

Histological Study on the Possible Ameliorating Effect of Platelet Rich Plasma on Ischemia/Reperfusion Injury in Testicular Torsion Model in Adult Albino Rat

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ABSTRACT

Introduction: Testicular torsion is a serious urologic problem that can cause dysfunction of the testis and fertility loss due to ischemia. Platelet rich plasma (PRP) with its high convergence of growth factor can possess a beneficial role in regenerative therapy particularly ischemia/reperfusion (I/R) injury.

Aim of the work: To evaluate the possible ameliorating effect of PRP in the I/R injury in testicular torsion rat model.

Materials and Methods: Thirty two adult male albino rats were used. 24 were assigned into three groups, with eight in each: Group I (Control group). Group II (Ischemia/reperfusion group) (I/R group): subjected to left testicular torsion for two hours followed by detorsion. Group III (PRP-treated I/R group) subjected to left testicular torsion for two hours followed by detorsion and PRP was injected into the testis parenchyma upon detorsion. The PRP was prepared from the remaining eight rats. Testicular sections were taken and stained with H&E, toluidine blue and immunohistochemical staining against PCNA & Bcl-2 and other samples were processed for transmission electron microscopic study. Western blot assay for caspase -3 and transforming growth factors- β (TGF- β) protein expressions. Malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH) measurement were performed. Blood samples are collected for testosterone hormone and Inhibin-B levels assays. Morphometric and statistical analysis were done.

Results: Light microscopic examination revealed testicular structural changes in I/R group, marked damage of the testis, defective spermatogenesis with wide areas of cell loss. Injection of PRP in group III resulted in improvement of testicular tissue changes. This was proved ultrastructurally and by the morphometric results, which showed significant changes in serum testosterone, Inhibin-B levels, MDA, GSH, and SOD measurement of group II when compared to the control group and was documented by Western blot assay for caspase -3 and TGF- β .

Conclusion: Platelet rich plasma could be promising to have an effective role in treatment for rescuing the testis from ischemia/reperfusion injury. PRP could use as a therapeutic plan for regeneration after I/R to avoided orchidectomy.

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INTRODUCTION

Testicular torsion (Torsion of the spermatic cord) is a urologic emergency could produce variable degrees of testicular damage and there is no agent to preserve testicular functions following detorsion and fixation. The annual incidence of testicular torsion is as four in 100.000 by the age of 18 years^[1]. It was recorded that unilateral testicular torsion/detorsion produced excessive damage to both testes with extra affection of the ipsilateral side. Proceedings was ensured to evaluate the true incidence of testicular atrophy after acute testicular torsion. In epidemiological studies, the range of orchidectomy has been reported as 41.9% in males subjecting to surgery for testicular torsion^[2].

As the testicular torsion is a pathologic case perform the testis ischemic, and the surgical intervention is usually prerequisite to reestablish the vascular flow^[3]. Time delay

is the most influential, factor in predicting clinical outcomes after essential critical care^[4].

Detorsion of the testis is important to improve testicular blood flow and it is not always effective due to reperfusion injury. Moreover detorsion and reperfusion to ischemic testis may increase the injury⁽³⁾. Previous studies have evaluated ischemia/reperfusion injury and effective treatment with therapeutic agents in torsion-detorsion with anti-inflammatory drugs, antioxidant drugs, free radical scavengers, non steroidal anti-inflammatory drugs, and nitric oxide donors^[5]. It is reported that, up to date, there is no clinical study of potential pharmaceutical therapeutic agents in human testicular torsion and to decrease I/R-induced oxidative stress has been performed. Induction of the therapeutic agents with surgical intervention could deliver the testes with better reperfusion harm^[6]. However,

the effectiveness of therapeutic agents after manual and surgical detorsion of testicular torsion is still have to preserve normal testicular functions^[7].

Platelet rich plasma (PRP), is a unique derivative of whole blood rich in a high convergence of vital growth factors and cytokines cell adhesion molecules. Platelet rich plasma has proven its role in regenerative medicine and clinical applications in cell therapy for its powerful healing properties^[8]. It is still needed to study its role in testicular torsion.

Administration of pharmacologic agents as adjuvant treatment to surgical therapy for restoring the testis from ischemia/reperfusion (I/R) injury is a clinically substantial target. Platelet rich plasma therapy has been recorded to be efficient in I/R organ models of some organs, in-vivo^[9]. In order to, growth factors like transforming growth factor beta 1 (TGF- β 1), insulin-like growth factor (IGF) and vascular/endothelial growth factor (VEGF) are generally released during I/R injury for various protective roles, these studies estimated PRP in ischemia/reperfusion organ models^[10&11]. The present study was to evaluate the possible ameliorating effects of PRP against testicular ischemia/reperfusion injury.

MATERIAL AND METHODS

Experimental animals thirty two adult male albino rats aged 8 weeks old and weighing 180-220 gm were obtained from the animal house colony of the national research center (Cairo, Egypt).

The experimental procedures were performed according to the Guide for Care and Use of Laboratory Animals Published by National Research Center, Cairo, Egypt. The rats were housed under the same environmental conditions and had free access to food and water ad libitum. After acclimatization, rats were divided into three groups, with eight in each. The remaining eight rats were used for the preparation of PRP. Each group underwent right orchietomy through a right subinguinal scrotal incision to prevent contribution of hormone production by right testis, providing equitable hormone level measurement.

- **Group I (Control group):** Were subdivided into 2 equal subgroups (4 animals each) as follows:
- **Subgroup 1-A:** Sham operated, control group.
- **Subgroup 1-B:** Subjected to intratesticular PRP injection into the left testis parenchyma 10 μ l (2000 x 10⁹/L) single dose^[12].
- **Group II (Ischemia/reperfusion group)(I/R):** Through the subinguinal scrotal incision the left testis was brought out and rotated twice clock wise, then reinserted and fixed to the tunica albuginea and subcutaneous tissue with 4/0 nylon sutures. The scrotal incision was closed with 4/0 nylon suture. By using the same incision, the scrotum was reopened after two hours and the testis was derotated to its natural position with closure of the scrotum^[13].

- **Group III (PRR-treated I/R group):** The left testis of each animal was exposed and rotated for two hours as in group II then the testis was counter-rotated to its normal position Then subjected to intratesticular PRP injection into the left testis parenchyma during detorsion at the same dose of subgroup 1-B. Surgical procedure was performed under anesthesia with ether inhalation.

In the sham-operated control group, animals subjected to the same surgical proceedings without torsion and detorsion. After four weeks, all animals were sacrificed after ether anesthesia. Blood samples were immediately collected to measure the serum testosterone and serum inhibin-B levels. The testicular tissues were cut transversely into 2 halves; half was preserved for histological study and the other half was taken for western blotting and biochemical assessment.

Platelet Rich Plasma Preparation

Eight age-matched healthy male rats were used as PRP donors. The whole blood samples of rats was withdrawn using the specific medical techniques to avoid hemolysis. Venous blood was taken via cardiac puncture and mixed acid citrate dextrose (ACD) at a blood/citrate ratio of 9/1. Samples test tubes transferred for laboratory analysis before centrifugation for platelet concentration. The blood was centrifuged at 1480 rpm using a soft spin for 6 minutes for separation of the plasma containing the platelets from erythrocytes. The plasma was drawn off the top and was transferred to another tube without anticoagulant, centrifuged again using a hard spin at a higher speed of 3400 rpm for 15 min to have a platelet concentrate. The upper 2/3 rd which consisted of platelet poor plasma (PPP) was removed. The lower 1/3 rd was platelet rich plasma (PRP) was allocated and frozen at -80oC for use. The average PRP was evaluated using a sysmex XT-1600i system. The platelet count was 2410x10³ platelets/UL. PRP was activated by addition of CaCL₂ 10% (0.8 mL of PRP + 0.3mL of CaCL₂ 10%) close to the moment of their therapeutic use, at time of detorsion^[14,15].

Biochemical Analysis

- Serum testosterone assays: Serum testosterone levels of the rats were measured by an Enzyme-Linked Immunosorbent Assay (ELISA) by using a specific kit for estimating testosterone hormone in the serum isolated from rat blood (Bio V-endor, Gunma, Japan). The experiment was done according to the manufacturer's instructions.

- Serum inhibin-B assays: Serum inhibin was estimated using rat inhibin-B (INH-B) Elisa kits (Cat. No: MBS162795). The data were expressed as ng/L. Serum inhibin B levels (a marker of Sertoli cell function and spermatogenesis).

- Measurement of superoxide dismutase (SOD) activity:

Superoxide Dismutase (SOD) activity in testicular tissue homogenate assay. SOD activity was determined

by using super oxide dismutase Kit (Bio-diagnostic Giza, Egypt) following to the instructions from the manufacture expressed as units/ml^[16].

- Measurement of malondialdehyde (MDA)& glutathione (GSH) levels:

Tissue malondialdehyde (MDA) level in the homogenized testicular tissue was calculated using a lipid peroxide (malondialdehyde) assay kit (Bio-diagnostic Giza, Egypt) according to the manufacture's instructions^[15].

Tissue glutathione (GSH) level in testicular tissue homogenate was determined by spectrophotometer using GSH reduced kit (Bio-diagnostic Giza, Egypt) according to the manufacture's instructions^[17].

Western blot analysis for caspase -3 and transforming growth factors- β protein expressions:

A small piece of testis parenchyma was suspended in homogenization buffer containing Tris-Hcl 50mM (pH: 7.4), 2mM/EDTA, 10mM β -glycerol phosphate, 10mM NaF, 1mM PMSF and complete protease inhibitor cocktail using polytron homogenizer in ice and then centrifuged at 12.000 Xg for 5 minutes at 4oC. Before taking the supernatants on ice, protein contents were determined using Bio-Rad Protein Assay Kit. Samples were then added with loading buffer to the same concentration. Briefly, samples containing equivalent amounts of 50 μ g of total protein were loaded to SDS-PAGE gel using the Bio-Rad minigel system and then transferred to PVDF membrane (Bio-Rad, Hercules, CA, USA) by electrophoresis.

At room temperature, blots were blocked with 3% non fat dry milk (Sigma,70166) in tris-buffered saline with tween TBST (TBScontaining0.1%Tween-20). Blotting membranes were probed overnight with specific primary antibodies (1:500 monoclonal rat anti TGF- β 1 sc-146; anti-caspase-3 sc-56055; and mouse monoclonal anti β -actin, 0.5 μ g/ml, sc-47778 from Santa Cruz Biotechnology). Membranes were washed 3 times with 0.1% Tween and TBST. Then labeled with secondary antibodies (goat-anti-rabbit IgG-H&L (horseradish peroxidase, HRP), Abcam, 1:20000; and goat-anti-mouse IgG-H&L (HRP), Abcam, 1:20000 respectively. Finally immunoreactive protein bands were visualized with an enhanced chemiluminescence reagent (ECL, Pierce, Rockford, IL, USA) and Alliance Gel-doc (Alliance 4.7 Gel doc, UV tec UK). UV tec software (UK) was used to semi quantify the density of each individual protein band. Signals were normalized with respect to β -actin as loading control^[18].

Histological Study

Samples of testicular tissue were fixed in bouin's solution and processed to obtain serial paraffin sections of 7 μ m thickness. The prepared histological slides were stained with the following:

1. Hematoxylin and eosin (H&E) for observation of histological changes^[19].

2. Immunohistochemical stains.

Sections were mounted on +ve charged slides for Immunohistochemical Staining for detection of bcl-2 an antiapoptotic factor (bcl-2) and proliferating cell nuclear antigen(PCNA) reaction in testicular tissue using an anti-bcl-2and anti- PCNA antibodies. Sections were incubated with the primary rabbit anti- bcl-2 (Sigma-Aldrich, USA) and anti-PCNA (Thermo scientific) over night at 4°C. Primary binding was detected using a horseradish peroxidase-conjugated goat anti-rabbit antibody (Vector Laboratories, Burlingame, CA, USA) and visualized by development with 3, 3-diaminobenzidine (DAB, Sigma). All sections were counterstained with hematoxylin. Negative control were obtained by skipping the step of applying the primary antibody^[20].

For electron microscopic study, Small pieces (1mm3) from the testes were excised and immediately fixed in 2.5% glutaraldehyde for 24 hours. Semi-thin sections (1 μ m) were stained with toluidine blue (TB) and examined with light microscope. Ultrathin sections (40-50 μ m) were stained with uranyl acetate and lead citrate then examined and photographed with a Jeol JEM-100 SX transmission electron microscope in the electron microscopic unit, Faculty of Medicine, Tanta University, Egypt^[21].

Morphometric Study

The following morphometric measurements were determined using "Leica Qwin 500 C" image analyzer computer system (Lieca imaging system Ltd., Cambridge,UK) (Cairo University):

1. Area % of Immunopositive reaction of anti - bcl-2 antibody.
2. Area % of Immunopositive reaction of anti - PCNA antibody.

Area % was estimated by measuring the area in randomly chosen ten Non overlapping field in each section at a magnification of x 100 .

Statistical Analysis

The values were represented as mean \pm SD. The data were analyzed, calculated and compared between different groups was made using analysis of variance followed by the post -hoc Tukey test .The P value was directly provided by the computer using SPSS software (version16; SAS Institute Inc., Cary, North Carolina, USA). Results were regarded statistically significant if *P value* was less than 0.05 .

RESULTS

Biochemical Results

Serum Testosterone Levels

The mean serum testosterone hormone level was significantly decreased in the I/R group in comparison to the control group ($P<0.001$). Moreover, the mean serum testosterone hormone level was found to be increased in

the IR/PRP group compared to the I/R group ($P<0.01$) (Table 1).

Serum Inhibin- β levels

The mean serum inhibin- β level was significantly lower in I/R group in comparison with control group ($P<0.001$). Moreover, the mean serum inhibin- β level revealed to be increased in PRP-treated I/R group compared to I/R group ($P<0.001$) (Table 2).

Oxidative Stress Parameters in Testis

As observed in (Figure 1A), MDA levels in testicular tissues of I/R group were significantly higher compared to the control group ($P<0.001$), while platelet rich plasma treated group showed significantly reduced MDA levels compared to the I/R group ($P<0.001$). GSH levels and superoxide dismutase activity were significantly reduced in the I/R group compared to the control group ($P<0.001$) and in the PRP treatment significantly reversed SOD ($P<0.001$) but not GSH levels compared to the I/R group (Figures 1B and C).

Caspase-3 and Transforming Growth Factor- β protein Expressions

The western blot analysis revealed that caspase-3 and TGF- β protein expression in the tissue of the thesis was significantly increase in I/R group in comparison to control group ($P<0.001$) and decreased with PRP treatment compared with I/R groups ($P<0.001$) (Figure 2).

Histological Results

Group 1 (Control group)

Examination of H&E and toluidine blue stained sections of testicular tissue of this group showed multiple rounded seminiferous tubules with regular outlines. They were lined by germinal epithelium at different stages of spermatogenesis. The flagella of mature sperms, were seen filling the lumina of the seminiferous tubules. The interstitial spaces between the tubules with Leydig cells. The lining epithelium consisted of sertoli cells and germinal cells. Sertoli cells appeared pyramidal in shape resting on the basement membrane. The germinal epithelium consisted of spermatogonia, spermatocytes, early rounded spermatids with acrosomal caps and late elongated spermatids (Figures 3 and 4).

Bcl-2 immunohistochemical result showed many brown positively stained cells of bcl-2 immunoreactions were found in the cytoplasm of the spermatogenic cells and interstitial Leydig cells (Figure 5). Testicular sections stained with anti-PCNA antibody showed positive immunoreactive nuclei of the spermatogonia and primary spermatocytes (Figure 6).

Electron microscopic examination of control testis revealed spermatogonia resting on the basement membrane with euchromatic nuclei close to Sertoli cell which showed normal appearance and exhibited triangular nucleus, well

developed nucleolus and the cytoplasm contained dense bodies, mitochondria, and ribosomes. Spermatocytes appeared as large cells, with rounded nuclei, dispersed granular chromatin and containing mitochondria and multiple ribosomes in the cytoplasm. Early spermatids with acrosomal cap were appeared. The mitochondrial cristae are irregular in outline and lie parallel instead of perpendicular to mitochondrial surface. So, their center appeared free of cristae and occupied by low density matrix (Figure 7-A and B). The rounded spermatids with rounded nuclei and vesicle spreading over the anterior hemisphere of the nucleus to form acrosomal cap and peripherally arranged mitochondria were also seen (Figure 8-A). Elongated spermatids showed elongated pyriform nuclei with condensed chromatin. The sperm middle piece showed central microtubules surrounded by nine coarse fibers with circumferential arranged mitochondria peripherally. Principle piece and end piece were also appeared (Figure 8-B). The interstitial tissue revealed blood vessels and interstitial cells of Leydig. The interstitial cells of Leydig exhibited large oval shaped nuclei with euochromatic chromatin. Their cytoplasm contained mitochondria, abundant smooth endoplasmic reticulum (SER), few profile of rough endoplasmic reticulum (RER), some lysosomes and lipid droplets and free ribosomes (Figure 9).

Group 2 (Ischemia/ Reperfusion (I/R) group)

Histological examination of H&E stained testicular tissue sections revealed disruption of the architecture with reduction in the thickness and disfigured germinal epithelium of the seminiferous tubules with irregular outlines and diminished spermatogenic cells. Some Spermatogonia revealed dark condensed nuclei and appeared detached from the basement membrane. The Iry spermatocytes exhibited hyper chromatic nuclei (Figure 10-A). Some tubules showed, with spaces of cellular loss, multiple vacuoles and most of spermatogenic cells showed dark small pyknotic nuclei. The interstitial space exhibited congested blood vessels and some Leydig cells, which were seen with an excess of acidophilic vacuolated exudate in-between (Figure 10-B). Toluidine blue-stained sections revealed disorganized spermatogenic cells with areas of cellular loss, marked vacuolation between the cells with sloughing of some cells to the lumen. Sertoli cell with vacuolated cytoplasm and some large cells with karyorrhexis. Areas of exudate in the widening interstitial spaces and congested blood vessels, with some of the Leydig cells have dark pyknotic nuclei were seen. (Figure 11-A and B).

Immunohistochemical stain of bcl-2 showed decreased positive immunoreactivity in the cytoplasm of germinal cells and Leydig cells when compared with the control group (Figure 12-A and B). Sections stained with anti-PCNA antibody showed nuclear immunopositive spermatogonia and some primary spermatocytes whereas other primary spermatocytes were found to be immunonegative (Figure 13-A and B).

Electron microscopic examination of the testis specimens, revealed Sertoli cell with highly indented nucleus and fine granular chromatin resting on the irregular basement membrane with cytoplasm contains mitochondria, clear vacuoles, and many dense bodies. Appearance of adjoining spermatogonia which has small nucleus with clumped chromatin with cytoplasmic dense bodies. (Figure 14-A and B and 15). Primary spermatocyte was appeared with dense clumps of heterochromatic nucleus while its cytoplasm showing dense aggregated ill-defined organelles, empty spaces and electron-dense granules. Shrunken nucleus is also seen in another spermatocyte. (Figure 15-A and B). Spermatids exhibited different forms of affections, some spermatids appeared with heterochromatic nucleus, complete loss of acrosomal cap and irregular distribution of the mitochondria. Nucleus with no acrosomal cap were appeared in another spermatid. The other one showed partial loss of acrosomal cap and vacuoles. Some deteriorated configured spermatids appear with deformed nuclei and defects in the acrosomal cap. Some abnormal elongated spermatids were also noticed (Figure 16-A and B).

The interstitial cells of Leydig showed variable significant findings, with small indented nucleus with peripherally clumped chromatin. The cytoplasm included extensive vacuoles, lysosomes, multiple lipid droplets of different density (Figure 17).

Group 3. (Platelet rich plasma-treated Ischemia/Reperfusion group):

Histological examination of H&E stained testicular tissue sections revealed that administration of Platelet rich plasma upon detorsion improved the general histological picture of the testis, where a nearly normal architecture for seminiferous tubules and the lining germinal epithelium with well-organized spermatogenic layers at different stages of spermatogenesis. Multiple rounded seminiferous tubules with abundant whorly appearance of tails of sperms appeared in their lumina and some Leydig cells in the interstitial spaces with little acidophilic vacuolated exudate in-between. Some tubules revealed some cytoplasmic vacuoles of spermatogenic cells (Figure 18). Toluidine blue-stained sections revealed the lining germinal epithelium showed spermatogenic cells at different stages of spermatogenesis. Sertoli cells were more or less similar to the control, apparently normal rounded spermatids with acrosomal caps and many elongated spermatids were also observed. Some exudate in the interstitial spaces and congested blood vessels could be also noticed. (Figure 19). Moderate increase in bcl-2 immunorexpression was observed in cytoplasm of germ cells and Leydig cells compared with the I/R group (Figure 20). The testicular sections stained with anti-PCNA antibody exhibited immunoreactivity localized at the nuclei of spermatogonia and primary spermatocytes (Figure 21).

Electron microscopic examination of the testicular sections revealed features that were nearly as those of the

control group. The Sertoli cell showed nucleus with fine granular chromatin resting on apparently normal basement membrane. The cytoplasm contains mitochondria and some dense bodies. The neighboring spermatogonia cell showed its nucleus close to the basement membrane. Primary spermatocytes preserved their normal appearance with their large spherical nuclei, dispersed chromatin and cytoplasm containing mitochondria and multiple ribosomes. (Figure 22). Rounded spermatids revealed spherical nuclei with fine granular chromatin and complete acrosomal cap. However, the other spermatids with distorted nuclei and complete loss of acrosomal cap were also observed (Figure 23-A and B).

Leydig cells appeared with small rounded nucleus and clumped marginated chromatin, the cytoplasm showed some lipid droplets, many normal mitochondria, ribosomes and dense bodies (Figure 24).

Morphometric results and statistical analysis

As regards the Group II, it showed a significant decrease in the mean area% of anti-Bcl2, and anti-PCNA antibodies ($P<0.05$) compared with the control group. As regards the Group III in which animals treated with PRP, it showed significant increase in the mean area% of anti-Bcl2, and anti-PCNA antibodies ($P<0.05$) compared with group II (Table 3).

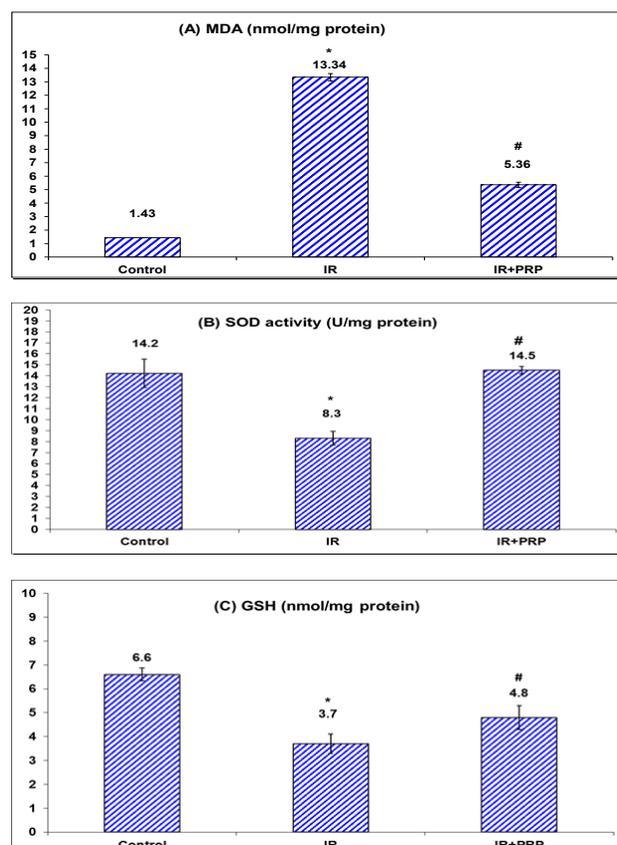


Fig. 1: (A) MDA (nmol/mg protein) in the studied groups. (B) SOD activity (U/mg protein) in the studied groups. (C) GSH(nmol/mg protein) in the studied groups. (n=8). Each vertical bar represents the mean \pm SD. *: Means $p<0.001$ (I/R group compared with control group). #: Means $p<0.001$ (I/R and PRP group compared with I/R group).

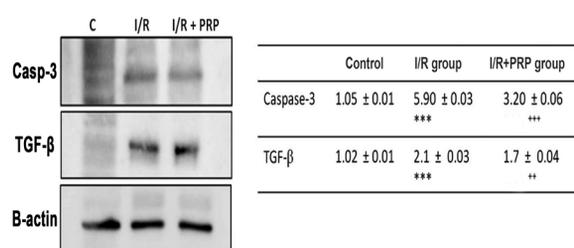


Fig. 2: Caspase-3 protein expression in testicular tissues of rats determined by Western blotting in nmol pNA/mg protein. C, control; I/R, ischemia/reperfusion (I/R) group; I/R PRP, PRP-treated I/R groups: *** $P < 0.001$: vs. control; ++ $P < 0.001$: vs. I/R.

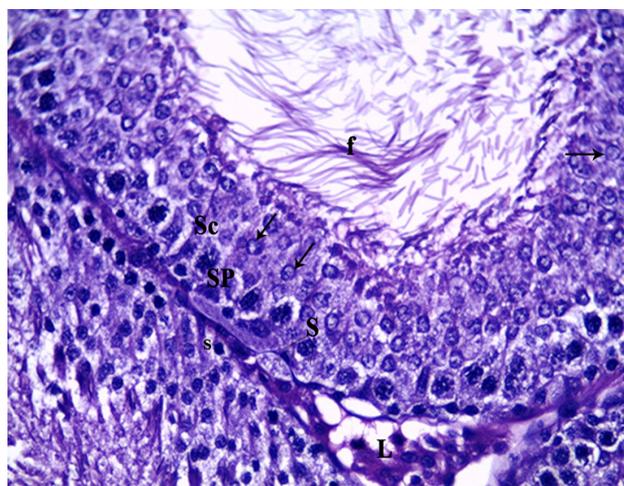


Fig. 3: A photomicrograph of a testicular section from the control group showing the seminiferous tubules with the interstitial spaces show Leydig cells (L). Notice the whorly appearance of sperm flagella, filling the lumina (f). Sertoli cell (S) appears pyramidal and resting on the basement membrane. Spermatogonia cells (Sp), Iry spermatocytes (Sc), and round spermatids with acrosomal caps (↑). (H & E x 400)

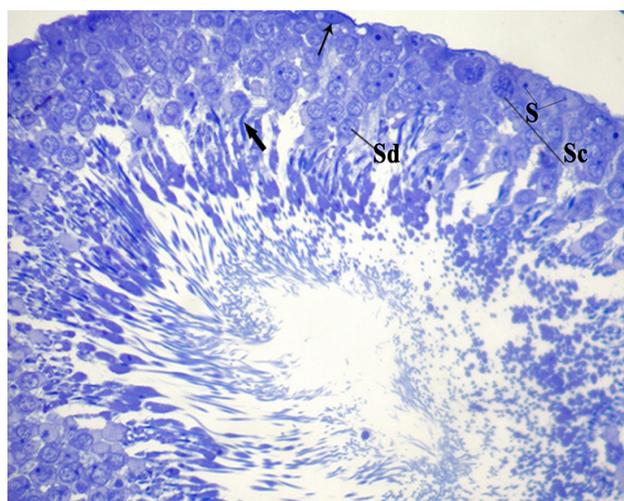


Fig. 4: A photomicrograph of a testicular section from the control group showing sertoli cells (S), spermatogonia cells (↑), Iry spermatocytes (Sc), rounded spermatids (Sd) with acrosomal caps and elongated spermatids (thick arrow). (Toluidine blue x 1000)

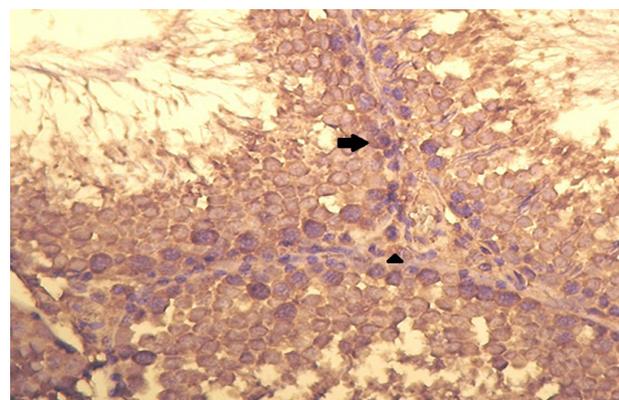


Fig. 5: A photomicrograph of a testicular section from the control group showing positive BCL2 immunoreactivity in the cytoplasm of the spermatogenic and Leydig cells. (Immunopositive BCL2, × 400)

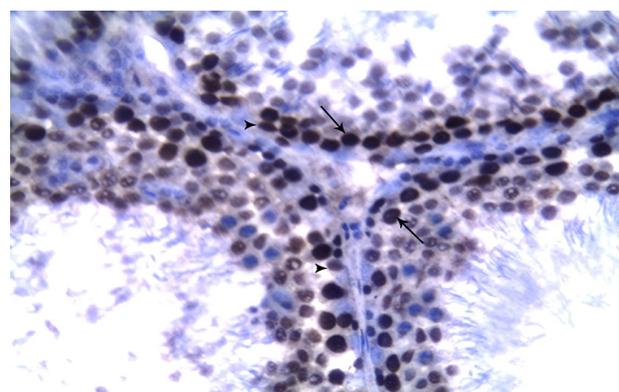


Fig. 6: A photomicrograph of a section of testis of the control group showing the seminiferous tubule lined by immunopositive nuclei of spermatogonia (arrow head) and primary spermatocytes (arrow). (Immunopositive PCNA, × 400)

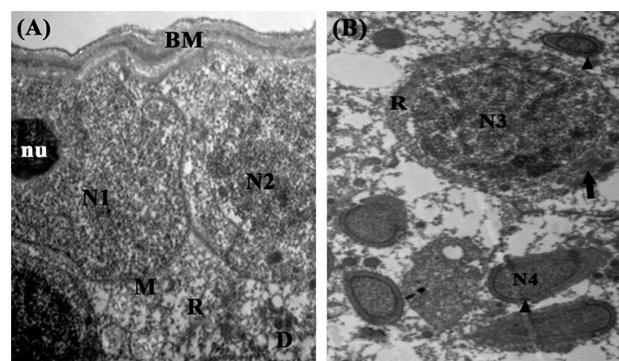


Fig. 7: An electron photomicrograph of a section of testis of control group showing (A) Sertoli cell appears with triangular nucleus (N1), and well developed nucleolus (nu), resting on the basement membrane (BM) with cytoplasm contains mitochondria (M), electron-dense bodies (D), and free ribosomes (R). Notice part of spermatogonia cells resting on the basement membrane with euchromatic nucleus (N2). (B) showing Iry spermatocyte cell with large rounded nucleus (N3) of dispersed granular chromatin and its cytoplasm contains mitochondria (thick arrow) and multiple ribosomes (R). Early spermatids with the nuclei (N4) appear with acrosomal cap (arrow head) spread over one pole. (TEM, (A) × 25000, (B) × 1500)

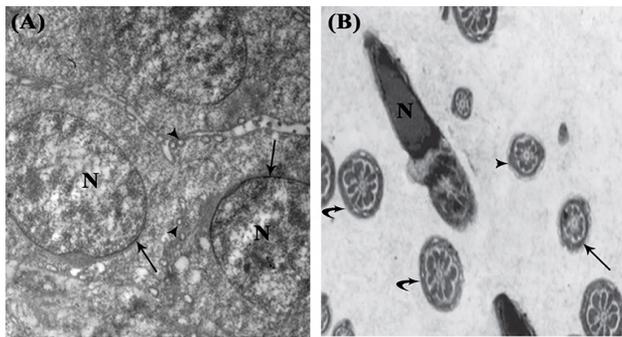


Fig. 8: (A) An electron photomicrograph of a section of testis of control group showing (A) rounded spermatid with rounded nuclei (N), acrosomal cap (arrow) spread over one pole and the cytoplasm contains multiple peripheral mitochondria (arrow head). (B) showing elongated spermatid with pyriform shaped nucleus and condensed chromatin (N), middle piece with central microtubules, coarse fibers, and peripheral mitochondria (curved arrow), principal piece (▲), and end piece (†). (TEM, (A) x 25000, (B) x 5000)

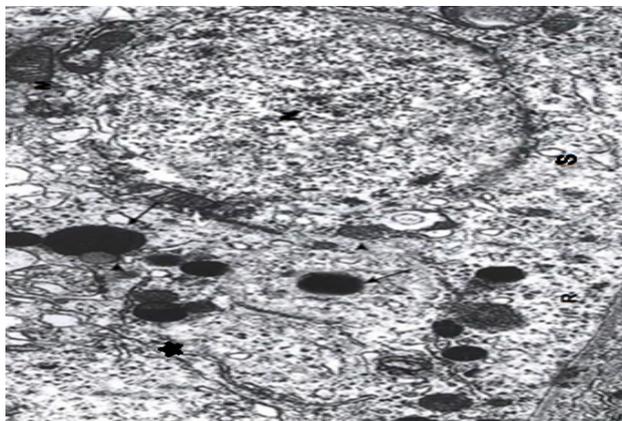


Fig. 9: An electron photomicrograph of a section of testis of control group showing Leydig cell with large oval euchromatic nucleus (N). The cytoplasm is rich in mitochondria (M), lipid droplets (†), free ribosomes (R), abundant SER (S), few profile of RER (star), and some lysosomes (arrow head). (TEM, x 3500)

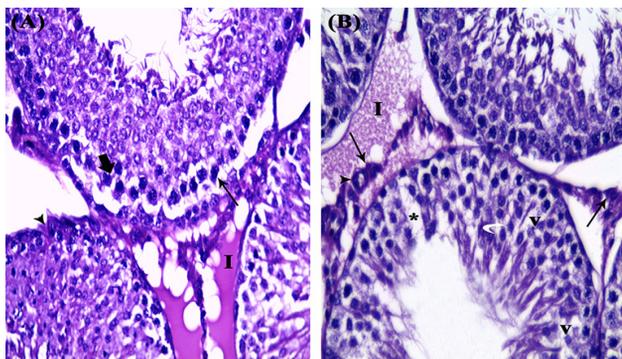


Fig. 10: (A) A photomicrograph of a testicular section from the I/R group showing apparent reduction in the thickness of the germinal epithelium, irregular borders of some seminiferous tubules (arrow heads), with acidophilic materials in the interstitial spaces (I), some spermatogonia cells detached from the basement membrane with condensed nuclei (†) and 1ry spermatocytes with hyperchromatic nuclei (thick arrow). (B) Some tubules show intercellular spaces of cellular loss (*) and multiple vacuoles (V). Congested blood vessels (arrow head), some Leydig cells (†), with an excess of acidophilic vacuolated exudate (I) in the interstitial space are seen. Notice, most of spermatogenic cells show dark small pyknotic nuclei (curved arrow). (H&E x 400)

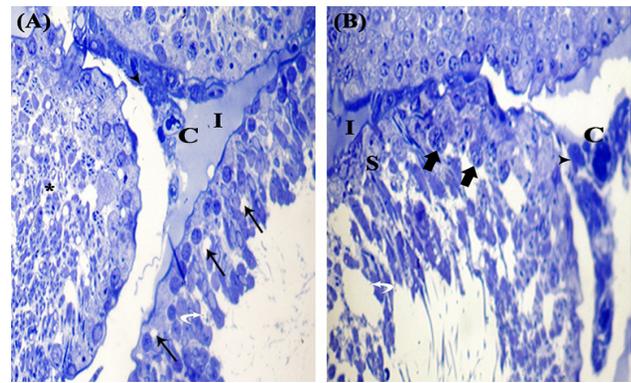


Fig. 11 A&B: A photomicrograph of a testicular section from the I/R group showing disorganized spermatogenic cells with areas of cellular loss (curved arrow) and multiple vacuoles (†) with sloughing of some cells to the lumen (*), Sertoli cell (S) with vacuolated cytoplasm and some large cells with karyorrhexis (thick arrow). Areas of exudate (I) in the widening interstitial spaces and congested blood vessels (C), with some of the Leydig cells have dark pyknotic nuclei (arrowhead) are seen. (Toluidine blue x 1000)

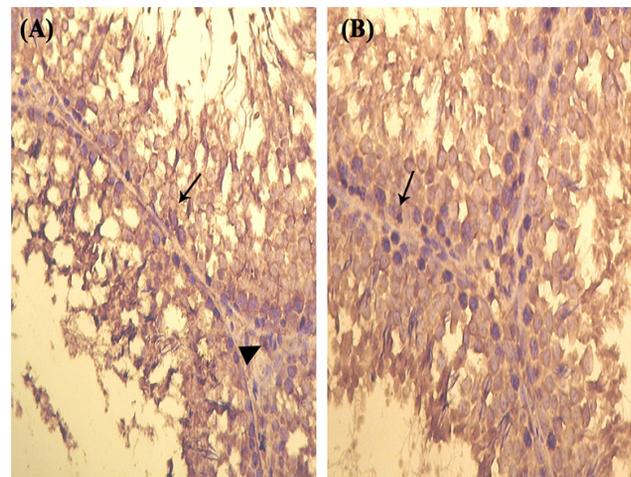


Fig. 12 A&B: A photomicrograph of a testicular section from the I/R group showing decreased positive immunoreactivity in the cytoplasm of germinal cells (†) and Leydig cells (arrow head). (Immunostaining for bcl-2, x 400)

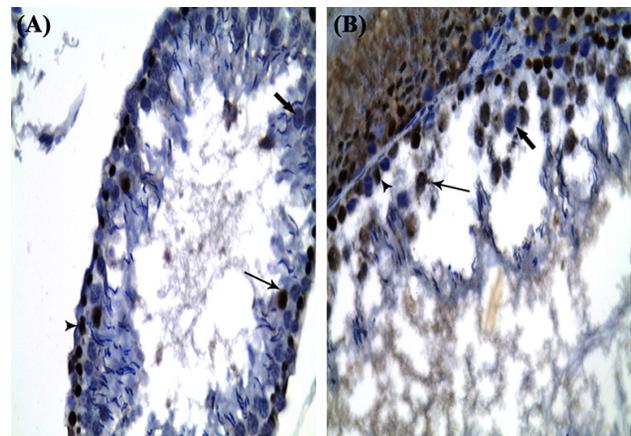


Fig. 13 A&B: A photomicrograph of a testicular section from the I/R group showing nuclear immunopositive spermatogonia (arrow head) and primary spermatocytes (†) whereas other primary spermatocytes appear to be immunonegative (thick arrow). (Immunopositive PCNA, x 400)

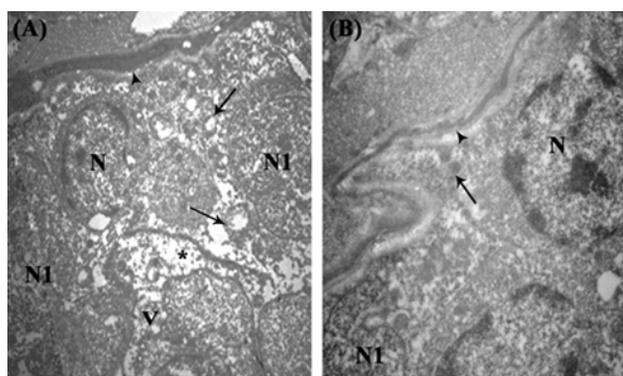


Fig. 14 A&B: An electron photomicrograph of a testicular section from the I/R group showing Sertoli cell with highly indented nucleus (N1) and fine granular chromatin resting on the irregular basement membrane (arrow head) with cytoplasm contains mitochondria (↑). Appearance of spermatogonia has small nucleus(N) with clumped chromatin. Notice, damaged spermatid with vacuolated cytoplasm (v) and empty space (*). (TEM, (A) x 1500, (B) x 3000)

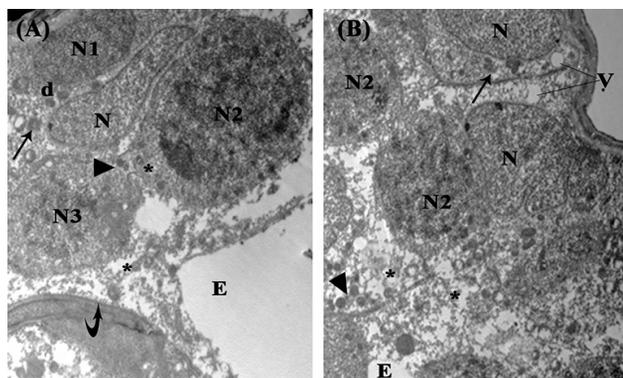


Fig. 15 A&B: An electron photomicrograph of a testicular section from the I/R group showing sertoli cell with irregular nucleus (N) , cytoplasmic clear vacuoles (v), and many dense bodies (↑). The adjoining spermatogonia cell has small nucleus(N1) with cytoplasmic dense bodies (d). Primary spermatocyte with dense clumps of heterochromatic nucleus (N2) with cytoplasm showing dense aggregated ill-defined organelles (*),empty spaces(E)and electron-dense granules (arrow head),shrunken nucleus (N3) is also seen in another spermatocyte. Notice, abnormal spermatid (curved arrow). (TEM, (A) x 1500, (B) x 1500)

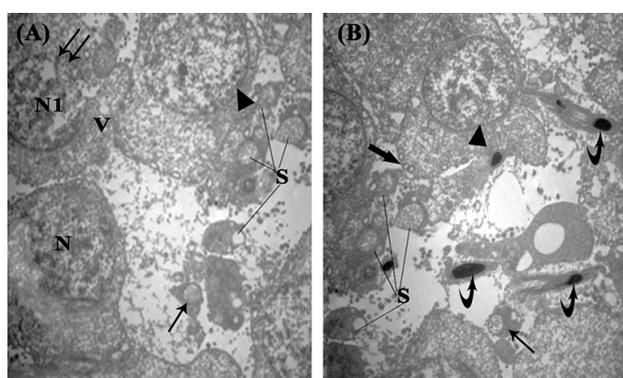


Fig. 16-A,B: An electron photomicrograph of a testicular section from the I/R group showing spermatids with heterochromatic nucleus and complete loss of acrosomal cap (arrow head) with irregular distribution of the mitochondria (thick arrow). Nucleus (N) with no acrosomal cap is also seen in another spermatid . The other one (N1) shows partial loss of acrosomal cap (double arrow) and vacuoles(v).Some deteriorated configured spermatids appear with deformed nuclei(S) and defects in the acrosomal cap(↑). Notice some abnormal elongated spermatids (curved arrow). (TEM, (A) x 1500, (B) x 1500)

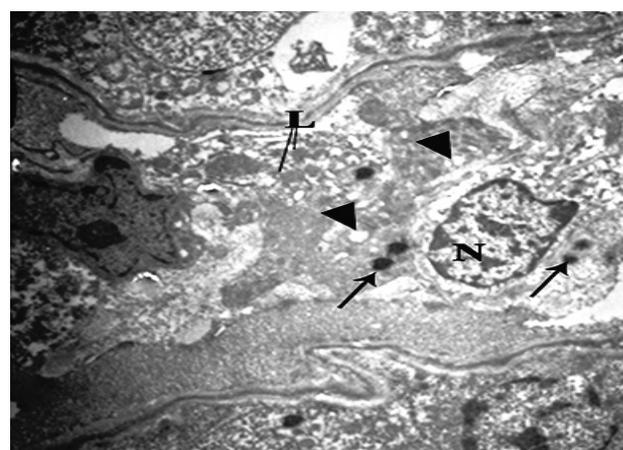


Fig. 17: An electron photomicrograph of a testicular section from the I/R group showing Leydig cells with small indented nucleus (N) with peripherally clumped chromatin. The cytoplasm contains vacuoles (arrow head), multiple lipid droplets (↑)and lysosomes (L). (TEM, x 1500)

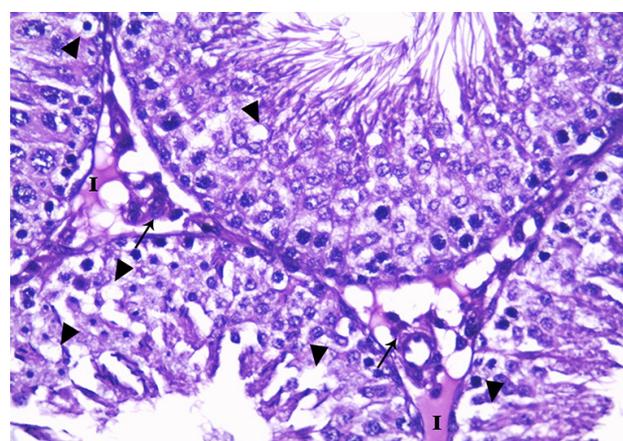


Fig. 18: A photomicrograph of a testicular section from the platelet rich plasma-treated Ischemia/ Reperfusion group showing rounded seminiferous tubules with abundant whorly appearance of sperm flagella(F) filling their lumina. Some tubules show cytoplasmic vacuoles of spermatogenic cells (arrow head). The interstitial space shows Leydig cells (↑) and few acidophilic vacuolated exudate in-between(I). (H & E x 400)

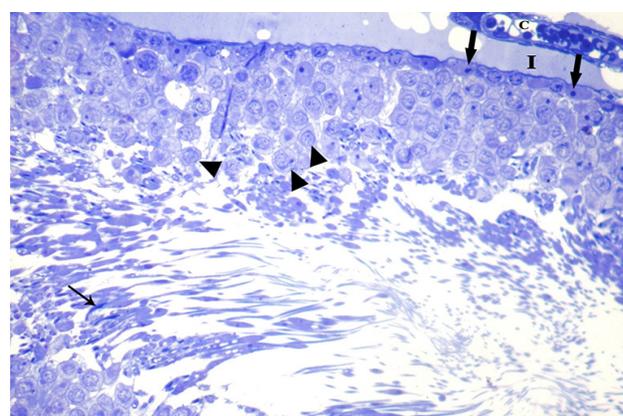


Fig. 19: A photomicrograph of a testicular section from the platelet rich plasma-treated Ischemia/ Reperfusion group showing apparently normal sertoli cell (thick arrow), rounded spermatids with acrosomal caps (arrow head) and elongated spermatids (arrow). Some exudate (I) in the interstitial spaces and congested blood vessels(C) are seen. (Toluidine blue x 1000)

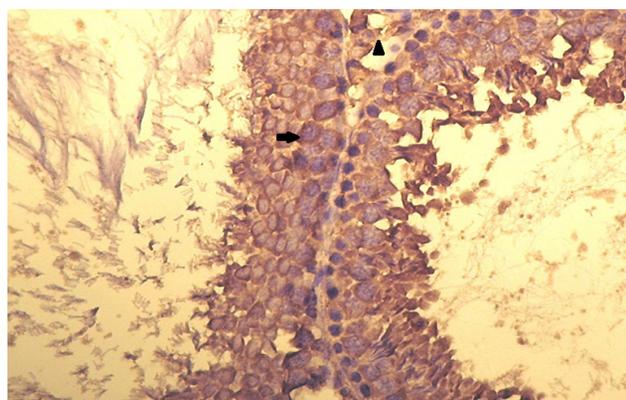


Fig. 20: A photomicrograph of a testicular section from the platelet rich plasma-treated Ischemia/ Reperfusion group showing positive BCL2 immunorexpression in the cytoplasm of the spermatogenic and Leydig cells. (Bcl-2, x 400)

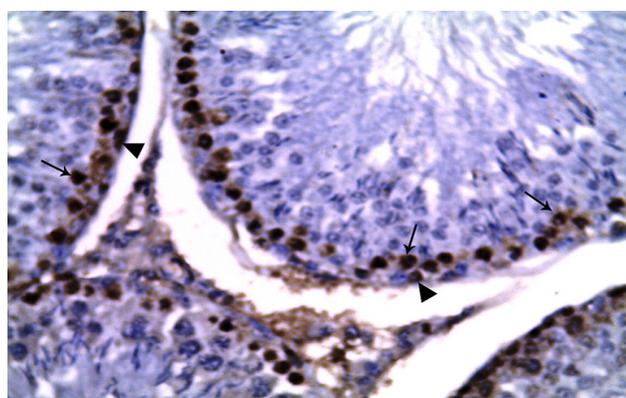


Fig. 21: A photomicrograph of a testicular section from the platelet rich plasma-treated Ischemia/ Reperfusion group showing the seminiferous tubule lined by immunopositive nuclei of spermatogonia (arrow head) and primary spermatocytes (†). (Immunopositive PCNA, × 400)

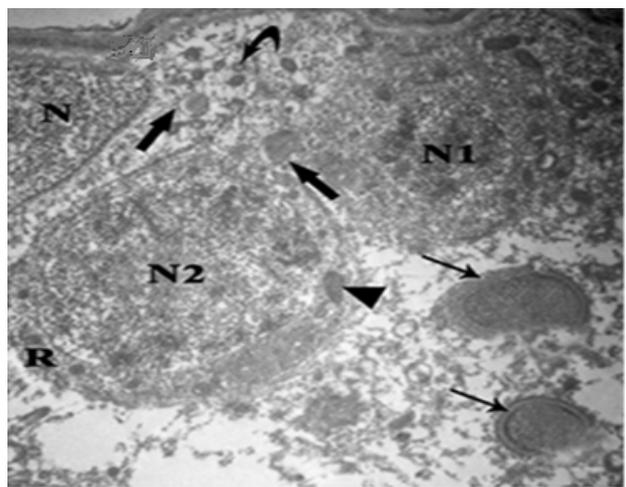


Fig. 22: An electron photomicrograph of a testicular section from the platelet rich plasma-treated Ischemia/ Reperfusion group showing part of Sertoli cell nucleus (N) with fine granular chromatin resting on apparently normal basement membrane. The cytoplasm contains mitochondria (thick arrow) and some dense bodies (curved arrow). The neighboring spermatogonia cell with its nucleus (N1) close to the basement membrane. The Iry spermatocytes shows spherical nuclei (N2), dispersed chromatin and cytoplasm containing mitochondria (arrow head) and multiple ribosomes (R). Notice, early spermatid and their their acrosomal cap ((†) (TEM, x 2000)

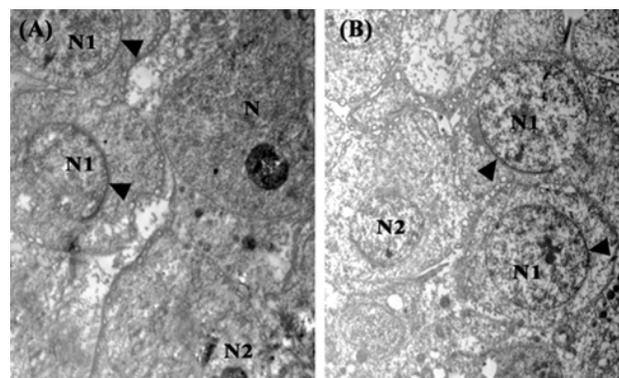


Fig. 23-A,B: An electron photomicrograph of a testicular section from the platelet rich plasma-treated Ischemia/Reperfusion group showing Sertoli cell with triangular nucleus and well developed nucleolus(N). Rounded spermatids showing spherical nuclei (N1) with fine granular chromatin and complete acrosomal cap (▶). Notice, the other spermatids with distorted nuclei (N2) and complete loss of acrosomal cap. (TEM, (A) x2000,(B)x 1500)

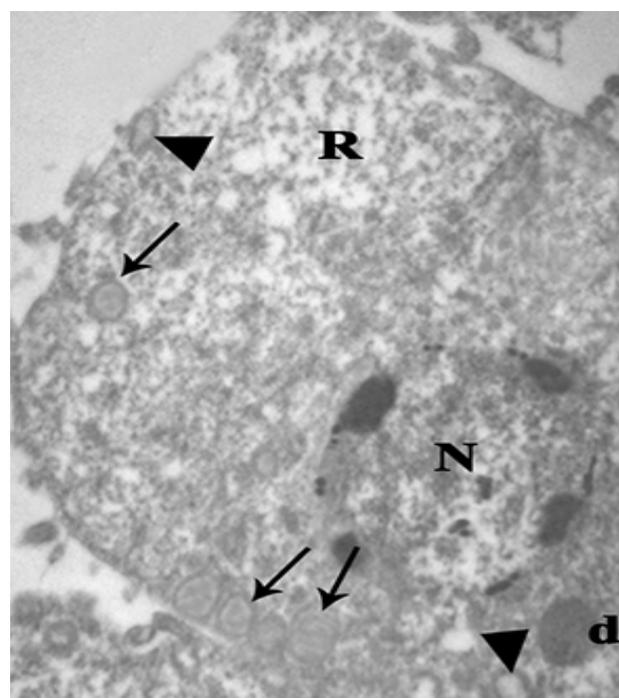


Fig. 24: An electron photomicrograph of a testicular section from the platelet rich plasma-treated Ischemia/ Reperfusion group showing Leydig cells with small rounded nucleus (N) and clumped marginated chromatin, the cytoplasm contains lipid droplets (d), normal mitochondria (arrow head) , and ribosomes (R).Notice, the cytoplasmic crystals(arrow). (TEM, x 3000)

Table 1: Comparison between the different studied groups according to the levels of testosterone

Groups	Testosterone (ng/ml)
G I control group	1.8 + 0.37
GII I/R group	0.55 + 0.09*
GIII PRP-treated I/R group	1.5 + 0.48#

Values are represented as mean + S.D.

* Means $P < 0.001$ significant compered to control.

Means $P < 0.001$ significant compared to group II.

Table 2: Comparison between the different studied groups according to the levels of Serum inhibin- β

Groups	Serum Inhibin- β (ng/L)
G I control group	53.69 + 7.19
GII I/R group	30.47 + 5.38*
GIII PRP-treated I/R group	43.32 + 6.28#

Values are represented as mean + S.D.

* Means $P < 0.001$ significant compared to control.

Means $P < 0.001$ significant compared to group II.

Table 3: Mean area% of anti-Bcl2, and anti-PCNA antibodies in all studied groups

Groups	Mean area% of anti-Bcl2 antibody	Mean area% of anti-PCNA antibody
Group I	4.75 \pm 1.43	4.235 \pm 1.739178
Group II	2.47 \pm 1.13 ^a	1.628 \pm 0.867881 ^a
Group III	3.67 \pm 2.91 ^b	3.034 \pm 0.901803 ^b

Bcl2 antiapoptotic factor; PCNA, proliferating cell nuclear antigen.

a. Significant ($P < 0.05$) vs. group I.

b. Significant ($P < 0.05$) vs. groups II.

DISCUSSION

Testicular torsion is a common urologic emergency that usually impact male newborns, children, and adolescents involved in testicular injury, mutata subfertility, infertility and hormone production. It results from a rotation of the spermatic cord and leads to disturb testicular blood flow. Testicular torsion is an acute progressive disease, with an incidence of 1 in 4000 males at age of 20 years^[22]. Rapid diagnosis and surgical detorsion are important to retrieve, blood flow to the testis to avoid testicular necrosis and to avoid loss of testicular function. If testicular torsion is left untreated within 4–6 hours, damage of germ cells will occur^[23].

In this study, we formed a 2-h testicular torsion model rotating left testis 720° to constitute a slight-to-moderate testicular damage. Events occurring during testicular torsion and detorsion are representative of an ischemia-reperfusion type of injury observed in other organs^[24]. Concomitant with reperfusion and despite return of blood flow after testicular detorsion, loss of spermatogenesis, and testicular atrophy may develop subsequently, depending on the duration and degree of torsion^[25].

The present study demonstrated that testicular torsion could cause physiological, histological, and functional alterations in rat testes. Also, torsion/detorsion (T/D) group revealed marked testicular damage and defected or failed spermatogenesis in testis. This result was consistent with the finding of other workers who reported that the pathophysiologic mechanism of testicular damage is an ischemia-reperfusion injury^[23]. Testicular ischemia-reperfusion leads to over generation of reactive oxygen species. Overproduced reactive oxygen species can damage lipids, proteins, and DNA, resulting in cellular dysfunction and even death^[26].

The present work evaluated oxidative tissue damage via MDA levels, an index of lipid peroxidation, and found MDA to be significantly high in the I/R group. These results are consistent with the results of previous studies^[27]. Malondialdehyde is a stable product of lipid peroxidation produced by reactive oxygen species and considered as one of the important indications of tissue injury due to oxidative stress^[28]. Enzymatic anti-oxidant defense systems, as SOD, a strong anti-oxidant and GSH a key antioxidant, were found to be significantly low in tissue of the I/R group compared with the control group.

In agreement with the I/R injury hypothesis and the role of free radicals in the disease process, numerous experimental animal studies have confirmed the efficacy of anti-oxidants. The protective role of antioxidant enzymes such as SOD, catalase and glutathione peroxidase against free radical attack is in balance under normal condition. This balance is disturbed under high oxidative stress such as reperfusion injury^[29].

In the current study, histological examination of testicular tissue sections of the I/R group showed marked testicular changes, distorted shrunken seminiferous tubules with wide interstitium in between. These tubules had marked reduction in the thickness of the germinal epithelium.

The presence of small dark nuclei in the spermatogonia and also the spermatids that had shrunken nuclei and abnormally distributed mitochondria may be attributed to apoptosis, which occurs because of oxidative stress that permeabilizes the mitochondrial outer membrane and releases apoptotic proteins for diffusion into the cytosol^[30]. Testicular reperfusion produces furthermore damage due to generation of toxic oxygen free radicals with the return of blood flow following testicular ischemia. The testicular regression and thinning of the epithelium leads to a reduction in sperm production^[31].

In this study, examination of the I/R group showed the interstitial cells associated with an excess of acidophilic vacuolated exudates, congested blood vessels in the interstitial spaces.

This acidophilic hyaline material could be attributed to an increase in vascular permeability^[32]. Also, it was in agreement with the results of some authors, who proved that areas of ischemia and reperfusion showed severe histological changes in the form of extensive widening of interstitial spaces, diffuse eosinophilic hyaline material, sloughing of some layers of the seminiferous tubules, atrophy with fibrosis, and complete absence of sperms^[33].

In the current study Leydig cells in I/R group, was smaller in size with peripherally clumped chromatin, small indented nuclei, and vacuolated cytoplasm. These results were consistent with other researchers who suggested that reperfusion/oxidative stress may play a role in Leydig cell dysfunction, and by acting instantly in germ cell apoptosis^[34]. However, other authors explained that Leydig

cells were preserved functionally after short periods of I/R despite of disturb and loss of germ cells^[35].

In the present results, electron microscopic examination of Sertoli cells in the I/R group revealed electron dense cytoplasm including, degenerated mitochondria, increased number of lysosomes and decreased number of ribosomes. These results were in agreement with investigators, who studied the ischemia and reperfusion on the testes of rats. They reported that testes showed histological perturbation, including degenerative changes within the seminiferous tubules and severe damage in some spermatogenic and Sertoli cells, which could explain the detached germ cells from the basement membrane^[36].

In the present study ultrastructural changes supported the result of the histological assessment, and same results were reported by researcher after the effect of torsion/detorsion on mice, they found rounded spermatids with loss of acrosomal caps and also found spermatids and spermatocytes were necrotized. These was explained as a result of ROS which could generate oxidative stress within cells by reacting with macromolecules leading to destruction of protein structure, mutations in DNA, with changes in gene expression and apoptosis^[37].

Testicular ischemia-reperfusion leads to alterations of testicular tissues of both humans and animals, with necrosis of spermatocytes, spermatids with significant decrease infertility. The susceptibility of spermatozoa to oxidative damage was attributed to the limited antioxidant capacity of spermatozoa, the high concentration of unsaturated fatty acids in membrane phospholipids, and the ability of spermatozoa to generate reactive oxygen species (ROS)^[38].

In agreement with previously mentioned results, the investigators stated that if reactive oxygen species, remain longer in the cellular environment they impacte the major biological molecules including membrane lipids and alert the cell from a normal oxidant state to a pro-oxidant state which referred to as oxidative stress^[39]. Furthermore, as testes being rich in polyunsaturated fatty acids and having less antioxidant protection, are oversensitive to peroxidation damage compared with other tissues^[40].

There are two main apoptotic pathways in cells, the death receptor-mediated pathway which is extrinsic and the mitochondria-mediated pathway which is intrinsic^[41]. The mitochondrial pathway can be divided into caspase dependent and caspase-independent pathways and is controlled by members of the bcl-2 family which have either anti-apoptotic as bcl-2 or pro-apoptotic properties. Members of bcl-2 can effectively increase or decrease apoptotic activity^[42]. The balance between anti- and pro-apoptotic proteins has an important role in apoptosis. In the testis, bcl-2 can present at any part in the cytoplasm or peri-nuclear area, as in the acrosomes of spermatids^[43].

In the present study, Immunohistochemical analysis was used to map the distribution of bcl-2 immunoreactivity in the testis. The expression of bcl-2 as an antiapoptotic

factor in the I/R group revealed minimum expression in the germ cells of rat testes, including spermatogonia, primary spermatocytes, spermatids, spermatozoa, and Sertoli cells. It was recorded that the differentiated spermatogonia are the most sensitive cells to any drug. In comparison, primary spermatocytes and spermatids showed to be resistant to apoptosis. However, there were clear apoptotic changes in the cytoplasm of the testicular cells^[41].

Examination of testicular sections taken from the control group stained with anti-PCNA antibody revealed immunopositive nuclei of the spermatogonia and primary spermatocytes, whereas those of I/R group revealed nuclear immunopositive of the spermatogonia and some primary spermatocytes while other primary spermatocytes were found to be immunonegative. Some investigators recorded that nuclear PCNA immunoreactivity were found in primary spermatocytes, while some primary spermatocytes were found to be immunonegative. This could be referred to their proliferative activity; so, proliferating stages of spermatocytes were PCNA immunoreactive^[44].

Morphometric results of I/R group in this experimental study revealed significant decrease in the mean area% of anti-Bcl2, and anti-PCNA antibodies ($P < 0.05$) compared with the control group indicating failure or arrest of spermatogenesis.

The present study found a significant increase in caspase-3 levels in the I/R group compared with the control group. Caspase-3 is part of the common pathway of apoptosis stimulation and overexpression of caspase-3 is interpreted as increased apoptosis. Previous researchers studied the apoptotic effect of testicular torsion compared to expression levels of active caspase-3, 8 and 9, and recorded statistically significant increases in expression of all caspase types following I/R injury in comparison of the control group. They also reported abnormal morphological changes, germ cell apoptosis, depending on formation of lipid peroxidation^[45].

This result was clarified with other authors who stated that testicular torsion leads to a significant increase in germ cell apoptosis and loss of spermatogenesis. They attributed the increase in germ cell apoptosis to the increase in testicular oxidative stress at reperfusion and the change in the activity of mitochondrial respiratory chain^[46].

In the present study, the I/R group showed significantly low testosterone levels compared with the control group following 2 h of testicular torsion. Among previous animal studies of testicular torsion, some authors consider that this difference denotes primary hypogonadism as result of testicular torsion and is based on the contralateral orchietomy as in the present model^[47]. Hand in hand with previously mentioned results, some studies revealed that testosterone appears to be responsible for preserve appropriate blood-testis barrier function^[48]. Some reporters suggested that decreased testosterone level in testicular ischemia-reperfusion could be referred to the oxidative stress generated in the testis^[49]. Consequently, other

reporters stated that disruption of any of the testosterone-dependent steps resulted in the failure of spermatogenesis and infertility^[50].

In the present study, the I/R group showed significantly low serum inhibin-B levels compared with the control group following 2 h of testicular torsion. The serum inhibin B levels, is suggested as a marker of Sertoli cell function and spermatogenesis, also Sertoli cells are the main producers of inhibin-B in the human body. Therefore, Sertoli cell function and quality of spermatogenesis are both closely related to inhibin-B concentrations^[51]. These results were also in line with those of some authors who reported that serum inhibin-B levels decrease following unilateral testicular torsion indicating contralateral testicular damage and measurement of inhibin-B levels to evaluate unilateral testicular damage after testicular torsion is more efficacious than histopathological examination^[52].

The present study investigate TGF- β expression in I/R injury in rat testis. TGF- β expression was found to be significantly higher in the I/R group compared with the control group, which similar to the results of previous researchers. Transforming growth factor - β plays an important role during development of the testis and in testicular function^[53]. Some authors concluded the study of TGF- β expression in ischemia/reperfusion injury in rat testis and examined the effects of amlodipine after testicular torsion/detorsion damage. While they recorded increased tumor necrosis factor α and TGF- β levels in the torsion and detorsion groups, they also reported a significant low levels of the inflammatory cytokines in the treatment groups^[54].

Platelet-rich plasma (PRP) has grown as an attractive biologic instrument in regenerative medicine for its powerful healing properties. It is believed as a source of growth factors that may produce tissue repairing and improve fibrosis^[55]. This product has proven its efficacy in many studies, but its effect on I/R injury has not yet been elucidated. The present investigation was performed to estimate the protective impact of platelet-rich plasma against testicular I/R injury in rats. Major histological changes occur following testicular torsion and were prominent in the I/R group, and PRP, to some extent, protected against these changes.

Platelet-rich plasma (PRP) has a potential effect on tissue repair through differentiation and proliferation of tissue progenitor cells^[56]. PRP a strong antioxidant has been reported to protect against ischemia-reperfusion injury in the brain, heart, and kidney. Furthermore, there is no adverse effect of PRP on male fertility^[57]. Therefore, we attempted to use PRP for the treatment of testicular ischemia-reperfusion injury. The present study showed that PRP treatment significantly attenuated malondialdehyde level and significantly improved spermatogenesis in the testes, compared with the ischemia-reperfusion group. These results suggest that PRP reduced testicular injury by decreasing reactive oxygen species level.

The present study evaluated oxidative tissue damage via MDA, GSH levels, and found MDA was significantly high, while GSH was significantly low in the I/R group. The present results support PRP treatment, as it effectively reduces the MDA levels and accomplish GSH levels. The anti-oxidant defense systems, such as SOD, were found to be significantly low in tissue in the I/R group compared with the control group and were higher in the RPR treated group compared with the I/R group.

Moreover, The PRP-treated group had significantly higher serum inhibin-B levels and significantly higher testosterone serum level compared with the I/R group, suggesting a protective effect of PRP on Leydig cell function, Sertoli cell function, and quality of spermatogenesis.

The present study revealed a significant increase in caspase-3 and Transforming growth factor β levels in the I/R group compared with the control group. However, the PRP-treated group

had significantly low caspase-3 and Transforming growth factor β levels compared with the I/R group.

Platelet-rich plasma, therefore, has a partial protective effect against apoptosis induced by I/R injury of testes. The rationale for the clinical use of platelet-rich plasma (PRP) is based on its ability to stimulate the generation and, accordingly, the increase in the secretion of proteins and in the concentration of growth factors which are induced maximum healing process at the cellular level^[5, 6, 55].

Furthermore, it is reported that the PRP performs increasing the proliferation and differentiation of the cells involved in the tissue regeneration^[7, 56]. PRP therapeutic use in regenerative medicine has proved proper effects and safe in experimental animals and in the clinical treatment of human patients. Therefore, new research are needed, with greater accuracy and experimental improvement, using a larger numbers of patients, and the definition of a approval to establish more exactly the clinical conditions for which the PRP should be properly and precisely employed^[8, 57].

CONCLUSIONS

Results of the present study suggest that PRP by inhibiting oxidative stress and increasing antioxidant defense exerts protective effects on testicular tissues against I/R.

CONFLICTS OF INTEREST

There are no conflicts of interest

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الملخص العربي

دراسة هستولوجية للتأثير المحسن المحتمل للبلازما الغنية بالصفائح الدموية على الافتقار وإعادة الإرواء الدموي في نموذج لانفتال الخصية في الجرذ الأبيض البالغ

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المقدمة: يعتبر إنفتال الخصية مشكله خطيره تؤدي إلى افتقار الخصية و فقدان الخصوبة. وتعتبر للبلازما الغنية بالصفائح الدموية عامل نموله دور فعال كعلاج تجديدي خاصه في حالات الاصابه بالافتقار وإعادة الإرواء الدموي الهدف من البحث: تقييم للدور المحسن المحتمل للبلازما الغنية بالصفائح الدموية على الافتقار وإعادة الإرواء الدموي في نموذج لانفتال الخصية في الجرذ الأبيض البالغ

مواد وطرق البحث: تم استخدام اثنان و ثلاثون جرذا من ذكور الجرذان البالغة وتم تقسيم اربعة وعشرون جرذا إلى ثلاث مجموعات رئيسية: مجموعة (١) ضابطة ومجموعة (٢) والتي تم تعريضها إلى إنفتال الخصية/ وإعادة تصليحه والمجموعة (٣) والتي تم تعريضها إلى إنفتال الخصية/ وإعادة تصليحه ثم تم حقنها بالبلازما الغنية بالصفائح الدموية داخل نسيج البرنثيمي للخصيه اثناء تصليح إنفتال الخصية. وقد استخدم الثمانية جرذا المتبقية كمصادر مانحة لتحضير البلازما الغنية بصفائح الدم وقد تم استخراج الخصيه وإعدادها للفحص بالميكروسكوب الضوئي والالكتروني الفائق الدقة. وقد تم استخدام صبغه الهيماتوكسيلين والايوسين و صبغه التولويدين الزرقاء و صبغة PCNA & Bcl-٢ الهستوكيميائية المناعية. وكذلك تم فحص بلطخه غرب بروتين الكاسباس-٣ وبروتين تحويل عوامل النمو – بيتا وايضا تم قياس مستوي هرمون التيستوستيرون والانهبين -بي في الدم ومستويات المالدندالدهيد , الجلوتاثيون و السوبراوكسيد ديسميوتاز في نسيج الخصيه . وتم عمل دراسة شكلية مترية لبعض القياسات ثم خضعت النتائج للتحليل الإحصائي.

النتائج: أوضحت النتائج أن إنفتال الخصية/ وإعادة تصليحه يؤدي إلى تلف بالغ في أنسجة الخصيه. وقد أظهرت الخصيه فشلا في عملية تصنيع الحيوانات المنوية و ظهور مناطق متسعة من فقد الخلايا و اختفاء النطفات المنوية الغير ناضجة. كما أوضح التحليل الإحصائي وذلك بالمقارنة بالمجموعة الضابطة. كما أوضح الفحص بالميكروسكوب الالكتروني الخصيه وجود فراغات سيتوبلازميه في خلايا سيرتولى مع خلل فى خاصية الترابط بين خلايا سيرتولى. بينما ظهرت أنويه خلايا المنى الأولية انكماشاً مع تكثف للكروماتين وتلف في عضيات الخلية بالسيتوبلازم. كما ظهرت الحيوانات المنوية الناضجة و الغير ناضجة بصورة غير طبيعية. وعلى النقيض وجد أن حقن البلازما الغنية بالصفائح الدموية اثناء إصلاح إنفتال الخصية كان له تأثير واقى و الذي ظهر جليا من خلال دراسة التركيب الفائق الدقة للخلايا و أيضا من خلال التحليل الإحصائي الذي أوضح.

الاستنتاج: العلاج بالبلازما الغنية بالصفائح الدموية اثناء إصلاح إنفتال الخصية قد يكون واعد كعلاج فعال من أجل إنقاذ الخصية من الافتقار الدموي وإعادة الإرواء. وعلى ذلك فانه يمكن تحاشي استئصال الخصية في حالات الإنفتال.