Original Article

Evaluation Intra-testicular Injection of Platelet-Rich Plasma in Restoration Male Rat Fertility Affected By Cisplatin

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Abstract

Cisplatin is considered the first-line chemotherapy, reducing its cytotoxicity is still unmet need. Platelet-rich plasma (PRP) has a potential effect on tissue repair through regeneration and differentiation of tissue progenitor cells. Here, we used PRP as a biomaterial to protect and regenerate testicular tissue against cisplatin toxicity. PRP was applied locally using intra-testicular injection as a single dose per week for three following the cisplatin treatment (intra-peritoneum injection). Cisplatin treatment alone and PRP intra-testicular injection alone were used as control groups. The results show that PRP treatment was able to restore sperm counts and sperm morphology following cisplatin exposure. PRP treatment significantly reintited reproductive hormonal balance in affected rats with cisplatin. The results also showed that PRP treatment minimized histological damaged in testicular tissue resulting by the cisplatin, however, also increasing the number of spermatocytes in all stages of spermatogenesis. As well as elevation in the number of leydig cells and thickness of interstitial tissue was restored. This indicates the potential role of PRP in tissue regeneration in addition to tissue repair which may provide a promising use of PRP in different aspects related to chemotherapy.

Keywords: Cisplatin, Intra-testicular injection, Platelet- Rich Plasma, Male, Fertility.

1. Introduction

Infertility considered a widespread issue so that researches and studies several therapeutic approaches are needed. Chemotherapy agent related to infertility as: Cisplatin, lead to temporal infertility according to treatment protocol [1, 2] .Cisplatin (CP) is known as antineoplastic medication used to treat a range of solid tumors and neoplasms. Cisplatin has high toxicity an alkylating DNA agent that destroys by a variety of processes, including damage of DNA, the formation of ROS (reactive oxygen species). In the addition to endocrine and exocrine systems were affected, leading to androgenesis, gonadal dysfunction and spermatogenesis. CP using causes diminished sperm motility and sperm abnormal morphology as well as cisplatin therapy include chromosomal abnormalities in spermatozoa [3, 4]. It is well- established, CP has many adverse effects, which include male reproductive system toxicity.

It has been noted a decline in reproductive function result in compromised fertility [5].

Oxidative damage caused by CP is thought to be the cause of these adverse effects Redox imbalance causes physiological and metabolic disturbances, and rise in peroxidation of lipid causes damage of germ cell [6].

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Access this article online

Quick Response Code:

Website: www.pnrjournal.com

DOI: 10.47750/pnr.2022.13.03.047

How to cite this article: Hazar Shakir Saleh, Samara Yahya Kraidi, Ahmed Salman Abdulhasan, Evaluation Intra-testicular Injection of Platelet-Rich Plasma in Restoration Male Rat Fertility Affected By Cisplatin, J PHARM NEGATIVE RESULTS 2022;13:295-301.

Platelet has tremendous of growth factors, such as platelet-derived growth factor (PDGF), transforming growth factor (TGF)-β1 and vascular endothelial growth factor (VEGF), which well-known about its ability to enhance cellular proliferation, differentiation, chemo taxis and angiogenesis. Actually, platelet rich plasma (PRP) has been used clinically to enhance wounds healing and used for spinal injuries [7].

Also, PRP is resulting in an increase of platelets concentration and growth factors leads to promote healing and stimulate regeneration. Subsequently, previous studies have revealed that germ cells generate "vascular endothelial growth factor" (VEGF), which is essential factor for spermatogonia stem cell survival [8]. Many growth factors have an essential role in spermatogenesis process such as Bone Morphogenic Protein 4 which plays a vital function in germ cell proliferation and differentiation. Essentially, growth factor has a positive influence in spermatogenesis [9]. Platelet derived growth factor which stimulates the germinal cell and regulates paracrine and autocrine functions [10]. These "growth factors" help to decrease testicular tissue ischemia, increase the width of seminal producing tubules which keep the epithelium of germinal healthy [11]. This study used rats as model to identify if a count and abnormalities of spermatozoa in addition to testicular tissue changed after CP treatment, and evaluate the ability of PRP to regenerate damaged structure of testicular tissue.

2. Methodology

2.1 Animal husbandry

Thirty-two adult male rats were used in this study, the animals were housed in clean cages at room temperature of 25 C. Food and water were given ad libitum. The study's design and animal experiments protocols were reviewed and approved by the department of Biology's Ethics Committee in accordance to the Authorized Guidelines of Care and use Animals in College of Education for Pure Science, University of Thi-Qar.

2.2 Experimental design

The animals were divided randomly into four groups 8 rats per group and considered as the following: Group I: control group was given 0.2 ml normal saline, intra testicular (I/T) injection as a single dose per week for 3 weeks. Group II: it was given 7 mg/kg of CP single dose, intra-peritoneal as single dose per week for three weeks [12]. Group III: : it was given 7 mg/kg of CP single dose, i.p and I/T of 10 µl PRP, single dose, per week for 3 weeks [13]. Group IV: it was given only 10 µl PRP I/T, single dose, per week for 3 weeks. After 3 weeks, using the heart puncture, blood samples were drawn, and the blood was directly collected in heparinized tubes. The blood samples then were centrifuged at 3000 rpm for 30 min and stored at −20 C0. All rats were decapitated and eviscerated testicular tissue to fix it in 10% formalin for histological preparation proceedings. Furthermore, spermatozoa collection from epididymis was prepared.

2.3 Spermatozoa collection (count and Morphological abnormalities)

For sperm count, caudal of epididymis was minced and then epididymis suspension was removed using a pipette (used for white blood cell counts). Then, the suspension was placed in a petri dish and diluted with 2 ml of normal saline. The spermatozoa were permitted to move freely in the fluid. The spermatozoa count was determined by using a neubauer's chamber through spreading the sample. It has been performed microscopic examination to the sperm at a magnification of x40. Calculation of average sperm number was in 8 squares measuring 0.1 cm2 and expressed as million/ml (Bordbar et al, 2013). For the morphological abnormalities analysis, spermatozoa were counted and then the percentage of normal and abnormal spermatozoa was evaluated. Abnormal spermatozoa were considered as double head, giant head, double tail, long tail, short tail, spermatid and pined tail.

2.4 Hormonal analysis

Serum level of LH, FSH, and testosterone were measured using ELISA kits and following the kit protocol.

2.5 Histological preparation

The testes tissues were immersed-fixed in 10% formalin for 24 hours. After fixation, the tissues were sliced with a sharp surgical blade and placed inside a histology cassette. Subsequent tissue processing was done in ascending grades of ethanol (70 %, 95 % and 100 %) for de-hydration the tissue. The tissue was placed in xylene for three changes and then embedded in fresh paraffin wax. Paraffin-embedded tissues were sectioning at 5 µm thickness using a microtome and mounted on slide. Tissue slides were then processed for hematoxylin and eosin (H&E) staining procedure. The stained sections were mounted with DPX and a cover slip was applied to preserve the section.

2.6 Estimation the diameter of the germinal epithelium

To estimate the seminiferous tubule diameter and germinal epithelium height, Image J software was used as previously described [15].

3. Statistical analyses

All data in this research represented as mean and SE. Results for serum hormones, sperm analysis and s histological biometric of testis were analyzed using multivariate analysis of variance (ANOVA) with post hoc test. Statistical significance was accepted at $p<0.05$.

4. Results

4.1 Assessment of a count and morphology spermatozoa

Spermatozoa were counted and the percentage of normal and abnormal spermatozoa was evaluated. there was a clear variety in spermatozoa count in different study groups as in was 15.27, 4.29, 10.13, 14.61 for control group, cisplatin group, (cisplatin +PRP) group, PRP group respectively. Sperm counts statistically significant increase in cisplatin +PRP group comparing to cisplatin group. Sperm counts were significantly lower in cisplatin group comparing to control (table.1). Percentage of morphology of Spermatozoa appeared with highly difference for all study groups (table.1).There was significantly elevated in percentage of abnormalities spermatozoa in cisplatin group comparing to control group. In the other hand, the percentage of sperm abnormalities in cisplatin +PRP group was decline comparing to cisplatin group (Table. 1).

Table 1. A count and Percentage of morphological abnormalities spermatozoa in experimental group

Groups	Spermatozoa count	Spermatozoa morphological abnormalities
Control	15.27 ± 0.64	5.44 ± 0.17
Cisplatin (7mg/kg)	4.29 ± 0.59	11.70 ± 0.16
Cisplatin + PRP	10.13 ± 0.52	8.89 ± 0.41
PRP	14.61 0.43	4.96± 0.19

4.2 Sex hormone analysis

The study results revealed that there was significantly increase of in concentration of FSH and LH in treated CP when compared with control group. As according to cisplatin +PRP group, FSH and LH a statistically significant reduction in serum concentration comparing to CP (Table 2). However, it has been shown that serum testosterone concentration was significantly decreased in concentration of in treated CP comparing to control group. On the other hand, it was increase in group treated with PRP + CP group as comparing to CP group (Table 2).

Table 2. Hormonal analysis of experimental groups.

Groups	FSH	LH	Testosterone
Control	0.24 ± 0.003	0.34 ± 0.008	1.02 ± 0.02
Cisplatin (7mg/kg)	0.83 ± 0.003	0.67 ± 0.004	0.77 ± 0.08
Cisplatin + PRP	0.45 ± 0.003	0.43± 0.006	0.91 ± 0.03
PRP	0.32 ± 0.003	0.29± 0.005	0.97 ± 0.03

4.3 Measuring diameter of seminiferous tubules and germinal epithelial height

The seminiferous tubules diameter and germinal epithelial height highly reduced in the CP –treated rats compared to control. Meanwhile they were significant increase in diameter for CP + PRP groups compared to CP-treated group shown in Table 3.

Table 3. Measuring of seminiferous tubules diameter and germinal epithelial height for all groups

Groups	Diameter of seminiferous tubules (um)	Germinal epithelium height (um)
Control	315.19±0.27	58.09±0.16
Cisplatin (7mg/kg)	109.04±0.70	36.46±0.22
Cisplatin + PRP	278.94±1.43	41.62±0.67
PRP	298.76±1.39	44.64±037

4.5 Histological examination

It was found that control group demonstrated normal testicular architecture with organized arrangement of spermatocytes in different stages therefore normal typical spermatogenesis has been observed. In addition, basement membrane of seminiferous tubules was distinct and lumen of seminiferous tubules filled with identified spermatids. Fine interstitial connective tissue separated intra spaces around seminiferous tubules (Figure 1).

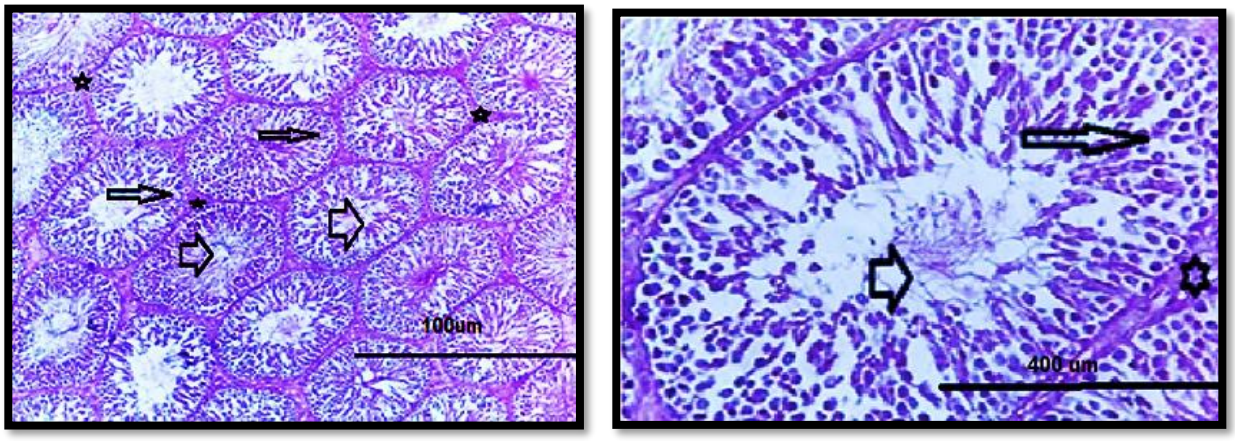

Fig1. Photo-micrograph of normal testicular histoarchitectures in Control group cells of spermatogenesis are shown by the thin arrow, lumen of semeniferous tubules by the head arrow and the interstitial stroma by asterisk

In CP-treated groups, findings demonstrated that testicular tissue included some seminiferous tubules were irregular and empty from spermatids; the germinal layer was detached from the basement membrane and dilated interstitial space with signs of edema due to degeneration in leydig cells and fibers of connective tissue. Many spermatocytes and sertoli cells appeared degenerative figures with clear vacuoles. Many seminiferous tubules appeared with abnormal spermatogenesis (Figure 2).

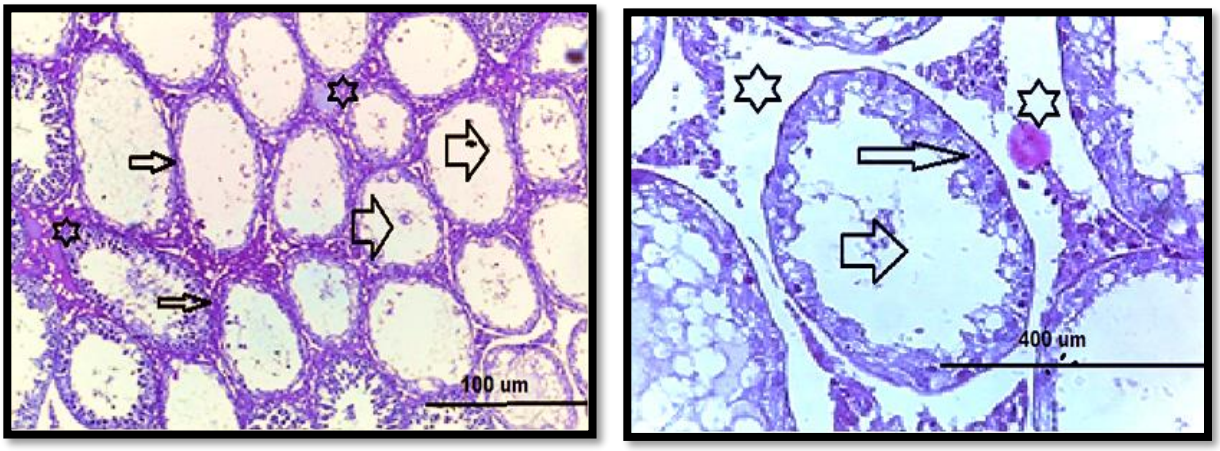

Fig 2. Photo-micrograph of abnormal testicular histoarchitectures in CP-treated rats cells of spermatogenesis are shown by the thin arrow, lumen of semeniferous tubules by the head arrow and the interstitial stroma by asterisk

In group received PRP+CP, testicular tissue sections showed significant reconstruction of histological architecture and almost appeared with organized as normal tissue. Also, seminiferous tubules showed regeneration of normal spermatocytes and sertoli cells. In addition, its lumen has contained evident spermatids. Restoration of interstitial connective tissue and leydig cells were observed near by a normal, moreover signs of edema were disappeared (Figure 3).

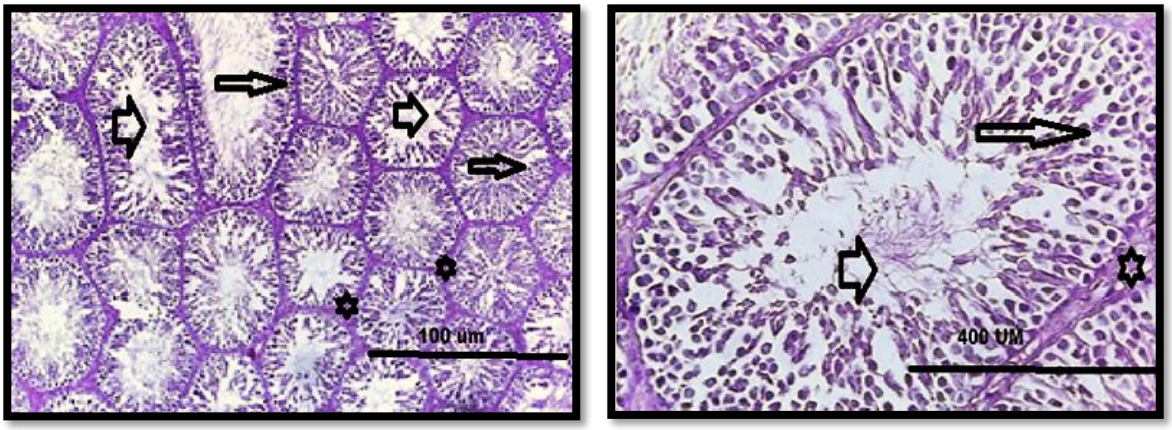

Fig 3. Photo-micrograph of testicular histoarchitectures in CP+PRP-treated rats cells of spermatogenesis are shown by the thin arrow, lumen of semeniferous tubules by the head arrow and the interstitial stroma by asterisk

The figure 4. represents the group which receives only PRP. It can be easily seen that normal tissue as similar to control group.

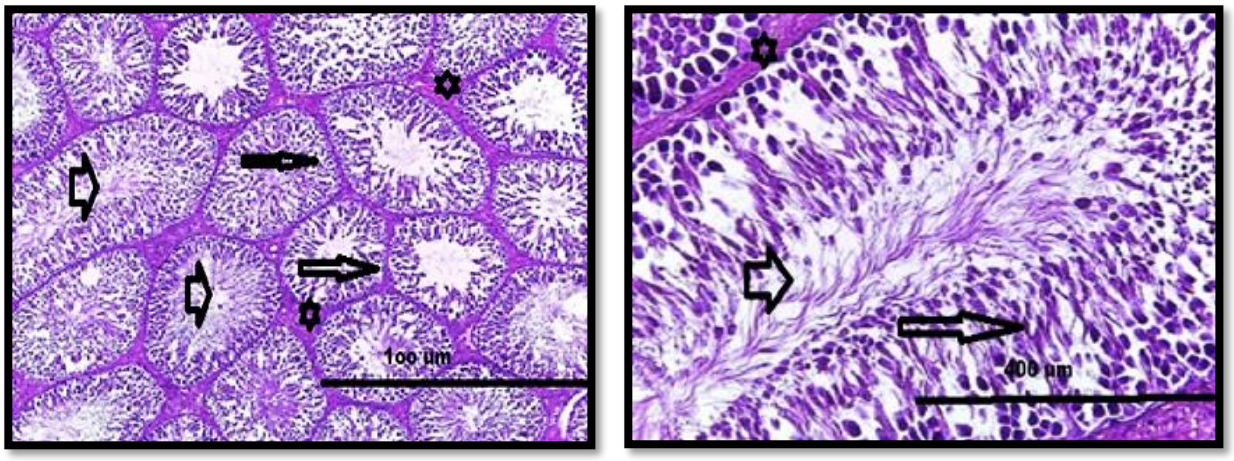

Fig 4. Photo-micrograph of testicular histoarchitectures in CP+PRP-treated rats cells of spermatogenesis are shown by the thin arrow, lumen of semeniferous tubules by the head arrow and the interstitial stroma by asterisk

5. Discussion

A malfunction in reproductive system could be due to dysfunctional and structural damage intra-testicular tissue. Essentially, evaluation Sperm account and morphology is also an evidence to determine fertility status. Therefore, this study disclosed that CP exposure diminished number and increase abnormalities of spermatozoa in rat as animal model. Thereby, intra-testicular PRP injection dramatically restored numbers and morphology of spermatozoa to near normal during CP treatment. Basically, fertility could not be achieved unless sperm has completely capable to fertilization so that it may suggest that PRP conferred sperm adequate ability to renew whether in number or morphology. In addition, current study focused on compromised fertility at sex hormonal level. After CP supplement, there was remarkable disturbances in main reproductive hormones, which are directly limited the fertility as it known.

However concurrent treatment PRP and CP give further evidence on re-function of testicular tissue and maintaining fertility throughout reinitiated reproductive hormonal balance (testosterone, FSH and LH). Moreover, it is take consideration that there was a correlation between sperm production and feed-back mechanism of sex hormonal balance. Consequently, all adverse effects of CP exposure which were mentioned above, it might attribute to study findings in bio-morphometric and histological changes which occurred in testicular tissue. Throughout decline in germinal epithelium height and seminiferous tubule diameters resulting in disappear of spermatogenesis as well as degeneration of spermatocytes and sertoli cells. In term of interstitial tissue surrounding tubules was disassembled and edematous. These deleterious changes in histo-structures of testis lead to impairment fertility. It is worthy mentioned, several previous studies showing comparable findings to the present results in CP treatment abused fertility at deferent levels [16, 17, 18, 19, 20].

Meanwhile intra testicular injection of PRP acts on reinstating functional tissue components according to our results via evident restoration the height of germinal epithelium and diameter of seminiferous tubules. In same context, it has been noted that PRP has evident role in regeneration of sertoli cells and complete development of spermatogenesis. These findings attributed to exist a various growth factors in PRP which play a crucial role in improving differentiation of stem cells and enhancing quantity of connective tissue [21, 22, 23].

Accordingly, previous Studies have also supported our study that fibroblast growth factor (FGF) and EGF stimulate the Sertoli cell to androgen-binding protein [24, 10]. As all above mention, our results indicated that there is a consistent finding with previous studies, in attempting protection male fertility throughout using natural products and antioxidant plant to minimize deleterious effects of CP [25, 26]. Nevertheless, those studies are limited on focusing in protective action only. Meanwhile, the essential key points regarding this study, it acts on enhancement of both preventative and regenerative processing to repair damaged tissue which may have promising future in participating in treatment of different health issues.

Overall, PRP has growth factors acts on regulate germinal cell division and maintain a healthy equilibrium between germ cell proliferation and differentiation [27, 28, 29, 30, 31, 32]. In same context, several studies suggested that CP treatment results in apoptotic activity in testicular tissue. When considering this reduction in height epithelium and Whereas prior study has been done by Dehghani et al, [13] showing incompatible results comparing to our study, when it be using PRP to improve fertility, however, it had referred that PRP has no evidently effects in repairing damage testicular tissue by using harmful chemical materials. This different points of view might be returned to different protocol has been use for each study.

6. Conclusions

In conclusion, the present study may consider as new point of view in application of PRP during chemotherapy exposure to protect male rat fertility. Eventually, PRP makes restoration and regeneration in number and morphology of sperm, maintaining sex hormonal balance and repairing testicular tissue.

Conflict of Interest

All authors do declare that there is no conflict of interest.

Acknowledgment

We would like to acknowledge the support which is produced by the College of Education for Pure Science, University of Thi-Qar, in this current study.

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