Effect of Erythropoietin on Experimental Unilateral Testicular Torsion Detorsion in Rat Model

Gehane M. Hamed[*1], Ramadan M. Ahmed [1], Maher M. Emara[2], and Manar H. Mahmoud [3]

[1]Physiology Dept., [2]Histology Dept., [3]Biochemistry Dept. Faculty of Medicine, Ain Shams University, Cairo, Egypt
[*]gihane_68@yahoo.com

Abstract: Testicular torsion is a common syndrome that could lead to infertility. In this study, we investigated the protective effect of erythropoietin on experimental unilateral testicular torsion detorsion in a rat model. Fourty male albino rats were divided into three groups: Group I (10): Sham operated rats, Group II (15) untreated torsion detorsion (T/D); torsion of the right testis by rotating the testis 720^{O} (2 times around the longitudinal axis of the spermatic cord) in a clockwise direction for 2 hours, and after 2 hours, the right testis was detorted by rotating the testis 2 times (720^{o}) in an anticlockwise fashion and then fixed in the right position for 30 days, Group III (15): treated torsion detorsion (T/D): the same surgical procedure was done as in group II, in addition EPO at a dose of 1000 IU/Kg was injected i.p, 3 times per week for 12 doses. After the end of the study, ECG was performed, abdominal aorta was exposed and blood samples were collected in plain tube and then centrifuged to obtain serum, to determine serum Testosterone, both testes were removed, weighed, then divided into 2 halves, one half for histopathological examination and the other half for determination of testicular malonyldialdehyde, testicular glutathione peroxidase (GPx), and testicular catalase levels. The results of the present study showed significant increase in Q wave voltage and ST segment slope in untreated T/D compared to sham operated rats. Biochemical parameters showed significant decrease in serum testosterone in untreated T/D rats compared to treated rats and to sham operated rats. In addition significant increase in right and left testicular MDA level and significant decreases in right and left testicular GPx and catalase levels compared to sham operated rats, these parameters were greatly ameliorated in treated T/D rats. These biochemical changes were further confirmed by histopathological examinations showing severe destruction of semineferous tubules of the right testes, left testes affection were also observed but to a lesser degree in untreated T/D. EPO treatment greatly ameliorated the damage of ipsilateral and contralateral testes of T/D model. In conclusion, the protective effect of erythropoietin on testicular torsion could serve as a promising intervention to oxidative stress associated infertility problems, such as testicular torsion.
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1. Introduction

Testicular torsion is an urological emergency that usually results from rotation of testis around the axis of the spermatic cord, and it is frequently observed in newlyborns, children and adolescents *(Ergur et al., 2008)*. Rapid diagnosis and immediate surgical treatment are essential to avoid permanent testicular damage. Delay or misdiagnosis and inappropriate treatment usually lead to male infertility *(Perotti et al., 2006).* The main pathophysiology of testicular torsion detorsion of spermatic cord is ischemia/ reperfusion (I/R) injury of the testis *(Turner et al., 1993).*

Under normal conditions, free radicals are produced and their effects are counterbalanced by the endogenous antioxidant system. When ROS generation exceeds the defense mechanisms capacity, oxidative stress is generated and contributes to reversible and irreversible cell injury *(Bulger & Maier, 2001).*

Erythropoietin (EPO) is a glycoprotein responsible for the formation of erythrocytes in the bone marrow and it is the drug of choice in treatment of anemia. There are several studies reported the beneficial effects of exogenous EPO administration on I/R of lung, eye, kidney, spinal cord in animals *(Akeora et al., 2007).* Moreover, EPO mRNA has been detected in brain, bone marrow, placenta, ovary and testis. In addition, functional EPO receptors (EPO-R) has been demonstrated in rodent and human placenta, brain, and kidney cells. In testis, there is basal expression of EPO mRNA which increased upon hypoxic exposure *(Akeora et al., 2007).* It has been reported that EPO influences rat Leydig cells steroidogenesis via stimulating production of testosterone and in humans, EPO administration intravenous increases testosterone in renal failure

(Yamamoto et al., 1997). In light of these data, we conducted this study in order to evaluate the protective effects of EPO on ischemic reperfusion injury in rat model subjected to torsion detorsion of the spermatic cord.

2. Materials and Methods:

This study was approved by the Ethics Committee FMASU 952/2011

Experimental animals:

This study was carried out on 40 young male albino rats weighing 120- 140 gm at start of the study, rats were purchased from Experimental Animal farm of Helwan and housed 3/cage in suspended wire-mesh cages and maintained in Physiology Department animal house, Faculty of Medicine, Ain Shams University under standard conditions of boarding, at room temperature and in controlled environment of 12h light-dark cycle. All rats were fed standard rat chow before starting the experiment. The standard rat chow diet (AIN-93 M diet formulated for adult rodents) prepared according to *Reeves et al. (1993).*

Experimental protocol:

The rats were allocated into 3 groups:

a- Group I included (n= 10) sham rats. Under ether anaesthesia, median scrotal incision was done, delivery of the right testis without twisting the testicle and fixed to the scrotum with chromic catgut sutures. Then they are submitted to a second sham under ether anaethesia, with incision of scrotal skin sutures and fixation sutures within the scrotum.

b-Group II included (n= 15) untreated torsion detorsion (T/D): Under ether anaesthesia, median scrotal incision was done, delivery of the right testis and after division of gubernaculums, then torsion was created by rotating the testis 720^{O} (2 times around the longitudinal axis of the spermatic cord) in a clockwise direction for 2 hours, and fixed to the scrotum with chromic catgut sutures. After 2 hours, the right testis was detorted by rotating the testis 2 times (720^{o}C) in an anticlockwise fashion after cutting the fixation sutures and then replaced into the scrotum and fixed in the right position for 30 days (modified from *Ergur et al., 2008).* 0.5 cc 0.9% Nacl solution was injected i.p. 1/2 an hour before the detorsion and 3 times in a week (for 12 doses).

c-Group III (n= 15): treated torsion detorsion (T/D): the same surgical procedure was done as in group II, in addition EPO at a dose of 1000 IU/Kg was injected i.p. 1/2 an hour before the detorsion and 3 times in a week (for 12 doses), as described by *Koseoglu et al. (2009).*

EPO was purchased from EPREX ®: is a sterile phosphate buffered Epoetinum alfa solution for parentral administration, graduated prefilled syringes with needle guard (PROTECS ^{TM}) containing 4000 IU/ 0.4 ml.

Experimental procedure:

At the end of the experimental period, all rats were fastened overnight, weighed and anaesthetized with intraperitoneal thiopental sodium (40 mg/Kg bw).

1-ECG recording:

Needle electrodes were placed under the skin of the 4 limbs of the animal near the paws, and connected through an ECG coupler to a 2 channel oscillograph (Cardimax FX 121, Fukuda Denshi Co, LTD). The electrocardiographic tracing was recorded using standard limbs. From lead II-ECG tracing with paper speed of 25 mm/sec, heart rate (HR), P-R interval, QRS duration, QT interval, Q wave voltage, R wave voltage and ST segment deviation were measured. The heart rate was calculated using the following formula:

$$HR = \frac{7500}{\text{Distance in mm between 6 successive peaks of R waves}}$$

2-Orchidectomy:

The scrotal skin was incised, sutures fixing the right testis were cut, the spermatic cord was clamped with forceps, transected and ligated with 2/0 chromic catgut sutures. The same procedure was done for the contralateral left testis. Each testis was weighed then cut transversely, one half is preserved in 10% formaline for histopathological examination, and the second half was preserved in parafilm and kept at – 80^{O}C for biochemical studies.

3- A midline incision was made, then the abdominal aorta was exposed and blood samples were collected in plain centrifuge tubes; to obtain serum after centrifugation at 4000 r.p.m. for 15 minutes for separation of serum and then stored at – 80^{o} till used for determination of biochemical measurements.

Biochemical measurements:

a- Serum Testosterone, Calbiotech Inc, ELISA; as described by ***Bricaire*** *et al. (1991).*

B-Testicular malonyldialdehyde (MDA), according to the method described by *Draper and Hadley (1990).*

c-Testicular glutathione peroxidase (GPx), Bio.diagnostic. com, UV method; as described by *Paglia and Valentine (1967).*

d-Testicular catalase, Bio.diagnostic. com, colorimetric method; as described by *Aebi (1984).*

4-Histopathological examinations:

Each testis was cut transversely into 2 halves by sharp incisor. The specimens were prepared for histological study, according to the method described by *Drury and Wallington (1980)*. They were fixed in 10% neutral buffered formaline, dehydrated cleared and embedded in paraplast. Paraffin sections were cut serially at 5 µm-thicknesses. Sections were stained by haematoxyline and eosin (Hx &E).

Statistical Analysis:

All statistical data and significance tests were performed by using SPSS (Statistical Program for Social Science) statistical package (SPSS Inc) version 8.0.1 *(Armitage & Berry, 1987)*. Statistical significance was determined by one-way ANOVA (analysis of variance) for differences between means of different groups; further analysis was made by LSD (least significance difference) multiple-range test to find intergroupal differences; a probability of $P< 0.05$ was considered statistically significant.

3. Results:

Concerning ECG parameters, significant increase ($P< 0.05$) in Q wave voltage and increase in ST segment slope were observed in untreated T/D rats compared to sham operated rats (Table 1).

Concerning testicular weights, significant decreases ($P< 0.05$) in right testicular weights were observed in group II compared to both group I and III, upon calculation of right testicular weight/ body weight ratio, the significance was still present between group II and III. While non significant differences were observed in left testicular weighs and left testicular weight/ body weight ratio among the 3 studied groups (Table 2).

Concerning lipid peroxidation, the untreated T/D rats promoted significant increases ($P< 0.05$) in right and left testicular MDA compared to treated T/D and to sham operated rats. Upon erythropoietin administration to T/D rats, significant increases ($P< 0.05$) were observed in right and left testicular GPx and catalase levels compared to untreated T/D rats (Table 3).

Concerning serum testosterone level, significant decrease ($P< 0.05$) in serum testosterone was observed in untreated T/D rats compared to sham operated rats. Upon treatment with EPO to T/D rats, serum testosterone showed significant increase ($P< 0.05$) compared to untreated T/D rats, although the level was still significantly lower than sham operated rats (table 4).

Histopathological examination of both right and left testes of sham operated group revealed multiple rounded seminiferous tubules with regular outlines. They were lined by multiple layers of germinal epithelium at different stages of spermatogenesis. The interstitial spaces between the tubules contained Leydig cells and some blood capillaries (Figure 2). In untreated T/D, histopathological examination of the testicular sections of right testes of this group showed seminiferous tubules with irregular outlines. Some tubules showed complete loss of the germinal epithelium with acidophilic exudates in the lumen. Other tubule showed few layers of germinal epithelium with multiple spaces in between the cells. The lumen showed complete loss of the sperm. The interstitial spaces were filled by cellular infiltration most probably fibroblast and fluid exudates (Figure 3). On the other hand, examination of sections of the left testes showed irregular outlines and destucted some seminiferous tubules. The germinal epithelium revealed diminution of spermatogenic cells with areas of cellular loss and intercellular spaces in between the cells. The lumen revealed very few spermatids and rare sperm. The interstitial space showed acidophilic exudates, congested blood vessels and some Leydig cells (Figure 4). Histopathological examination of testicular sections of right testes of treated T/D rats showed some seminiferous tubules appearing with regular lining, multiple layers of spermatogenic cells, abundant whorly appearance of sperm flagella filling their lumina and some Leydig cells in the interstitial spaces. However, some tubules showed cellular infiltration in their lumina. The most prominent observation were the presence of enlarged congested blood vessels in the interstitial spaces (Figure 5). On the other hand, examination of the sections of the left testes of this group showed multiple seminiferous tubules with regular outline and spermatogenic cells at different stages of spermatogenesis. Also, enlarged congested blood vessels and cellular exudates in the interstitial spaces were also seen (Figure 6).

4. Discussion:

The results of the present study demonstrated that I/R or torsion of the right testes for 2 hours followed by 30 days detorsion caused testicular necrosis and marked destruction of semineferous tubules and decreased testicular weight in ipsilateral testes, as well as diminution of spermatogenic cells with areas of cellular loss and intercellular spaces in between the cells in contralateral left testes compared to sham operated rats. While treatment with EPO provided a beneficial effect to the ipsilateral and contralateral testes by decreasing necrosis and atrophy and improving spermatogenesis as shown by histopathological examinations and further proved by significant increases in serum testosterone level in treated T/D rats compared to untreated T/D rats.

Table (1): Changes in ECG parameters; heart rate (HR, beats/min), P-R interval (msec), QRS wave (msec), QT (msec), Q wave (μv), R wave (μv), and ST segment elevation (μv) in the three studied groups.

Groups	HR (beats/min)	P-R (msec)	QRS (msec)	QT (msec)	Q (μv)	R (μv)	S (μv)
-Sham (10)	377 ± 22	54 ± 2.7	40 ± 2.9	80 ± 3.7	43 ± 3.8	575 ± 37.5	55 ± 3.3
-Untreated T/D (15)	387 ± 10.7	58 ± 1.5	39 ± 0.7	88 ± 2.6	62 ± 5.4*	547 ± 23.6	73 ± 4.5*
-Treated T/D (15)	359± 13.2	53 ± 3.2	41± 2.3	90 ± 4.4	53 ± 3.3	560 ± 21.4	58 ± 3.2**
P	NS	NS	NS	NS	< 0.05	NS	< 0.05

P: Significance by 1-way ANOVA among the 3 studied groups.
**: Significance by LSD at P< 0.05 from sham-operated group **: Significance by LSD at P< 0.05 from untreated T/D group.*
In parenthesis is the number of rats. Results are expressed as Mean ± SEM. NS: not significant.

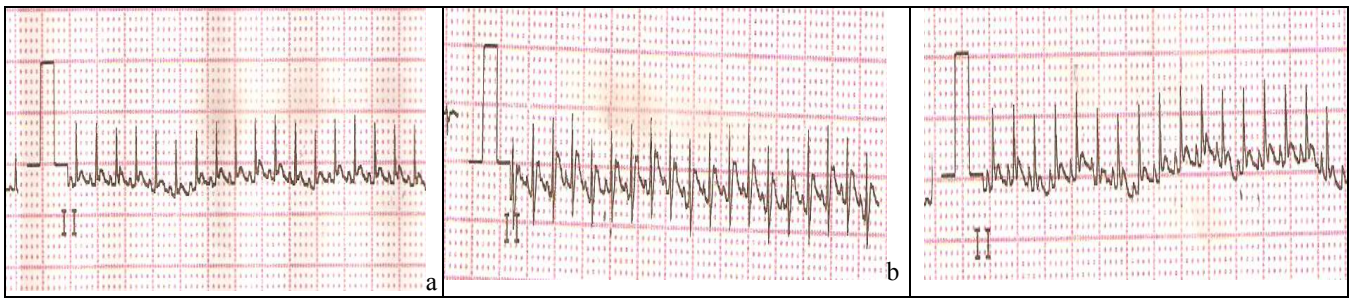


Figure (1): ECG records (lead II) of: a) Sham-operated b) Untreated T/D c) Treated T/D.

Table (2): Changes in right testis weight (RTW, g), right testis weight/ body weight (RTW/BW, mg/g), left testis weight (LTW, g), left testis weight/ body weight (LTW/BW, mg/g) in the three studied groups.

Groups	RTW (g)	RTW/BW (mg/g)	LTW (g)	LTW/BW (mg/g)
-Sham (10)	0.62 ± 0.05	3.1 ± 0.2	0.64 ± 0.49	3.2 ± 0.3
-Untreated T/D (15)	0.53 ± 0.02*	2.7 ± 0.1	0.65 ± 0.01	3.3 ± 0.07
-Treated T/D (15)	0.64 ± 0.01**	3.2 ± 0.08**	0.68 ± 0.01	3.4 ± 0.06
P	*< 0.05*	*< 0.05*	NS	NS

*P:Significance by 1-way ANOVA among the 3 studied groups. *: Significance by LSD at P< 0.05 from sham-operated group.*
***: Significance by LSD at P< 0.05 from untreated T/D group.*
In parenthesis is the number of rats. Results are expressed as Mean ± SEM. NS: not significant.

Table (3): Effect of ischemia/reperfusion injury and EPO on malondialdehyde (MDA, umol/g tissue), glutathione peroxidase (GPx,mU/mg protein), and catalase (CAT,U/mg protein) levels in ipsilateral (right= R) and contralateral (left= L) testicular tissues in the three studied groups.

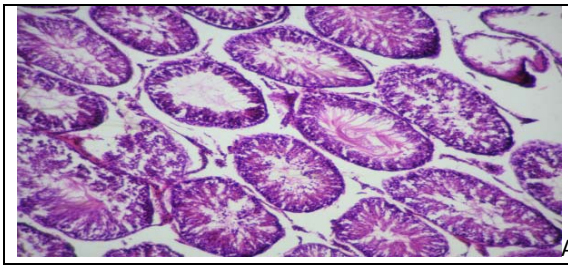
Groups	MDA (umol/g) R	MDA (umol/g) L	GPx (mU/mg) R	GPx (mU/mg) L	CAT (U/mg) R	CAT (U/mg) L
-Sham (10)	5.3 ± 0.2	5 ± 0.2	7.5 ± 0.3	7.5 ± 0.3	64.3 ± 4.5	62.9 ± 3.8
-Untreated T/D (15)	9.6 ± 0.5*	6.4 ± 0.4*	4.2 ± 0.3*	5.9 ± 0.3*	33.6 ± 2.3*	41.9 ± 2.3*
-Treated T/D (15)	6.6 ± 0.4 **	5.3 ± 0.3 **	6.4 ± 0.2*,**	7.3 ± 0.3**	45.3 ± 2.8*,**	53.9 ± 3*,**
P	< 0.001	< 0.05	< 0.001	< 0.05	< 0.001	< 0.001

*P:Significance by 1-way ANOVA among the 3 studied groups. *: Significance by LSD at P< 0.05 from sham-operated group.*
***: Significance by LSD at P< 0.05 from untreated T/D group.*
In parenthesis is the number of rats. Results are expressed as Mean ± SEM. NS: not significant.

Table (4): Changes of serum testosterone levels in the three studied groups.

Groups	Testosterone level (pg/ml)
-Sham (10)	2.9 ± 0.2
-Untreated T/D (15)	1.1 ± 0.07*
-Treated T/D (15)	1.8 ± 0.09*,**
P	*< 0.001*

*P:Significance by 1-way ANOVA among the 3 studied groups. *: Significance by LSD at P< 0.05 from sham-operated group.*
***: Significance by LSD at P< 0.05 from untreated T/D group.*
In parenthesis is the number of rats. Results are expressed as Mean ± SEM. NS: not significant.



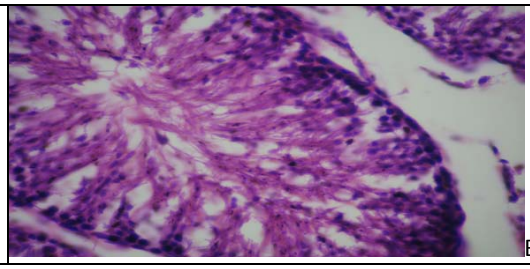
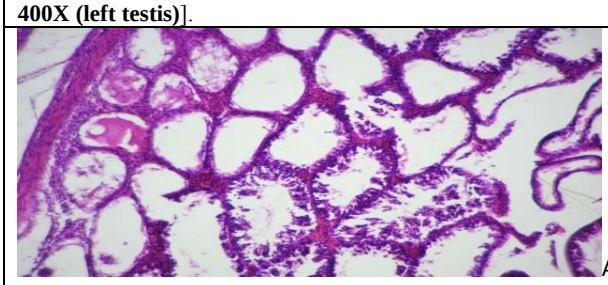


Fig.2: A photomicrograph of a T.S in the testis of sham operated group **[Hx & E, magnification A 100X (right testis) & B 400X (left testis)**].



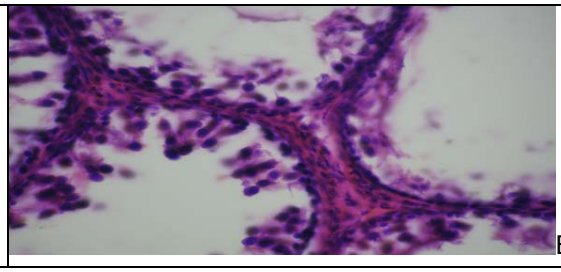
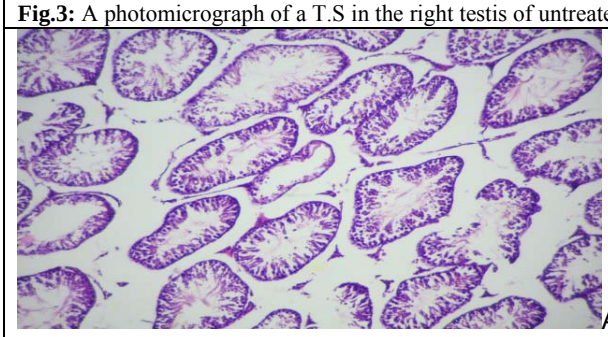


Fig.3: A photomicrograph of a T.S in the right testis of untreated T/D group [**Hx & E, magnification A 100X & B 400X]**.



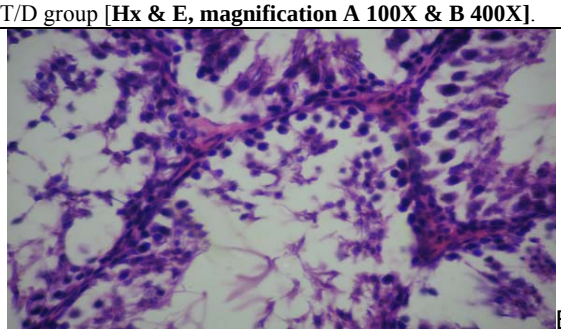
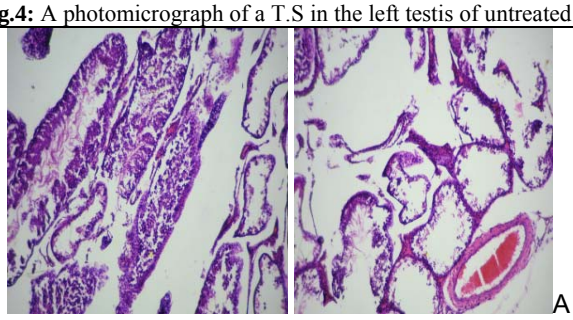


Fig.4: A photomicrograph of a T.S in the left testis of untreated T/D group [**Hx & E, magnification A 100X & B 400X]**.



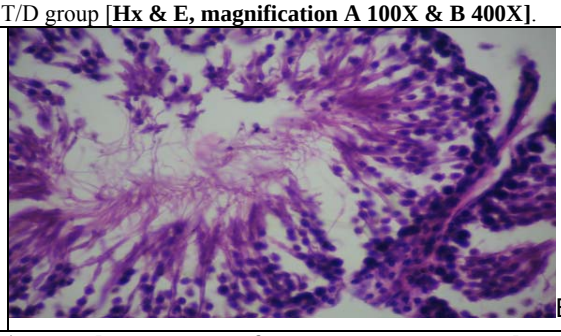
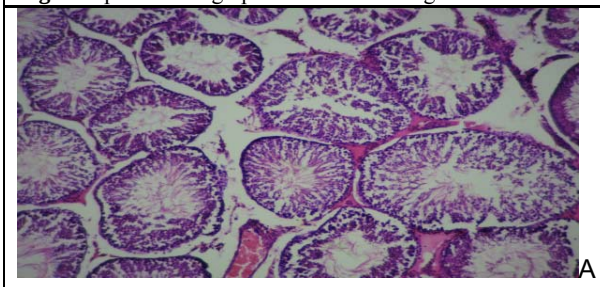


Fig.5: A photomicrograph of a T.S in the right testis of treated T/D group **[Hx & E, magnification A 100X & B 400X]**.



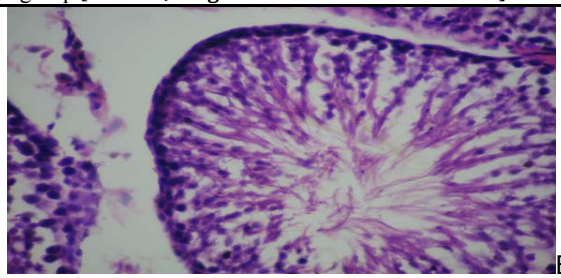


Fig.6: A photomicrograph of a T.S in the left testis of treated T/D group **[Hx & E, magnification A 100X & B 400X]**.

The ECG is one of clinical tests used for diagnosis of cardiovascular diseases *(Okin et al., 2004).* Abnormalities in the ECG were detected in untreated T/D rats such as significant elevation in the slope of ST segment compared to both sham-operated and treated T/D rats, as well as significant increase in Q wave voltage in untreated T/D rats compared to sham operated rats; which are important in diagnosis of ventricular injury or infarction. This observation indicates that testosterone confers cardioprotection against ischaemic insult. This finding is also in agreement with a previous observation that testosterone is associated with a decreased susceptibility to myocardial ischaemia *(Callies et al., 2003).* The study of *Tsang et al. (2008)* showed that the protective effect of testosterone is due to its direct action on the myocardium in addition to the beneficial effects of androgen, such as dilation of the coronary artery *(Weidemann and Hanke, 2002).* Moreover, testosterone enhances the effects of stimulation of both α1- and β1-adrenoceptors, thus enhancing both cardioprotection and injury, respectively. It has been shown that endogenous testosterone induces cytoprotection via activating cardiac mitochondrial ATP-sensitive K^+ channels *(Er et al., 2004).* Two studies have shown that chronic administration of testosterone at physiological concentrations enhances Ca^{2+} influx via the L-type Ca^{2+} channel in neonatal *(Michels et al., 2006)* and adult *(Er et al., 2007)* rat ventricular myocytes. In the adult ventricular myocyte, testosterone also increases the Ca2+ spark, indicating an increase in Ca^{2+} release from the sarcoplasmic reticulum. It has also been shown that testosterone increases the Na^+–Ca^{2+} exchange mRNA level in the heart, suggesting increased activity of the exchanger, which is responsible for Ca^{2+} removal *(Golden et al., 2004).* So testosterone may alter Ca^{2+} homeostasis, thus attenuating the $[Ca^{2+}]i$ overload in response to ischaemic insult and conferring cardioprotection *(Tsang et al., 2008).*

Torsion of the testis initially results in obstruction of venous blood flow of the spermatic cord with secondary edema and haemorrhage, then edema results in ischaemia and arterial obstruction leading to necrosis *(Koseoglu et al., 2009).* A possible mechanism of torsion detorsion is ischemic reperfusion injury. In fact, although that restoration of the blood flow to the testis is essential for tissue salvage, but it may have detrimental effects *(Minutoli et al., 2005).* As it has been reported that reperfusion of an ischemic tissue leads to ROS generation which arise from xanthine oxidase activation or from leukocytes penetration in the interstitial tissue *(Granger & Korthuis, 1995).* Also, activation of NOS which can cause testicular damage and atrophy and stimulation of proinflammatory cytokines *(Filho et al., 2004)*. Normally, they are counterbalanced by the endogenous antioxidant system. When ROS production exceeds the capacity of the defense mechanisms, cell damage occurs *(Lysiak et al., 2002).* Moreover, upon reperfusion of the tissue, oxygen needed for the conversion of hypoxanthine to uric acid becomes available resulting in generation of enormous amount of free radicals which react with lipid in the cell and mitochondrial membranes leading to disruption of integrity of the cell *(Reilly et al., 1991).*

As previously known, sperms are highly sensitive to oxidative stress and particularly to lipid peroxidation due to their high content of polyunsaturated fatty acids in the plasma membrane. The fatty acids are an essential requirement for the male germ cell to maintain sperm functions *(Henkel, 2005).* ROS can also decrease the enzymatic denfenses of the spermatozoa *(Michael et al., 2009).* Increased oxidative stress damages the sperm membranes, proteins and DNA *(Sanoka and Kurpisz, 2004).* Also, oxidative stress induces peroxidative damage to the plasma membrane of sperm *(Visser and Heynes, 2003),* which causes loss of sperm function *(Kato et al., 2001).* The effect of oxidative stress of I/R of right testes are demonstrated by significant increases in right and left testicular MDA levels and significant decreases in right and left testicular glutathione peroxidase and catalase levels compared to both sham and treated T/D rats. These results are in agreement with the study of *Ergur et al. (2008)* who reported that darbepoetin α; a novel EPO protein caused similar effects in torsioned testes. The significant increase in MDA level (indirect method for measurement of ROS activity) in untreated T/D rats compared to both sham and treated rats proved that I/R of the testes promotes severe cell membrane peroxidation *(Guimaraes et al., 2007).* The antioxidant effect of EPO is previously supported by the findings that EPO protects against oxidative damage via inhibition of lipid peroxidation and restoration of cytosolic catalase and GPx activities in erythrocytes. Erythropoietin also increases free radical scavengers activities in cultured mouse astrocytes. Thus, EPO may serve the role of direct antioxidant by scavenging oxyradicals and indirect antioxidant by stimulating the other antioxidant defensive mechanisms *(Palmer et al., 1990).* It is also possible that EPO exerts EPO-R independent cytoprotective actions via antioxidation *(Akeora et al., 2007).*

EPO is a hormone secreted in response to hypoxia mainly by the kidneys, also the liver has a significant role in erythropoietin mRNA formation *(Koseglu et al., 2009)*. Moreover, it has been reported

that testosterone production was stimulated by EPO in rat Leydig cells and this finding might be the cause of suggestion that EPO can act directly on human Leydig cells *(Buemi et al., 2002)*. Furthermore, erythropoietin is important in the development of new blood vessels, for example, in the muscle of atheletes training at high altitudes, also it is found in ovary, uterus and testis *(Lappin, 2003).* In the present study EPO treatment caused an increase in vascularization in between semineferous tubules and this may suggest a vasoproliferative and neoangiogenic ability of EPO as previously described by *Koseglu et al. (2009).*

Histopathological examination of sections of right testes in untreated T/D rats showed loss of germinal epithelium, loss of spermatid, absence of the sperm which denotes absence of spermatogenisis. Dark (deep stained) nuclei indicating inactive cells. Also, presence of cellular infiltration, exudates, congested blood vessels are further proofs of the dysfunction of the right testes. Contralateral left testes affection are also observed, one theory postulates decrease in blood supply of contralateral testis as a reflex to an afferent stimulus *(Andiran et al., 2000)* , other theories proposed autoimmune reaction, release of acrosomal enzymes from contralateral testis *(Uguralp et al., 2004*), and apoptosis *(Pampal et al., 2010)*. 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 histopathological damage of the contralateral side, or using a therapy to decrease the injurious effect of I/R. In the present study, EPO treated T/D rats showed marked improvement of the general histopathological picture of the right T/D as well as of the left contralateral testes which may be explained by the direct stimulating effect of EPO on germ cell proliferation and its antiapoptotic signaling. The exact mechanism of the protective effect of EPO against I/R injury is not fully understood. *Akeora et al.* in their study reported that EPO protection on brain tissues exposed to oxidative stress is secondary to the inhibition of caspases 3, 8 and 9 and hence an antiapoptotic effect of EPO *(Akeora et al., 2007).* Both sperm concentration and motility were reduced on 30 days after one hour torsion in rats in the study of Payabvash *et al. (2008).* The detrimental effects of IR on the sperm concentration suggest an interference with spermatogenesis; ischemia may induce necrotic degeneration of testicular tissues, which may contribute to loss of spermatogenesis in testis.

In conclusion we can suggest that erythropoietin treatment can be used beside surgical correction as a novel therapeutic approach in T/D of the testis to ameliorate the injurious effect of ischemia reperfusion, and to be an alternative therapy to T/D instead of removal of ipsilateral testicular torsion.

Corresponding author:
Gehane M. Hamed
Physiology Dept.**,** Faculty of Medicine, Ain Shams University, Cairo, Egypt
gihane_68@yahoo.com

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