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Thymoquinone Attenuates Immune Mediated Liver Injury Induced by Concanavalin A in Mice

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Abstract

Autoimmune hepatitis is an inflammatory disease and its incidence has been increasing. The features of hepatitis are the release of inflammatory cytokines, the elevation of AST and ALT, and hepatocyte apoptosis and necrosis. Concanavalin A considered as essential model represents the acute immune-mediated liver damage in rodents. Thymoquinone is well known herbal compound that exert hepatoprotective, anti-inflammatory, and antioxidant activity. In this study, we focus on the immunoregulatory and liver protective effect of thymoquinone in a mouse model of concanavalin A-induced liver injury.

Twenty-four male mice were randomly divided into four groups each containing six animals: Negative control group, concanavalin A model group, thymoquinone 15mg/kg group, and thymoquinone 30mg/kg group. Blood was collected to detect the activities of alanine transaminase (ALT) and aspartate transaminase (AST) as well as liver tissue for the detection of tumor necrosis factor α (TNF α), NF-kB, and interferon γ (IFN γ) levels, after 8 hours of concanavalin A injection.

Injecting mice with concanavalin resulted in a dramatic increase in serum level of both ALT and AST which indicate severe liver tissue damage with a significant increase in inflammatory cytokines TNF α, and INF γ levels, with a notable increase in NF-kB gene expression in mice liver tissue homogenate. Thymoquinone pretreatment revealed a dose-dependent increase in liver tissue protection.

Conclusion: Thymoquinone has hepatoprotective and immune modulatory effects in concanavalin A induced immune mediated liver damage. Thymoquinone exerted its effect through attenuating NF-κB and its downstream effectors TNF α and INF γ in a dose dependent manner.

Keywords: Concanavalin A, Liver injury, Thymoquinone, ALT, AST, TNF-α, IFN-γ, NF-KB.

الثيموكينون يخفف من إصابة الكبد المناعية المستحثه بالكونكانافالين أ لدى الفئران

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الخلاصة

التهاب الكبد المناعي الذاتي هو مرض التهابي .تتمثل سمات التهاب الكبد المناعي في إطلاق السيتوكينات ، الالتهابية ، وأرتفاع مستوى انزيمات الكبد (الانين امينو ترانسفيراس واسبارتات امينوترانسفيراس) وموت الخلايا المبرمج والنخر. كونكانافالين أ يعتبر نموذجاً اساسيا ليمثل تلف الكبد المناعي في القوارض. الثيموكينون هو مركب عشبي معروف جيدًا له فوائد كثيرة كحماية الكبدً و مضادًا للالتهابات ومضادًا للأكسدة, وفي دراستنا نركز على التأثير المناعي والوقائي لمادة اللثيموكينون في نموذج فأر مصاب بمرض التهاب الكبد المناعي والتي يسببها حقن مادة الكونكانافالين أ.

تم تقسيم أربعة وعشرين فأر من الذكور بشكل عشوائي إلى أربع مجموعات تحتوي كل مجموعة على ستة حيوانات ، مجموعة السيطرة السلبي، مجموعة النموذج لمرض التهاب الكبد المناعي حقنت بمادة الكونكانافالين أ ، مجموعة ثيموكينون ١٥ مجم / كجم ، مجموعة ثيموكينون ٣٠ مجم / كجم. تم جمع الدم للكشف عن أنشطة ترانس أمينيز ألانين(ALT) وأسبارتات أمينيز(AST), وكذلك أنسجة الكبد للكشف عن عامل نخر الورم(TNF α) , و NF-κB و interferon-(IFN γ) بعد ٨ ساعات من حقن كونكانافالين أ.

يؤدي حقن الفئران بمادة الكونكانافلين أ إلى زيادة كبيرة في مستوى المصل لكل من ALT و AST مما يشير إلى تلف شديد في أنسجة الكبد مع زيادة ملحوظة في السيتوكينات الالتهابية TNF α و INF ، مع زيادة ملحوظة في التعبير الجيني NF-κB في أنسجة كبد الفئران, كشفت المعالجة المسبقة بالثيموكينون عن زيادة في حماية أنسجة الكبد.

الأستنتاج: الثايموكوينون له تأثيرات مناعية و وقائية للكبد في حالة تلف الكبد المناعي الذي يسببه الكونكانافالين أ . يمارس الثايموكوينو ن تأثيره من خلال تقليل التعبير الجيني للنيوكليرفاكتر كابا بي وبالتالي التقليل من مستويات التيومر نكروسز فاكتر الفا والانتيرفيرون كاما بطريقة تعتمد على الجرعة.

الكلمات المفتاحية: كونكانافالين أ ، إصابة الكبد ، ثيموكينون ، ALT، AST، TNF α، IFN γ، NF-B

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Introduction

The liver in the human body considered as the largest hard organ, approximately making 2% of body weight in adult, the liver is responsible for many important tasks that play a critical role in all physiological systems and supporting the function of many organs. The exclusive vasculature of the liver allows it to perform the removal of exogenous antigens and pathogens from the systemic circulation, and the degradation of waste products and toxins [(1)]. Several essential functions of the liver are local and systemic host defense including inflammatory reaction and both innate immunity and the more specific adaptive immunity [(2)].

Hepatitis is defined as inflammation of the liver. Hepatic inflammation can be caused by alcohol, viruses, medication use, or exposure to toxins, and autoimmune diseases [(3)]. In Immune-mediated hepatitis, the distinct distribution of hepatic chemokine receptors and its secretion that regulate the accumulation of immune response cells, and the obvious anatomical parts of the liver with its unique liver sinusoids and its paired portal and arterial blood supply permit the mobilization of distinct populations of circulating cells that present large surface for the interaction of lymphocyte and local cell, all these features in enable liver to carry an efficient immune response by recruitment of the standard lymphocytes [(4)].

The innate immune system always activated before the adaptive immune system [(5)]. The liver is highly perfused with innate immune cells, like natural killer (NK) cells, NKT cells, Kupffer cells, and dendritic cells (DC), which result in exclusive immune responses against microbial pathogens [(6)(7)]. Macrophages (Kupfer cells) in innate immune response display outstanding plasticity and can differentiate into macrophage 1 (M1 that linked with type 1 cytokines such as IL-6, TNF α, and INF γ) and macrophage 2 (M2 subsets show the potent pro-inflammatory response) [(8)]. Adaptive immunity which is the cellular immunity plays an essential role in the pathogenesis of HBV related acute liver failure. The clearance of the virus depends principally on two ways: the straight killing of infected hepatocytes by cytotoxic T lymphocytes (CTLs), or by the release of IFN γ[(9)]. T helper cell responses and CTL responses are of significant importance in immune-mediated liver damage and viral control [(10)].

The concanavalin A model is a distinctive and well-established model for inspecting T-cell and macrophage dependent liver damage in mice, which closely resemble the pathological changes and pathogenesis mechanisms of autoimmune hepatitis patients, and is considered as the best experimental model for immune-mediated liver damage study up to now [(11)]. The predominant and extremely special activation of T cells of concanavalin A provides a suitable model for straight exploratory mechanisms of T cell-mediated hepatitis, which holds similarities to human autoimmune or acute viral hepatitis along with immune-mediated drug hepatotoxicity and may therefore help to explore new treatment options [(12)].

In this study, evaluation of the liver protective effect of thymoquinone. Thymoquinone is a principal active constituent of black seed oil *Nigella sativa*. The chemical formula is 2-isopropyl-5-methyl1,4-benzoquinones. Thymoquinone is richly present in volatile oil in addition to fixed oil in *N. sativa* seeds [(13)]. The important medical effect of thymoquinone can be linked to suppression of the nuclear factor-kappa B (NF-κB) signaling. Most studies showed that thymoquinone suppresses TNF α and NF-κB activation with dose-dependent manner [(14)].

Material and Method

Reagents

Concanavalin A, and thymoquinone were purchased from (Hangzhou Hyper Chemicals) to induce immune mediated liver injury. TNF α and INF γ ELISA kites were purchased from Beijing Solarbio Science/china, and used to measure these mediators in liver tissue. NF-κB mRNA expression detected using real time PCR, the primer was purchased from Macrogen, Korea, and the primer sequence used showed in table (1). ALT and AST measured using randox kits using colorimetric method.

Table 1. Reverse and forward primer sequence

Primer	Sequence
Nfkb1-F	5`-AAGACAAGGAGCAGGACATG-3`
Nfkb1-R	5`-AGCAACATCTTCACATCCCC-3`
YWHAZ-F	5`-GATGAAGCCATTGCTGAACTTG-3`
YWHAZ-R	5`-GTCTCCTTGGGTATCCGATGTC-3`

Animals

Twenty-four adults' albino male mice weighing (20-30) gram were purchased from the Animal House of College of Pharmacy/University of Baghdad and maintained under normal conditions of temperature, humidity, and light/dark cycle. The animals were fed commercial pellets and tap water throughout the experimental period. The study was approved by the Scientific and Ethical-committees of the College of the Pharmacy/University of Baghdad.

Experimental protocol

Twenty-four mice were divided into 4 groups, each group with 6 mice as follows:

Negative control group: Animals in this group received 0.1 ml distilled water orally for 4 days starting day 1, with a single dose of 0.1 ml normal

saline on day 4 by retro-orbital IV route and sacrificed after 8 hours. This group served as a negative control group.

Model control group: Animals in this group received 0.1 ml distilled water orally for 4 days starting day 1, with a single dose of 0.1 ml concanavalin A 20mg/kg on day 4 by retro-orbital IV route and sacrificed after 8 hours. This group served as an immune mediated liver damage model group.

Thymoquinone 15mg/kg group: Received 0.1ml thymoquinone (15 mg/Kg dissolved in corn oil) by oral gavage for 4 successive days starting day 1, with a single dose of concanavalin A 20mg/kg on day 4 and sacrificed after 8 hours.

Thymoquinone 30mg/kg group: Received 0.1ml thymoquinone (30 mg/Kg) by oral gavage for 4 successive days starting day 1, with a single dose of concanavalin A 20mg/kg on day 4 and sacrificed after 8 hours.

Samples collection

Blood and serum samples collection

On day 5 and exactly 8 hrs. after concanavalin A injection, about 1.5 ml of blood was drawn from the retro-orbital area of mice. Blood were let to stand in room temperature for 30 mins for serum collection. Blood centrifuged for 15 min. at 5000 rpm in cold centrifuge at 4 ˚C. The serum was collected and frozen for ALT and AST determination.

Preparation of liver tissue homogenate

After euthanization, and dissection of each mouse, liver was quickly excised, rinsed in ice-cold normal saline. After that, 100 mg of liver tissue were placed in Eppendorf tube containing 1ml of TRIzol™ Reagent and gently mixed by vortex and kept frozen for later use to measure mRNA NF-κB level. Take 150 mg from the remaining liver tissues were placed in Eppendorf tube containing 1.5 ml of phosphate buffer saline. The liver tissues were homogenized by electrical homogenizer. Then the homogenate centrifuged with a cold centrifuge for 15 minutes at 5000 rpm and the supernatant fluid collected and kept frozen for later use to measure TNF α and INF γ.

Statistical analysis

The numeric data presented in the study were expressed as mean ± standard error of the mean (SE). All statistical analyses were carried out using the Statistical Package of Social Science (SPSS) software version 25. Intergroup comparisons were made using (Student's t test). Differences between the means were considered significant at P<0.05.

Results

Effect of thymoquinone pretreatment on serum ALT and AST level

Data obtained from this study showed that concanavalin A administration in a dose 20mg /kg causes a significant and dramatic elevation of serum ALT (122.14 ± 3.33 IU/L) and AST(183.65 ± 5.11 IU/L) levels ($P < 0.05$), indicating severe liver injury in comparison to the negative control group ALT (3.895 ± 1.05 IU/L) and AST (56.5 ± 4.56 IU/L) levels as shown in table (2) and figures (1) and (2). When mice pretreated with thymoquinone 15mg /kg resulted in significant decrease in ALT (37.09±10.74 IU/L) and AST(113.84 ± 13.86 IU/L) levels ($P < 0.05$) in comparison to the model control group received concanavalin A, group treated with thymoquinone dose 30mg /kg resulted in significant and massive reduction of ALT (31.12 ± 3.82 IU/L) and AST (80.1 ± 11.27 IU/L) levels ($P < 0.05$) in comparison to the model control group, as shown in the table (1) and figures (1) and (2).

Effect of thymoquinone pretreatment on TNF α level.

Analysis of data of present study shows that the administration of concanavalin A 8 hours before animal sacrificing results in significant ($P< 0.05$) and dramatic elevation of TNF α level (44.59 ± 2.05 pg/mg) in comparison to the negative control group (12.79 ± 0.53 pg/mg). This result in 3fold duplication of tissue TNF α level, as shown in figure (3) and table (2). Pretreatment of animals with thymoquinone 15 mg/kg 4 days before injection with concanavalin A resulted in significant ($P<0.05$) lowering in TNF α (24.27 ± 2.1pg/mg) in comparison to the model group (44.59 ± 2.05 pg/mg), doubling up the dose of thymoquinone to 30 mg /kg resulted in significant ($P<0.05$) attenuation of TNF α level (17.67 ± 1.58 pg/mg) in comparing to the lower dose, these results indicate the dose dependent reduction effect of thymoquinone on tissue TNF α level as shown in table (1) and figure (3).

Effect of thymoquinone pretreatment on INF γ level.

Mice administered concanavalin A 20mg /kg by the retro-orbital rout in this study showed significant ($P<0.05$) and notable elevation of the immunological marker INF γ by 4 folds (2.23 ± 0.052 pg/mg) in comparison to the negative control group which administered normal saline only (0.61 ± 0.046 pg/mg), as shown in table (2) and figure (4). The groups which were treated with thymoquinone showed dose-dependent attenuation of INF γ tissue level, 15mg /kg dose of thymoquinone was given to the mice showed a significant ($P<0.05$) decrease in INF γ level (1.39 ± 0.02 pg/mg) in comparison to the model group (2.23 ± 0.052 pg/mg), doubling the dose of thymoquinone to 30mg /kg result in even more significant ($P<0.05$) depletion of INF γ level (0.81 ± 0.018 pg/mg) as shown in table (1) and figure (4).

Effect of thymoquinone pretreatment on NF-κB gene expression

As demonstrated in table (2) and figure (5), concanavalin A administration resulted in significant ($P<0.05$) increase in hepatic gene

expression of NF-κB by 3 folds (3.56±0.55 fold) compared to the non-treated negative control group (1.11±0.34 fold). On the other hand, treatment with thymoquinone (15mg/kg) showed a significant (P<0.05) attenuation of NF-κB mRNA expression (2.03±0.29 fold) compared to experimental model group (3.56±0.55 fold). Further escalation in thymoquinone dose to (30mg/kg) resulted in more significant (P<0.05) drop in NF-κB mRNA level (1.6±0.16 fold) compared to experimental model group (3.56±0.55 fold), in this case the gene expression of NF-κB reduced by (30%). These data clearly revealed a dose-dependent attenuation of thymoquinone against NF-κB surge due to Con A.

Table 2. Effect of thymoquinone pretreatment on serum ALT and AST levels, and levels of INF γ, TNF α, and NF-κB in liver tissue homogenate of mice:

Group	Type of treatment	Tissue INF γ (pg /mg) Mean ± SEM	Tissue TNF α (pg/mg) Mean ± SEM	Serum ALT (IU/L) Mean ±SEM	Serum AST (IU/L) Mean ± SEM	mRNA NF-κB level (fold) Mean ±SEM
Negative control group	Normal saline	0.61 ± 0.05	12.79 ± 0.53	3.895 ± 1.05	56.5 ± 4.56	1.11± 0.34
Con A group	Con A	2.23 ± 0.05 #	44.59 ± 2.05 #	122.14±3.33 #	183.65±5.11 #	3.58 ± 0.54 #
TQ 15mg/kg group	Con A +TQ 15mg/kg	1.39 ± 0.02 *	24.27 ± 2.1 *	37.09±10.74 *	113.84 ± 13.86 *	2.02 ±0.29*
TQ 30mg/kg group	Con A +TQ 30 mg/kg	0.81 ± 0.018 *	17.67 ± 1.58 *	31.12 ± 3.82 *	80.1 ± 11.27 *	1.59 ±0.16*

Data are expressed as mean ± standard error of means (SEM). (#) Significant difference (P<0.05) compared to the negative control group, (*) Significant difference (P<0.05) compared to the model control group, Con A: concanavalin A, TQ: thymoquinone, TNF α: tumor necrosis factor-alpha, INF γ: interferon-gamma, NF-κB: nuclear factor kappa B.

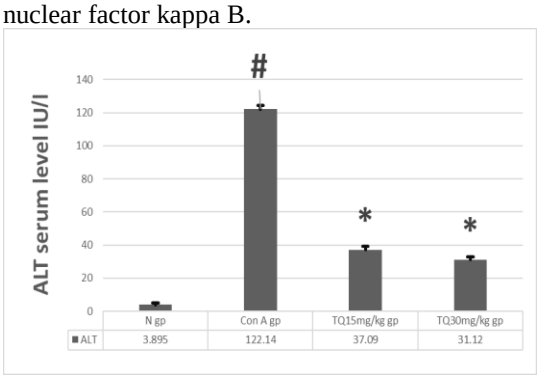

Figure 1.Effect of thymoquinone pretreatment on serum ALT level.

(#) Significant difference (P<0.05) compared to the experimental negative control group, (*): Significant difference ($P<0.05$) compared to the model control group, Con A: concanavalin A, TQ: thymoquinone, N: negative control group

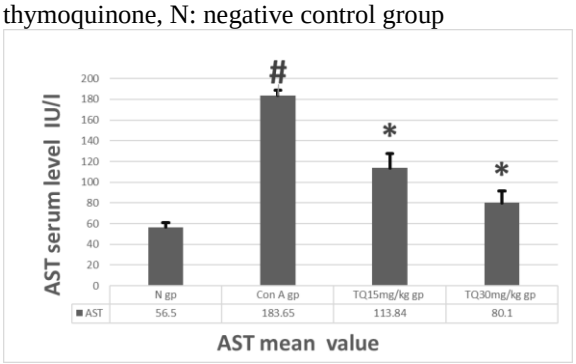

Figure 2.Effect of thymoquinone pretreatment on serum AST level.

(*): Significant difference ($P<0.05$) compared to the model group, (#): Significant difference (P<0.05) compared to the negative control group, Con a: concanavalin A, TQ: thymoquinone, N: negative control group

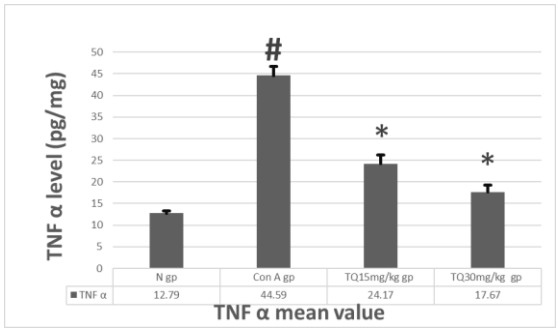

Figure 3.Effect of thymoquinone pretreatment on TNF α level.

(*): Significant difference ($P<0.05$) compared to the model control group, (#): Significant difference (P<0.05) compared to the negative control group, Con A: concanavalin A, TQ: thymoquinone, N: negative control group .

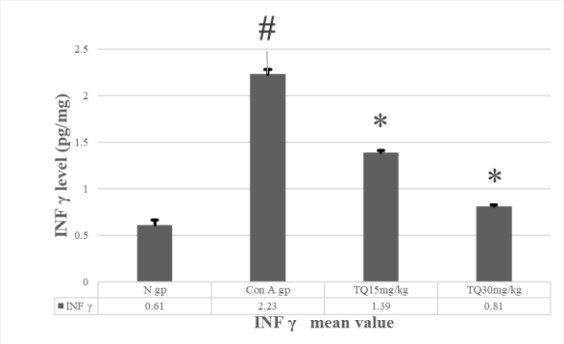

Figure 4. Effect of thymoquinone pretreatment on INF γ level.

(*): Significant difference (*P<0.05*) compared to its corresponding model control group, (#): Significant difference (P<0.05) compared to the negative control group, N: negative control group, Con A: concanavalin A, TQ: thymoquinone, INF γ: interferon gamma.

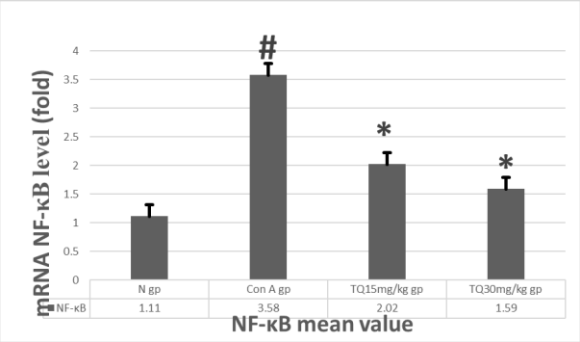

Figure 5.Effect of thymoquinone pretreatment on NF-κB level.

(*): Significant difference (*P<0.05*) compared to the model control group, (#) Significant difference (P<0.05) compared to the negative control group, N: negative control group, Con A: Concanavalin A, TQ: thymoquinone, NF-κB: nuclear factor kappa B

Discussion

Hepatitis is defined as inflammation of the liver. Hepatic inflammation can be caused by alcohol, viruses, medication use, exposure to toxins, or autoimmune diseases [15]. Both innate and adaptive immunity contribute to the pathogenesis of immune-mediated liver injury [9]. Concanavalin A is a plant agglutinin extracted from Brazilian rubber beans that were used to study liver injury. Concanavalin A model is a representative and easily built model of autoimmune hepatitis, it was shown to bind to mannosyl sugar residues of the insulin receptor in the liver, in addition the binding of concanavalin A to hepatocytes, endothelial cells, and Kupffer cells is also reported [16]. Concanavalin A can modify the major histocompatibility complex (MHC) structure to produce inflammatory reactions, by activating macrophages and CD4+ T cells, which release TNF α, IL-1β, IL-6, and other inflammatory factors that damage hepatic cells [17]. Thus, concanavalin A treatment is a good simulation of the clinical onset of autoimmune hepatitis (AIH) and viral hepatitis. The model also has the advantages of utilizing a simple extraction and causing liver-specific damage (injury to other organs is not obvious), thus providing a reliable animal model for clinical research in basic immunology [18]. Many research efforts recently oriented to explore the potential therapeutic activity of natural products develop more effective therapeutic strategies. In the present study, the work dedicated to investigate the immunomodulatory and liver protective effect of herbal active constituent thymoquinone using concanavalin A as a model of immune induced hepatitis. Thymoquinone is one of the major bioactive components of the volatile oil of *N. sativa* seeds. Studies showed that thymoquinone has immunomodulatory and anti-inflammatory effects, that prevent the biosynthesis of important mediators in inflammatory processes and reduce proinflammatory cytokines such as interleukins (ILs) and TNF α. Besides, thymoquinone showed the immunomodulatory role in cellular and humoral immunity [19][20]. Analysis of data obtained from the current study revealed that concanavalin A administration by retro-orbital route result in a remarkable elevation in both AST and ALT serum activities in comparison to the negative control group which received normal saline through retro-orbital rout. Injection of concanavalin A result in liver damage which can alter the permeability of the membrane, and cause the release of some hepatic specific enzymes. Therefore, abnormally high levels of serum ALT and AST indicate hepatic damage. These observations are in agreement with the studies of other researchers. Previous study has shown that even doses as low as 1.5mg/kg are already capable of inducing mild immune-mediated hepatitis with slightly elevated transaminase levels but no further manifestation of liver disease [12]. In another study, even a dose of 10mg/kg gives a significant result and rising the serum activity of ALT and AST with obvious manifestations of immune-mediated liver damage [21]. Concerning thymoquinone treatment, results obtained from the present study revealed a thymoquinone hepatoprotective effect. This hepatoprotective effect concluded from the remarkable attenuation in ALT and AST serum activities that were elevated by concanavalin A administration. This result is the first to demonstrate a hepatoprotective effect of thymoquinone in immune mediated liver damage, that means an immune modulatory activity has been proposed in the present study. However, other studies also showed hepatoprotective activity of thymoquinone using other experimental models, these models were induced liver damage in way that did not include a direct immune induction activity. These models include lipopolysaccharide (LPS)-, sepsis-, methotrexate-, metal-, induced liver damage among other models [22][23][24][25]. Each model induces liver damage in a unique mechanism but not through

direct immune activation as Con A does. The importance of immune mediated liver damage is to simulate human autoimmune and viral hepatitis, which considered an upgrowing health issue globally [26]. For example, thymoquinone exerts a hepatoprotective effect against CCl4-induced liver damage. In this model the suggested mechanism of protection is antioxidant activity of thymoquinone as CCL4 induced the damage through oxidative stress mechanism [27]. In another model, acetaminophen-induced liver damage is also reversed by administration of thymoquinone. Similarly, the suggested mechanism of protection is the antioxidant activity of thymoquinone [28]. Furthermore, liver damage is also induced by NAFLD (non-alcoholic fatty liver disease) where thymoquinone also exerted a beneficial effect through metabolic modulation and antioxidant activity [29]. Antioxidant activity of thymoquinone is a well-known effect observed from many studies [30]. The dose dependent hepatoprotective effect of thymoquinone were also observed in metal-induced liver damage. Thymoquinone showed an attenuating effect on ALT and AST in Pb-induced hepatotoxicity that might be clinically useful in Pb intoxication [24]. However, Pb-induced liver damage is through direct effect on enzymes and oxidative stress. However, the present study is the first to provide a clue that thymoquinone have immune mediated hepatoprotective activity as the damage induced by concanavalin A is via direct immune induction [11].

In the present study effect from taking concanavalin A on TNF α 8 hours before animal sacrificing results in dramatic elevation TNF α value. This suggests that the pro-inflammatory cytokine TNF α play important role in the progression of concanavalin A-induced hepatitis. Concanavalin A attachment to the endothelial lining of hepatocytes that mainly rich in Kupffer cell (liver macrophage) and liver sinusoidal endothelial cells (LSEC), therefor T cells become activated to CD4+ and secreting different immunogenic cytokines mostly TNF alpha [31]. Similar observations have been made by other study that revealed that serum level of TNF α dramatically increased after 1 hour from concanavalin A injection in dose 15mg/kg [32]. Results obtained from the analysis of data of the present study about thymoquinone groups improve its dose-dependent effect on immunomodulatory cytokine TNF α, that 15mg/kg dose of thymoquinone give significant reduction, 30mg/kg dose of thymoquinone dose resulted in a more remarkable decrease in TNF α in comparison to the model control group. Other researcher approved that thymoquinone may be a potential small-molecule inhibitor to modulate TNF α induced signaling in rheumatoid arthritis synovial fibroblast (RA-FLS), the molecular mechanism through which thymoquinone modulates TNF α response in RA-FLS, is through thymoquinone effect on TNF α-induced MAPK pathways, which are integral signaling mediators of TNF α-induced downstream inflammatory proteins in RA pathogenesis, that thymoquinone selectively inhibits TNF α-induced phosphorylation of p38 and JNK in a dose-dependent manner [33]. Mice administered concanavalin A in this study showed notable elevation of the immunological marker INF γ in comparison to the negative control group which administered normal saline only. Results of this study were consistent with those of other authors who improved that concanavalin A administration results in a significant and remarkable increase in stimulation of NF-κB followed by liberation of inflammatory cytokine IFN γ which have a great role in inducing and maintaining inflammatory induced liver injury [34] [11]. The animal groups which were treated with thymoquinone showed dose-dependent attenuation of INF γ tissue value, doubling the dose of thymoquinone result in even more significant depletion of INF γ in comparison to the concanavalin A model group. Results of the current study were consistent with another study, where they utilized guinea pig instead of mice as an animal model of asthma, and they provide that single dose of thymoquinone (3 mg/kg injected intraperitoneally) results in a significant decrease of INF γ level [35]. Concanavalin A-induced hepatic injury is associated with the release of pro-inflammatory cytokines such as TNF α and IFN γ. Production of these and other inflammatory mediators mainly depends upon activation of NF-κB, a ubiquitous transcription factor that regulates several genes involved in inflammation. A recent report has also shown that the expressions of the NF-kB gene significantly decreased by thymoquinone administration in both dose and time-dependent manner [14].

Conclusion

According to the observed data in the current study, a conclusion can be comprehended that thymoquinone has hepatoprotective and immunomodulatory effects against concanavalin A induced immune mediated liver damage. Thymoquinone exerted its hepatoprotective and immunomodulatory activity through downregulating the transcription factor NF-κB and its downstream effectors TNF α and IFN γ.

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