Egyptian Journal of Basic and Clinical Pharmacology
December 2017, Vol. 7, No. 2: 37-46
http://www.ejbcp.eg.net/

Original Article

Effect of Telmisartan on Ischemia Reperfusion Induced Testicular Injury in Rats

Marwa M. M. Refaie[1], Seham A. Abdel-Gaber[2*], and Rehab K. Mohammed[3]

[1,2]Department of Pharmacology, Faculty of Medicine, Minia University, 61511, Minia, Egypt

[3]Department of Pathology, Faculty of Medicine, Minia University, 61511, Minia, Egypt.

A B S T R A C T

Torsion/detorsion of the testis is considered as surgical urologic emergency that affects newborn, infant and adolescent males that leads to testicular ischemia-reperfusion (TIR) injury. TIR can lead to testicular necrosis with subfertility or infertility. We investigated the effect of angiotensin II receptor blocker Telmisartan (TEL) (10 mg/kg i.m. 30 min before the reperfusion) on experimental TIR injury in rats. Rats were divided into four groups. Sham operated control group, TEL treated control group, TIR non-treated group, TEL treated TIR group. Testicular injury was evaluated by testicular weight changes and serum testosterone level and confirmed by histopathology. Testicular content of malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD) and nitric oxide (NO) were evaluated. Moreover; the testicular immunohistochemical expression of the inflammatory marker tumor necrosis factor-alpha (TNF-α) was evaluated. The study showed that TEL has a protective effect against TIR injury. The protective effect of TEL was associated with modulation of testicular weight, serum testosterone level and the histopathological changes. TEL corrected the testicular oxidative stress parameters MDA, GSH, SOD, and NO with reduction of the testicular inflammatory marker TNF-α immunoexpression. The protective effect of TEL in TIR was attributed, at least in part, to its antioxidant and anti-inflammatory characters.

Key Words: Telmisartan; Testicular ischemia-reperfusion; Testosterone; Oxidative stress.

Corresponding Author: ***Seham A. Abdel-Gaber*** **Email:** *sehamabdelwakeel@yahoo.com*

1. INTRODUCTION

Testicular or spermatic cord torsion is an emergency state of newborn and adolescent males (Unsal et al., 2004). If it is undiagnosed or inappropriately treated, it causes testicular injury, which may lead to subfertility (Ringdahl and Teague, 2006).40-60% of these patients may develop testicular atrophy and infertility even after successful surgical intervention (Sharp et al., 2013).

Spermatic cord rotation initially results in the interruption of testicular venous then arterial blood flow, which consequently leads to testicular ischemia (Kostakis et al., 2017). After testicular detorsion, tissues reperfusion occurs which leads to testicular damage. This harmful effect is more severe than that induced by ischemia (Unsal et al., 2006). TIR injury occurs when testicular blood flow is restored following an episode of acute ischemia (Arena et al., 2017).

Testicular ischemia causes germ cell death, which is mainly due to the reduction of the oxygen supply relative to metabolic demands. This effect causes accumulation of the toxic metabolites and depletion of the stored ATP (Turner et al., 2004). The testicular reperfusion following distortion increases the production of various proinflammatory cytokines, including interleukin-1β and TNF-α, which recruit macrophages and neutrophils that infiltrate the testicular parenchyma (Lysiak, 2004). Neutrophils which are activated during inflammatory response are a potential source of reactive oxygen species (ROS) in ischemic injury (Lipton, 1999).The increased production of ROS damages the proteins, lipids, and DNA of testicular cells (Altavilla et al., 2012).

Stimulation of angiotensin II receptors leads to production of proinflammatory cytokines such as interleukin-6 and TNF-α (Han et al., 1999; Kalra et al., 2002) from monocytes (Hahn et al., 1994) or induces production of ROS (Zhang et al., 1999; Nickenig and Harrison, 2002). TEL is angiotensin II receptor (type I) blockers and is widely used for treatment of hypertension to prevent organ damage such as renal and cardiac remodeling (Kumtepe et al., 2010).

It was reported that TEL suppressed the superoxide production from the vessel wall and prevented atherosclerosis via its antioxidant effects (Takaya et al., 2006). Furthermore, it was also reported that TEL

protected the testes of diabetic rats by downregulating the level of oxidative stress and inflammatory cytokines (Guo et al., 2016).

Despite of the proven antioxidant activity of TEL, its role in TIR injury has not yet been investigated. In this study, we examined whether TEL administration can protect the rats from TIR injury or not and exploring the main mechanisms of its action.

2. MATERIALS AND METHODS

2.1. Chemicals

The used chemicals were: Telmisartan (Boehringeringelheim, Egypt). Monoclonal primary anti-rat TNF-α antibody (Thermo Fisher Scientific Inc./Lab Vision Corporation, Fremont, CA, USA).Copper sulphate (El Nasr Pharmacutical Company, Egypt). Cadmium ganules (Fluka, Switzerland). Sulphanilamide (El-Gomhoria Pharmacutical Company, Egypt). Thiobarbituric acid (Sigma, USA). Trichloroacetic acid (El-Gomhoria Pharmacutical Company, Egypt). Pyrogallol (Sigma USA).

2.2. Animals

24Adult male albino rats (230-300 g) were obtained from the animal house in Giza, Egypt. We kept animals in standard housing conditions in cages, 3 rats/cage, and animals were left to acclimatize for one week. Animals were supplied with chow and tap water. The experiment was conducted with adherence to the ethical standards approved by the faculty board committee of Faculty of Medicine, Minia University, Egypt.

2.3. Experimental design

2.3.1. Induction of TIR

The rats were fasted overnight with free access to water before the experiment. All procedures were performed under sterile conditions. Rats were anesthetized by urethane hydrochloride (1 g/kg i.p.). In TIR rats, a vertical paramedian scrotal incision was done in one side with exteriorization of the testis. The testis was torsioned 1080° clockwise for induction of ischemia for 1 hour and then detorsioned for reperfusion for 2 hours. The sham-operated control animals were subjected to the same protocol without torsion/detorsion technique.

2.3.2. Grouping

Rats were divided into 4 groups (n=6). Group 1: Sham-operated control group received vehicle (i.m. injection of normal saline). Group 2: TEL treated control group received TEL (10 mg/kg i.m. dissolved in normal saline) (Goyal et al., 2011). First and second groups are subjected to anesthesia, scrotal incision, and testicular exteriorization without ischemia reperfusion. Group 3: TIR-non treated group-received vehicle. Group 4: TEL+TIR group received TEL (10 mg/kg i.m. dissolved in normal saline) 30 min before reperfusion.

2.4. Sample collection

At the end of experimental period, animals were sacrificed. Blood samples were collected from the neck vessels and centrifuged for serum collection. Serum is used for detection of serum testosterone level. Then the testicles were dissected and weighed on Mettler Toledo scale, Swizerland. Testicular samples were prepared for biochemical analysis by homogenization in10% (weight/volume) ice cold phosphate buffer (0.01 M, pH 7.4) with centrifugation at 3000 rpm for 20 min. The clear supernatant was kept at -80°C for biochemical analysis. Testicular specimens were prepared separately for histopathological evaluation and immunohistochemichal assay of TNF-α.

2.5. Biochemical analysis

2.5.1. Serum testosterone level

Serum testosterone level is evaluated according to the testosterone enzyme-linked immune-sorbent assay (ELISA) kit instructions (Cayman Chemicals., USA).

2.5.2. Testicular GSH level

Reduced glutathione evaluation is based on the reaction between the sulfhydryl groups of GSH with5, 5-dithio-bis-2-nitrobenzoic acid (Ellman's reagent) which gives a yellow colored 5-thio-2-nitrobenzoic acid. The color density was detected using Beckman DU-64 UV/VIS spectrophotometer, USA at 405nm (Beutler et al., 1963).

2.5.3. Testicular SOD activity

Superoxide dismutase activity evaluation depends on the ability of SOD enzyme to inhibit the phenazinemethosulphate-mediated reduction of nitrobluetetrazolium dye (Nishikimi et al, 1972).

2.5.4. Testicular MDA level

Malondialdehyde is a measure of lipid peroxidation and was evaluated spectrophotometricaly by method that is based on the reaction between MDA and thiobarbituric acid (Buege and Aust, 1978).

2.5.5. Testicular NO level

Nitrite (NO_2^-) and nitrate (NO_3^-) are stable oxidation end products of NO, were used as indicators of NO production. NO_2^-/ NO_3^- levels were estimated by Griess method. The main principle of this assay is the reduction of nitrate to nitrite by copperized cadmium granules, followed by color development with Griess reagent (sulfanilamide and *N*-naphthylethylenediamine) in acidic medium (Soguta et al., 2003).

2.6. Testicular histopathology

Testis samples were fixed in Bouin's fixative for 24–48 h and embedded in paraffin. Tissue sections were stained by the routine histological stain hematoxylin/eosin (H&E) and observed under a light microscope (magnification X200). Slides were examined by an experienced pathologist who was blinded to the study groups. Testicular biopsy was assessed for testicular injury and spermatogenesis according to Johnsen`s scoring system (Johnsen, 1970).

Johnsen`s score

10	Complete spermatogenesis with perfect tubules.
9	Many spermatozoa present but disorganized spermatogenesis.
8	Only a few spermatozoa present.
7	No spermatozoa but many spermatids present.
6	Only a few spermatids present.
5	No spermatozoa or spermatids but many spermatocytes present.
4	Only a few spermatocytes present.
3	Only spermatogonia present.
2	No germ cells, sertoli cells only.
1	Neither germ nor sertoli cells (No seminiferous epithelium).

2.7. Immunohistochemical expression of testicular TNF-α

Immunohistochemical examination of testicular TNF-α was performed using monoclonal anti-rat antibody(Lab Vision Laboratories; USA).Sections were dewaxed in xylene overnight then rehydrated in descending graded alcohol. Rehydrated sections were then immersed in a 10% solution of hydrogen peroxide for 45 minutes at room temperature then in PBS for 5 min. Immersion of the slides in citrate buffer solution for 20 minutes at 80°C, then slides were allowed to cool and reach room temperature then washed with PBS. Sections were then incubated at 4 ºC with primary antibody overnight, and then slides were put in PBS for 5 min. Secondary biotinylated antibody was added for each slide for 45 min at room temperature. The slides then were put in PBS for 5 min. Streptavidin reagent was applied to cover each section for 45 min at room temperature then slides were placed in PBS for 5 minutes. DAB substrate-chromogen solution was applied on sections, and then slides were left at room temperature for 10 minutes. Slides were counterstained in haematoxylin stain then dehydrated in ascending grades of alcohol, mounted and covered. A homogenous brown staining of the cytoplasm revealed positive cells.

Each slide was individually examined and scored. TNF-α was scored as follow: Negative (0) for <10% of cells stained: low (1) >10%and <30% of cells stained; moderate (II) > 30% and <70 of cells stained; and high (III) for > 70 % of stained cells (Skondras et al., 2015).

Statistical Analysis

Data were analyzed by one-way ANOVA followed by Tukey's post hoc test. Statistical analysis was done using GraphPad Prism software (version 6). The values are represented as means ± SEM. P values less than 0.05 were considered significantly different.

3. RESULTS

3.1. Effect of TIR and TEL on testicular weight and serum testosterone level

Testicular weight and serum testosterone level significantly decreased by the effect of induced TIR in comparison to sham operated control group. This effect significantly improved by TEL treatment in comparison to TIR non treated group (table 1).

3.2. Effect of TIR and TEL on testicular parameters of oxidative stress

The antioxidant parameters in the form of testicular SOD activity and GSH level significantly reduced however the oxidative stress parameters in the form of testicular MDA and NO levels significantly increased by TIR in comparison to sham operated control group. Treatment of rats by TEL significantly elevated the testicular antioxidant parameters and decreased the oxidative stress parameters in comparison of TIR non treated rats (table 2).

3.3. Histopathological findings and scoring

Histopathological score (fig. 1 E) showed significant testicular injury in TIR group (fig. 1C) in the form of disorganization, slough formation and loss of maturation of germ cells with absence of spermatozoa. Treatment with TEL to TIR induced group is associated with improvement of histopathological changes (fig. 1 D) in comparison to TIR group. There is no significant histopathological changes in TEL treated control group (fig. 1B) in comparison to the normal sham operated control group (fig. 1A).

3.4. Immunohistochemical expression of TNF-α in the testis

Fig. 2 E showed that there is a significant increase in TNF-α immunoexpression in TIR non treated group (fig. 2 C) in comparison to sham operated control group (fig. 2A). This effect is significantly ameliorated by treatment of TIR rats by TEL (fig. 2D). There is no significant

expression of TNF-α in TEL treated control group (fig. 2B) in comparison to sham operated control group.

Table (1): Effect of TIR and TEL on testicular weight and serum testosterone level

Groups	Testicular weight (g)	Serum testosterone (nmol/ml)
Sham	1.98±0.06	2.38±0.12
TEL	2.03±0.09	1.95±0.07
TIR	1.45±0.12[a]	0.18±0.01[a]
TEL+TIR	1.95±0.08[b]	2.03±0.15[b]

Values represents means ± S.E.M (n=6). Groups are compared by Tukey's post hoc test. [a]significance from sham control group ($p < 0.05$). [b]significance from TIR group ($p < 0.05$).TEL: telmisartan; TIR: testicular ischemia reperfusion.

Table (2): Effect of TIR and TEL on testicular parameters of oxidative stress

Group	GSH (nmol/g tissue)	SOD (unit/g tissue)	MDA (nmol/g tissue)	NO (nmol/g tissue)
Sham	367.5±16.72	33309±2838	51.85±4.82	52.58±2.4
TEL	390.8±29.39	38595±3133	49.77±4.099	50.62±1.58
TIR	213.7±15.16[a]	20078±1846[a]	124.7±8.44[a]	73.40±2.70[a]
TEL +TIR	346.1±41.10[b]	36524±2592[b]	65.24±6.37[b]	41.16±3.0[ab]

Values represents means ± S.E.M (n=6). Groups are compared by Tukey's post hoc test. [a]significance from sham control group ($p < 0.05$). [b]significancefromTIR group ($p < 0.05$). TEL: telmisartan; TIR: testicular ischemia reperfusion, GSH: reduced glutathione, SOD: superoxide dismutase, MDA: malondialdehyde, NO: nitritc oxide.

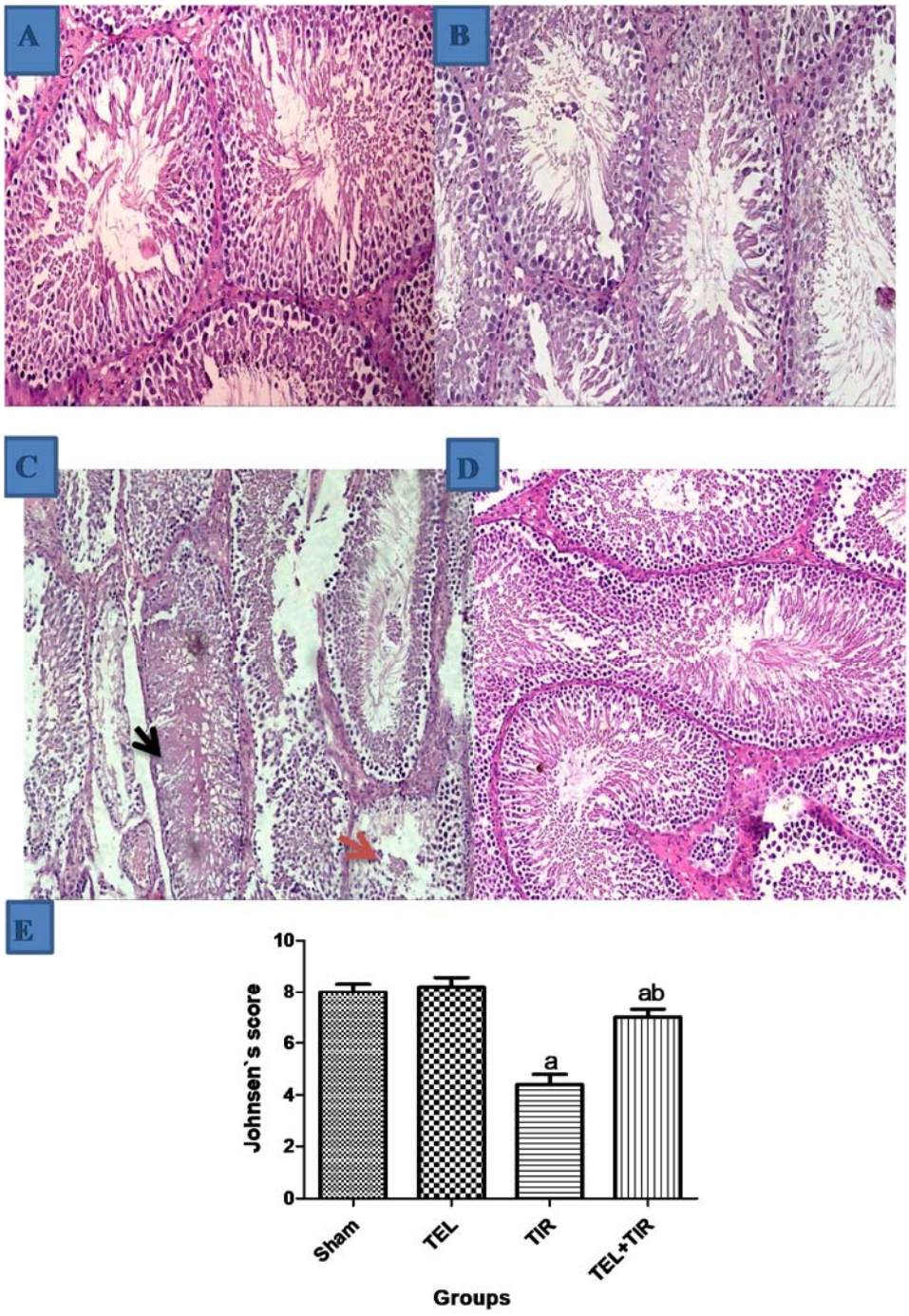

Fig 1: Effect of TIR and TEL on testicular histopathology

Light microscope of rat testicular sections (magnification 200X, stained with H&E). **A:** Normal histological structures of seminiferous tubules and the interstitial area. **B:** TEL treated control group shows normal testicular morphology. **C:**Epithelial disruption, decreased number of spermatozoa (red arrow)and debris in lumen (black arrow). **D:** TEL+TIR group shows mild histological changes. **E:**Semi quantitative analysis of the histopathological score. Data are expressed as means ± S.E.M. (n=6). [a]significance from sham control group ($p < 0.05$). [b]significance from TIR group ($p < 0.05$). TEL: telmisartan; TIR: testicular ischemia reperfusion.

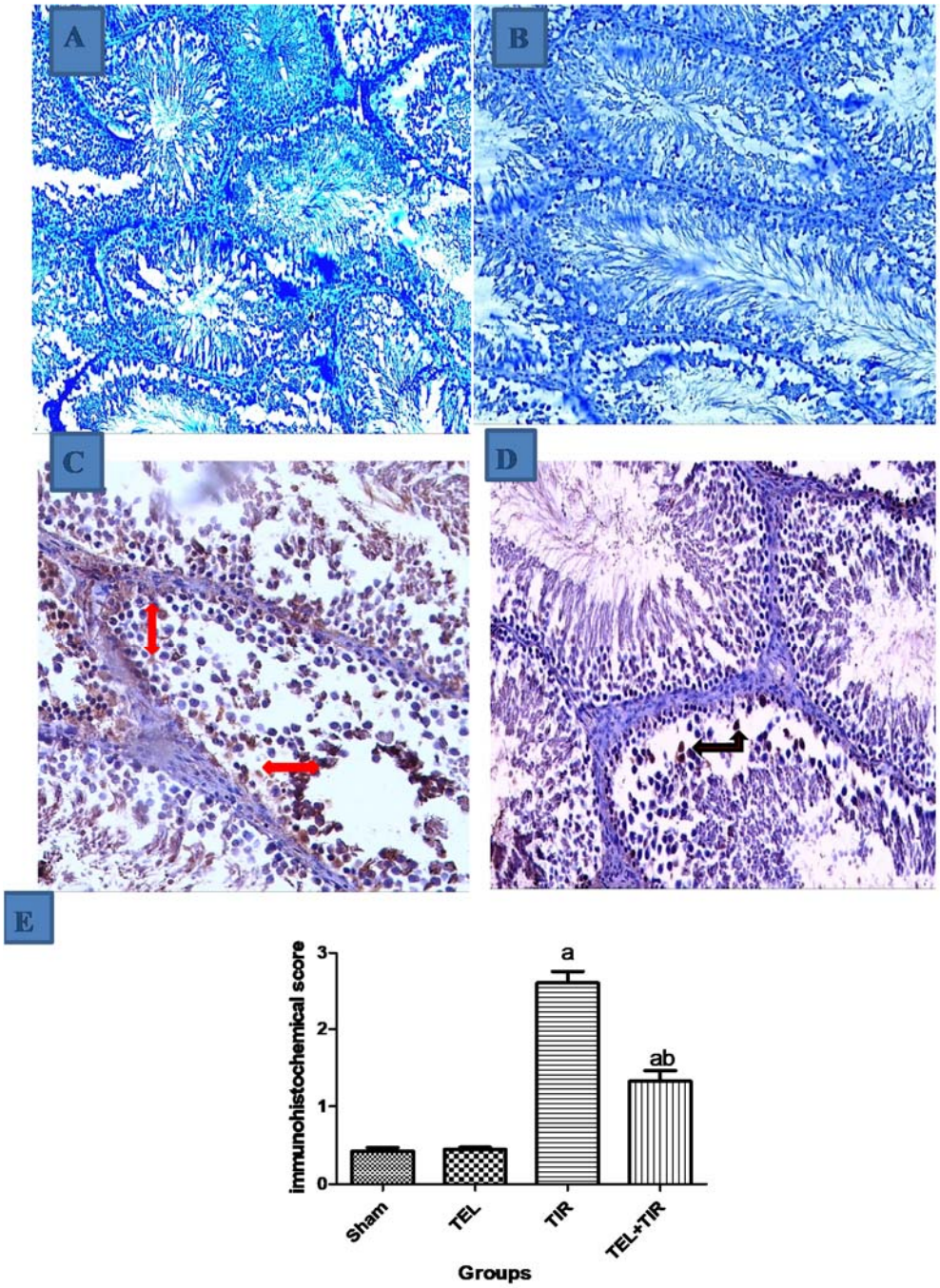

Fig 2: Effect of TIR and TEL on testicular TNF-α immunoexpression
Immunoexpression of TNF-α is indicated by brown color. **A:** sham control group shows no expression. **B:** TEL treated control group shows no expression. **C:** TIR group shows extensive expression (red arrows). **D:** TEL+TIR group shows mild expression (black arrow). **E:** Semi quantitative analysis of optical density. Data are expressed as means ± S.E.M. (n=6). [a] significance from sham control group ($p < 0.05$). [b] significance from TIR group ($p < 0.05$). TEL: telmisartan; TIR: testicular ischemia reperfusion.

4. DISCUSSION

Testicular torsion/detorsion is a serious urological emergency. It is associated with ischemia reperfusion injury of the testis and leads to male infertility (Arena et al., 2017). TIR is considered as a surgical emergency. Understanding the molecular mechanisms of TIR is essential to optimize therapeutic intervention. Overproduction of ROS and RNS are the main cause of tissue injury after TIR (Dokmeci, 2006).

In the current study, TIR was associated with significant testicular damage which led to decrease in both testicular weight and serum testosterone level. These results are in accordance with the previously published results. Zhang et al. (2013) evaluated the therapeutic utility of hyperbaric oxygen therapy in TIR injury. They found marked decrease in testicular weight in TIR induced group. Ahmed et al. (2016) studied the effect of Ginkgo Biloba on induced TIR. They found significant decrease of both testicular weight and serum testosterone level in TIR group.

Testicular damage was confirmed by the histopathological changes in tubular architecture in the form of testicular epithelial atrophy, tubular congestion, necrosis with debris formation and loss of sperms formation with subsequent significant decrease in testicular Johnsen's score. The histological findings are in accordance with Kemahli et al. (2016) who studied the effect of pyrrolidinedithiocarbamate on TIR in rats. They found epithelial irregularity, decreased number of spermatozoa and significant reduction in Johnsen's score.

Telmisartan is an angiotensin receptor blocker that is highly selective for angiotensin II receptors type 1. It is used in treatment of mild to moderate hypertension (Sharpe et al., 2001). Results of the represent study showed the protective effect of TEL in TIR as evidenced by significant increase in testicular weight and serum testosterone level and confirmed by histopathology. TEL treatment significantly restored the testicular architecture with maturation of spermtogonia and improvement of spermatogenesis that is confirmed by the increase in Johnsen's score.

In the present study, TIR was associated with elevation of testicular tissue content of oxidative stress biomarkers in the form of elevation of tissue contents of MDA and NO levels with reduction of GSH level and SOD activity. Our findings are similar to the previously reported results. Tamamura et al. (2010) reported that TIR was associated with increase in both testicular MDA and NO levels. Also Kara et al. (2016) found that induced TIR in rats was associated with significant decrease in both GSH and SOD activity in testicular tissue.

Neutrophils that attract to the injury site secrete ROS, which causes the TIR injury (Kara et al., 2016). ROS react with membrane lipids and result in lipid peroxidation and loss of cellular components of the tissue. Membrane lipid peroxidation changes membrane permeability and cell integrity (Tuncer et al., 2007). MDA is the product of lipid peroxidation and is a sensitive parameter for determining the increased free radical formation in TIR tissue injury (Takhtfooladi et al., 2013). SOD is an oxygen radical scavenger enzyme protecting the cells against damage caused by ROS (Soumya et al., 2014). SOD metabolizes the superoxide to hydrogen peroxide (Miller, 2012). SOD is one of the testicular antioxidant defense systems (Celik et al., 2016). Reduced glutathione is one of the free radicle scavengers, which helps to restore the cell membrane's physiological structure. It is an important factor for detoxification of oxygen metabolites mostly the hydrogen peroxide and lipid hydroperoxide (Elshaari et al., 2011).

Nitric oxide is highly diffusible free radical (Ibrahim et al., 2014) which at high level can be toxic as it reacts with the superoxide anion to produce the highly reactive peroxynitrite. Peroxynitrite can damages the DNA, RNA, proteins, and lipids (Karaguzel et al., 2014).

In the present work, TEL treatment restored the testicular antioxidant capacity that was disturbed by TIR. TEL significantly increased both testicular GSH level and SOD activity and decreased both testicular MDA and NO levels. The antioxidant activity of TEL was previously reported in different tissues. Czechowska et al. (2016) reported that TEL ameliorated the thioacetamide induced liver fibrosis via its antioxidative and anti-inflammatory properties. The protective antioxidant activity of TEL was also proven in renal ischemia-reperfusion (Kocak et al., 2016), arsenic testicular injury (Fouad et al., 2015) and induced inflammatory bowel disease (Arab et al., 2015).

Tumor necrosis factor-α is a proinflammatory, proapoptotic and immunoregulatory cytokine. It is reported that murine model of TIR increases the production of TNF-α after reperfusion (Lysiak et al., 2003). It is reported that TNF-α expression was increased after one hour of the reperfusion in a rat model of experimental TIR, reached its maximum increase after three hours then returned to its basal level after 5 hours of the reperfusion (Minutoli et al., 2005). In the present study, TIR significantly increased the testicular immunohistochemical expression of TNF-α in which is supported by the previously published results (Yazihan et al., 2007; Okur et al., 2017). TEL treatment significantly decreased the testicular TNF-α immunoexpression which confirmed its anti-inflammatory activity that was previously reported in different tissues. It was confirmed in rat liver (Czechowska et al., 2016), rat airway (Abdel-Fattah et al., 2015), human neuronal cells (Saravanan et al., 2015) and rat gums (Araújo et al., 2013). It was

reported also that TEL protected the testis from diabetic injury (Guo et al., 2016) and cadmium injury (Fouad and Jresat, 2013) via its antioxidant and anti-inflammatory activity.

5. CONCLUSION

Our results revealed that the selective angiotensin II receptor type I blocker TEL has a testicular protective activity against experimentally induced testicular ischemia-reperfusion at least in part by its antioxidant and anti-inflammatory activities.

Conflicts of interest

The authors reported no conflict of interest regarding the publication of this article.

6. REFERENCES

Abdel-Fattah, M.M., Salama, A.A., Shehata, B.A., Ismaiel, I.E., 2015. The potential effect of the angiotensin II receptor blocker telmisartan in regulating OVA-induced airway remodeling in experimental rats.Pharmacol Rep 67(5):943-951.

Ahmed, A.I., Lasheen, N.N., El-Zawahry, K.M., 2016. Ginkgo Biloba Ameliorates Subfertility Induced by TesticularIschemia/Reperfusion Injury in Adult WistarRats: A Possible New Mitochondrial Mechanism. Oxid Med Cell Longev 2016:6959274.

Altavilla, D., Romeo, C., Squadrito, F., Marini, H., Morgia, G., Antonuccio, P. Minutoli, L., 2012. Molecular pathways involved in the early and late damage induced by testis ischemia: evidence for a rational pharmacological modulation. Curr Med Chem 19(8): 1219-1224**.**

Arab, H.H., Al-Shorbagy, M. Y., Abdallah, D.M., Nassar, N.N., 2014. Telmisartan attenuates colon inflammation, oxidative perturbations and apoptosis in a rat model of experimental inflammatory bowel disease. PLoS One 9(5):e97193.

Araújo, A.A., Souza, T.O., Moura, L.M., Brito, G.A., Aragão, K.S., Araújo, L.S., Medeiros, C.A., Alves, M.S., Araújo, R.F. Jr., 2013. Effect of telmisartan on levels of IL-1, TNF-α, down-regulated COX-2, MMP-2, MMP-9 and RANKL/RANK in an experimental periodontitis model.JClinPeriodontol 40(12):1104-1111.

Arena, S., Iacona, R., Antonuccio, P., Russo, T., Salvo, V., Gitto, E., Impellizzeri,P., Romeo, C., 2017. Medical perspective in testicular ischemia-reperfusion injury.ExpTher Med 13(5):2115-2122.

Beutler, E., Duron, O., Kelly, B.M., 1963. Improved method for the determination of blood glutathione.J.LabClin.Med61:882-888.

Buege, J.A., Aust, S.D., 1978. Microsomal lipid peroxidation.Methods Enzymol 52, 302–310.

Celik, E., Oguzturk, H., Sahin, N., Turtay, M.G., Oguz, F., Ciftci, O., 2016. Protective effects of hesperidin in experimental testicularischemia/reperfusion injury in rats. Arch Med Sci12(5):928-934.

Czechowska, G., Celinski, K., Korolczuk, A., Wojcicka, G., Dudka, J., Bojarska, A., Madro, A., Brzozowski, T., 2016. The effect of the angiotensin II receptor, type 1 receptor antagonists, losartan and telmisartan, on thioacetamide-induced liver fibrosis in rats. J PhysiolPharmacol 67(4):575-586.

Dokmeci D., 2006. Testicular torsion, oxidative stress and the role of antioxidant therapy.Folia Med (Plovdiv) 48(3-4):16-21.

Elshaari, F.A., Elfagih, R.I., Sheriff, D.S.,Barassi, I.F., 2011. Oxidative and antioxidativedefensesystem in testiculartorsion/detorsion. Indian J Urol 27(4):479-484.

Fouad, A.A., Albuali, W.H., Al-Mulhim, A.S., Jresat, I., 2015. Protective effect of telmisartan treatment against arsenic-induced testicular toxicity in rats. Z NaturforschC 70(7-8):175-181.

Fouad, A.A., Jresat, I., 2013. Captopril and telmisartan treatments attenuate cadmium-induced testicular toxicity in rats. FundamClinPharmacol 27(2):152-160.

Goyal, S.N., Bharti, S., Bhatia, J., Nag, T.C., Ray, R., Arya, D.S., 2011. Telmisartan, a dual ARB/partial PPAR-γ agonist, protects myocardium from ischemic reperfusion injury in experimental diabetes. Diabetes ObesMetab 13(6):533-541.

Guo, Z., Yan, X., Wang, L., Wu, J., Jing, X., Liu, J. 2016. Effect of Telmisartan or Insulin on the Expression of Adiponectin and its Receptors in the Testis of Streptozotocin-Induced Diabetic Rats. HormMetab Res 48(6):404-412.

Hahn, A.W., Jonas, U., Buhler, F.R., Resink, T.J, 1994. Activation of human peripheral monocytes by angiotensin II. FEBS Lett 347(2-3):178–180.

Han, Y., Runge, M.S., Brasier, A.R., 1999. Angiotensin II induces interleukin-6 Transcription in vascular smooth muscle cells through pleiotropic activation of nuclear factor-kappa B transcription factors. Circ Res 84(6):695–703.

Ibrahim, M.A., Abdel-Gaber, S.A., Amin, E.F., Ibrahim, S.A., Mohammed, R.K., Abdelrahman, A.M., 2014. Molecular mechanisms contributing to the protectiveeffect of levosimendan in liverischemia-reperfusion injury.Eur J Pharmacol741:64-73.

Johnsen, S.G., 1970. Testicular biopsy score count--a method for registration of spermatogenesis in human testes: normal values and results in 335 hypogonadal males. Hormones 1(1):2-25.

Kalra, D., Sivasubramanian, N., Mann, D.L., 2002. Angiotensin II induces tumor necrosis factor biosynthesis in the adult mammalian heart through a protein kinase C–dependent pathway. Circulation 105(18):2198-2205.

Kara, Ö., Sari, E., Akşit, H., Yay, A., Akşit, D., Dönmez, M.I., 2016. Effects of selenium on ischaemia-reperfusion injury in a rat testis model. Andrologia 48(10):1267-1273.

Karaguzel, E., Kadihasanoglu, M., Kutlu, O., 2014. Mechanisms of testicular torsion and potential protective agents. Nat Rev Urol 11(7):391-399.

Kemahli, E., Yildiz, M., Firat, T., Özyalvaçli, M.E., Üyetürk, U., Yilmaz, B., Gücük, A., 2016. An experimental study on effects of pyrrolidinedithiocarbamate on ischemia-reperfusion injury in testis. Can Urol Assoc J 10(3-4):E104-109.

Kocak, C., Kocak, F.E., Akcilar, R., Bayat, Z., Aras, B., Metineren, M.H., Yucel, M., Simsek, H., 2016. Effects of captopril, telmisartan and bardoxolone methyl (CDDO-Me) in ischemia-reperfusion-induced acute kidney injury in rats: an experimental comparative study. ClinExpPharmacolPhysiol 43(2):230-241.

Kostakis, I.D., Zavras, N., Damaskos, C., Sakellariou, S., Korkolopoulou, P., Misiakos, E.P., Tsaparas, P., Vaos, G., Karatzas, T., 2017. Erythropoietin and sildenafil protect against ischemia/reperfusion injury following testicular torsion in adult rats. ExpTher Med 13(6):3341-3347.

Kumtepe, Y., Odabasoglu, F., Karaca, M., Polat, B., Halici, M.B., Keles, O.N., Altunkaynak, Z., Gocer, F., 2010. Protective effects of telmisartan on ischemia/reperfusion injury of rat ovary: biochemical and histopathologic evaluation FertilSteril 93(4):1299-1307.

Lipton P., 1999. Ischemic cell death in brain neurons.Physiol Rev 79(4): 1431-1568.

Lysiak, J.J., 2004. 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. Endocrino 10;2:9.

Lysiak, J.J., Nguyen, Q.A., Kirby, J.L., Turner, T.T., 2003. Ischemia-reperfusion of the murine testis stimulates the expression of proinflammatory cytokines and activation of c-jun N-terminal kinase in a pathway to E-selectin expression. BiolReprod 69(1):202-210.

Miller, A.F., 2012. Superoxide dismutases: ancientenzymes and newinsights. FEBS Lett 2012;586(5):585-595.

Minutoli, L., Antonuccio, P., Romeo, C., Nicòtina, P.A, Bitto, A., Arena, S., Polito, F., Altavilla, D., Turiaco, N., Cutrupi, A., Zuccarello, B., Squadrito, F., 2005. Evidence for a role of mitogen-activated protein kinase3/mitogen-activated protein kinase in the development of testicular ischemia-reperfusion injury.BiolReprod73(4):730-736.

Nickenig, G., Harrison, D.G., 2002. The AT(1)-type angiotensin receptor in oxidative stress and atherogenesis: part II: AT(1) receptor regulation. Circulation 105(4):530-536.

Nishikimi, M., Appaji, N.,Yagi, K., 1972. The occurrence of superoxide anion in the reaction of reduced phenazinemethosulfate and molecular oxygen.Biochem.Bioph. Res. Common 46(2), 849-854.

Okur, M.H., Arslan, S., Aydogdu, B., Zeytun, H., Basuguy, E., Arslan, M.S., Ibiloglu, I., Kaplan, I., 2017. Protective Effect of Cordycepin on Experimental Testicular Ischemia/Reperfusion Injury in Rats.J Invest Surg 12, 1-8.

Ringdahl, E., Teague, L., 2006. Testicular torsion. Am Fam Physician 74 (10): 1739-1743.

Saravanan, P.B., Shanmuganathan, M.V., Ramanathan, M., 2015. Telmisartan attenuated LPS-induced neuroinflammation in human IMR-32 neuronal cell line via SARM in AT1R independent mechanism. Life Sci130:88-96.

Sharp, V.J., Kieran, K., Arlen, A.M., 2013. Testicular torsion: diagnosis, evaluation, and management. Am Fam Physician 88(12):835-840.

Sharpe, M., Jarvis, B., Goa, K,L., 2001. Telmisartan: a review of its use in hypertension. Drugs 61(10):1501-1529.

Skondras, I., Lambropoulou, M., Tsaroucha, A., Gardikis, S., Tripsianis, G., Simopoulos, C., Vaos, G., 2015. The role of Apigenin in testicular damage in experimental ischemia-reperfusion injury in rats. Hippokratia 19(3):225-230.

Soguta, S., Zoroglu, S.S., Ozyurt, H., Yılmaz, H.R., Zugurlu, F.O.,Sivasli, E.,Yetkin, O.,Yanık, M., Tutkun, H., Savas, H.A., Tarakcoglu, M. Akyol, O., 2003. Changes in nitric oxide levels and antioxidant enzyme activities may have a role in the pathophysiological mechanisms involved in autism. ClinChimActa331, 111-117.

Soumya, R.S., Vineetha, V.P., Salin Raj, P., Raghu, K.G., 2014. Beneficial properties of selenium incorporated guar gum nanoparticles against

ischemia/reperfusion in cardiomyoblasts (H9c2). Metallomics 6(11):2134-2147.

Takaya, T., Kawashima, S., Shinohara, M., Yamashita, T., Toh, R., Sasaki, N., Inoue, N., Hirata, K., Yokoyama, M., 2006. Angiotensin II type 1 receptor blocker telmisartan suppresses superoxide production and reduces atherosclerotic lesion formation in apolipoprotein E-deficient mice. Atherosclerosis 186(2):402-410.

Takhtfooladi, M.A., Jahanshahi, A., Sotoudeh, A., Daneshi, M.H., Khansari, M., Takhtfooladi, H.A., 2013. The antioxidant role of N-acetylcysteine on the testicular remote injury after skeletal muscle ischemia and reperfusion in rats.Pol J Pathol 64(3):204-209.

Tamamura, M., Saito, M., Kinoshita, Y., Shimizu, S., Satoh, I., Shomori, K., Dimitriadis, F., Satoh, K., 2010. Protective effect of edaravone, a free-radical scavenger on ischaemia-reperfusion injury in the rat testis. BJU Int 105(6):870-876.

Tuncer, M.C., Ozturk, H., Buyukbayram, H., Ozturk, H., 2007. Interaction of L-arginine-methylester and Sonic hedgehog in liver ischemia-reperfusion injury in the rats. World J Gastroenterol 13(28):3841-6.

Turner, T.T., Bang, H.J., Lysiak, J.L., 2004. The molecular pathology of experimental testicular torsion suggests adjunct therapy to surgical repair. J Urol 172 (6 Pt 2): 2574-2578.

Unsal, A., Devrim, E., Guven, C., Eroglu, M., Durak, I., Bozoklu, A., Balbay, M.D., 2004. Propofol attenuates reperfusion injury after testicular torsion and detorsion. World J Urol 22(6): 461-465.

Unsal, A., Eroglum, M., Avci, A., Cimentepe, E., Guven, C., DeryaBalbay, M., Durak, I., 2006. Protective role of natural antioxidant supplementation on testicular tissue after testicular torsion and detorsion. Scand J UrolNephrol 40(1): 17-22.

Yazihan, N., Ataoglu, H., Koku, N., Erdemli, E., Sargin, A.K., 2007. Protective role of erythropoietin during testicular torsion of the rats. World J Urol25(5):531-536.

Zhang, H., Schmeisser, A., Garlichs, C.D., Plotze, K., Damme, U., Mugge, A., Daniel, W.G., 1999. Angiotensin II-induced superoxide anion generation in human vascular endothelial cells: role of membrane-bound NADH-/NADPH-oxidases. Cardiovasc Res 44(1):215–222.

Zhang, Y., Lv, Y., Liu, Y.J., Yang, C., Hu, H.J., Meng, X.E., Li, M.X., Pan, S.Y., 2013. Hyperbaric oxygen therapy in rats attenuates ischemia-reperfusion testicular injury through blockade of oxidative stress, suppression of inflammation, and reduction of nitric oxide formation. Urology 82(2):489.e9-489.e15.