

## Effects of thymoquinone on alpha-amanitin induced hepatotoxicity in human C3A hepatocytes

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Thymoquinone (TQ) has shown hepatoprotective effects in various experimental studies. We aimed to investigate the possible beneficial effects of TQ regarding its prevention of alpha-amanitin induced hepatotoxicity in human C3A hepatocytes. After administering alpha-amanitin in a concentrations of 1 and 10 µg/mL on the cells in a hepatocyte cell line, TQ was administered in various concentrations (10, 5, 1, 0.5, 0.1, 0.05, 0.01, 0.005 µg/mL). The MTT test was used to determine cell viability. For the groups given only TQ at various concentrations, the cell viability rates at 48 hours post-administration were found at 82.6, 98.3, 102.1, 102.5, 99.4, 99.4, 101.9 and 106.3%, respectively. For the group with 1 µg/mL alpha-amanitin and various TQ concentrations, the cell viability rates were found at 74.6, 88.5, 87.4, 88.7, 85.7, 86.8, 88.4, and 92.9%, respectively. For the group with 10 µg/mL alpha-amanitin and various TQ concentrations, the cell viability rates for each TQ subgroup were found at 65.2, 79.2, 81.4, 81.1, 81.8, 81.8, 82.2 and 91.9%, respectively. Our study is the first *in vitro* study that investigates TQ's effects on alpha-amanitin induced hepatotoxicity. Although TQ had beneficial effect in low doses did not significantly increase cell viability in liver damage due to alpha-amanitin toxicity.

**Keywords:** Thymoquinone. Alpha-amanitin. Hepatotoxicity. MTT assay. C3A human hepatocyte cell line.

### INTRODUCTION

Mushroom-related poisoning is common, but serious toxicities are rarely encountered. Mushrooms that contain cyclopeptide amatoxins are the most toxic species known, and more than 90% of the fatal incidents are due to this type of mushroom poisoning (Karlson-Stiber, Persson 2003; Yilmaz *et al.*, 2015). Amatoxins are present in several *Amanita* mushroom species, which include several members of the *Lepiota* and *Gallerina* mushrooms (Kaya *et al.*, 2013; Kose *et al.*, 2015). Amatoxins can be divided into different subgroups, including alpha-amanitin, beta-amanitin, gamma-amanitin, epsilon-amanitin, amanullinic acid, amanullin, proamanullin, amanin and amaninamide (Vetter, 1998); the toxin constituents vary among and

within different species. Furthermore, the quantity of toxins has also been found to vary within different parts of the mushroom and during different growth stages (Yilmaz *et al.*, 2014; Kaya *et al.*, 2013). From the various toxin subgroups, the toxic effect of alpha-amanitin is considered the most potent (Kaya *et al.*, 2014). Here, alpha-amanitin is a strong hepatotoxin made from a cyclic peptide containing eight amino acids. And although alpha-amanitin is primarily held responsible for lethal mushroom poisonings, beta-amanitin and gamma-amanitin have also been shown to have similar cytotoxic actions (Kaya *et al.*, 2014; Bakirci *et al.*, 2015b). These amatoxins cause severe mushroom poisoning by irreversibly binding to RNA polymerase II, which results in the inhibition of DNA transcription and impairment of protein synthesis processes, causing cell death. In this perspective, the mushroom species *Amanita phalloides* contains these amatoxins, and its ingestion is mainly held responsible for the majority of casualties: caused by delayed-onset hepatocellular necrosis

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and renal cell injury (Kaplan, Larsson, Kornberg, 2008; Yilmaz *et al.*, 2015). Although various hepatoprotective agents are currently available—as monotherapies or in combination with other agents found in experimental studies and clinics—there is still no effective drug for treatment (Kaya *et al.*, 2016; Vetter, 1998).

*Nigella sativa* (also known as black cumin) is a common seasoning that has traditionally been used as a herbal remedy for the treatment of various diseases including fever, cough, bronchitis, asthma, chest congestion, migraine, dizziness, hemiplegia, back pain, dysmenorrhea, diabetes, infection and inflammation, rheumatism, hypertension, and gastrointestinal disorders such as flatulence, dyspepsia, diarrhea. (Gholamnezhad *et al.*, 2016). Thymoquinone (TQ, chemical name: 2-isopropyl-5-methyl-1,4-benzoquinone) is the most bioactive component in the oil and extracts of the herb *Nigella sativa* and has anti-inflammatory, immunomodulatory, anti-histaminic, anti-microbial, analgesic, spasmolytic, bronchodilating, anti-diabetic, anti-hypertensive, anti-tumor, and antioxidant properties (Mabrouk *et al.*, 2016; Darakhshan *et al.*, 2015; Gholamnezhad *et al.*, 2016). More importantly, in various experimental studies, TQ has shown hepatoprotective effects against hypervitaminosis A (Al-Suhaimi, 2012); tert-butyl hydroperoxide toxicity (Daba, Abdel-Rahman, 2004); carbon tetrachloride- (Turkdogan *et al.*, 2003; Kanter, Coskun, Budancamanak, 2005), aflatoxin B(1)- (Nili-Ahmadabadi *et al.*, 2011), and diazinon-induced hepatotoxicity (Nili-Ahmadabadi *et al.*, 2018); surgery-induced hepatic ischemia-reperfusion injury (Tekbas *et al.*, 2018); isoniazid- (Hassan *et al.*, 2012) and paracetamol-induced liver toxicity (Yesmin *et al.*, 2013); high-dose atorvastatin-induced hepatic oxidative injury (Hassan *et al.*, 2018); and cisplatin (Al-Malki, Sayed, 2014), cyclophosphamide (Laskar *et al.*, 2016), and methotrexate-induced hepatotoxicity (Sayeed *et al.*, 2017). However, to the best of our knowledge, there are currently no available studies that investigate the effects of TQ against alpha-amanitin-induced liver damage. As such, the aim of this study was to investigate the possible beneficial effects of TQ regarding its prevention of alpha-amanitin induced hepatotoxicity in human C3A hepatocytes cell line.

## MATERIAL AND METHODS

### Extraction and purification of alpha-amanitin

The alpha-amanitin was obtained from *A. phalloides* mushroom as purification. Carpophores of *A. phalloides* were gathered in Duzce (Turkey). Taxonomic classification of the mushrooms was made accordingly via microscopic and macroscopic definitions (Kaya *et al.*, 2013). Refinement of alpha-amanitin was performed as described previously (Kaya *et al.*, 2012; 2014). Briefly, the mushrooms were dried and pulverized followed by incubation in 50% methanol at 25°C for 12 h to extract the alpha-amanitin. The dissolvent of the obtained extract was then vaporized. Next, the dried extract was dissolved in mobile phase solution [50 mM ammonium acetate + acetonitrile; (90/10; v/v) (both purchased from Sigma-Aldrich, St. Louis, MO)] and the alpha-amanitin purified using the preparative high-performance liquid chromatography (HPLC) system (Shimadzu, Japan) with a C18 column (Advanced Chromatography Technologies Ltd, Aberdeen, Scotland). The obtained extract was purified twice in total with the above HPLC method. The purity and amount of obtained alpha-amanitin toxin were compared with the alpha-amanitin standard (Sigma-Aldrich, St. Louis, MO) using the analytic HPLC system (Shimadzu, Japan).

### Cell culture

In this study, the cell culture procedures were performed as described previously (Bakirci *et al.*, 2015a,b). Briefly, the C3A human hepatocyte cell line was obtained from the American Type Culture Collection. DMEM/F12 (Dulbecco's modified Eagle's medium: Nutrient mix F-12, Invitrogen, Carlsbad, CA) was used as culture medium and fetal bovine serum as serum supplementation (Invitrogen, Carlsbad, CA). The cells were grown in a humidified incubator maintaining 37°C with 5% CO<sub>2</sub>. The culture medium was refreshed every three days, and the hepatocytes divided twice a week using trypsin-EDTA (ethylenediaminetetraacetic acid) (Sigma-Aldrich, St. Louis, MO) dissociation.

## Experimental procedures

For experimental purposes, the cells were placed in 96-well-plate wells at  $3 \times 10^4$  cells/100  $\mu$ L per well after the dissociation procedure. They were then placed in the incubator and allowed to adhere overnight to the well's surface. The wells containing cells were divided into multiple groups. The control group was not treated with alpha-amanitin or TQ. The number of living cells of the control group was determined and set as 100% for normalization purposes.

The other groups contained eight TQ subgroups at various concentrations of TQ (obtained from Sigma-Aldrich, St. Louis, MO): 10, 5, 1, 0.5, 0.1, 0.05, 0.01, and 0.005  $\mu$ g/mL. Alpha-amanitin subgroups at different concentrations: 10 and 1  $\mu$ g/mL. Finally, the main study group was divided into sixteen subgroups. The first half received 10  $\mu$ g/mL of alpha-amanitin, while the other received 1  $\mu$ g/mL. After 4 hours, both halves received various concentrations of TQ: 10, 5, 1, 0.5, 0.1, 0.05, 0.01, and 0.005  $\mu$ g/mL.

## Cell viability assessment

The overall functional integrity and viability of the groups were assessed with the MTT assay (Sigma-Aldrich, St. Louis, MO) [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide] by determining the cell viability 48 hours after the experimental procedure (Bakirci *et al.*, 2015a,b). To this end, enough MTT solution was added to each well to obtain an end concentration of approximately 10% MTT. After 4 hours, formazan (Sigma-Aldrich, St. Louis, MO) was added—dissolved in 100  $\mu$ L dimethyl sulfoxide (Sigma-Aldrich, St. Louis, MO)—to each well. The plates were then incubated overnight in a moisturized drying oven at 37°C. Finally, the absorbance of each well was measured with a microplate ELISA reader using a detection wavelength

of 570 nm (after the reference wavelength was adjusted to 690 nm).

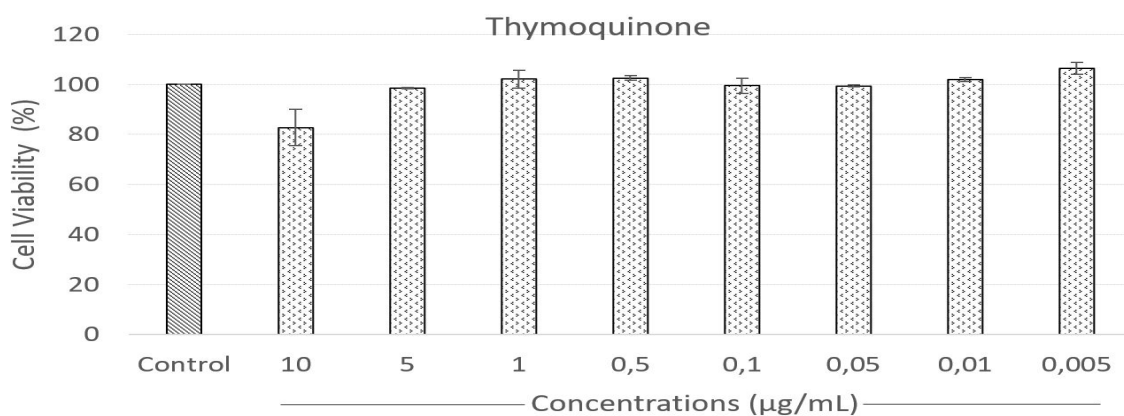
## Statistical analysis

The average absorbance values of the control group were normalized to 100%, while the other samples were calculated by comparing them to the control groups. Data are given as the mean value  $\pm$  standard error mean. The statistical analysis for the viability values was assessed between the dual groups by using the Mann-Whitney U Test. All values with  $P < 0.05$  were considered significant.

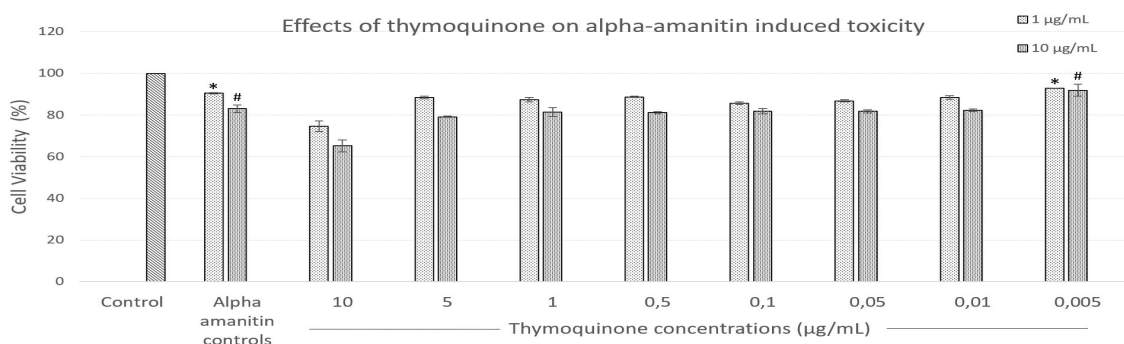
## RESULTS

According to the MTT assay; for the groups given only TQ at various concentrations (10, 5, 1, 0.5, 0.1, 0.05, 0.01, and 0.005  $\mu$ g/mL), the cell viability rates at 48 hours post-administration were found at 82.6, 98.3, 102.1, 102.5, 99.4, 99.4, 101.9, and 106.3%, respectively (Figure 1). The cell viability rates at 48 hours post-administration of the groups with only 1 and 10  $\mu$ g/mL alpha-amanitin were found at 90.5 and 83.1%, respectively (Figure 2).

Finally, for the group with 1  $\mu$ g/mL alpha-amanitin and various TQ concentrations (10, 5, 1, 0.5, 0.1, 0.05, 0.01, and 0.005  $\mu$ g/mL), the cell viability rates were found at 74.6, 88.5, 87.4, 88.7, 85.7, 86.8, 88.4, and 92.9%, respectively. For the group with 10  $\mu$ g/mL alpha-amanitin and various TQ concentrations (10, 5, 1, 0.5, 0.1, 0.05, 0.01, and 0.005  $\mu$ g/mL), the cell viability rates for each TQ subgroup were found at 65.2, 79.2, 81.4, 81.1, 81.8, 81.8, 82.2, and 91.9%, respectively (Figure 2). Overall, 0.005  $\mu$ g/mL of TQ showed the best efficacy on cell viability, and when this concentration was compared in proportion to the control alpha-amanitin groups, the differences between them were not statistically significant ( $P=0.102$ ,  $P=0.05$ ).



**FIGURE 1** - Effects of thymoquinone on cell viability.



**FIGURE 2** - The ratio of cell viability after thymoquinone (TQ) treatment. The cell line stimulated by alpha-amanitin at 1 µg/mL and 10 µg/mL of concentrations and was treated with TQ 4 hours after toxin administration. After incubating 48 hours, the MTT assay was done to assess the cell viability. \*:  $P=0.102$  TQ (0.005 µg/mL) group with the highest viability group against control 1 µg/mL alpha-amanitin group. #:  $P=0.05$  TQ (0.005 µg/mL) group with the highest viability group against control 10 µg/mL alpha-amanitin group.

## DISCUSSION

Over the years, several treatment methods such as cimetidine, penicillin, N-acetyl cysteine, and silibinin have been evaluated for their properties to prevent hepatocyte and renal cell injury after alpha-amanitin poisoning. Despite these efforts, no effective and clear treatment protocol has been defined yet (Tong *et al.*, 2007; Ward *et al.*, 2013; Magdalan *et al.*, 2011).

Oxidative stress is described as the disruption of the equilibration between reactive oxygen species and the antioxidant capacity of living organisms. Here, the increased role of oxidative stress has been verified with various *in vitro* models using methotrexate, organophosphate, and paracetamol (Ranjbar *et al.*, 2005; Akbulut *et al.*, 2014; Saritas *et al.*, 2012). Recent experimental studies have indicated that alpha-amanitin

potentially causes hepatotoxicity by oxidative stress (Magdalan *et al.*, 2011; Nikolova *et al.*, 2010). To further analyze the treatment options, primary human hepatocyte cultures are an appropriate model to investigate various antidotes without the need to consider any of the aforementioned clinical factors.

In regards to hepatotoxicity and oxidative stress, TQ has shown to be a strong antioxidant with protective properties against oxidative organ injury caused by free radical-generating agents (Nagi *et al.*, 2010; Houghton *et al.*, 1995; Bayrak *et al.*, 2008). For example, a rapid assessment for antioxidants, using two thin-layer chromatography screening methods, showed that TQ and the components carvacrol, t-anethole and 4-terpineol showed remarkable radical scavenging effect. In a study by Burits and Bucar (2000), TQ demonstrated antioxidant effects by reducing the oxidative radical



diphenyl-picrylhydrazyl. In a different study, TQ was also found to have protective effects toward an array of free radical creating compounds caused by doxorubicin treatment (Nagi, Mansour, 2000). In another study, TQ was formulated with a proniosomal formulation and evaluated for its efficacy against hepatotoxicity caused by methotrexate in rats (Sayeed *et al.*, 2017). The results showed that the formulation significantly inhibited the elevated levels of serum marker enzymes such as alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and improved histopathological deformities. Next, in a study by Turkdogan *et al.* (2003) the hepatoprotective effect of *Nigella sativa* in rabbits treated with carbon tetrachloride was investigated. The results showed that the hepatocellular degenerative and necrotic changes were mild for the *Nigella sativa*-treated group and that there were no indications of fibrosis or cirrhotic processes. In a similar study by Kanter, Coskun, Budancamanak, (2005), their results showed that the addition of TQ improved the protecting process against lipid peroxidation caused by carbon tetrachloride-induced hepatotoxicity. Nagi *et al.* (2010) reported protective effects via antioxidant mechanisms with the prophylactic use of TQ against hepatotoxicity, which was caused by acetaminophen. In another example, Nili-Ahmadahabi *et al.* (2011) showed that AST, ALT, and ALP levels dropped significantly in mice treated with TQ compared to the ones treated with Aflatoxin B1 (AFB1). Histopathologically, the liver of the AFB1-exposed mice showed inflammation, necrosis, Kupffer cell hyperplasia; and mononuclear cell infiltration, disruption of hepatocytes, and dilation of sinusoids, whereas treatment with TQ helped to normalize the liver structure according to biochemical findings. Here, TQ was found to have an optimal protective effect in mice suffering from AFB1 hepatotoxicity at a dosage of 9 mg/kg. Additionally, Nili-Ahmadabadi *et al.* (2018) recently showed that TQ attenuated liver toxicity (caused by diazinon) and oxidative damage and improved the antioxidant state of the tissue. They also showed that the therapeutic effects of low TQ dosages (1.25 mg/kg) were relatively better than the ones with higher doses (5 mg/kg), which are results that resemble the ones in our current study.

## CONCLUSION

In conclusion, to the best of our knowledge, our study is the first *in vitro* study that investigates TQ's effects on alpha-amanitin induced hepatotoxicity. Even though various animal studies have shown the protective antioxidant effects of TQ, our treatment did not significantly increase cell viability in liver damage due to alpha-amanitin toxicity. However, in our study, we showed that the TQ group with the lowest dose (0.005 µg/mL) had the highest viability compared to the control one, indicating that a lower TQ concentration might have a larger effect *in vivo* as well.

## CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

## REFERENCES

- Akbulut S, Elbe H, Eris C, Dogan Z, Toprak G, Otan E, et al. Cytoprotective effects of amifostine, ascorbic acid and N-acetylcysteine against methotrexate-induced hepatotoxicity in rats. *World J Gastroenterol.* 2014;20(29):10158-65.
- Al-Malki AL, Sayed AA. Thymoquinone attenuates cisplatin-induced hepatotoxicity via nuclear factor kappa-β. *BMC Complement. Altern Med.* 2014;14:282.
- Al-Suhaimi EA. Hepatoprotective and immunological functions of *Nigella sativa* seed oil against hypervitaminosis A in adult male rats. *Int J Vitam Nutr Res.* 2012;82(4):288-97.
- Bakirci S, Bayram R, Kaya E, Yaykasli KO, Yilmaz I. What is the restoring role of chrysin in alpha amanitin toxicity? *Acta Med. Mediterr.* 2015a;31(6):1181.
- Bakirci S, Bayram R, Yilmaz I, Yaykasli KO, Bayram S, Kaya E. Purification and in vitro toxicity of gamma amanitin. *Toxin Rev.* 2015b;34(4):200-5.
- Bayrak O, Bavbek N, Karatas OF, Bayrak R, Catal F, Cimentepe E, et al. *Nigella sativa* protects against ischaemia/reperfusion injury in rat kidneys. *Nephrol Dial Transplant.* 2008;23(7):2206-12.
- Burits M, Bucar F. Antioxidant activity of *Nigella sativa* essential oil. *Phytother Res.* 2000;14(5):323-8.
- Daba MH, Abdel-Rahman MS. Hepatoprotective activity of thymoquinone in isolated rat hepatocytes. *Toxicol Lett.* 1998;95(1):23-9.

- Darakhshan S, Bidmeshki Pour A, Hosseinzadeh Colagar A, Sisakhtnezhad S. Thymoquinone and its therapeutic potentials. *Pharmacol Res.* 2015;95-96:138-58.
- Gholamnezhad Z, Havakhah S, Boskabady MH. Preclinical and clinical effects of *Nigella sativa* and its constituent, thymoquinone: A review. *J Ethnopharmacol.* 2016;190:372-86.
- Hassan AS, Ahmed JH, Al-Haroon SS. A study of the effect of *Nigella sativa* (Black seeds) in isoniazid (INH)-induced hepatotoxicity in rabbits. *Indian J Pharmacol.* 2012;44(6):678-2.
- Hassan SS, Razzaque A, Ahmad Z, Pazdernik V, Amin SN. Does posttreatment thymoquinone reverse high-dose atorvastatin-induced hepatic oxidative injury in rats? *Can J Physiol Pharmacol.* 2018;96(1):51-9.
- Houghton PJ, Zarka R, de las Heras B, Houtl JR. Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. *Planta Med.* 1995;61(1):33-6.
- Kanter M, Coskun O, Budancamanak M. Hepatoprotective effects of *Nigella sativa* L and *Urticadioica* L on lipid peroxidation, antioxidant enzyme systems and liver enzymes in carbon tetrachloride-treated rats. *World J Gastroenterol.* 2005;11(42):6684-8.
- Kaplan CD, Larsson KM, Kornberg RD. The RNA polymerase II trigger loop functions in substrate selection and is directly targeted by alpha-amanitin. *Mol Cell.* 2008;30(5):547-56.
- Karlson-Stiber C, Persson, H. Cytotoxic fungi-an overview. *Toxicon.* 2003;42(4):339-49.
- Kaya E, Bayram R, Yaykaşli KO, Yilmaz I, Bayram S, Yaykasli E, et al. Evaluation and comparison of alpha- and beta-amanitin toxicity on MCF-7 cell line. *Turk J Med Sci.* 2014;44(5):728-32.
- Kaya E, Yaykasli KO, Karahan S, Bayram R, Saritas A, Yaykasli E. Purification of high purity alpha amanitin using preparative HPLC method. *Konuralp Med J.* 2012;4(3):35-41.
- Kaya E, Yilmaz I, Admis O, Oktay M, Bayram R, Bakirci S, et al. Effects of erdosteine on alpha amanitin-induced hepatotoxicity in mice. *Toxin Rev.* 2016;35(1-2):4-9.
- Kaya E, Yilmaz I, Sinirlioglu ZA, Karahan S, Bayram R, Yaykasli KO, et al. Amanitin and phallotoxin concentration in *Amanita phalloides* var. *alba* mushroom. *Toxicon.* 2013;76:225-33.
- Kose M, Yilmaz I, Akata I, Kaya E, Guler K. A Case Study: Rare *Lepiota brunneoincarnata* Poisoning. *Wilderness Environ Med.* 2015;26(3):350-4.
- Laskar AA, Khan MA, Rahmani AH, Fatima S, Younus H. Thymoquinone, an active constituent of *Nigella sativa* seeds, binds with bilirubin and protects mice from hyperbilirubinemia and cyclophosphamide-induced hepatotoxicity. *Biochimie.* 2016;127:205-13.
- Mabrouk A, Bel Hadj Salah I, Chaieb W, Ben Cheikh H. Protective effect of thymoquinone against lead-induced hepatic toxicity in rats. *Environ Sci Pollut Res Int.* 2016;23(12):12206-15.
- Magdalan J, Piotrowska A, Gomułkiewicz A, Sozański T, Szeląg A, Dziegiel P. Influence of commonly used clinical antidotes on antioxidant systems in human hepatocyte culture intoxicated with alpha-amanitin. *Hum Exp Toxicol.* 2011;30(1):38-43.
- Nagi MN, Almakki HA, Sayed-Ahmed MM, Al-Bekairi AM. Thymoquinone supplementation reverses acetaminophen-induced oxidative stress, nitric oxide production and energy decline in mice liver. *Food Chem Toxicol.* 2010;48(8-9):2361-5.
- Nagi MN, Mansour MA. Protective effect of TQ against doxorubicin- induced cardiotoxicity in rats: a possible mechanism of protection. *Pharmacol Res.* 2000;41(3):283-9.
- Nikolova G, Karamalakova Y, Hadjibojeva P, Georgiev Ts, Tolekova A, Gadjeva V, et al. Severe mushroom toxin alpha amanitin causes generation of reactive oxygen species in liver tissues of mice-a comparative study by two different instrumental methods. *Trakia J Sci.* 2010;8(2):149-154.
- Nili-Ahmadabadi A, Alibolandi P, Ranjbar A, Mousavi L, Nili-Ahmadabadi H, Larki-Harchegani A, et al. Thymoquinone attenuates hepatotoxicity and oxidative damage caused by diazinon: an in vivo study. *Res Pharm Sci.* 2018;13(6):500-8.
- Nili-Ahmadabadi A, Tavakoli F, Hasanzadeh G, Rahimi H, Sabzevari O. Protective effect of pretreatment with thymoquinone against Aflatoxin B1 induced liver toxicity in mice. *Daru.* 2011;19(4):282-7.
- Ranjbar A, Solhi H, Mashayekhi FJ, Susanabdi A, Rezaie A, Abdollahi M. Oxidative stress in acute human poisoning with organophosphorus insecticides; a case control study. *Environ Toxicol Pharmacol.* 2005;20(1):88-91.
- Saritas A, Kandis H, Baltaci D, Yildirim U, Kaya H, Karakus A, et al. N-Acetyl cysteine and erdosteine treatment in acetaminophen-induced liver damage. *Toxicol Ind Health.* 2012;30(7):670-8.
- Sayeed S, Imam SS, Najmi AK, Aqil M, Akhtar M. Nonionic surfactant based thymoquinone loaded nanoprionosomal formulation: in vitro physicochemical evaluation and in vivo hepatoprotective efficacy. *Drug Dev Ind Pharm.* 2017;43(9):1413-20.

Tekbas A, Huebner J, Settmacher U, Dahmen U. Plants and Surgery: The protective effects of thymoquinone on hepatic injury-a systematic review of in vivo studies. *Int J Mol Sci.* 2018;19(4):1085.

Tong TC, Hernandez M, Richardson WH 3rd, Betten DP, Favata M, Riffenburgh RH, et al. Comparative treatment of alpha-amanitin poisoning with N-acetylcysteine, benzylpenicillin, cimetidine, thioctic acid, and silybin in a murine model. *Ann Emerg Med.* 2007;50(3):282-8.

Turkdogan MK, Ozbek H, Yener Z, Tuncer I, Uygan I, Ceylan E. The Role of *Urtica dioica* and *Nigella sativa* in the Prevention of Carbon Tetrachloride induced Hepatotoxicity in Rats. *Phytother Res.* 2003;17(8):942-6.

Vetter J. Toxins of *Amanita phalloides*. *Toxicon.* 1998;36(1):13-24.

Ward J, Kapadia K, Brush E, Salhanick SD. Amatoxin poisoning: case reports and review of current therapies. *J Emerg Med.* 2013;44(1):116-21.

Yesmin F, Rahman Z, Dewan JF, Helali AM, Islam Z., Abdu I RNI, et al. Hepatoprotective effect of aqueous and N-hexane extract of *Nigella sativa* in paracetamol (acetaminophen) induced liver disease of rats: a histopathological evaluation. *Int Res J Pharm.* 2013;4(7):90-4.

Yilmaz I, Ermis F, Akata I, Kaya E. A Case Study: What Doses of *Amanita phalloides* and Amatoxins Are Lethal to Humans? *Wilderness Environ Med.* 2015;26(4):491-6.

Yilmaz I, Kaya E, Sinirlioglu ZA, Bayram R, Surmen MG, Colakoglu S. Clinical importance of toxin concentration in *Amanita verna* mushroom. *Toxicon.* 2014;87:68-75.

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