# Effects of thymoquinone on some cytokine levels in cerulein-induced acute pancreatitis

Research Article

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## ABSTRACT

In this study, it was aimed to evaluate the possible effects of thymoquinone administration on some cytokine levels in rats with experimental acute pancreatitis. No application was made the animals in group K. TQ group animals were intraperitoneally given 20 mg/kg thymoquinone daily for 9 days. In the AP group animals, acute pancreatitis was induced by intraperitoneal administration of cerulein as first dose 50 µg/kg and 2 hours later 25 µg/kg second dose on the 7th day of the study. Animals in the AP+TQ group were intraperitoneally administered 20 mg/kg thymoquinone daily for 9 days. On the 7th day of the study, after 2 hours from thymoquinone administration, acute pancreatitis was induced by intraperitoneal administration of cerulean as 50 µg/kg and 2 hours later 25 µg/kg. TNF-α, IL-6, IL-8, IL-10, AST and ALT levels were determined in the blood samples taken from all animals. In the study, TNF-α level was found to be importantly higher in the acute pancreatitis group compared to the control group, while TNF-α level was significantly lower in the acute pancreatitis group treated with thymoquinone than the acute pancreatitis group. IL-6 and IL-8 levels were higher in the acute pancreatitis group compared to the control group. IL-6 and IL-8 levels were found to be significantly lower in rats with acute pancreatitis treated with thymoquinone compared to the group with acute pancreatitis. While AST and ALT levels in the acute pancreatitis group were significantly increased when compared with the control group, both enzyme levels in the acute pancreatitis group treated with thymoquinone administration were found to be significantly lower than the rats with acute pancreatitis. In the study, the findings obtained in rats with acute pancreatitis which were pre-treated with thymoquinone can be evaluated as that thymoquinone alleviates inflammation due to pancreatitis.

**Keywords:** Cerulein, Cytokine, Rats, Thymoquinone

## INTRODUCTION

The incidence of acute pancreatitis (AP) is increasing globally, and mortality can be high in case of organ failure and severe necrosis. Acute pancreatitis is an acute inflammatory disease of the pancreas and mainly characterized by acinar cells atrophy, necrosis, activation of digestive enzymes (amylase and lipase), and inflammatory cell aggregation in the pancreas. In addition, intrapancreatic/acinar activation of trypsin causes a series of varying degrees of severity, which include multiple organ failure and death (Petrov et al., 2010). In the early stage of acute pancreatitis, there are release of proinflammatory cytokines (TNF-α, IL-1β, and IL-6) and the activation of zymogens in acinar cells. The degrees of systemic inflammatory response determine serious multiorgan dysfunction and mortality (Banks et al., 2013). Reversing of proinflammatory cytokine activation and cytokine storm play a crucial role in prognosis of acute pancreatitis (Zhang et al., 2018).

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In recent years, the use of herbal substances for the treatment or prevention of many ailments has been very popular. As one of these plants, Nigella sativa is medicinal plant belonging to family Ranunculaceae. Many cultures have used seeds of Nigella sativa to treat different disease for centuries. In many studies, it was investigated the pharmacological properties and potential medicinal benefits. The well-known properties of Nigella sativa are hepatoprotective, immunomodulatory, gastroprotective, anti-inflammatory, renal protective and antioxidant effects (Al Rowais, 2002). Thymoquinone is the fundamental constituent of the black seed oil which has shown strong anti-inflammatory effects by suppressing prostaglandins, leukotrienes and modulating cytokines (Salem, 2005; Arjumand et al., 2019).

In the light of these data, we evaluated the antiinflammatory effect of Thymoquinone in rats with cerulein-induced acute pancreatitis by measuring some proinflamatory and antiinflamatory cytokines.

## MATERIAL and METHOD

In the study, 32 healthy adult male Wistar Albino rats were used. During the study, the suitable living conditions (heat, humidity and light) for the rats were provided. All animals were fed with Purina brand standard rat diet during the experiment. The animals were allowed to drink water while fasting for 16 hours before the start of the study. Animals were divided into four groups.

Group I (K) (n=6): No application was made the animals in group K.

Group II (TQ) (n=6): Animals in TQ group were intraperitoneally given 20 mg/kg thymoquinone daily for 9 days.

Group III (AP) (n=10): In the AP group animals, acute pancreatitis was induced by intraperitoneal administration of cerulein as first dose 50 µg/kg and 2 hours later 25 µg/kg second dose on the 7th day of the study.

Group IV (AP+TQ) (n=10): Animals in the AP+TQ group were intraperitoneally administered 20 mg/kg thymoquinone daily for 9 days. On the 7th day of the study, acute pancreatitis was induced by intraperitoneal administration of cerulein as 50 µg/kg and 2 hours later 25 µg/kg after 2 hours from thymoquinone administration.

At the end of the 9th day of the study, blood samples taken from animals in all groups. TNF-α, IL-6, IL-8, IL-10, AST and ALT levels were determined in the blood samples taken from all animals. In plasma samples, TNF-α, IL-6, IL-8 and IL-10 levels were determined with ELISA (Biotek ELx800, Biotek Instrumentations, Inc, Winooski, VT, USA) using sandwich enzyme-linked immunosorbent method via commercial kits (Elabscience), while it was determined AST and ALT levels in the Siemens Centaur CP autoanalyzer using Siemens kits.

The data obtained from the study were analyzed by one-way ANOVA (SPSS). Differences among the groups were determined by Duncan's multiple range test. Differences were considered significant at $p<0.05$.

## RESULTS

In the study, the effects of thymoquinone administration on TNF-α, IL-6, IL-8 and IL-10 levels in rats with cerulein-induced acute pancreatitis are given in Table 1, AST and ALT levels are given in Table 2.

**Table 1.** The effects of thymoquinone on TNF-α, IL-6, IL-8 and IL-10 levels in rats with cerulein-induced acute pancreatitis (Mean±SE).

| | TNF-α (pg/ml) | IL-6 (pg/ml) | IL-8 (pg/ml) | IL-10 (pg/ml) |
|---|---|---|---|---|
| **K** | 46,65±6,26$^{c}$ | 38,34±3,68$^{b}$ | 141,34±6,98$^{c}$ | 61,37±6,62$^{b}$ |
| **TQ** | 43,84±5,83$^{c}$ | 40,95±4,63$^{b}$ | 132,01±6,65$^{c}$ | 63,56±7,13$^{b}$ |
| **AP** | 113,19±5,09$^{a}$ | 67,07±4,35$^{a}$ | 329,57±20,03$^{a}$ | 78,15±4,55$^{ab}$ |
| **AP+TQ** | 79,34±5,94$^{b}$ | 51,58±4,04$^{b}$ | 268,71±24,19$^{b}$ | 81,42±4,65$^{a}$ |

a-c The difference between mean values with different superscripts in the same column are significant ($p<0.05$)

**Table 2.** The effects of thymoquinone on AST and ALT levels in rats with cerulein-induced acute pancreatitis (Mean±SE).

| | AST (U/L) | ALT (U/L) |
|---|---|---|
| **K** | 84,17±6,83$^{c}$ | 23,50±3,57$^{c}$ |
| **TQ** | 79,33±6,14$^{c}$ | 24,67±2,94$^{c}$ |
| **AP** | 146,10±4,39$^{a}$ | 51,20±2,89$^{a}$ |
| **AP+TQ** | 115,40±6,34$^{b}$ | 38,90±3,14$^{b}$ |

a-c The difference between mean values with different superscripts in the same column are significant ($p<0.05$)

## DISCUSSION

Acute pancreatitis is a rapidly developing inflammatory disease in which peripancreatic tissues and other organ systems is often involved (Bhatia et al., 2005). Although the development and progression of the disease has not been fully explained, severe necrotic tissue cases result in a very high morbidity and mortality rate (Pandol et al., 2007).

The systemic inflammatory response syndrome and multi-organ failure syndrome in acute pancreatitis are associated with the severity of pancreatitis. This response to pancreatitis includes increments in leukocyte activation and production of various cytokines such as TNF-α, IL-1, IL-6, IL-8, IL-10 and IL-18 (Wereszczynska-Siemiatkowska et al., 2003; Štimac et al., 2006).

Although TNF-α is produced by many cells, its main source is macrophages and monocytes. TNF-α has a leading role in the development of the inflammatory response (Lane et al., 2001). It has shown that TNF production in mononuclear cell cultures taken from patients with acute pancreatitis is increased compared to healthy ones (McKay et al., 1996). While it has been reported that elevated circulating TNF-α levels might cause shock in many conditions, including acute pancreatitis, TNF-α is now considered one of the most important mediators of shock. It has been reported that elevations in TNF concentration are directly related to the severity of inflammation and pancreatic damage (Norman et al., 1995; Bhatia et al., 2000).

IL-10, synthesized by Th2 cells, monocytes, and B cells, is a naturally occurring anti-inflammatory cytokine. IL-10 reduces the release of Th1 proinflammatory cytokines (Fiorentino et al., 1991). In experimental studies, IL-10 has been shown to reduce the levels of inflammatory markers and the severity of pancreatitis (Kusske et al., 1996; Rongione et al., 1997). It has been reported that pre-application of synthetic IL-10 agonists reduces mortality and lung damage caused by experimental pancreatitis (Osman et al., 1998). However, IL-10 levels in humans have been shown to be related with the severity of pancreatitis and tissue damage. Pezzilli et al. (1997) have stated that IL-10 levels in healthy individuals were so low that it cannot be determined, while it increased in the first day of acute pancreatitis and gradually decreased in the following days.

IL-6 is produced by immunologically active cells such as monocytes/macrophages, endothelial cells and fibroblasts in response to stimuli. It was stated that IL-6 is produced in pancreatic tissue by periacinar myofibroblasts in response to TNF-α and IL-1β after experimentally induced pancreatitis (Norman et al., 1997; Shimada et al., 2002). This event is considered to play important role in the regulation of the generation and secretion of acute phase reactants by hepatocytes (Geiger et al., 1988; Castell et al., 1989). High levels of IL-6 are a marker of the severity of pancreatitis and also serve to determine the course of the disease (Galloway and Kingsnorth, 1994; Kingsnorth et al., 1995).

IL-8 is a potent chemokine that has neutrophil chemoattractant properties (Baggiolini et al., 1989). Following stimulation by proinflammatory mediators, IL-8 is released by wide cell types such as monocytes/macrophages, endothelial cells, neutrophils, fibroblasts, epithelial cells, keratinocytes, T cells, NK cells, and chondrocytes (Jeannin et al., 1994). IL-8 is known to be an important mediator in many inflammatory diseases. The increments in IL-8 level of the circulation in systemic inflammatory diseases help in predicting morbidity and mortality (Hack et al., 1992).

Throughout history, people have used various herbs and plants to prevent or treat diseases (Majdalawieh and Fayyad, 2015). Such plants are considered as an excellent treatment option with their easy obtaining, nutritious and synergistic effects (Donaldson, 1998). Treatment approaches based on the properties and effects of natural plants have played an important role in the development of conventional medicine and modern medicines.

Nigella Sativa is botanically a dicotyledonous flowering plant belonging to the Ranunculaceae family and is commonly known as black seed or black cumin (Ali and Blunden, 2003; Salem, 2005). Nigella Sativa seeds are traditionally used as a food preservative, nutritional supplement or spice in various cultures. Thymoquinone with a chemical formula of C10H12O2 and a molecular weight of 164.2 g/mol is the most important bioactive phytochemical found in Nigella Sativa oil and extracts (Woo et al., 2012; AbuKhader, 2013; Schneider-Stock et al., 2014). It is claimed that some of the medical benefits of Nigella Sativa and thymoquinone are related to its antihistaminic, anti-inflammatory, antihypertensive, hypoglycemic, anticancer and immune-enhancing effects (Ahmad et al., 2013; Shabana et al., 2013; Rahmani et al., 2014; Schneider-Stock et al., 2014).

It is reported that Nigella Sativa extracts suppress IL-6, TNF-α and NO expressions in a dose-dependent manner (Majdalawieh et al., 2010). It has been supported by various findings that Nigella Sativa has anti-inflammatory and antioxidant effects in patients with rheumatoid arthritis (Hadi et al., 2016). It is stated that these findings are consistent with the findings that NO production in mouse macrophages is inhibited by Nigella Sativa (Mahmood et al., 2003).

Increases in glutathione levels appear to be a mechanism underlying the beneficial effects of thymoquinone against inflammatory disorders. Nuclear Factor Kappa Beta (NF-κB) is a eukaryotic transcription factor critically involved in various biological processes, including inflammation (Majdalawieh and Ro, 2010). It has been shown that thymoquinone administration inhibits NF-κB signaling in the medulla spinalis and brains of Experimental Allergic Encephalitis (EAE) induced rats. This suggests that NF-κB is potentially a target molecule for thymoquinone (Mohamed et al., 2005). It is reported that thymoquinone suppresses Advanced Glycation End Product (AGE)-induced NF-κB activation and IL-6 expression (Sayed and Morcos, 2007). In another study, it was determined that

thymoquinone administration significantly suppressed Lipopolysaccharide (LPS)-induced TNF-α production in the basophil cell line (El Gazzar, 2007). Consistent with above data, Chehl et al. (2009) reported that thymoquinone significantly decreased the expressions of IL-1β, TNF-α, monocyte chemoattractant protein 1 (MCP-1), and cyclooxygenase 2 in pancreatic ductal adenocarcinoma depending on dose and time. On the other hand, it has been reported that LPS-induced NF-κB and p38-mitogen activating protein kinase (MAPK) activation is blocked by thymoquinone, resulting in suppression of the expression of proinflammatory mediators such as IL-1β and TNF-α (Vaillancourt et al., 2011). It has been reported that thymoquinone reduces serum levels of proinflammatory cytokines such as IL-1β, IL-6 and TNF-α (Badr et al., 2011).

In our study, while TNF-α level, which is a proinflammatory cytokine and plays a key role in inflammatory events, was found to be significantly higher in the acute pancreatitis group than in the control group ($p<0.05$, Table 1), this difference was expected situation and was consistent with the above data. In this study, the significantly lower TNF-α level in the acute pancreatitis group with thymoquinone administration compared to the pancreatitis group ($p<0.05$, Table 1) supports the reports that thymoquinone suppresses the increments in TNF-α levels in various inflammatory conditions. The levels of IL-6 and IL-8, which are proinflammatory cytokines, increased in parallel with the changes in TNF-α levels in the acute pancreatitis group ($p<0.05$, Table 1). IL-8 levels were found to be significantly lower in rats with acute pancreatitis due to thymoquinone administration compared to the group with acute pancreatitis ($p<0.05$, Table 1). IL-6 level in acute pancreatitis group treated with thymoquinone was different from the group with acute pancreatitis and it was observed that it approached the control group values. The increase in the level of IL-10, an anti-inflammatory cytokine, was not statistically significant in the acute pancreatitis group compared to the control group. This increase became more evident with the administration of thymoquinone to rats with acute pancreatitis ($p<0.05$, Table 1). The changes in proinflammatory cytokine levels determined by the thymoquinone administration to rats with acute pancreatitis are consistent with the reports that thymoquinone inhibits the NF-κB signal, an inflammation transcription factor, and suppresses the expression of cytokines such as IL-1β, IL-6 and TNF-α. In the study, the significant increase in IL-10 level determined by the application of thymoquinone to rats with acute pancreatitis can be evaluated as thymoquinone increases IL-10 production, which suppresses proinflammatory cytokines against inflammation.

It has been shown that pancreatic enzymes spread into the systemic circulation as a result of inflammatory changes in the pancreas and retroperitoneum. These enzymes cause damage to its own tissue and the other organs such as the liver by producing various inflammatory mediators (Gallagher et al., 2004; Rahimian et al., 2017). In this study, increases in AST and ALT levels showed that cerulein administration also caused liver damage, while thymoquinone administration to the acute pancreatitis group significantly limited the increase in hepatic enzyme levels such as AST and ALT ($p<0.05$, Table 2). Increases in serum AST, ALT and total bilirubin levels are accepted as an indicator of hepatic damage in experimental acute pancreatitis. While it has been reported that TNF-α and IL-6 levels increase in acute pancreatitis, it is suggested that apoptosis-related gene expression is upregulated in parallel to the increases in these cytokines. It has also been suggested that this increase in hepatic cell apoptosis may be the main cause of liver dysfunction in the early stages of severe acute pancreatitis (Sha et al., 2008). In the study, the significant changes in AST and ALT

levels in the acute pancreatitis group with thymoquinone administration can be considered as a result of the positive reflection on liver damage by suppressing of inflammatory mediators due to the antiinflammatory and antioxidant effects of thymoquinone.

## CONCLUSION

Depend on the obtained changes in the cytokine levels and liver enzymes levels may be considered that thymoquinone showed beneficial effect in rats with acute pancreatitis in this study.

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Conflict of interest: The author declares no conflict of interest.

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