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ORIGINAL ARTICLE

Beneficial effects of thymoquinone and omega-3 on intestinal ischemia/reperfusion-induced renal dysfunction in rats

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Abstract Intestinal ischemia–reperfusion (II/R) is a complex phenomenon causing local and remote tissue destruction and multiple-organ dysfunction. The technique has been used by many authors to produce certain organ dysfunctions. This study is to investigate the possible beneficial effects of thymoquinone and omega-3 separately in II/R-induced renal dysfunction in rats. Sixty-four Wistar albino rats were randomly allocated into four experimental groups namely sham control, II/R control, thymoquinone and omega-3 each group pre-treated separately. II/R model was established by clamping the superior mesenteric artery (SMA) for 30 min followed by 60 min reperfusion. Serum level of creatinine was measured. Renal tissue contents of malodialdehyde (MDA) and reduced glutathione (GSH) as well as myeloperoxidase (MPO), tumor necrosis factor alpha (TNF-α) and superoxide dismutase (SOD) activities were measured. Apoptosis in renal tissue cells was determined by immunohistochemical analysis of caspase-3. Renal histopathological examination was carried out.

II/R elevated serum creatinine level. In-addition, renal tissue content of GSH and SOD activity were decreased. However renal tissue content of MDA and MPO activity were increased. Immunohistochemical analysis showed remarkable activation of caspase-3 in renal tissue. Histopathological examination revealed definite alterations after II/R.

Pre-treatment with thymoquinone and omega-3 resulted in increased renal tissue content of GSH and SOD activity. The results revealed significant decrease in renal tissue content of MDA as well as MPO activity. Test drugs decreased the activity of caspase-3 through immunohistochemical examination. Thymoquinone and omega-3 corrected the reported histopathological changes induced by II/R.

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Depending on the obtained results in the present study it could be concluded that thymoquinone and omega-3 have beneficial effects on II/R-induced renal dysfunction in rats. The protective potential could be attributed to the antioxidant, antiapoptotic and anti-inflammatory effects of test drugs.

1. Introduction

Ischemia/reperfusion (I/R) is a pathological condition characterized by an initial restriction of blood supply to an organ followed by the subsequent restoration of perfusion and concomitant re-oxygenation.[1] The absence of oxygen and nutrients from blood during the ischemic period and reperfusion results in inflammation and oxidative damage through the induction of oxidative stress. Surprisingly, restoration of blood flow and re-oxygenation is frequently associated with tissue injury and a profound inflammatory response called reperfusion injury.[2]

Intestinal ischemia/reperfusion (II/R) leads to systemic inflammation and multiple organ failure in clinical and laboratory settings.[3] II/R injury includes production of reactive oxygen species (ROS), inflammatory cell infiltration, and cytokine production.[4] Increased oxidative stress and inhibition of cellular antioxidant defense mechanisms are associated with apoptotic cell death.[5] Malondialdehyde (MDA) is one of the final products of lipid peroxidation. MDA can be found in both tissue and blood, and its concentration is directly proportional to the cell damage caused by free radicals.[6] Antioxidant enzymes, including reduced glutathione (GSH) and superoxide dismutase (SOD), protect tissues from reperfusion injury by destroying ROS.[7]

One of the main orchestrators of II/R injury is the proinflammatory cytokine tumor necrosis factor (TNF-α).[8] Pharmacological modulation of TNF-α production is a promising strategy in the prevention of II/R injury.[9] II/R activates various programs of cell death, which can be categorized as necrosis, apoptosis or autophagy-associated cell death. Apoptosis induced by II/R involves an orchestrated caspase signaling cascade that induces a self-contained program of cell death, characterized by the shrinkage of the cell and its nucleus.[10]

Thymoquinone (TQ), a component of Nigella sativa is known to have a wide range of anti-inflammatory activities and attenuates allergic inflammation. TQ attenuates the proinflammatory response mainly by modulating nuclear transactivation of NF-kappa B and TNF-α production.[11]

Omega-3 fatty acid is one of the major constituents of fish oil. It has been shown that omega-3 has beneficial effects in multiple disease states that involve an inflammatory process. The effects of omega-3 were reported to be through modulation of inflammatory mediators such as TNF-α as well as inhibition of apoptotic marker caspase-3.[12] The present study is constructed in order to explore the effects of TQ and omega-3 in II/R-induced renal dysfunction in rats.

2. Materials and methods

2.1. Animals

Adult male Wistar albino rats, weighing 150–180 g were used in the present study. Animals were purchased from the National Institute of Ophthalmology, Cairo, Egypt. Animals were kept in the animal center under appropriate conditions of temperature, humidity and light. The study was carried out according to the approval of ethics committee for animal experimentation at the faculty of pharmacy, Cairo University. Animals were fed standard pellet chow (El-Nasr Chemical Co., Cairo, Egypt) and were allowed water *ad libitum*.

2.2. Drugs

Thymoquinone (Sigma–Aldrich Co, USA) and Omega-3 (Metagenics, INC., Norway), each were separately used in this study.

2.3. Experimental design

Rats were randomly allocated into 4 groups (16 rats each) as follows:

- Group 1: Rats exposed to sham operation and served as sham control.
- Group 2: Rats received distilled water orally for 14 days and then exposed to II/R operation and served as II/R control.
- Group 3: Rats received thymoquinone (10 mg/kg) dissolved in distilled water orally[13,14] for 14 days and then exposed to II/R operation.
- Group 4: Rats received omega-3 (300 mg/kg) orally[15] for 14 days and then exposed to II/R operation and served as reference standard.

2.4. Induction of II/R

The II/R rat model was established according to the method described by Cheng et al.[16] Superior mesenteric artery was clamped for 30 min followed by reperfusion for 60 min. At the end of the experiment, blood samples were collected via the retro-orbital plexus under light anesthesia (di-ethyl ether), serum was separated, for biochemical parameters. Animals were sacrificed by cervical dislocation, kidney was rapidly isolated and washed with ice cold saline. One kidney was homogenized in phosphate buffer saline (pH 7.5). The other kidney was embedded in 10% formalin for histopathological examination and immunohistochemical staining.

2.5. Determination of MDA, GSH and SOD

Renal tissue contents of MDA and GSH as well as SOD activity in renal tissue were determined spectrophotometrically using kits supplied by Biodiagnostic, Egypt.

2.6. Determination of serum creatinine

The serum level of creatinine was measured spectrophotometrically using kit supplied by Biodiagnostic, Egypt.

2.7. Determination of TNF-α and MPO

Renal tissue activities of TNF-α and MPO were measured by ELISA using kits obtained from Assay Pro and Hycult Biotechnology, USA, respectively.

2.8. Histopathological and immunohistochemical examination

Kidney specimens were fixed in 10% formalin and embedded in paraffin. Tissue sections (4 μm) were stained with hematoxylin and eosin (H&E). Immunohistochemistry for caspase-3 was performed in sections prepared from formalin-fixed, paraffin-embedded tissue using the avidin–biotin immunodetection complex method according to manufacturer's instruction (Labvision, USA). Interpretation of results was done semiquantitatively by evaluating both intensity and distribution of positive cells. Cytoplasmic staining for caspase-3 was carried in renal cells. The intensity of caspase-3 immunostaining was assessed as follows: none = 0, mild = 1, moderate = 2 and strong = 3. The immunohistochemical histological score (H-score) was then calculated by multiplying the intensity by the percentage of renal cells showing positive staining for caspase-3, creating a range of possible scores of 0–300.

2.9. Statistical analysis

Results were expressed as means ± standard error of mean (SEM). Comparisons between different groups were carried out by One-Way Analysis of Variance (ANOVA) followed by the Tukey–Kramer multiple comparison test. The level of significance was set at $p < 0.05$. Graphpad software instat (version 2) was used to carry out statistical analysis.

3. Results

3.1. Effect of TQ and omega-3 separately on renal tissue MDA, GSH and SOD

II/R resulted in significant increase in MDA content and significantly decreased renal tissue content of GSH and renal tissue SOD activity. Pre-treatment of TQ and omega-3 significantly decreased renal tissue content of MDA. The two test drugs significantly increased renal tissue content of GSH and renal tissue SOD activity (Table 1).

3.2. Effect of TQ and omega-3 separately on serum creatinine

II/R resulted in significant increase in serum creatinine. Pre-treatment with TQ and omega-3 resulted in significant decrease in serum creatinine (Table 2).

3.3. Effect of TQ and omega-3 separately on renal TNF-α

No significant changes occurred in renal tissue activity of TNF-α neither after II/R nor after administration of test drugs (Fig. 1a).

3.4. Effect of TQ and omega-3 separately on renal MPO

II/R resulted in significant increase in renal tissue activity of MPO. Pre-treatment with TQ and omega-3 resulted in significant decrease in renal tissue activity of MPO (Fig. 1b).

3.5. Effect of TQ and omega-3 separately on renal histopathological finding

Rats subjected to II/R showed inflammation in the lining endothelium of the glomerular tuft. Rats treated with TQ showed few inflammations in the perivascular area of the dilated blood vessels. Rats treated with omega-3 showed normal tubules in the endothelial cell lining of the glomerular tuft (Fig. 2).

3.6. Effect of TQ and omega-3 separately on renal immunohistochemical staining of caspase-3

Immunohistochemical staining of caspase-3 in the sham control group was mild positive in 30% of renal cells (H-score = 30). While it was strongly positive in 50% of renal cells in the II/R control group (H-score = 150). Rats given TQ were moderately positive caspase-3 in 30% of renal cells (H-score = 60) and rats given omega-3 were strongly positive caspase-3 in 10% of renal cells (H-score = 30) (Fig. 3).

4. Discussion

Intestinal ischemia/reperfusion (II/R) is considered to be a serious and triggering event in the development of local and distant organ dysfunction[17], which involves renal diseases.[18]

Table 1 Effect of fourteen day daily dose administration of TQ and omega-3 separately on renal tissue contents of MDA and GSH as well as SOD activity.

Groups	MDA (nmol/g.tissue)	GSH (mg/g.tissue)	SOD (U/mg.tissue)
Sham control	78.23 ± 6.699	114.65 ± 10.029	28.12 ± 2.011
II/R control	139.45 ± 11.369[a]	50.83 ± 2.633[a]	9.66 ± 0.83[a]
TQ + II/R	85.69 ± 7.678[b]	100.78 ± 8.484[b]	18.97 ± 1.237[b]
Omega-3 + II/R	81.23 ± 6.960[b]	103.98 ± 9.073[b]	20.45 ± 1.879[b]

Thymoquinone (10 mg/kg; P.O.) as well as omega-3(300 mg/kg; P.O.) was administered for 14 days before II/R.
Data are presented as the mean ± SEM.
[a] Significantly different from sham control at $P < 0.05$.
[b] Significantly different from II/R control at $P < 0.05$.

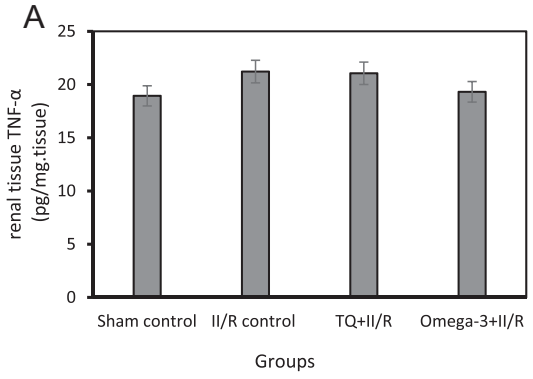
Table 2 Effect of fourteen day daily dose administration of TQ and omega-3 separately on serum creatinine.

Groups	Creatinine (mg/dl)
Sham control	0.633 ± 0.052
II/R control	1.882[a] ± 0.144
TQ + II/R	1.066 [b] ± 0.110
Omega-3 + II/R	0.931[b] ± 0.087

Thymoquinone (10 mg/kg; P.O.) as well as omega-3(300 mg/kg; P.O.) was administered for 14 days before II/R.
Data are presented as the mean ± SEM.
[a] Significantly different from sham control at $P < 0.05$.
[b] Significantly different from II/R control at $P < 0.05$.

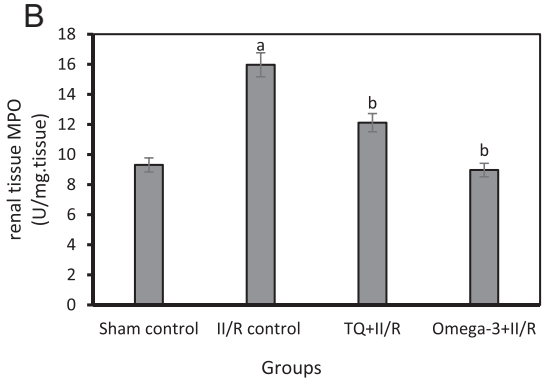
II/R-induced renal dysfunction is confirmed biochemically by decreased renal tissue content of GSH and the activity of SOD along with increased renal tissue content of MDA and MPO activity. These findings are in agreement with Zhao et al.[17] who reported that the activity of SOD in the tissue decreased after ischemia/reperfusion. He stated that tissue content of GSH significantly decreased after ischemia/reperfusion. SOD is the major enzyme for scavenging ROS, and its activity can reflect the functional status. Glutathione plays an important role in the prevention of oxidative damage as a direct scavenger.[19] Under normal conditions, free radicals are neutralized by endogenous antioxidants such as SOD and GSH.[20] However, oxidative stress occurs when the production of oxidants exceeds the capacity of the antioxidant defense systems of the cell, or when the effectiveness of the antioxidant defense system decreases.[18] The elevation in MDA renal tissue content and MPO renal tissue activity is in accordance with Pergel et al.[21] who stated that II/R significantly increased the tissue MDA levels and Zhao et al.[17] who reported that tissue MPO activity increased significantly after II/R.

Increased MPO activity is indicative of neutrophil activation because MPO is found almost exclusively within neutrophils.[22] Activated neutrophils release more ROS and various enzymes.[7,23,24] Also, oxygen radical-initiates lipid peroxidation



Thymoquinone (10 mg/kg; P.O.) as well as omega-3(300 mg/kg; P.O.) was administered for 14 days before II/R.
Data are presented as the mean ± SEM.

Figure 1a Effect of fourteen day daily dose administration of TQ and omega-3 on renal tissue activity of TNF-α.



Thymoquinone (10 mg/kg; P.O.) as well as omega-3(300 mg/kg; P.O.) was administered for 14 days before II/R.
Data are presented as the mean ± SEM.
a: significantly different from sham control at P<0.05.
b: significantly different from II/R control at P<0.05.

Figure 1b Effect of fourteen day daily dose administration of TQ and omega-3 on renal tissue activity of MPO.

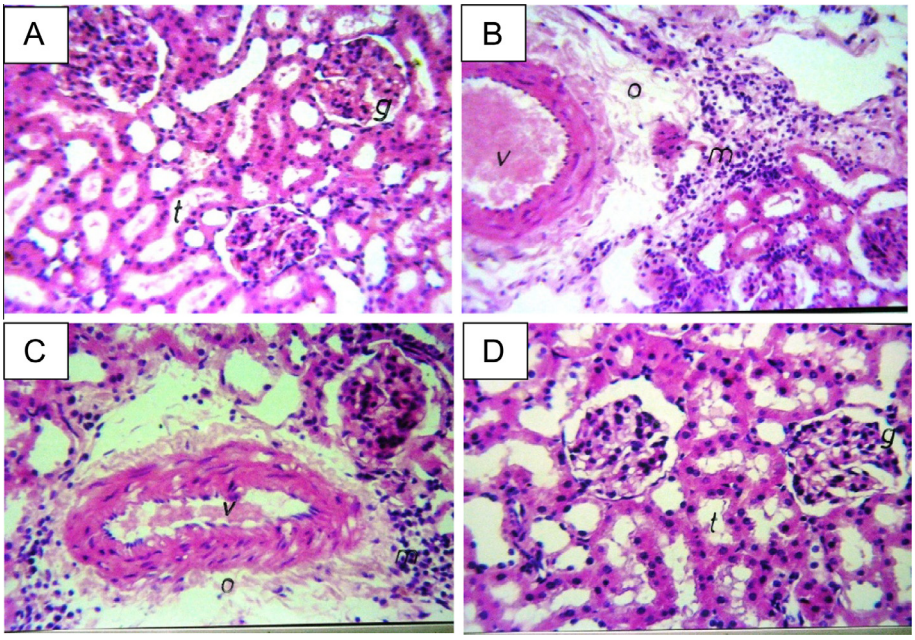


Figure 2 Renal histological findings of the sham control group showed normal glomeruli and tubules [A]. Those from the II/R control group [B] showed swelling and vacuolization in the lining endothelium of the glomerular tuft and inflammatory cell infiltration. Those treated with thymoquinone [C] showed few inflammatory cell infiltrations. Those treated with omega-3 [D] showed normal tubules (t).

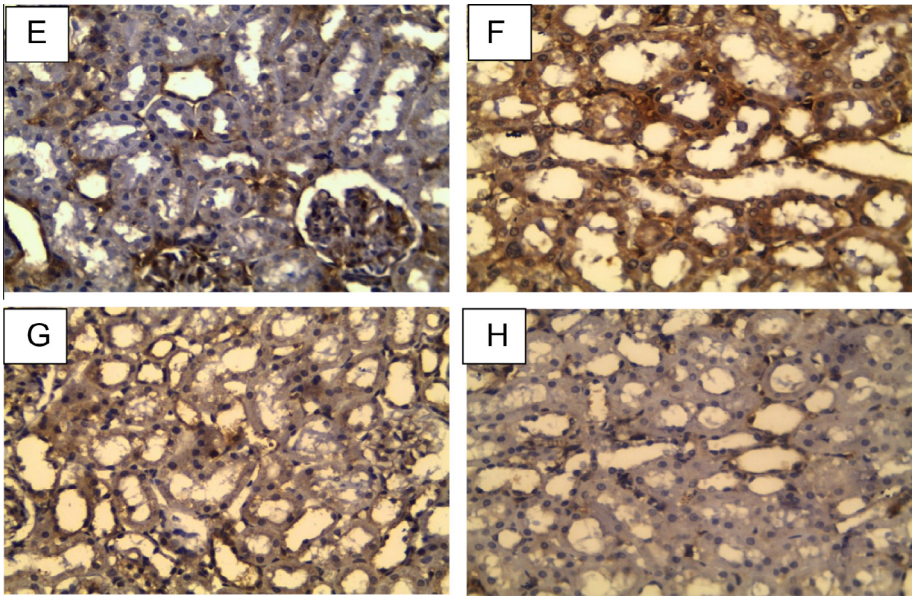


Figure 3 Renal immunohistochemical staining of caspase-3 in the sham control group was mild positive in 30% of renal tissue [E]. Those from the II/R control group, caspase-3 was strongly positive in 50% of renal tissue [F]. Those treated with thymoquinone caspase-3 was moderately positive in 30% of renal tissue [G]. Those treated with omega-3 caspase-3 was strongly positive in 10% of renal tissue [H].

and protein damage may contribute to the impaired cellular function and necrosis associated with reperfusion.[25] MDA is one of the final products of lipid peroxidation and can be measured in both tissue and blood, and its concentration is directly proportional to the cell damage caused by free radicals.[26] Antioxidants, including GSH and SOD, protect tissues from reperfusion injury by destroying ROS.[27,28]

In the present study TQ and omega-3 separately increased renal tissue content of GSH and SOD activity. Similar results have been reported by Fouda et al.[13] who stated that thymoquinone protected kidney damage by preventing renal GSH depletion and have a beneficial effect in restoring declined SOD activity due to ischemic insult. Romieu et al.[29] reported that supplementation with omega-3 was related to an increase in SOD activity and an increase in GSH plasma levels. Moreover test drugs decreased MDA content and MPO activity these results are in accordance with Umar et al.[30] who reported that TQ was effective in bringing significant reduction in MDA

and MPO. Bento et al.[31] reported that omega-3 has been demonstrated to reduce MPO activity in mouse colon. Benson and Devi[32] stated that omega-3 decreased MDA significantly.

The effect of TQ on GSH, SOD, MDA and MPO could be attributed to the antioxidant power of thymoquinone in various models of oxidative stress. Thymoquinone acts as a scavenger of superoxide, hydroxyl radical and singlet molecular oxygen.[33] Previous studies have shown that pre-treatment with thymoquinone protected organs against oxidative damage induced by a variety of free radical generating agents, including cisplatin,[34] carbon tetrachloride[35] and doxorubicin.[36] Moreover, thymoquinone supplementation has recently been shown to prevent deterioration of the biochemical and histological indices of gentamicin-induced nephrotoxicity, which coincide with the increase in the total antioxidant status in renal cortex, including GSH concentration.[37] The strong antioxidant potentials of thymoquinone may be related to the redox properties of the quinone structure of thymoquinone molecule and its unrestricted crossing of morphophysiological barriers, and easy access to subcellular compartments facilitates the ROS scavenging effect.[38] Brunmark et al.[39] have shown that quinone reductase, catalyzes the two electron reduction of quinones to hydroquinones, preventing their participation in redox cycling, and subsequent generation of ROS. Quinone reductase activity was found in all rat tissues including the kidney and can catalyze the two electron reduction of thymoquinone to form dihydro thymoquinone.[40]

Omega-3 administration could cause its beneficial effects by its known anti-inflammatory properties through displacement of arachidonic acid from the cellular membrane, shifting of prostaglandin E2 and leukotriene B4 production.[41] Although the exact mechanism of cytoprotection by Omega-3-fattyacids remains unresolved, certain effects of omega-3 suggest that this substance could serve as an antioxidant that makes the tissues less susceptible to the damaging action of noxious agents.[42]

In the current study no significant changes occurred in the renal tissue content of TNF-α. This result is in accordance with the data of Donnahoo et al.[43] who found that 30 min of ischemia followed by 1 h of reperfusion did not increase renal tissue content of TNF-α. However, TNF-α content increased significantly when the animals were exposed to 1 h of ischemia followed by 1 h of reperfusion.

A valuable and more selective tool to identify apoptotic cells in solid tissues may be the use of antibodies that specifically detect the cleaved subunits of caspases, because activation of these enzymes constitutes a key molecular event during the process of apoptosis. In fact, the utility of this approach has been proven in previous studies by other groups.[44–46] The present data showed that immunostaining for caspase-3, as a reliable marker of apoptosis, in rat kidney caspase-3 was strongly activated in 50% renal cells in the II/R control group compared to mild activation in 30% renal tissues in sham control rats. This result is in agreement with El Gazzar et al.[11] and Giakoustidis et al.[47], who stated that activated caspace-3 was widely activated after ischemia/reperfusion, although a very limited amount was detected in the sham operation animals. Activation of caspase-3 may explain the injury caused after II/R. Thymoquinone administration in this study reduced apoptosis through decreasing the activation of caspase-3. This is in agreement with Fouda et al.[13] who reported that apoptosis and proliferative reactions are reduced by thymoquinone. Administration of omega-3 in the present study also reduced caspase-3 activity. This is in agreement with El-Ansary et al.[12] and could be attributed to the powerful antioxidant effect of TQ and omega-3.

Histopathological examination showed swelling and vacuolization in the lining endothelium of the glomerular tuft and inflammatory cell infiltration in the II/R control group. Thymoquinone showed a marked improvement in the renal cell structure and omega-3 showed a normal histopathological picture. TQ protective effect could be attributed to the potent superoxide anion scavenging abilities, and inhibiting iron-dependent microsomal lipid peroxidation.[48] Beltz et al.[49] attributed the beneficial action of omega-3 to its anti-inflammatory effect. Moreover Song et al.[50] reported that omega-3 decreased the production of pro-inflammatory cytokines, which is another possible explanation for omega-3 action on renal tissue.

In conclusion, the present study revealed that the protective effect of TQ and omega-3 against renal dysfunction induced by intestinal ischemia/reperfusion is attributed, at least in part, to their profound anti-inflammatory and antioxidant activities.

5. Conflicts of interest

The authors declare that they have no potential conflicts of interest.

References

1. Yellon DM, Hausenloy DJ. Myocardial reperfusion injury. *N Engl J Med* 2007;**357**(11):1121–35.
2. Takizawa Y, Kitazato T, Kishimoto H, Tomita M, Hayashi M. Effects of antioxidants on drug absorption in *in vivo* intestinal ischemia/reperfusion. *Eur J Drug Metab Pharmacokinet* 2011;**35**(3–4):89–95.
3. Santora RJ, Lie ML, Grigoryev DN, Nasir O, Moore FA, Hassoun HT. Therapeutic distant organ effects of regional hypothermia during mesenteric ischemia-reperfusion injury. *J Vasc Surg* 2010;**52**(4):1003–14.
4. Paterno F, Longo WE. The etiology and pathogenesis of vascular disorders of the intestine. *Radiol Clin North Am* 2008;**46**(5):877–85, v.
5. Hayes JD, McLellan LI. Glutathione and glutathione-dependent enzymes represent a co-ordinately regulated defence against oxidative stress. *Free Radic Res* 1999;**31**(4):273–300.
6. Halliwell B, Gutteridge JM. Role of free radicals and catalytic metal ions in human disease: an overview. *Methods Enzymol* 1990;**186**:1–85.
7. Deshmukh DR, Mirochnitchenko O, Ghole VS, Agnese D, Shah M, Reddell M, et al. Intestinal ischemia and reperfusion injury in transgenic mice overexpressing copper-zinc superoxide dismutase. *Am J Physiol* 1997;**273**(4 Pt 1):C1130–5.
8. Pascher A, Klupp J. Biologics in the treatment of transplant rejection and ischemia/reperfusion injury: new applications for TNFalpha inhibitors? *BioDrugs* 2005;**19**(4):211–31.
9. Camara-Lemarroy CR, Guzman-de la Garza FJ, Alarcon-Galvan P, Cordero-Perez P, Munoz-Espinosa LE, Fernandez-Garza NE. Effects of thalidomide and pentoxyphylline over local and remote organ injury after intestinal ischemia/reperfusion. *Transplant Proc* 2010;**42**(5):1624–6.
10. Hotchkiss RS, Strasser A, McDunn JE, Swanson PE. Cell death. *N Engl J Med* 2009;**361**(16):1570–83.
11. El Gazzar MA, El Mezayen R, Nicolls MR, Dreskin SC. Thymoquinone attenuates proinflammatory responses in lipopolysaccharide-activated mast cells by modulating NF-kappaB nuclear transactivation. *Biochim Biophys Acta* 2007;**1770**(4):556–64.

12. El-Ansary AK, Al-Daihan SK, El-Gezeery AR. On the protective effect of omega-3 against propionic acid-induced neurotoxicity in rat pups. *Lipids Health Dis* 2011;**10**:142–51.
13. Fouda AM, Daba MH, Dahab GM, Sharaf El-Din OA. Thymoquinone ameliorates renal oxidative damage and proliferative response induced by mercuric chloride in rats. *Basic Clin Pharmacol Toxicol* 2008;**103**(2):109–18.
14. Padhye S, Banerjee S, Ahmad A, Mohammad R, Sarkar FH. From here to eternity – the secret of Pharaohs: therapeutic potential of black cumin seeds and beyond. *Cancer Ther* 2008;**6**(b):495–510.
15. Kavakli A, Kose E, Kus I, Zararsiz I, Akpolat N, Sarsilmaz M. Protective effect of omega-3 fatty acids in a rat focal cerebral ischemia-reperfusion model. *Neurosciences (Riyadh)* 2007;**12**(3):198–201.
16. Cheng CH, Lin HC, Lai IR, Lai HS. Ischemic postconditioning attenuate reperfusion injury of small intestine: impact of mitochondrial permeability transition. *Transplantation* 2013;**95**(4):559–65.
17. Zhao HD, Zhang F, Shen G, Li YB, Li YH, Jing HR, et al. Sulforaphane protects liver injury induced by intestinal ischemia reperfusion through Nrf2-ARE pathway. *World J Gastroenterol* 2010;**16**(24):3002–10.
18. Sasaki M, Joh T. Oxidative stress and ischemia-reperfusion injury in gastrointestinal tract and antioxidant, protective agents. *J Clin Biochem Nutr* 2007;**40**(1):1–12.
19. Kayanoki Y, Fujii J, Islam KN, Suzuki K, Kawata S, Matsuzawa Y, et al. The protective role of glutathione peroxidase in apoptosis induced by reactive oxygen species. *J Biochem* 1996;**119**(4):817–22.
20. Cerqueira NF, Hussni CA, Yoshida WB. Pathophysiology of mesenteric ischemia/reperfusion: a review. *Acta Cir Bras* 2005;**20**(4):336–43.
21. Pergel A, Kanter M, Yucel AF, Aydin I, Erboga M, Guzel A. Anti-inflammatory and antioxidant effects of infliximab in a rat model of intestinal ischemia/reperfusion injury. *Toxicol Ind Health* 2012;**28**(10):923–32.
22. Mullane KM, Kraemer R, Smith B. Myeloperoxidase activity as a quantitative assessment of neutrophil infiltration into ischemic myocardium. *J Pharmacol Methods* 1985;**14**(3):157–67.
23. Horton JW, White DJ. Free radical scavengers prevent intestinal ischemia-reperfusion-mediated cardiac dysfunction. *J Surg Res* 1993;**55**(3):282–9.
24. Schoenberg MH, Beger HG. Oxygen radicals in intestinal ischemia and reperfusion. *Chem Biol Interact* 1990;**76**(2):141–61.
25. Zimmerman BJ, Granger DN. Oxygen free radicals and the gastrointestinal tract: role in ischemia-reperfusion injury. *Hepatogastroenterology* 1994;**41**(4):337–42.
26. Gutteridge JM, Halliwell B. The measurement and mechanism of lipid peroxidation in biological systems. *Trends Biochem Sci* 1990;**15**(4):129–35.
27. Deshmukh MD. Pulmonary tuberculosis, its incidence, prevalence and management. *J Assoc Physicians India* 1977;**25**(11):777–88.
28. Dunning S, Ur Rehman A, Tiebosch MH, Hannivoort RA, Haijer J, Woudenberg J, et al. Glutathione and antioxidant enzymes serve complementary roles in protecting activated hepatic stellate cells against hydrogen peroxide-induced cell death. *Biochim Biophys Acta* 2013;**1832**(12):2027–34.
29. Romieu I, Garcia-Esteban R, Sunyer J, Rios C, Alcaraz-Zubeldia SR, Velasco SR, et al. The effect of supplementation with omega-3 polyunsaturated fatty acids on markers of oxidative stress in elderly exposed to PM(2.5). *Environ Health Perspect* 2008;**116**(9):1237–42.
30. Umar S, Zargan J, Umar K, Ahmad S, Katiyar CK, Khan HA. Modulation of the oxidative stress and inflammatory cytokine response by thymoquinone in the collagen induced arthritis in Wistar rats. *Chem Biol Interact* 2012;**197**(1):40–6.
31. Bento AF, Claudino RF, Dutra RC, Marcon R, Calixto JB. Omega-3 fatty acid-derived mediators 17(*R*)-hydroxy docosahexaenoic acid, aspirin-triggered resolvin D1 and resolvin D2 prevent experimental colitis in mice. *J Immunol* 2011;**187**(4):1957–69.
32. Benson MK, Devi K. Influence of omega-6/omega-3 rich dietary oils on lipid profile and antioxidant enzymes in normal and stressed rats. *Indian J Exp Biol* 2009;**47**(2):98–103.
33. Kruk I, Michalska T, Lichszteld K, Kladna A, Aboul-Enein HY. The effect of thymol and its derivatives on reactions generating reactive oxygen species. *Chemosphere* 2000;**41**(7):1059–64.
34. 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**(12):1356–61.
35. Mansour MA. Protective effects of thymoquinone and desferrioxamine against hepatotoxicity of carbon tetrachloride in mice. *Life Sci* 2000;**66**(26):2583–91.
36. Nagi MN, Mansour MA. Protective effect of thymoquinone against doxorubicin-induced cardiotoxicity in rats: a possible mechanism of protection. *Pharmacol Res* 2000;**41**(3):283–9.
37. Verstrepen WA, Nouwen EJ, Zhu MQ, Ghielli M, De Broe ME. Time course of growth factor expression in mercuric chloride acute renal failure. *Nephrol Dial Transplant* 1995;**10**(8):1361–71.
38. Daba MH, Abdel-Rahman MS. Hepatoprotective activity of thymoquinone in isolated rat hepatocytes. *Toxicol Lett* 1998;**95**(1):23–9.
39. Brunmark A, Cadenas E, Segura-Aguilar J, Lind C, Ernster L. DT-diaphorase-catalyzed two-electron reduction of various p-benzoquinone- and 1,4-naphthoquinone epoxides. *Free Radic Biol Med* 1988;**5**(3):133–43.
40. Nagi MN, Alam K, Badary OA, al-Shabanah OA, al-Sawaf HA, al-Bekairi AM. Thymoquinone protects against carbon tetrachloride hepatotoxicity in mice via an antioxidant mechanism. *Biochem Mol Biol Int* 1999;**47**(1):153–9.
41. Beyazit Y, Purnak T, Kekilli M. Role of nitric oxide in the treatment of non-alcoholic fatty liver by omega-3 fatty acids. *Aliment Pharmacol Ther* 2010;**32**(2):303–4.
42. Meganathan M, Gopal KM, Sasikala P, Mohan J, Gowdhaman K, Balamurugan K, et al. Evaluation of hepatoprotective effect of omega 3-fatty acid against paracetamol induced liver injury in albino rats. *Global J Pharmacol* 2011;**5**(1):50–3.
43. Donnahoo KK, Meng X, Ayala A, Cain MP, Harken AH, Meldrum DR. Early kidney TNF-alpha expression mediates neutrophil infiltration and injury after renal ischemia-reperfusion. *Am J Physiol* 1999;**277**(3 Pt 2):R922–9.
44. Tanaka M, Miyazaki T, Tanigaki S, Kasai K, Minegishi K, Miyakoshi K, et al. Participation of reactive oxygen species in PGF2alpha-induced apoptosis in rat luteal cells. *J Reprod Fertil* 2000;**120**(2):239–45.
45. Gamonal J, Bascones A, Acevedo A, Blanco E, Silva A. Apoptosis in chronic adult periodontitis analyzed by in situ DNA breaks, electron microscopy, and immunohistochemistry. *J Periodontol* 2001;**72**(4):517–25.
46. Gown AM, Willingham MC. Improved detection of apoptotic cells in archival paraffin sections: immunohistochemistry using antibodies to cleaved caspase 3. *J Histochem Cytochem* 2002;**50**(4):449–54.
47. Giakoustidis AE, Giakoustidis DE, Koliakou K, Kaldrymidou E, Iliadis S, Antoniadis N, et al. Inhibition of intestinal ischemia/repurfusion induced apoptosis and necrosis via down-regulation of the NF-kB, c-Jun and caspace-3 expression by epigallocatechin-3-gallate administration. *Free Radical Res* 2008;**42**(2):180–8.
48. Badary OA, Taha RA. Gamal el-Din AM and Abdel-Wahab MH. Thymoquinone is a potent superoxide anion scavenger. *Drug Chem Toxicol* 2003;**26**(2):87–98.
49. Beltz BS, Tlusty MF, Benton JL, Sandeman DC. Omega-3 fatty acids upregulate adult neurogenesis. *Neurosci Lett* 2007;**415**(2):154–8.
50. Song C, Zhang XY, Manku M. Increased phospholipase A2 activity and inflammatory response but decreased nerve growth factor expression in the olfactory bulbectomized rat model of depression: effects of chronic ethyl-eicosapentaenoate treatment. *J Neurosci* 2009;**29**(1):14–22.