch. Clin. Exp. Med. 4 (2) (2019) 60–65.
[90] S. Nennig, J. Schank, The role of NFkB in drug addiction: beyond inflammation, Alcohol Alcohol. 52 (2) (2017) 172–179.
[91] T. Liu, L. Zhang, D. Joo, S.-C. Sun, NF-κB signaling in inflammation, Signal Transduct. Target. Ther. 2 (1) (2017) 1–9.
[92] S. Pai, R. Thomas, Immune deficiency or hyperactivity-Nf-κb illuminates autoimmunity, J. Autoimmun. 31 (3) (2008) 245–251.
[93] S.-C. Sun, J.-H. Chang, J. Jin, Regulation of nuclear factor-κB in autoimmunity, Trends Immunol. 34 (6) (2013) 282–289.
[94] Z. Han, D. Boyle, A. Manning, G. Firestein, AP-1 and NF-kB regulation in rheumatoid arthritis and murine collagen-induced arthritis, Autoimmunity 28 (4) (1998) 197–208.
[95] H.K. Wong, G.M. Kammer, G. Dennis, G.C. Tsokos, Abnormal NF-κB activity in T lymphocytes from patients with systemic lupus erythematosus is associated with decreased p65-RelA protein expression, J. Immunol. 163 (3) (1999) 1682–1689.
[96] D. Gveric, C. Kaltschmidt, M.L. Cuzner, J. Newcombe, Transcription factor NF-KB and inhibitor lκBα are localized in macrophages in active multiple sclerosis lesions, J. Neuropathol. Exp. Neurol. 57 (2) (1998) 168–178.
[97] C. Mc Guire, M. Prinz, R. Beyaert, G. van Loo, Nuclear factor kappa B (NF-κB) in multiple sclerosis pathology, Trends Mol. Med. 19 (10) (2013) 604–613.
[98] M. Hofmann, S. Schiekofer, B. Isermann, M. Kanitz, M. Henkels, M. Joswig, A. Treusch, M. Morcos, T. Weiss, V. Borcea, Peripheral blood mononuclear cells isolated from patients with diabetic nephropathy show increased activation of the oxidative-stress sensitive transcription factor NF-kB, Diabetologia 42 (2) (1999) 222–232.
[99] Z.U. Mollah, S. Pai, C. Moore, B.J. O'Sullivan, M.J. Harrison, J. Peng, K. Phillips, J.B. Prins, J. Cardinal, R. Thomas, Abnormal NF-κB function characterizes human type 1 diabetes dendritic cells and monocytes, J. Immunol. 180 (5) (2008) 3166–3175.
[100] A. Mohamed, D. Afridi, O. Garani, M. Tucci, Thymoquinone inhibits the activation of NF-kappaB in the brain and spinal cord of experimental autoimmune encephalomyelitis, Biomed. Sci. Instrum. 41 (2005) 388–393.
[101] G. Sethi, K.S. Ahn, B.B. Aggarwal, Targeting nuclear factor-κB activation pathway by thymoquinone: role in suppression of antiapoptotic gene products and enhancement of apoptosis, Mol. Cancer Res. 6 (6) (2008) 1059–1070.
[102] M.A. El Gazzar, R. El Mezayen, M.R. Nicolls, S.C. Dreskin, Thymoquinone attenuates proinflammatory responses in lipopolysaccharide-activated mast cells by modulating NF-kappaB nuclear transactivation, Biochimica et Biophysica Acta (BBA)-General Subjects 1770 (4) (2007) 556–564.
[103] M.J. Hossen, W.S. Yang, D. Kim, A. Aravinthan, J.-H. Kim, J.Y. Cho, Thymoquinone: an IRAK1 inhibitor with in vivo and in vitro anti-inflammatory activities, Sci. Rep. 7 (1) (2017) 1–12.
[104] S.Z. Josefowicz, R.E. Niec, H.Y. Kim, P. Treuting, T. Chinen, Y. Zheng, D. T. Umetsu, A.Y. Rudensky, Extrathymically generated regulatory T cells control mucosal TH 2 inflammation, Nature 482 (7385) (2012) 395–399.
[105] Y.-c. Qiao, J. Shen, X.-z. Hong, L. Liang, C.-s. Bo, Y. Sui, H.-l. Zhao, Changes of regulatory T cells, transforming growth factor-beta and interleukin-10 in patients with type 1 diabetes mellitus: a systematic review and meta-analysis, Clin. Immunol. 170 (2016) 61–69.
[106] T. Gaafar, R. Farid, H. Raafat, F. Bayoumi, B. Gerges, D. Rasheed, The TH17/Treg imbalance in rheumatoid arthritis and relation to disease activity, J. Clin. Cell. Immunol. 6 (381) (2015) 2.
[107] J.H. Lee, L.C. Wang, Y.T. Lin, Y.H. Yang, D.T. Lin, B.L. Chiang, Inverse correlation between CD4+ regulatory T-cell population and autoantibody levels in paediatric patients with systemic lupus erythematosus, Immunology 117 (2) (2006) 280–286.
[108] A.B. Glick, A. Wodzinski, P. Fu, A.D. Levine, D.N. Wald, Impairment of regulatory T-cell function in autoimmune thyroid disease, Thyroid 23 (7) (2013) 871–878.
[109] S.M. Lieberman, P.A. Kreiger, G.A. Koretzky, Reversible lacrimal gland-protective regulatory T-cell dysfunction underlies male-specific autoimmune dacryoadenitis in the non-obese diabetic mouse model of Sjögren syndrome, Immunology 145 (2) (2015) 232–241.
[110] J.A. Bluestone, J.H. Buckner, M. Fitch, S.E. Gitelman, S. Gupta, M.K. Hellerstein, K.C. Herold, A. Lares, M.R. Lee, K. Li, Type 1 diabetes immunotherapy using polyclonal regulatory T cells, Sci. Transl. Med. 7 (315) (2015) 315ra189.
[111] P. Desreumaux, A. Foussat, M. Allez, L. Beaugerie, X. Hébuterne, Y. Bouhnik, M. Nachury, V. Brun, H. Bastian, N. Belmonte, Safety and efficacy of antigen-specific regulatory T-cell therapy for patients with refractory Crohn's disease, Gastroenterology 143 (5) (2012) 1207–1217, e2.
[112] M. Romano, G. Fanelli, C.J. Albany, G. Giganti, G. Lombardi, Past, present, and future of regulatory T cell therapy in transplantation and autoimmunity, Front. Immunol. 10 (2019) 43.
[113] S. Paust, H. Cantor, Regulatory T cells and autoimmune disease, Immunol. Rev. 204 (1) (2005) 195–207.
[114] S. Kheirouri, V. Hadi, M. Alizadeh, Immunomodulatory effect of Nigella sativa oil on T lymphocytes in patients with rheumatoid arthritis, Immunol. Invest. 45 (4) (2016) 271–283.
[115] M.S. Guida, A. Abd El-Aal, Y. Kafafy, S.F. Salama, B.M. Badr, G. Badr, Thymoquinone rescues T lymphocytes from gamma irradiation-induced apoptosis and exhaustion by modulating pro-inflammatory cytokine levels and PD-1, Bax, and Bcl-2 signaling, Cell. Physiol. Biochem. 38 (2) (2016) 786–800.
[116] G. Badr, S. Alwasel, H. Ebaid, M. Mohany, I. Alhazza, Perinatal supplementation with thymoquinone improves diabetic complications and T cell immune responses in rat offspring, Cell. Immunol. 267 (2) (2011) 133–140.
[117] G. Pearson, F. Robinson, T. Beers Gibson, B.-e. Xu, M. Karandikar, K. Berman, M. H. Cobb, Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions, Endocr. Rev. 22 (2) (2001) 153–183.
[118] M. Soares-Silva, F.F. Diniz, G.N. Gomes, D. Bahia, The mitogen-activated protein kinase (MAPK) pathway: role in immune evasion by trypanosomatids, Front. Microbiol. 7 (2016) 183.
[119] A.S. Dhillon, S. Hagan, O. Rath, W. Kolch, MAP kinase signalling pathways in cancer, Oncogene 26 (22) (2007) 3279–3290.
[120] D. Owens, S. Keyse, Differential regulation of MAP kinase signalling by dual-specificity protein phosphatases, Oncogene 26 (22) (2007) 3203–3213.
[121] J.M. Kyriakis, J. Avruch, Mammalian MAPK signal transduction pathways activated by stress and inflammation: a 10-year update, Physiol. Rev. (2012).
[122] S.S. Afrose, M. Junaid, Y. Akter, M. Tania, M. Zheng, M.A. Khan, Targeting kinases with thymoquinone: a molecular approach to cancer therapeutics, Drug Discov. Today (2020).
[123] D. Thummuri, M.K. Jeengar, S. Shrivastava, H. Nemani, R.N. Ramavat, P. Chaudhari, V. Naidu, Thymoquinone prevents RANKL-induced osteoclastogenesis activation and osteolysis in an in vivo model of inflammation by suppressing NF-KB and MAPK Signalling, Pharmacol. Res. 99 (2015) 63–73.
[124] M.A. Kandeil, M.O. Mahmoud, A.-R.H. Abdel-Razik, S.B. Gomaa, Thymoquinone and geraniol alleviate cisplatin-induced neurotoxicity in rats through downregulating the p38 MAPK/STAT-1 pathway and oxidative stress, Life Sci. 228 (2019) 145–151.
[125] J. Tabeshpour, S. Mehri, K. Abnous, H. Hosseinzadeh, Role of oxidative stress, MAPKinase and apoptosis pathways in the protective effects of thymoquinone against acrylamide-induced central nervous system toxicity in rat, Neurochem. Res. 45 (2) (2020) 254–267.
[126] E. Bousoik, H. Montazeri Aliabadi, "Do we know jack" about JAK? A closer look at JAK/STAT signaling pathway, Front. Oncol. 8 (2018) 287.
[127] J.J. O'Shea, R. Plenge, JAK and STAT signaling molecules in immunoregulation and immune-mediated disease, Immunity 36 (4) (2012) 542–550.
[128] J.J. O'Shea, D.M. Schwartz, A.V. Villarino, M. Gadina, I.B. McInnes, A. Laurence, The JAK-STAT pathway: impact on human disease and therapeutic intervention, Annu. Rev. Med. 66 (2015) 311–328.
[129] P. Isomäki, I. Junttila, K.-L. Vidqvist, M. Korpela, O. Silvennoinen, The activity of JAK-STAT pathways in rheumatoid arthritis: constitutive activation of STAT3 correlates with interleukin 6 levels, Rheumatology 54 (6) (2015) 1103–1113.
[130] X. Hu, J. Ma, V. Vikash, J. Li, D. Wu, Y. Liu, J. Zhang, W. Dong, Thymoquinone augments cisplatin-induced apoptosis on esophageal carcinoma through mitigating the activation of JAK2/STAT3 pathway, Dig. Dis. Sci. 63 (1) (2018) 126–134.
[131] F. Li, P. Rajendran, G. Sethi, Thymoquinone inhibits proliferation, induces apoptosis and chemosensitizes human multiple myeloma cells through suppression of signal transducer and activator of transcription 3 activation pathway, Br. J. Pharmacol. 161 (3) (2010) 541–554.
[132] W.-Q. Zhu, J. Wang, X.-F. Guo, Z. Liu, W.-G. Dong, Thymoquinone inhibits proliferation in gastric cancer via the STAT3 pathway in vivo and in vitro, World J. Gastroenterol. 22 (16) (2016) 4149.
[133] L. Wang, N. Wang, Q. Zhao, B. Zhang, Y. Ding, Pectolinarin inhibits proliferation, induces apoptosis, and suppresses inflammation in rheumatoid arthritis fibroblast-like synoviocytes by inactivating the phosphatidylinositol 3 kinase/protein kinase B pathway, J. Cell. Biochem. 120 (9) (2019) 15202–15210.
[134] S.N. Zarneshan, S. Fakhri, M.H. Farzaei, H. Khan, L. Saso, Astaxanthin targets PI3K/Akt signaling pathway toward potential therapeutic applications, Food Chem. Toxicol. (2020) 111714.
[135] A. Dirican, H. Atmaca, E. Bozkurt, C. Erten, B. Karaca, R. Uslu, Novel combination of docetaxel and thymoquinone induces synergistic cytotoxicity and apoptosis in DU-145 human prostate cancer cells by modulating PI3K–AKT pathway, Clin. Transl. Oncol. 17 (2) (2015) 145–151.
[136] A. García-Sánchez, A.G. Miranda-Díaz, E.G. Cardona-Muñoz, The role of oxidative stress in physiopathology and pharmacological treatment with pro-and antioxidant properties in chronic diseases, Oxid. Med. Cell. Longev. 2020 (2020).
[137] S. Ramani, A. Pathak, V. Dalal, A. Paul, S. Biswas, Oxidative stress in autoimmune diseases: an under dealt malice, Curr. Protein Pept. Sci. 21 (6) (2020) 611–621.
[138] A.R. Sofy, R.A. Dawoud, M.R. Sofy, H.I. Mohamed, A.A. Hmed, N.K. El-Dougdoug, Improving regulation of enzymatic and non-enzymatic antioxidants and stress-related gene stimulation in Cucumber mosaic cucumovirus-infected cucumber plants treated with glycine betaine, chitosan and combination, Molecules 25 (10) (2020) 2341.
[139] P. Conigliaro, M. Chimenti, P. Triggianese, F. Sunzini, L. Novelli, C. Perricone, R. Perricone, Autoantibodies in inflammatory arthritis, Autoimmun. Rev. 15 (7) (2016) 673–683.
[140] F. Forouzanfar, H. Hosseinzadeh, Protective Role of Nigella sativa and Thymoquinone in Oxidative Stress: a Review, Nuts and Seeds in Health and Disease Prevention, Elsevier, 2020, pp. 127–146.
[141] D. Allison, D. Ditor, Immune dysfunction and chronic inflammation following spinal cord injury, Spinal Cord 53 (1) (2015) 14–18.
[142] H. Khither, W. Sobhi, A. Khenchouche, A. Mosbah, M. Benboubetra, In-vitro antioxidant effect of thymoquinone, Annu. Res. Rev. Biol. (2018) 1–9.
[143] H. Zeinvand-Lorestani, A. Nili-Ahmadabadi, F. Balak, G. Hasanzadeh, O. Sabzevari, Protective role of thymoquinone against paraquat-induced hepatotoxicity in mice, Pestic. Biochem. Physiol. 148 (2018) 16–21.

[144] U. Sener, R. Uygur, C. Aktas, E. Uygur, M. Erboga, G. Balkas, V. Caglar, B. Kumral, A. Gurel, H. Erdogan, Protective effects of thymoquinone against apoptosis and oxidative stress by arsenic in rat kidney, Ren. Fail. 38 (1) (2016) 117–123.
[145] N.M. Hamdy, R.A. Taha, Effects of Nigella sativa oil and thymoquinone on oxidative stress and neuropathy in streptozotocin-induced diabetic rats, Pharmacology 84 (3) (2009) 127–134.
[146] M.F. Zafeer, M. Waseem, S. Chaudhary, S. Parvez, Cadmium-induced hepatotoxicity and its abrogation by thymoquinone, J. Biochem. Mol. Toxicol. 26 (5) (2012) 199–205.
[147] C.S. Williams, M. Mann, R.N. DuBois, The role of cyclooxygenases in inflammation, cancer, and development, Oncogene 18 (55) (1999) 7908–7916.
[148] J.V. Ryn, G. Trummlitz, M. Pairet, COX-2 selectivity and inflammatory processes, Curr. Med. Chem. 7 (11) (2000) 1145–1161.
[149] A.F. Majdalawieh, M.W. Fayyad, Immunomodulatory and anti-inflammatory action of Nigella sativa and thymoquinone: a comprehensive review, Int. Immunopharmacol. 28 (1) (2015) 295–304.
[150] P.J. Houghton, R. Zarka, B. de las Heras, J. Hoult, Fixed oil of Nigella sativa and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation, Planta Med. 61 (01) (1995) 33–36.
[151] M. Mansour, S. Tornhamre, Inhibition of 5-lipoxygenase and leukotriene C4 synthase in human blood cells by thymoquinone, J. Enzyme Inhib. Med. Chem. 19 (5) (2004) 431–436.
[152] M. El Gazzar, R. El Mezayen, M.R. Nicolls, J.C. Marecki, S.C. Dreskin, Downregulation of leukotriene biosynthesis by thymoquinone attenuates airway inflammation in a mouse model of allergic asthma, Biochimica et Biophysica Acta (BBA)-General Subjects 1760 (7) (2006) 1088–1095.
[153] R.J. Al Wafai, Nigella sativa and thymoquinone suppress cyclooxygenase-2 and oxidative stress in pancreatic tissue of streptozotocin-induced diabetic rats, Pancreas 42 (5) (2013) 841–849.
[154] R. El Mezayen, M. El Gazzar, M.R. Nicolls, J.C. Marecki, S.C. Dreskin, H. Nomiyama, Effect of thymoquinone on cyclooxygenase expression and prostaglandin production in a mouse model of allergic airway inflammation, Immunol. Lett. 106 (1) (2006) 72–81.
[155] G. Lal, N. Zhang, W. Van Der Touw, Y. Ding, W. Ju, E.P. Bottinger, D.E. Levy, J. S. Bromberg, Epigenetic regulation of Foxp3 expression in regulatory T cells by DNA methylation, J. Immunol. 182 (1) (2009) 259–273.
[156] D.U. Lee, S. Agarwal, A. Rao, Th2 lineage commitment and efficient IL-4 production involves extended demethylation of the IL-4 gene, Immunity 16 (5) (2002) 649–660.
[157] M.A. Khan, M. Zheng, J. Fu, Epigenetic Modification of Oncogenes or Tumor Suppressor Genes by Thymoquinone in Triple Negative Breast Cancer, AACR, 2019.
[158] M.A. Khan, M. Tania, J. Fu, Epigenetic role of thymoquinone: impact on cellular mechanism and cancer therapeutics, Drug Discov. Today 24 (12) (2019) 2315–2322.
[159] J. Pang, N. Shen, F. Yan, N. Zhao, L. Dou, L.-C. Wu, C.L. Seiler, L. Yu, K. Yang, V. Bachanova, Thymoquinone exerts potent growth-suppressive activity on leukemia through DNA hypermethylation reversal in leukemia cells, Oncotarget 8 (21) (2017) 34453.
[160] J. Li, M. Khan, C. Wei, J. Cheng, H. Chen, L. Yang, I. Ijaz, J. Fu, Thymoquinone inhibits the migration and invasive characteristics of cervical cancer cells SiHa and CaSki in vitro by targeting epithelial to mesenchymal transition associated transcription factors Twist1 and Zeb1, Molecules 22 (12) (2017) 2105.
[161] M. Alhosin, A. Abusnina, M. Achour, T. Sharif, C. Muller, J. Peluso, T. Chataigneau, C. Lugnier, V.B. Schini-Kerth, C. Bronner, Induction of apoptosis by thymoquinone in lymphoblastic leukemia Jurkat cells is mediated by a p73-dependent pathway which targets the epigenetic integrator UHRF1, Biochem. Pharmacol. 79 (9) (2010) 1251–1260.
[162] S.A. Qadi, M.A. Hassan, R.A. Sheikh, O.A. Baothman, M.A. Zamzami, H. Choudhry, A.L. Al-Malki, A. Albukhari, M. Alhosin, Thymoquinone-induced reactivation of tumor suppressor genes in cancer cells involves epigenetic mechanisms, Epigenetics insights 12 (2019), 2516865719839011.
[163] C. Iside, M. Scafuro, A. Nebbioso, L. Altucci, SIRT1 activation by natural phytochemicals: an overview, Front. Pharmacol. 11 (2020).
[164] Y. Lu, Y. Feng, D. Liu, Z. Zhang, K. Gao, W. Zhang, H. Tang, Thymoquinone attenuates myocardial ischemia/reperfusion injury through activation of SIRT1 signaling, Cell. Physiol. Biochem. 47 (3) (2018) 1193–1206.
[165] L. Liu, P. Wang, X. Liu, D. He, C. Liang, Y. Yu, Exogenous NAD+ supplementation protects H 9c2 cardiac myoblasts against hypoxia/reoxygenation injury via S irt1-p53 pathway, Fundam. Clin. Pharmacol. 28 (2) (2014) 180–189.
[166] R. Velagapudi, A. El-Bakoush, I. Lepiarz, F. Ogunrinade, O.A. Olajide, AMPK and SIRT1 activation contribute to inhibition of neuroinflammation by thymoquinone in BV2 microglia, Mol. Cell. Biochem. 435 (1-2) (2017) 149–162.
[167] L. Zhang, H. Wu, M. Zhao, C. Chang, Q. Lu, Clinical significance of miRNAs in autoimmunity, J. Autoimmun. (2020) 102438.
[168] Q. Luo, Z. Ren, L. Zhu, Y. Shao, Y. Xie, Y. Feng, B. Li, Y. Chen, Involvement of microRNA-146a in the inflammatory response of s tatus epilepticus rats, CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders) 16 (6) (2017) 686–693.
[169] S. Imani, C. Wei, J. Cheng, M.A. Khan, S. Fu, L. Yang, M. Tania, X. Zhang, X. Xiao, X. Zhang, MicroRNA-34a targets epithelial to mesenchymal transition-inducing transcription factors (EMT-TFs) and inhibits breast cancer cell migration and invasion, Oncotarget 8 (13) (2017) 21362.
[170] I. Meral, M. Pala, F. Akbas, S. Ustunova, C. Yildiz, M.H. Demirel, Effects of thymoquinone on liver miRNAs and oxidative stress in Ehrlich acid mouse solid tumor model, Biotech. Histochem. 93 (4) (2018) 301–308.