Bull. Egypt. Soc. Physiol. Sci. *34 (2)*, 187-199

Bull. of Egyp. Soc. Physiol. Sci.

(Official Journal of Egyptian Society for Physiological Sciences)

(ISSN: 1110-0842)

Protective effect of Thymoquinone and vitamin E on unilateral testicular torsion/detorsion in rats.

Elhussainy M.A. Elhussainy

Department of Physiology, Faculty of Medicine, Kafr-Elsheikh University, Egypt.

Received: June 12, 2014
Accepted: Sept 15, 2104
Available online: Nov 1, 2014

Keywords

- Thymoquinone
- Vitamin E
- Testicular torsion/detorsion
- Antioxidants

Abstract

Aim: this work was designed to investigate the effects of Thymoquinone and vitamin E pretreatment after 2 h of unilateral testicular torsion and their possible mechanism of action. **Methods:** the present study was performed on fifty male albino rats which were divided into five groups. Thymoquinone treated torsion/detorsion group, received pretreatment with Thymoquinone 100 mg/kg intraperitoneally 30 minutes before detorsion. Vitamin E treated torsion/detorsion group); received pretreatment with vit.E 100 mg/kg intraperitoneally 30 minutes before detorsion. Combined Thymoquinone and vit.E treated torsion/detorsion group received pretreatment in a dose of 100 mg/kg for each intraperitoneally 30 minutes before detorsion. **Results:** thymoquinone and vitamin E pretreatment caused significant decrease of malondialdehyde and nitric oxide levels and significant increase in reduced glutathione and glutathione peroxidase levels compared with untreated torsion/detorsion group. They improved testicular structures as they caused significant decrease in congestion, edema, and damage of seminiferous tubules in the ipsilateral and contralateral testis histologically when compared with torsion/detorsion group. **Conclusion:** the results suggest that early administration of Thymoquinone and vitamin E might have a protective effect for preventing testicular injury caused by testicular torsion probably through their antioxidant effects.

Corresponding author: Corresponding author: Elhussainy Mohammed Abouelez Elhussainy, Department of Physiology, Faculty of Medicine, Kafr-Elsheikh University, Egypt. E-mail:elhussainy1970@yahoo.com. Mob.: 01145350937

INTRODUCTION

It is well known that ischemia/reperfusion (I/R) generates metabolic and structural tissue damage, which may be due to trauma or sepsis. Reactive oxygen species (ROS) are over-produced on reperfusion, and play a critical role in the injury caused by ischemia-reperfusion (1). Oxidative stress is generated and contributes to reversible and irreversible cell injury (2).

Testicular torsion is a common emergency especially among children and adolescents. Testicular torsion and detorsion induces biochemical and morphological changes caused by IR injury in the testicular tissue (3).

Many mechanisms like in I/R injuries, ROS, and activation of inflammatory cytokines have been implicated in the testicular damage following torsion and detorsion process (4). I/R injuries are associated with the activation of neutrophils and inflammatory cytokines and injury starts in the ischemia period and exacerbates during reperfusion (5).

The rate of testicular injury is directly proportional to the duration of torsion, and early diagnosis followed by detorsion is the current management for the preservation of spermatogenesis and fertility (6). Although reperfusion is essential for the survival of an ischemic tissue, there is good evidence that reperfusion itself causes the pathophysiological cascades including an activation of neutrophils, inflammatory cytokines, and adhesion molecules with increased thrombogenicity, release of massive intracellular Ca2+, and ROS generation (7,8). ROS cause DNA damage, endothelial damage, and germinal cell necrosis.

Testicular torsion is the twisting of the spermatic cord, which cuts off the blood supply to the testicle and surrounding structures within the scrotum. In these circumstances, there is an increased risk of decreased sperm production, fertility, and atrophy of testis (9).

Experimental testicular torsion models showed that it induces apoptosis and damage in the ipsilateral and contralateral testes (10), although some authors suggested that the injury occurs only in the ipsilateral testis while the contralateral one is not affected (11).

Thymoquinone is one of the most important active principles of Nigella sativa seeds. Protection against reperfusion injury can be induced by assorted treatments including administration of antioxidants and anti-inflammatory drugs. Various therapeutic effects, such as antioxidant, anti-inflammatory, anticancer, antihistaminic, and antibacterial effects, have been described for Nigella sativa seeds (12,13). Additionally, it has been shown that Nigella sativa may have a possible protective effect against IR injury to various organs (14,15,16).

Vitamin E is the most prevalent nutritional antioxidant and has been shown to retard atherosclerosis in animal models (17). In addition to its antioxidant properties, vitamin E is anticancer and reduces the cytotoxic effect of oxidized lipoproteins, smooth muscle cell proliferation, platelet adherence and aggregation, inflammation, beside improving endothelial function (18). Vitamin E antioxidant activity has been suggested to scavenge ROS and attenuate IR injury (19).

MATERIALS AND METHODS

Animals

Fifty adult male albino rats weighing 200 - 250 g were provided by the Animal House of Zoology Department, Faculty of Science, Tanta University. The rats were housed in an animal room maintained at 23 ± 1 °C with a 12 h light/dark cycle, and they were allowed free access to water and food. The rats were acclimatized to housing conditions for at least 4 days before surgery.

Surgical procedure and drug administration

The rats were randomly divided into five groups. **Group 1** (control group) with sham operation this group included (n= 10) sham rats. Under ether anesthesia, median scrotal incision was done, delivery of the right testis without twisting the testicle and then fixed within the scrotum.

Group 2 (n=10 rats) underwent 2 h of testicular torsion then detorsion; (untreated T/D group), torsion was created by rotating the testis 720° in a clockwise direction for 2 h. Then, the right testis was detorted and replaced into the scrotum.

Group 3 (T/D + Thymoquinone - TQ, Sigma/Aldrich, Deisenhofen, Germany, TQ group), torsion and detorsion as in group 2 but group 3 received pretreatment with Thymoquinone 30 minutes before detorsion in a single dose of 100 mg/kg by intraperitoneal injection.

Group 4 (T/D + vitamin E group);as in group 2 but group 4 received pretreatment with vit. E 30 minutes before detorsion in a single dose of 100 mg/kg by intraperitoneal injection.

Group 5 (T/D + Thymoquinone and vit. E treated group) torsion and detorsion as in group 2 but group 5 received pretreatment with Thymoquinone and vit. E before detorsion in a single dose of 100 mg/kg for each by intraperitoneal injection.

The animals were sacrificed and the blood was collected to evaluate serum testosterone hormone level by biochemical analysis. Malondialdehyde, nitric oxide, reduced glutathione and glutathione peroxidase levels were determined in testicular tissues as follow:

Biochemical analyses

Sample preparation

Testes tissues for the estimation of tissue antioxidant levels were prepared at 4°C. Tissues were weighted and cut into small pieces. 10% homogenate was made in ice-cold potassium phosphate buffer solution (PBS, pH 7.4) containing 5 mM ethylene-diamine-tetraacetic acid (EDTA) using a glass homogenizer. The homogenate was centrifuged at 20000g for 10 minutes at 4°C and the supernatant was obtained for measurement estimation of different antioxidants and lipid-peroxides. The total protein levels for homogenates were estimated by the Biuret method (20).

Testicular tissue GSH determination

Reduced GSH was determined by the method of Beutler et al. (1963) (21). The supernatants were mixed with 4 ml phosphate buffer and 0.01 M dinitro2, 2-dithiobenzoic acid (DTNB). After shaking, its absorbance was measured at 412 nm within 10 min of the addition of DTNB against blank. The quantity of GSH in tissue samples was calculated using the standard GSH and results were given in mg GSH/g tissue using a concurrently run standard curve.

Testicular glutathione peroxidase (GPx)

Glutathione peroxidase in testicular tissue was determined by the method of Paglia and Valentine (1967)(22).

Testicular tissue MDA determination

MDA levels in the samples were determined indirectly by a previously described method of Draper and Hadley (1990)(23). Briefly,0.5 mL of sample was pipetted into a 10 mL centrifuge tube and 2.5 mL of trichloro-acetic acid (20%) and 1.0 mL of thiobarbituric acid (0.6%) solution was added. The tubes were heated for 30 min in a boiling water bath and the reaction mixture was then cooled in an ice-bath followed by the addition of 4.0 mL of n-butanol. The tubes were mixed with a vortex and centrifuged at 1500 g for 10 minutes. The absorbance of the organic layer was measured at 535 nm.

Testicular tissue NO determination

The production of NO was determined indirectly by measuring the nitrite levels based on the Griess reaction. Samples were initially deproteinized with 75 mmol/L ZnSO4. After clean up, an aliquot of the sample was treated with copperized cadmium in glycine buffer at pH 9.7 to reduce nitrate to nitrite. The concentration of nitrite in this aliquot thus represented the total nitrate plus nitrite. In the Griess reagent, a chromophore with a strong absorbance at 545 nm is formed by the reaction of nitrite with a mixture of naphthyl-ethylendiamine and sulfanilamide (24).

Serum Testosterone

Serum testosterone level was measured according to Bricaire et al. (1991) (25).

Histopathological examination

Bilateral orchiectomies were performed for histo-pathological examination in all groups. All testes were fixed in formalin and embedded in paraffin blocks. Tissue sections were stained with hematoxylin–eosin (H&E). The light microscope histological examination was done using 100 or 200 magnification power.

Statistical analysis

Statistical analyses were accomplished using the Statistical Product and Service Solutions (SPSS) computer program (version 13.0). All results were reported as means + SD. The comparison of the results from the various experimental groups and their corresponding controls was carried out using a one-way analysis of variance (ANOVA) followed by multiple comparison procedures (Tukey test).

RESULTS

Oxidative stress markers (MDA, GPx , GSH and NO):

Concerning lipid peroxidation and tissue malondialdehyde (MDA), reduced glutathione (GSH) and glutathione peroxidase (GPx) (Fig.1 A, B, & C), the untreated T/D rats in group 2 promoted significant increases in right and left testicular malondialdehyde (MDA) ($P < 0.001$) and significant decrease in right and left testicular in the levels of reduced glutathione and glutathione peroxidase ($P < 0.001$) compared to sham operated rats.

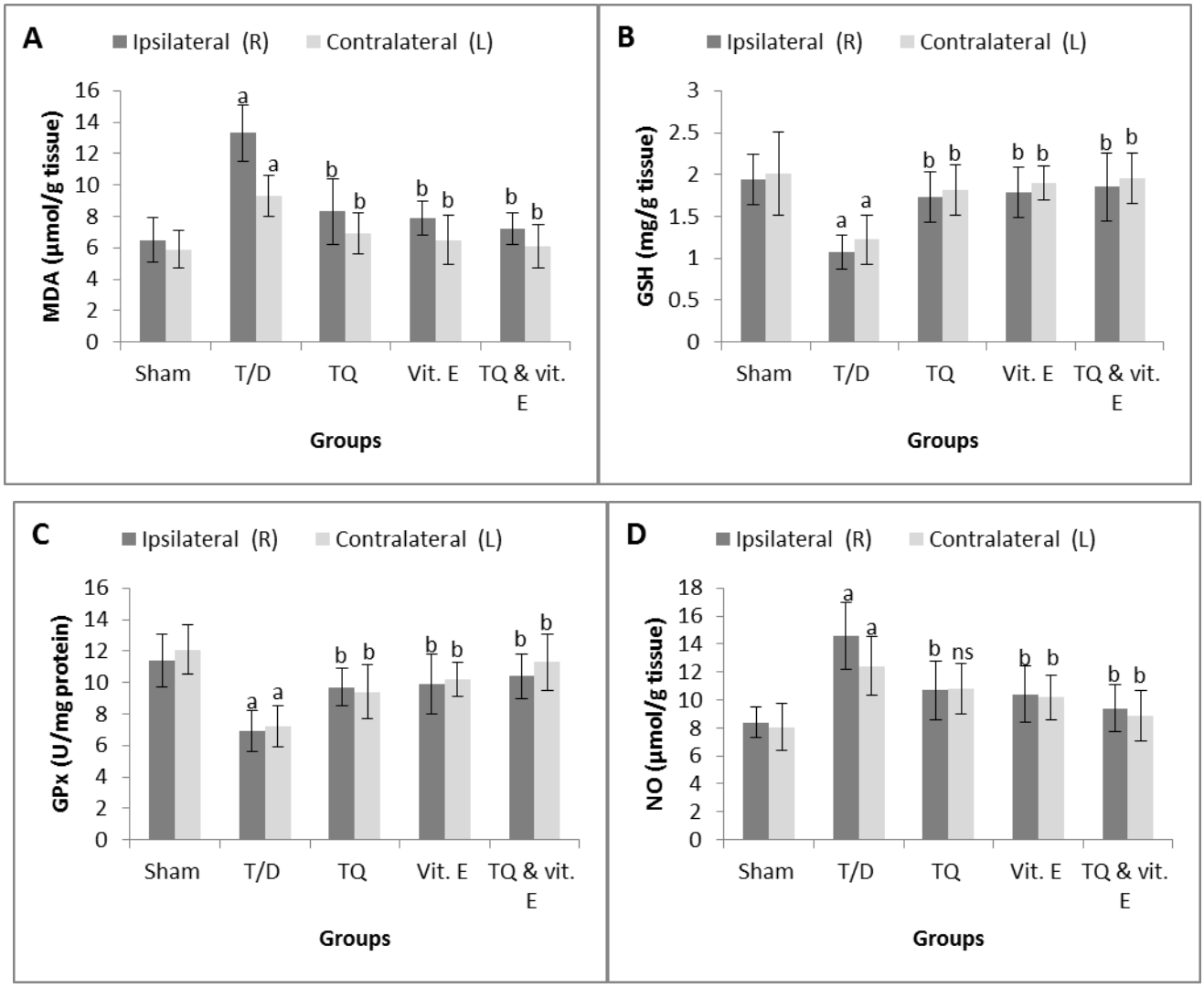

Figure 1. Effect of torsion/detorsion, TQ, vit. E and combined TQ & vit. E on malondialdehyde (A), reduced glutathione (B), glutathione peroxidase (C) and nitric oxide (D) levels in ipsilateral and contralateral testicular tissues in the studied groups. "a" shows significant difference with the sham group in the same series. "b" shows significant difference with the torsion/detorsion group in the same series. "ns" shows no significant difference (all at $p < 0.05$).

Concerning Thymoquinone administration to T/D rats in group 3 (Fig.1 A, B, & C), significant increase ($P < 0.001$) was observed in ipsilateral (right) and in contralateral (left) ($P < 0.01$) testicular glutathione peroxidase (GPx) and reduced (GSH) glutathione levels and significant decreases in MDA levels in right testis ($P < 0.001$) and left testis ($P < 0.01$) compared to untreated T/D rats.

Concerning Vit. E administration to T/D rats in group 4 (Fig.1 A, B, & C), significant increase ($P < 0.001$) was observed in right and left testicular GPx and GSH levels and significant decreases in MDA level ($P < 0.001$) in ipsilateral testis and ($P < 0.01$) were observed in contralateral testis compared to untreated T/D rats.

Concerning Thymoquinone and vit. E pretreatment to T/D rats in group 5, significant increase ($P < 0.001$) was observed in right and left testicular GPx and GSH levels and significant decrease in

right and left testicular MDA level ($P < 0.001$) compared to untreated T/D rats were observed in group 2 (Fig.1 A, B, & C).

Concerning tissue nitric oxide (NO) levels (Fig.1 D), the nitrite levels of both ipsilateral (right) and contralateral (left) testes in group 2 increased significantly compared with sham group ($P < 0.001$). In ipsilateral testis, TQ administration in group 3 significantly decreased (NO) production before detorsion procedure compared to T/D group ($P < 0.001$). Vit. E pretreatment in group 4 significantly decreased NO production compared to T/D group ($P < 0.001$). Combined TQ & vit. E pretreatment in group 5 significantly decreased NO production before detorsion procedure compared to T/D group 2 ($P < 0.001$).

In contralateral testis, TQ administration non-significantly decreased (NO) compared to T/D group ($P < 0.05$; Fig.1 D). Vit. E pretreatment significantly decreased NO production compared to T/D group ($P < 0.05$; Fig. 2). While TQ & Vit. E pretreatment in group 5 significantly decreased NO production compared to T/D group ($P < 0.01$; Fig. 1 D).

Concerning serum testosterone level (Fig. 2), a significant decrease ($P < 0.001$) in serum testosterone was observed in untreated T/D rats compared to sham operated rats. Upon pretreatment with TQ to T/D rats, serum testosterone showed significant increase ($P < 0.01$) compared to untreated T/D rats, although the level was still lower than sham operated rats. Pretreatment with vit. E to T/D rats, serum testosterone showed insignificant increase ($P < 0.05$) compared to untreated T/D rats. Pretreatment with combination of TQ & vit. E to T/D rats, serum testosterone showed significant increase ($P < 0.001$) compared to untreated T/D rats.

Histological examination

(Group 1) sham or control group showed normal seminiferous epithelium with Sertoli cells and spermatogenic cells. The regions between the seminiferous tubules are occupied by highly vascularized loose connective tissue and interstitial cells (Fig. 3).

(Group 2) torsion/detorsion group showed severe damage in seminiferous tubules with acidophilic exudate in their lumina and destruction of basal membrane of some tubules with absence of epithelial lining, severe inter-tubular edema, and severe congestion in the right or ipsilateral testis (Fig.4 A). The torsioned testis and the contralateral testis showed empty vacuolar spaces between Sertoli cells and germ cells in multilayered seminiferous epithelium. The contralateral testis (left testis) showed mild congestion and edema (Fig. 5 A).

(Group 3) Thymoquinone treated group showed marked improvement in testicular tissue. Ipsilateral testis showed mild vacuolization in seminiferous epithelium and germ cell and slight inter-tubular edema and congestion (Fig.4 B). The contralateral testis showed slight congestion and edema (Fig. 5 B).

(Group 4) Vit. E treated group, ipsilateral testis, showed moderate vacuolization and inter-tubular edema. Also, there were moderate damage of tubular lining of epithelial and spermatogenic cells with acidophilic exudate, moderate congestion and

inflammatory cellular infiltrates (Fig. 4 C). The contralateral testis showed decrease lining of spermatogenic cells and edema (Fig. 5 C).

(Group 5) Combined TQ & vit. E treated group, ipsilateral testis, showed damage of some spermatogenic cells but most tubules looks normal with sperms in their lumina (Fig. 4 D). Intraepithelial vacuolization and inter-tubular edema in the ipsilateral and contralateral testis were minimal (Fig.4 D, 5 D).

.

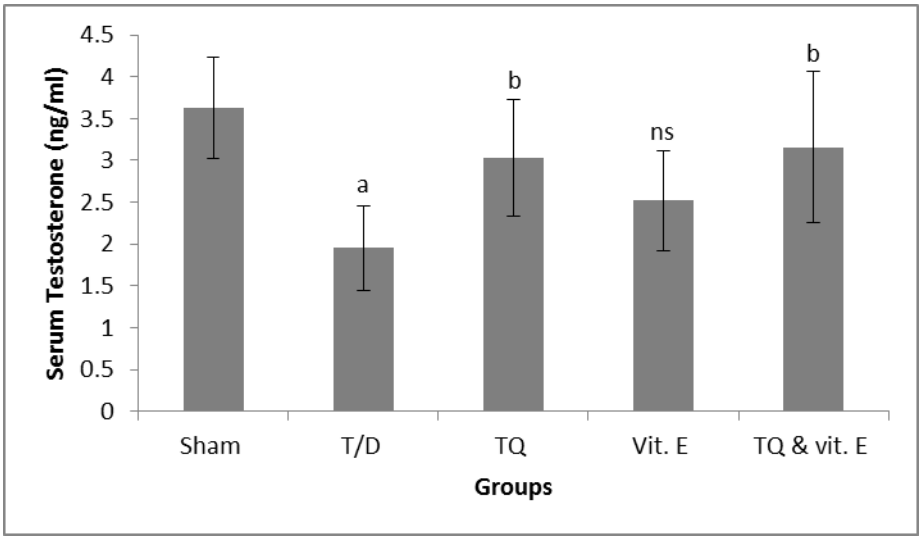

Figure 2: Effect of torsion/detorsion, TQ, vit. E and combined TQ & vit. E on serum testosterone level in the studied groups. "a" shows significant difference with the sham group. "b" shows significant difference with the torsion/detorsion group. "ns" shows no significant difference (all at $p < 0.05$).

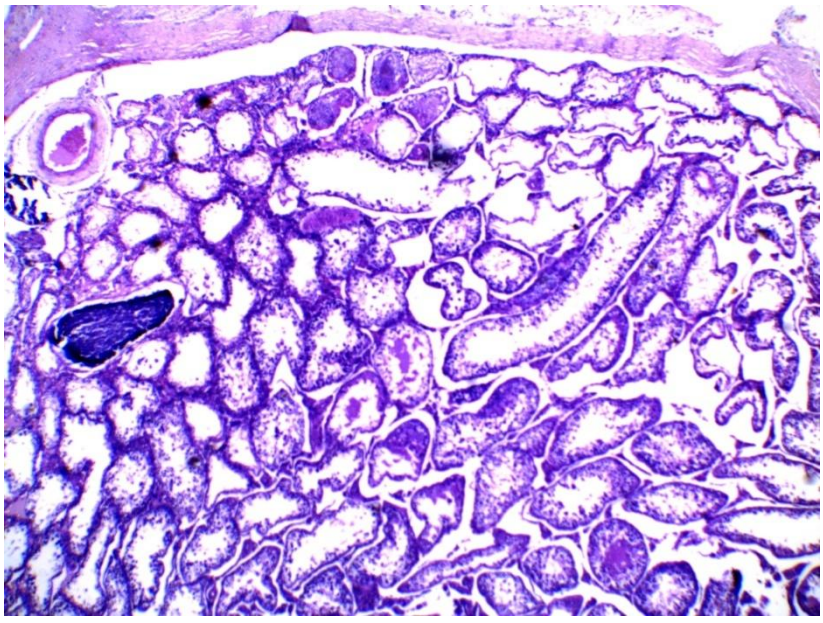

Figure 3: Normal testicular tissue stained with Haematoxiline & Eosine (magnification is 100x).

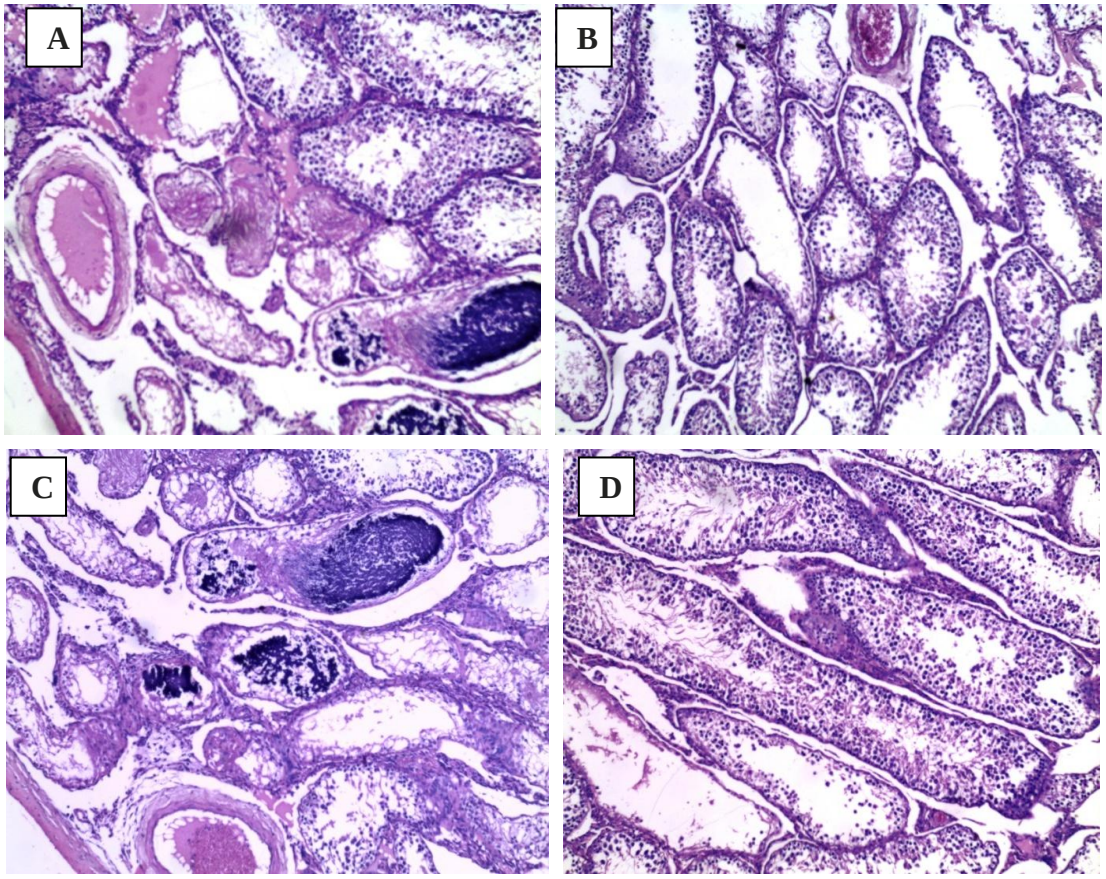

Figure 4: Effect of torsion/detorsion (A), TQ (B), vit. E (C) and combined TQ & vit. E (D) on ipsilateral testis in studied groups stained by Haemtoxiline & Eosine (magnification is 200x).

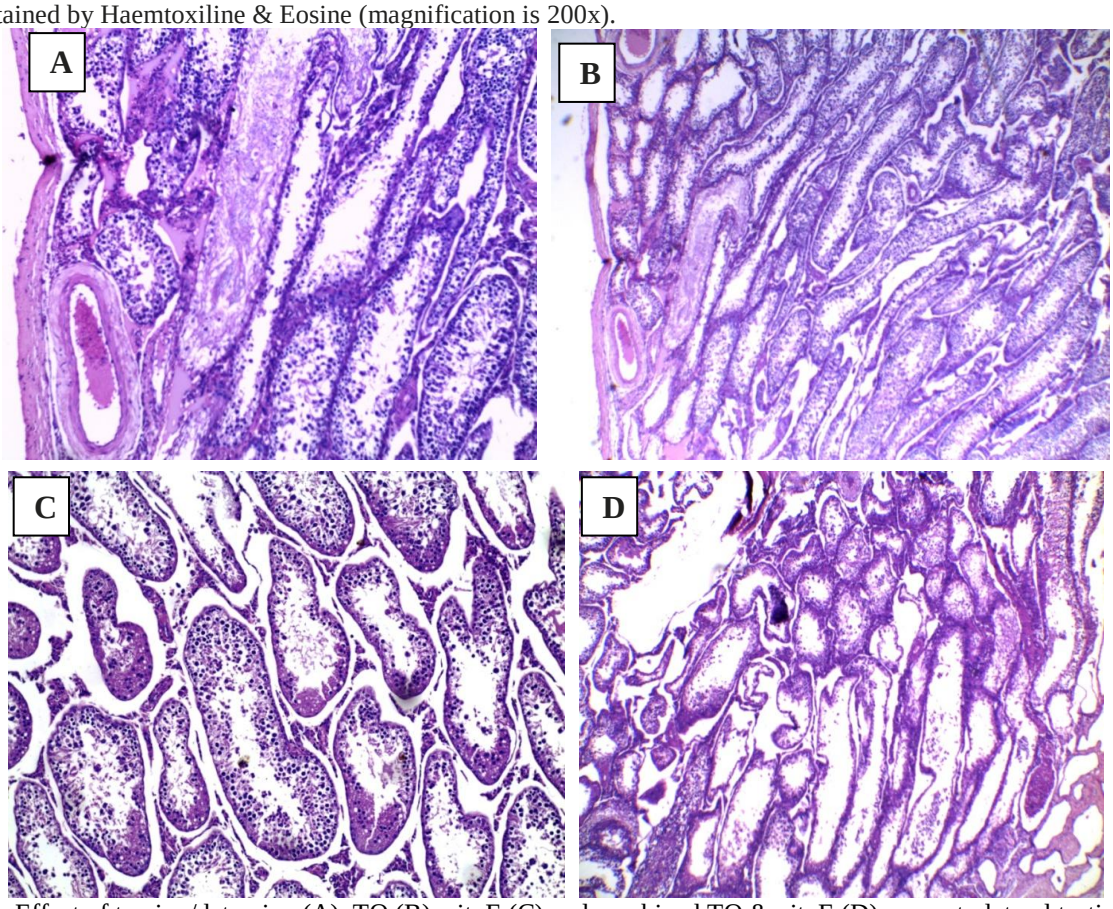

Figure 5. Effect of torsion/detorsion (A), TQ (B), vit. E (C) and combined TQ & vit. E (D) on contralateral testis in studied groups stained by Haemtoxiline & Eosine (magnification is 200x).

DISCUSSION

Results of the present study indicate that testicular T/D induces progressive biochemical and histological changes as a consequence of I/R injury. In our study, data revealed a significant increase in testicular lipid peroxidation products (TBARS and MDA), nitric oxide (NO) contents and histological damage of spermatogenic cells. The effect of oxidative stress of I/R in right testes are demonstrated by significant increases in right and left testicular MDA levels and significant decreases in right and left testicular glutathione peroxidase, as well as decreases in reduced glutathione levels compared to sham operated rats. These results are in agreement with the study of Ergur et al. (2008) who reported that I/R caused similar effects in torsioned testes. The significant increase in MDA levels in untreated T/D rats compared to both sham and treated rats indicated that I/R of the testes promotes severe cell membrane peroxidation (3). Also, oxidative stress induces per-oxidative damage to the plasma membrane of sperm, which causes loss of sperm function (6,26). The antioxidant effects of TQ and vit. E was previously supported by the findings that both protect against oxidative damage via inhibition of lipid peroxidation and restoration of reduced glutathione levels, and glutathione peroxidase activity in erythrocytes (15).

The effect of unilateral torsion on the contralateral testes was controversial. Turner (1987) reported that ipsilateral torsion does not result in contralateral testicular damage in rats (11). However, previous studies demonstrated that biochemical and histologic parameters of the contralateral testes did not reveal any statistical differences (27). The results of the present study are against these findings, because unilateral testicular torsion had an adverse effect on the contralateral testis, which has been previously explained as immunologic and oxidative mechanisms (1). Although still little is known about mechanisms on the effect of ischemia on the contralateral testes, our findings indicates that unilateral testicular IR produces contralateral damage, congestion and edema with significant decrease in reduced glutathione levels and glutathione peroxidase activity and significant increase in lipoperoxidation.

Testicular damage increased after detorsion procedure in both testes of untreated group explained by multiple factors that are known to induce testicular apoptosis during testicular torsion. Neutrophils and macrophages recruited during reperfusion have been implicated as mediators of parenchymal injury, and because of their ability to release a variety of toxic materials such as ROS and inflammatory cytokines, they are able to induce testicular cell apoptosis. Also, damaged spermatozoa can trigger further oxidative damage and cause expansion of damage and apoptosis (10). The increased levels of cytokines indicate tissue inflammation. Secretion of TNF-α acts as pro-inflammatory cytokines that induce and activate IL-6 and stress-related intracellular and extracellular pathways. In this regard, it was shown that these cytokines were produced from testicular cells as well as activated interstitial macrophages (5).

In our study we found that the testes of the I/R group had a high level of inter-tubular edema, congestion and inflammation which was correlated with high levels of nitrite. Also, in the same group nitrite levels in ipsilateral and contralateral testes were significantly higher than the sham operated group. The high nitrite levels may have been a result of activation of nitric oxide synthase and stimulation of pro-inflammatory cytokines, secretion of tumour necrosis factor alpha (TNF-α) and interleukin 6 (IL-6).

The present study confirmed that TQ pretreatment caused significant decrease in nitrite level in ipsilateral testis, while Vit. E pretreatment significantly decreased nitrite levels in both ipsilateral and contralateral testes. Furthermore, administration of TQ and Vit. E in combination caused significant decrease in nitrite levels in ipsilateral and contralateral testes as well.

Histopathological examination of sections of ipsilateral right testes in untreated T/D rats showed loss of germinal epithelium, absence of the sperm which denotes absence of spermatogenisis. Also, presence of cellular infiltration, exudates, congested blood vessels are further proofs of the dysfunction of the right testes. Contralateral testes affection are also observed, one theory postulates decrease in blood supply of contralateral testis as a reflex to an afferent stimulus, other authors proposed autoimmune reaction, release of acrosomal enzymes from contralateral testis, and apoptosis (28,29,30). So the preservation of twisted testes by the detorsion procedure might cause further deterioration by I/R injury, indicating the importance of the removal of the damaged testis to decrease histo-pathological damage of the contralateral side, or using a therapy to decrease the injurious effect of I/R. Thymoquinone either alone or in combination with vit. E after testicular torsion and before detorsion improved the ipsilateral and contralateral testes as also was observed by the histopathological analyses.

In our study, concerning serum testosterone level significant decrease in serum testosterone was observed in untreated T/D rats compared to sham operated rats. Upon pretreatment with TQ to T/D rats, serum testosterone showed significant increase compared to untreated T/D rats, although the level was still lower than sham operated rats. After pretreatment with vit.E to T/D rats, serum testosterone showed insignificant increase compared to untreated T/D rats, while pretreatment with combination of TQ & vit. E to T/D rats, a significant increase in serum testosterone levels when compared to untreated T/D rats was found. In conclusion, the results suggest that Thymoquinone and vitamin E have a potential protective effect for preventing testicular injury caused by testicular torsion. So, Thymoquinone either alone or in combination with vit. E after testicular torsion and before detorsion have a protective role in the early period of the testicular biochemical changes associated with I/R injury. They probably have anti-inflammatory and antioxidant effects against I/R injuries of testicular tissues.

Acknowledgements: This study was supported by Dr. Karema El-Desouky and Dr. Safenaz El-Shurbagy, department of pathology faculty of Medicine Tanta University. I would like to thank

the editor and two reviewers for their valuable comments and suggestions which improved the manuscript.

REFERENCES

1. **Akgur FM, Kilinc K, Tanyel FC, Buyukpamukcu N, Hicsonmez A:** Ipsilateral and contralateral testicular biochemical acute changes after unilateral testicular torsion and detorsion. Urology **44**: 413–418, 1994.
2. **Bulger EM, Maier RV:** Antioxidants in critical illness. Arch Surg. **136**: 1201-1207, 2001.
3. **Ergur BU, Kiray M, Pekcetin C, Bagriyanik HA, Erbil G:** Protective effect of erythropoietin pretreatment in testicular ischemia-reperfusion injury in rats. J Pediatr Surg. **43**: 722-728, 2008.
4. **Guimarães SB, Aragão AA, Santos JM, Kimura Ode S, Barbosa PH,Vasconcelos PR:** Oxidative stress induced by torsion of the spermatic cord in young rats. Acta Circ Bras. **22**: 30-33, 2007.
5. **Lysiak JJ:** The role of tumor necrosis factor-alpha and interleukin-1 in the mammalian testis and their involvement in testicular torsion and autoimmune orchitis. Reprod Biol Endocrinol **10:** 2-9, 2004.
6. **Visser AJ, Heyns CF:** Testicular function after torsion of the spermatic cord. BJU Int. **92**: 200–203, 2003.
7. **Cuzzocrea S, Riley DP, Caputi AP, Salvemini D:** Antioxidant therapy: a new pharmacological approach in shock, inflammation, and ischemia/reperfusion injury. Pharmacol Rev. **53**:135–159, 2001.
8. **Lysiak JJ, Nguyen QA, Kirby JL, Turner TT:** Ischemia-reperfusion of the murine testis stimulates the expression of pro-inflammatory cytokines and activation of c-jun N-terminal kinase in a pathway to E-selectin expression. Biol Reprod. **69**: 202–210, 2003.
9. **Filho DW, Torres MA, Bordin AL, Crezcynski-Pasa TB, Boveris A:** Spermatic cord torsion, reactive oxygen and nitrogen species and ischemia-reperfusion injury. Mol Aspects Med. **25**: 199–210, 2004.
10. **Payabvash S, Kiumehr S, Tavangar SM, Dehpour AR:** Ethyl pyruvate reduces germ cell specific apoptosis and oxidative stress in rat model of testicular torsion/ detorsion. J Pediatr Surg. **43:** 705-712, 2008.
11. **Turner TT:** On unilateral testicular and epidymal torsion: no effect on the contralateral testis. J Urology **138**: 1285–1290, 1987.
12. **Kanter M, Coskun O, Uysal H:** The antioxidative and antihistamine effect of Nigella sativa and its major constituent, thymoquinone on ethanol-induced gastric mucosal damage. Arch Toxicol. **80**: 217-224, 2006.
13. **Morsi NM:** Antimicrobial effect of crude extracts of Nigella sativa on multiple antibiotics-resistant bacteria. Acta Microbiol Pol. **49**: 63-74, 2000.
14. **Bayrak O, Bavbek N, Karatas OF, Bayrak R, Catal F, Cimentepe E, Akbas A, Yildirim E, Unal D, Akcay A:** Nigella sativa protects against ischemia/reperfusion injury in rat kidneys. Nephrol Dial Transplant. **23**: 2206-2212, 2008.
15. **Hosseinzadeh H, Parvardeh S, Asl MN, Sadeghnia HR, Ziaee T:** Effect of

thymoquinone and Nigella sativa seeds oil on lipid peroxidation level during global cerebral ischemia- reperfusion injury in rat hippocampus. Phytomedicine **14**: 621-627, 2007.

16. **El-Abhar HS, Abdallah DM, Saleh S:** Gastroprotective activity of Nigella sativa oil and its constituent, thymo- quinone, against gastric mucosal injury induced by ischemia/reperfusion in rats. J Ethnopharmacol **84**:251-258, 2003.
17. **Dong YH, Guo YH, Gu XB:** Anticancer mechanisms of vitamin E succinate. Chin J Cancer 28: 1114-1118, 2009.
18. **Ricciarelli R, Zingg JM, Azzi A:**The 80th anniversary of vitamin E: beyond its antioxidant properties. Biol Chem. **383:** 457-465, 2002.
19. **Altavilla D, Deodato B, Campo GM, Arlotta M, Miano M, Squadrito G:** IRFI 042, a novel dual vitamin E-like antioxidant, inhibits activation of nuclear factor-kappa B and reduces the inflammatory response in myocardial ischemia-reperfusion injury. Cardiovasc Res. **47**: 515-528, 2000.
20. **Doumas BT, Bayse DD, Carter RJ, Peters T Jr, and Schaffer R.A: C**andidate reference method for determination of total protein in serum. I. Development and validation. Clin Chem. **27**: 1642–1650, 1981.
21. **Beutler E, Duron O, Kelly BM:** Improved method for the determination of reduced glutathione. J Lab Clin Med. **61**: 882–888, 1963.
22. **Paglia DE, Valentine WN:** Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med. **10:** 158-169, 1967.
23. **Draper HH, Hadley M:** Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol. **186**: 421-431, 1990.
24. **Cortas NK, Wakid NW:** Determination of inorganic nitrate in serum and urine by a kinetic cadmium-reduction method. Clin Chem. **36**: 1440–1443, 1990.
25. **Bricaire C, Raynaud A, Benotmane A, Clair F, Paniel B, Mowszowicz I, right F, Moreau JF, Kuttenn F, Mauvais-Jarvis P:** Selective venous catheterization in the evaluation of hyperandrogenism. J Endocrinol Invest. **14:** 946-956, 1991.
26. **Kato M, Makino S, Kimura H, Ota T, Furuhashi T, Nagamura Y:** Sperm motion analysis in rats treated with adriamycin and its applicability to male reproductive toxicity studies. J Toxicol Sci. **26**: 51-59, 2001.
27. **Tunckiran A, Cayan S, Bozlu M, Yilmaz N, Acar D, Akbay E:**Protective effect of vascular endothelial growth factor on histologic changes in testicular ischemia-reperfusion injury. Fertil Steril **84**: 468–473, 2005.
28. **Andiran F, Okur DH, Kilink A, Gedikoglu G, Kilink K, Tanyel FC:** Do experimentally induced ipsilateral testicular torsion, vas deferens obstruction, intra-abdominal testis or venous obstruction damage the contralateral testis through a common mechanism. BJU Intern. **85**: 330-335, 2000.
29. **Uguralp S, Karabulut AB, Mizrak B, Kaymaz F, Kiziltay A, Hasirci N:**The effect of sustained and local administration of epidermal growth factor on improving bilateral

testicular tissue after torsion. Urol Res.**32:** 323-331, 2004.

30. **Pampal A, Ozen IO, Ekingen G, Demirogullari B, Helvacioglu F, Take G:** The morphological evaluation of ipsilateral and contralateral vasa deferentia in a rat model of unilateral spermatic cord torsion. Pediatr Surg Int. **26**: 287-292, 2010.