doi: 10.5262/tndt.2013.1002.09

Modulation of Nrf2/HO-1 by Thymoquinone During Cisplatin-Induced Nephrotoxicity

Sisplatin Nefrotoksisitesinde Nrf2/HO-1'nin Thymoquinone Tarafından Modülasyonu

Ramazan ULU[1]
Ayhan DOĞUKAN[1]
Mehmet TUZCU[2]
Ali GÜREL[1]
Irfana MUQBİL[3]
Ramzi M MOHAMMAD[3]
Kazım ŞAHİN[4]

1 Firat University School of Medicine, Department of Nephrology, Elazig, Turkey
2 Firat University Faculty of Science, Department of Biology, Elazig, Turkey
3 Wayne State University School of Medicine, Department of Oncology, Detroit, USA
4 Firat University Faculty of Veterinary Science, Department of Animal Nutrition, Elazig, Turkey

ABSTRACT

OBJECTIVE: Side effects of cisplatin, such as nephrotoxicity, limit its use in chemotherapeutic regimens and indicate an agent that suppresses its toxicity. Thymoquinone (TQ), the predominant bioactive constituent present in black seed oil (Nigella sativa), has antiinflammatory, antioxidant and antitumor effects. We propose a protective mechanism of of TQ on cisplatin-nephrotoxicity in rats that is through modulation of Nrf2-mediated antioxidant induction and reduced inflammation.

MATERIAL and METHODS: Twenty-eight male Wistar rats (8 weeks-old) were divided into four groups; Control (vehicle; 0.9% saline, 1 ml/kg body wt., p.o.), TQ (10 mg/kg body weight/day in drinking water for 5 days), cisplatin (a single injection of 7mg/kg body wt, i.p.) and TQ for 5 days in drinking water then a single injection of cisplatin. On day 10, all rats were sacrificed by cervical dislocation, kidneys were removed, and serum urea and creatinine were collected.

RESULTS: Serum urea and creatinine levels were significantly higher in cisplatin-treated rats compared with control rats. TQ-treatment significantly decreased serum urea and creatinine levels. Cisplatin-treatment caused significant downregulation of the nuclear NF-E2-related factor-2 (Nrf2), heme oxygenase-1(HO-1) and caused an increase in the levels of nuclear factor-kappa B (NF-κB). Interestingly, TQ supplementation significantly improved the changes associated with cisplatin nephrotoxicity by increasing the levels of Nrf-2 and HO-1 and decreasing the levels of NF-κB.

CONCLUSION: This study demonstrates the TQ targets NRF2/HO-1 and can be used as a potential agent against cisplatin-induced nephrotoxicity.

KEY WORDS: Cisplatin, Kidney, Nrf2; HO-1, Thymoquinone

ÖZ

AMAÇ: Sisplatinin nefrotoksisite gibi sitotoksik etkileri, kemoterapi rejimlerinde kullanımını sınırlamakta ve toksik etkilerini baskılayacak bir ajana gereksinim doğmaktadır. Thymoquinone (TQ) çörek otu yağında bulunan biyoaktif bileşen olup, antiinflamatuvar, antioksidan ve antitümör etkileri vardır. Bu çalışmada, ratlarda TQ'nin Nrf2 indüksiyonu ve antiinflamatuvar etki yoluyla sisplatin nefrotoksisitesi üzerine olan koruyucu etkisini araştırdık.

GEREÇ ve YÖNTEMLER: Bu çalışmada, TQ'in sisplatin aracılı nefrotoksisiteye etkisini ve bu süreçte in vivo olarak inflamasyonu baskılama mekanizmalarını araştırdık. Sekiz haftalık 28 erkek Wistar rat 4 gruba ayrıldı; kontrol (1 mg/kg oral %0,9'luk tuz çözeltisi), TQ (5 gün boyunca içme suyuna 10 mg/kg/gün), sisplatin (7 mg/kg, i.p. tek doz) ve içme suyuyla 5 günlük TQ uygulaması sonrası tek doz sisplatin enjeksiyonu yapılan grup. Onuncu günde tüm ratlardan servikal dislokasyon sonrası böbrek dokuları ile üre ve kreatinin ölçümü amaçlı serumları alındı.

BULGULAR: Çalışmamız kontrol grubuna kıyasla sisplatin grubunda üre ve kreatinin değerlerinin anlamlı şekilde yüksek olduğunu göstermektedir ($p<0,0001$). TQ tedavisi serum üre ve kreatinin düzeylerini anlamlı şekilde azaltmıştır ($p<0,001$). Sisplatin tedavisi anlamlı biçimde ($p<0,05$) nükleer NF-E2 ilişkili faktör-2 (Nrf2) ve hem oksijenaz-1 (HO-1) downregülasyonuna neden olarak nükleer faktör-kappa B (NF-kB p65) düzeylerinde artmaya neden olmuştur. İlginç biçimde TQ desteği Nrf-2 ve HO-1 artışı ve NF-kB düzeylerinde anlamlı ($p<0,05$) azalmaya neden olarak sisplatin nefrotoksisitesine bağlı değişiklikleri düzeltmiştir.

SONUÇ: Bu çalışma TQ'in NRF2/HO-1'i hedef aldığını ve sisplatin aracılı nefrotoksisiteye karşı potansiyel koruyucu bir ajan olarak kullanılabileceğini ortaya koymaktadır.

ANAHTAR SÖZCÜKLER: Sisplatin, Böbrek, Nrf2, HO-1, Thymoquinone

Received : 29.11.2012
Accepted : 18.12.2012

Correspondence Address:
Ayhan DOĞUKAN
Fırat Üniversitesi Tıp Fakültesi,
Nefroloji Bilim Dalı, Elazığ, Turkey
Phone : + 90 424 233 35 55
E-mail : aydogukan@hotmail.com

INTRODUCTION

Cisplatin (cis-diamminedichloroplatinum II) and other platinum analouges are among the most effective antineoplastic drugs widely used in the treatment of human tumors, including prostate, breast, testicular, ovarian, lung, bladder, colon, brain, and head and neck (1). However, these compounds exhibit specific dose-dependent toxicity, limiting their therapeutic potential. The major dose-limiting factors in the therapy are nephrotoxicity, gastrointestinal toxicity, and neurotoxicity (2).

Following cisplatin treatment nephrotoxicity occurs in 25–33% of single dose patients and in 50–75% of multiple dose patients (3,4), and results in necrosis of proximal tubule (5). Cisplatin causes tubular injury through multiple mechanisms, including oxidative stress, inflammation apoptosis and DNA damage (6). Oxidative stress is caused by oxygen free radicals in the kidney, including the superoxide anion, the hydrogen peroxide and the hydroxyl radical (7,8). Cisplatin-induced acute renal failure is closely associated with enhanced lipid peroxidation in the kidney and with inhibitory activity against antioxidant defenses (7,9). Cisplatin-induced nephrotoxicity was also shown to be associated with an increase in the NF-κB protein and a decrease in the transcription factor Nrf2 (10,11). Additionally, significant interactions among these various pathways may occur in cisplatin injury. Transcription factor Nrf2 plays a central role in the regulation of phase II genes. Under basal conditions, Nrf2 is bound to Keap1 in the cytoplasm due to an interaction between a single Nrf2 protein and a Keap1 (12). Exposure to a number of stressors and inducing agents leads to dissociation of Nrf2 from Keap1 thereby rescuing Nrf2 from proteasomal degradation and allowing for entry into the nucleus. Thus, activation of Nrf2 is considered an important molecular target of cytoprotective agents (12–14).

Previous studies demonstrated that some of the dietary phytochemicals can downregulate Nrf2/HO-1 pathway, thereby sensitizing drug-resistant kidney cells to chemotherapeutic drug-induced apoptosis (10,11). Dietary phytochemical thymoquinone (TQ) is the main active ingredient of the volatile oil of *Nigella sativa* Linn, derived from the black seed. The black seed is an annual plant that has been widely used in the Indian subcontinent, Arabian countries and Europe for culinary and medicinal purposes (15, 16). TQ acts by various mechanisms such as: (i) anti-oxidant (15), (ii) anti-neoplastic effects (17), (iii) anti-histaminic (18), (iv) anti-inflammatory (19). TQ has also been shown ameliorated the nephrotoxicity from cisplatin and also improved cisplatin therapeutic effects in both mouse and rats (20). Earlier our group has demonstrated that TQ can augment chemotherapeutic effects of gemcitabine and oxaliplatin in pancreatic tumor models through down-regulation of the NF-kB pathway and activation of apoptotic signaling (21).

Here, we propose a protective mechanism of of TQ on cisplatin-nephrotoxicity in rats that is through modulation of Nrf2-mediated antioxidant induction and reduced inflammation.

MATERIALS and METHODS

Animals

Male Wistar rats (n=28, 8 weeks-old), weighing 180–245 g, were obtained from our University Research Center. The rats were kept in an environmentally controlled room with a constant temperature and humidity. The animals were exposed to a 12 h long light-dark cycle. The animals had free access to water and standard rodent diet ad libitum. All procedures involving rats were conducted in strict compliance with relevant laws, the Animal Welfare Act, Public Health Services Policy, and guidelines established by the Institutional Animal Care and Use Committee of the University.

Experimental Design

Nephrotoxicity was provoked by a single intraperitoneal (i.p.) injection of cisplatin. Cisplatin (Sigma Chemical Co, USA) was administered (7 mg/kg body weight) by i.p. injection as a single dose in 0.9% saline (1 ml/100 g BW, i.p.) (22,23). Rats were divided into four groups: 1) control group (no treatment; n=7) received a vehicle only (0.9% saline, 1 ml/kg body wt., p.o.); 2) TQ group (n=7) received TQ for 5 days (10 mg/kg body weight/day in drinking water); 3) cisplatin group (n=7) and 4) cisplatin and TQ group (n=7). After 5 days of TQ treatment at a dose of 10 mg/kg body weight/day in drinking water, rats were treated with a single i.p. injection of cisplatin (7 mg/kg body weight). The dose of TQ was selected based on preliminary and published data (24,25). On day 10 (5 days after the cisplatin treatment), all rats were sacrificed by cervical dislocation. Blood samples were taken and the kidneys were removed.

Western Blot

Protein extraction was performed by homogenizing the rat kidney in 1 ml ice-cold hypotonic buffer A, containing 10 mM HEPES (pH 7.8), 10 mM KCl, 2 mM MgCl2, 1 mM DTT, 0.1 mM EDTA, and 0.1 mM phenylmethylsulfonyl-fluoride (PMSF). To the homogenates 80 μl of 10% Nonidet P-40 (NP-40) solution was added and the mixture was centrifuged for 2 min. at 14,000g. The supernatant was collected as a cytosolic fraction for HO-1. The precipitate, containing nuclei, were washed once with 500 μl of buffer A plus 40 μl of 10% NP-40, centrifuged, resuspended in 200 μl of buffer C [50 mM HEPES (pH 7.8), 50 mM KCl, 300 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.1 mM PMSF, 20% glycerol], and centrifuged for 5 min at 14,800g. The supernatant containing nuclear proteins was collected for Nrf2 and NF-κB p65 (13). Equal amounts of protein (50 μg) were electro-phoresed and subsequently transferred to nitrocellulose membrane (Schleicher and Schuell Inc., Keene, NH, USA). The antibody against Nrf-2, HO-1 and NF-κB p65 were the purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Primary antibody was diluted (1:1000) in the same buffer containing 0.05% Tween-20. Protein loading was controlled using a monoclonal mouse antibody

against β-actin antibody (A5316; Sigma). Bands were analyzed densitometrically using an image analysis system (Image J; National Institute of Health, Bethesda, USA).

Laboratory Analyses

Blood samples were centrifuged at 3000 g for 10 min and sera were collected. Serum urea-N and creatinine were measured using biochemical analyzer (Olympus AU-660, Tokyo, Japan).

Statistical Analysis

Data are given as mean±SEM. Sample size was calculated based on a power of 85% and a p value of 0.05. Given that assumption, a sample size of seven per treatment was calculated. The data were analyzed using the GLM procedure of SAS (26). The treatments were compared using ANOVA and student's unpaired t test; $P < 0.05$ was considered statistically significant. Between group differences in latencies were analyzed by the analysis of variance for repeated measurements (ANOVAR) followed by Fisher's post hoc test for all groups.

RESULTS

Effect of TQ on renal function

As can be seen from results of Table I the levels of both serum urea-N and creatinine values were significantly elevated in cisplatin-treated rats compared with control rats, indicating renal injury was evoked by cisplatin administration ($P < 0.0001$). However, the changes in urea-N and creatine levels in cisplatin-treated rats were significantly reduced by administration of TQ in cisplatin-treated rats ($P < 0.001$) (Table I). Treatment with TQ alone did not affect the levels of urea-N and creatinine (Table I).

Expression of NF-ϰB p65, Nrf2 and HO-1

The expressions of NF-ϰB p65 was measured in kidneys at 5 days after cisplatin injection. The protein expressions of NF-ϰB p65 was decreased significantly in the kidneys of the TQ plus cisplatin treated group compared to the expression levels in the cisplatin-treated group ($P < 0.05$) (Figure 1). The expression of Nrf2 and HO-1 were decreased significantly in the kidneys of rats treated with cisplatin compared with the expression in the kidneys from control and TQ-only-treated rats ($P < 0.001$). TQ administration significantly increased the expression of Nrf2 and HO-1 in cisplatin-treated rats ($P < 0.05$) (Figure 1).

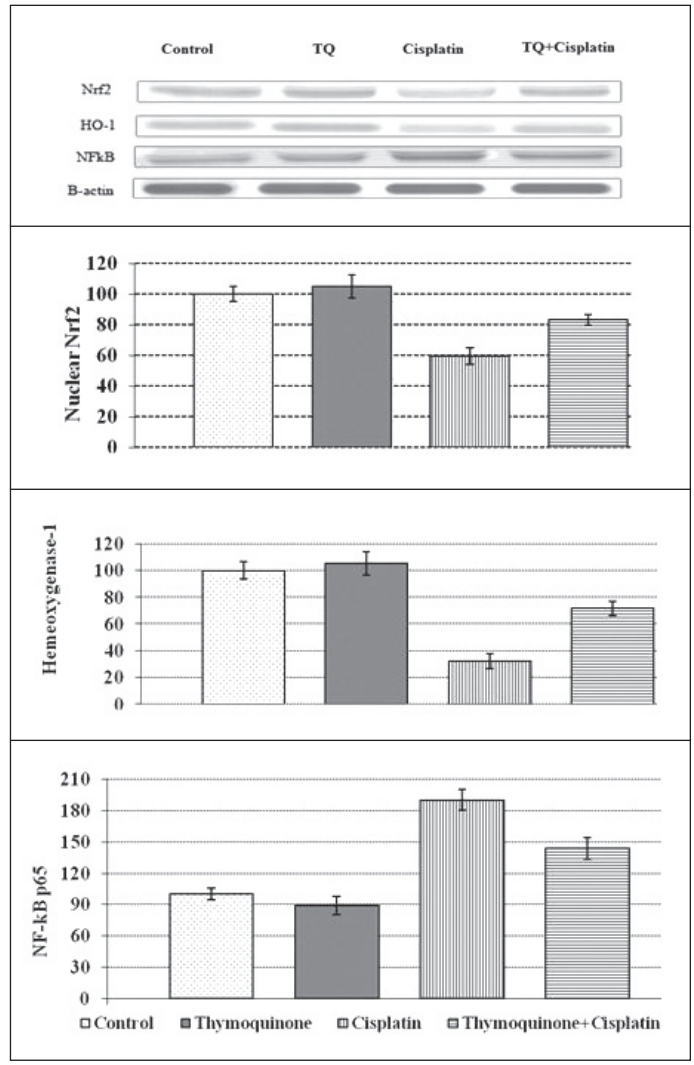

Figure 1: *Effect of TQ on nuclear erythroid 2-related factor 2 (Nrf2) (A), hemeoxygenase-1 (HO-1) (B) and nuclear factor-kappa B (NF-ϰB p65) (C) expressions in rats with cisplatin-induced kidney injury. The intensity of the bands was quantified by densitometric analysis. The bar represents the standard error of the mean. Blots were repeated at least 3 times (n=3) and a representative blot is shown. Actin was included to ensure equal protein loading. Data points with different superscripts are significantly different at the level of P < 0.05 by Fisher's multiple comparison test.*

DISCUSSION

In this study, we found that cisplatin causes increase in the levels of urea-N, creatine and hepatic nuclear factor-kappa B (NF-ϰB p65), a decrease in the hepatic expression nuclear levels of NF-E2-related factor-2 (Nrf2) and the heme oxygenase-1(HO-1). Interestingly, TQ supplementation significantly ($p<0.05$) reduced the changes associated with cisplatin nephrotoxicity by increasing the levels of Nrf-2 and HO-1 and decreasing the levels of NF-ϰB. This study for the first time demonstrates the role of Nrf2/HO-1 in TQ mediated preventive mechanisms against cisplatin-induced nephrotoxicity in rats.

The therapeutic effects of cisplatin, a potent anticancer drug, can be significantly improved by dose escalation. However, high-dose cisplatin therapy is not recommended because of the assocaited nephrotoxicity (27). Although several studies have been carried out to elucidate the molecular mechanism of cisplatin nephrotoxicity, the reasons accountable for this toxicity are not fully understood. Recently, it has been suggested that oxidative

Table I: The effect of TQ supplementation on urea-N and creatinine levels in kidney of experimental rats (n=7).

Item	Groups			
	Control	TQ	Cisplatine	TQ+Cisplatine
Urea-N (mg/dl)	53.5±15.8^{c}	55.1±2.4^{c}	152±10.5^{a}	104.1±2.71^{b}
Creatinine (mg/dl)	0.43±0.08^{c}	0.43±0.01^{c}	1.46±0.18^{a}	0.71±0.18^{b}

Values are mean±SD of 7 rats from each group.
a,b,c: means in the same row not sharing a common superscript are significantly different ($P < 0.05$) between groups.

stress and reactive oxygen species (ROS) are involved in the pathogenesis of cisplatin-induced nephrotoxicity (28). Based on these and other observations, various antioxidant agents have been studied for their effects against lipid peroxidation, cisplatin nephrotoxicity with positive outcome (9,29,30). Previously, we were able to demonstrate that lycopene and EGCG can have a preventive effect against cisplatin-induced nephrotoxicity in rats (10,11). In this study we investigated a potent natural compound ,TQ, extracted from Black Caraway seeds of *Nigella sativais*, that has been shown to exert anti-oxidant, anti-inflammatory, and anti-neoplastic effects both *in vitro* and *in vivo* (15,21). Earlier investigations have shown that oral administration of TQ at 50 mg/L in drinking water for 5 days before and after single injections of cisplatin of 5 mg/kg, i.v. can ameliorate cisplatin-induced nephrotoxicity in rats (20). In this study, we were able to demonstrate the effects of TQ on NF-κB and Nrf2/HO-1 in the kidneys of cisplatin-treated rats. Results show that cisplatin treatment is associated with changes in renal function which indicate nephrotoxicity. Conversely, treating animals with TQ prior to cisplatin administration significantly prevented the increase in nephrotoxicity by cisplatin, suggesting that TQ may have a potential protective effect against cisplatin-induced renal dysfunction through down regulation of NF-κB and a decrease serum urea and creatinine. These data are in agreement with previous report, which showed that TQ-induced amelioration of cisplatin nephrotoxicity (20).

Our data shows that cisplatin treatment has an inverse correlation between the transcription factors and are in agreement with previous studies (10,11,31,32). Components of the cell signaling network, especially those which converge on the ubiquitous eucaryotic redox-sensitive transcription factor NF-κB, have been implicated in pathogenesis of many inflammation-associated disorders. NF-κB is an oxidative stress sensitive transcription factor that plays a critical role in the regulation of a variety of genes important in cellular homeostasis and cell death (33). Nrf2 is present in the cytoplasm as an inactive complex with the inhibitory protein subunit, in the case Keap1. Nrf2 protects the cell against oxidative stress through ARE-directed induction of several phase-2 detoxifying and antioxidant enzymes, particularly the HO-1 (13,34–36). Moreover, NF-κB inhibitors have shown protection against cisplatin induced nephrotoxicity (32). TQ seems to exert its chemotherapeutic effects but the cellular mechanisms for this effect have not been characterized yet (37). In the present study, TQ supplementation inhibited NF-κB, and increased Nrf2 that was accompanied by an increase in HO-1. There are no studies investigating the effects of TQ supplementation on the Nrf2/HO-1 pathway in kidney. Earlier we reported that lycopene and EGCG can modulate Nrf2-Keap1 signaling, thereby potentiating cellular antioxidant capacity of facilitating detoxification of carcinogens and other toxicants (10,11). Similar to our results, Jafri et al. (38) reported that TQ down regulated NF-κB expression which may explain its various cellular activities and this activity may prove useful in overcoming cisplatin resistance that is primarily due over expression of NF-κB. It has also been reported that TQ can suppress expression of NF-κB activation pathway through modulation of p65 subunit of NF-κB and inhibition of IκBα kinase (IKK) expression in human chronic myeloid leukemia cells (39). Additionally**,** Banerjee et al. (21) reported that TQ can augment anti-tumor activity of gemcitabine and oxaliplatin in pancreatic cancer by down regulation of NF-κB. These investigations support our findings that indeed TQ mediated suppression of NF-kB can substantially suppress cisplatin nephrotoxicity. Indeed deeper understanding of the protective effect of TQ on cisplatin induced nephrotoxicity is necessary. Investigations on the mode of NF-kB suppression are critical to our understanding of this novel protective mechanism. In conclusion, we have demonstrated that increase in HO-1 through Nrf2 activation by TQ significantly inhibits inflammation mediated by NF-κB in rat with cisplatin-induced kidney injury. This study demonstrates the TQ targets NRF2/HO-1 and can be used as a potential agent against cisplatin-induced nephrotoxicity.

Acknowledgements

The authors declare that there are no conflicts of interest. All authors participated in data collection, laboratory analyses, and

organization of the study and preparation of the manuscript. All authors read and approved the final manuscript. Authors thank FUBAP for supporting this project.

REFERENCES

1. Rabik CA, Dolan ME: Molecular mechanisms of resistance and toxicity associated with platinating agents. Cancer Treat Rev 2007; 33: 9-23
2. Arany I, Safirstein RL: Cisplatin nephrotoxicity. Semin Nephrol 2003; 23: 460-464
3. Goldstein RS, Mayor GH: A minireview. The nephrotoxicity of cisplatin. Life Sci 1983; 32: 685-690
4. Lee KW, Jeong JY, Lim BJ, Chang YK, Lee SJ, Na KR, Shin YT, Choi DE: Sildenafil attenuates renal injury in an experimental model of rat cisplatin-induced nephrotoxicity. Toxicology 2009; 257: 137-143
5. Yao X, Panichpisal K, Kurtzman N, Nugent K: Cisplatin nephrotoxicity: A review. Am J Med Sci 2007; 334: 115-124
6. Pabla N, Dong Z: Cisplatin nephrotoxicity: Mechanisms and renoprotective strategies. Kidney Int 2008; 73: 994-1007
7. Kotnis MS, Patel P, Menon SN, Sane RT: Renoprotective effect of Hemidesmus indicus, a herbal drug used in gentamicin-induced renal toxicity. Nephrology (Carlton) 2004; 9: 142-152
8. Rao NK, Nammi S: Antidiabetic and renoprotective effects of the chloroform extract of Terminalia chebula Retz. seeds in streptozotocin-induced diabetic rats. BMC Complement Altern Med 2006; 7: 6-17
9. Shimeda Y, Hirotani Y, Akimoto Y, Shindou K, Ijiri Y, Nishihori T, Tanaka K: Protective effects of capsaicin against cisplatin-induced nephrotoxicity in rats. Biol Pharm Bull 2005; 28: 1635-1638
10. Sahin K, Tuzcu M, Gencoglu H, Dogukan A, Timurkan M, Sahin N, Aslan A, Kucuk O: Epigallocatechin-3-gallate activates Nrf2/HO-1 signaling pathway in cisplatin-induced nephrotoxicity in rats. Life Sci 2010; 87: 240-245
11. Sahin K, Tuzcu M, Sahin N, Ali S, Kucuk O: Nrf2/HO-1 signaling pathway may be the prime target for chemoprevention of cisplatin-induced nephrotoxicity by lycopene. Food Chem Toxicol 2010; 48: 2670-2674
12. Sriram N, Kalayarasan S, Sudhandiran G: Epigallocatechin-3-gallate augments antioxidant activities and inhibits inflammation during bleomycin-induced experimental pulmonary fibrosis through Nrf2-Keap1 signaling. Pulm Pharmacol Ther 2009; 22: 221-236
13. Farombi EO, Shrotriya S, Na HK, Kim SH, Surh YJ: Curcumin attenuates dimethylnitrosamine-induced liver injury in rats through Nrf2-mediated induction of heme oxygenase-1. Food Chem Toxicol 2008; 46: 1279-1287
14. Kim J, Cha YN, Surh YJ: A protective role of nuclear factor-erythroid 2-related factor-2 (Nrf2) in inflammatory disorders. Mutat Res 2010; 690: 12-23
15. Gali-Muhtasib H, Roessner A, Schneider-Stock R: Thymoquinone: A promising anti-cancer drug from natural sources. Int J Biochem Cell Biol 2006; 38: 1249-1253
16. Arafa el-SA, Zhu Q, Shah ZI, Wani G, Barakat BM, Racoma I, El-Mahdy MA, Wani AA: Thymoquinone up-regulates PTEN expression and induces apoptosis in doxorubicin-resistant human breast cancer cells. Mutat Res 2011; 706: 28-35
17. Swamy SM, Tan BK: Cytotoxic and immunopotentiating effects of ethanolic extract of Nigella sativa L. seeds. J Ethnopharmacol 2000; 70: 1-7
18. Chakravarty N: Inhibition of histamine release from mast cells by nigellone. Ann Allergy 1993; 70: 237-242
19. Abdel-Fattah AM, Matsumoto K, Watanabe H: Antinociceptive effects of Nigella sativa oil and its major component, thymoquinone, in mice. Eur J Pharmacol 2000; 400; 89-97
20. Badary OA, Nagi MN, al-Shabanah OA, al-Sawaf HA, al-Sohaibani MO, al-Bekairi AM: Thymoquinone ameliorates the nephrotoxicity induced by cisplatin in rodents and potentiates its antitumor activity. Can J Physiol Pharmacol 1997; 75: 1356-1361
21. Banerjee S, Kaseb AO, Wang Z, Kong D, Mohammad M, Padhye S, Sarkar FH, Mohammad RM: Antitumor activity of gemcitabine and oxaliplatin is augmented by thymoquinone in pancreatic cancer. Cancer Res 2009; 69(13): 5575-5583
22. Jones MM, Basinger MA, Beaty JA, Holscher MA: The relative nephrotoxicity of cisplatin, cis-[Pt(NH3)2(guanosine)2]2+, and the hydrolysis product of cisplatin in the rat. Cancer Chemother Pharmacol 1991; 29: 29-32
23. Saleh S, Ain-Shoka AA, El-Demerdash E, Khalef MM: Protective effects of the angiotensin II receptor blocker losartan on cisplatin-induced kidney injury. Chemotherapy 2009; 55: 399-406
24. Nagi MN, Mansour MA: Protective effect of thymoquinone against doxorubicin-induced cardiotoxicity in rats: A possible mechanism of protection. Pharmacol Res 2000; 41: 283-289
25. Awad AS, Kamel R, Sherief MA: Effect of thymoquinone on hepatorenal dysfunction and alteration of CYP3A1 and spermidine/spermine N-1-acetyl-transferase gene expression induced by renal ischaemia-reperfusion in rats. J Pharm Pharmacol 2011; 63: 1037-1042
26. SAS User's guide: Statistics. Cary, North Carolina, USA: SAS institute, Inc., 2002
27. O'Dwyer PJ, Stevenson JP, Johnson SW: Clinical Status of Cisplatin, Carboplatin and Other Platinum-Based Antitumor Drugs. In: Lippert B, (ed). Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug. Zurich: Wiley-VCH, 1999: 29-70
28. Dogukan A, Tuzcu M, Agca CA, Gencoglu H, Sahin N, Onderci M, Ozercan IH, Ilhan N, Kucuk O, Sahin K: A tomato lycopene complex protects the kidney from cisplatin-induced injury via affecting oxidative stress as well as Bax, Bcl-2, and HSPs expression. Nutr Cancer 2011; 63: 427-434
29. Al-Majed AA, Abd-Allah AR, Al-Rikabi AC, Al-Shabanah OA, Mostafa AM: Effect of oral administration of Arabic gum on cisplatin-induced nephrotoxicity in rats. J Biochem Mol Toxicol 2003; 17: 146-153

30. Sung MJ, Kim DH, Jung YJ, Kang KP, Lee AS, Lee S, Kim W, Davaatseren M, Hwang JT, Kim HJ, Kim MS, Kwon DY, Park SK: Genistein protects the kidney from cisplatin-induced injury. Kidney Int 2008; 74: 1538-1547

31. Francescato HD, Costa RS, Júnior FB, Coimbra TM: Effect of JNK inhibition on cisplatin-induced renal damage. Nephrol Dial Transplant 2007; 22: 2138-2148

32. Francescato HD, Costa RS, Scavone C, Coimbra TM: Parthenolide reduces cisplatin-induced renal damage. Toxicology 2007; 230: 64-75

33. Syed DN, Afaq F, Kweon MH, Hadi N, Bhatia N, Spiegelman VS, Mukhtar H: Green tea polyphenol EGCG suppresses cigarette smoke condensate-induced NF-kappaB activation in normal human bronchial epithelial cells. Oncogene 2007; 26: 673-682

34. Prawan A, Kundu JK, Surh YJ: Molecular basis of heme oxygenase-1 induction: Implications for chemoprevention and chemoprotection. Antioxid Redox Signal 2005; 7: 1688-1703

35. McNally SJ, Harrison EM, Ross JA, Garden OJ, Wigmore SJ: Curcumin induces heme oxygenase 1 through generation of reactive oxygen species, p38 activation and phosphatase inhibition. Int J Mol Med 2007; 19: 165-172

36. Surh YJ, Kundu JK, Na HK: Nrf2 as a master redox switch in turning on the cellular signaling involved in the induction of cytoprotective genes by some chemopreventive phytochemicals. Planta Med 2008; 74: 1526-1539

37. Shoieb AM, Elgayyar M, Dudrick PS, Bell JL, Tithof PK: In vitro inhibition of growth and induction of apoptosis in cancer cell lines by thymoquinone. Int J Oncol 2003; 22: 107-113

38. Jafri SH, Glass J, Shi R, Zhang S, Prince M, Kleiner-Hancock H: Thymoquinone and cisplatin as a therapeutic combination in lung cancer: In vitro and in vivo. J Exp Clin Cancer Res 2010; 1: 29-87

39. Sethi G, Ahn KS, Aggarwal BB: Targeting nuclear factor-kappa B activation pathway by thymoquinone: Role in suppression of antiapoptotic gene products and enhancement of apoptosis. Mol Cancer Res 2008; 6: 1059-1070