

ORIGINAL ARTICLE

Plasma rich-platelet (PRP) potentially attenuates tamoxifen spermatogenic dysfunction in rat's model: sperm quality, histomorphometric, and apoptosis assessment

HOSSEIN HAMALI^{1,*}, FATEMEH MASOUMI¹, BEHZAD BARADARAN², GHOLAMREZA HAMIDIAN³

¹Department of Clinical Sciences, Faculty of Veterinary Medicine, Tabriz University, Tabriz, Iran

²Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

³Department of Basic Sciences, Faculty of Veterinary Medicine, Tabriz University, Tabriz, Iran

Abstract

Background: Tamoxifen induces testicular atrophy and azoospermia. Conversely, platelet-rich plasma (PRP) is a distinctive analog yield enriched with cytokines and growth factors for improving tissue regeneration. Thus, this present study explored the roles model of PRP on production of sperm via apoptosis of tamoxifen-induced toxicity.

Methods: Animals at 48 days' post-normal saline treatment and intra-testicular injection of normal saline (0.2 ml, left testis) as a single dose per week for two weeks. Infertility was induced by the oral administration of tamoxifen [0.6 mg/kg b.wt/day], and was then allocated into three groups: tamoxifen: oral administration of tamoxifen for 14 consecutive days, and intra-testicular injection normal saline; PRP: intra-testicular of 10 μ l PRP, single dose; and tamoxifen + PRP.

Results: Tamoxifen-injured rats showed significantly lower body weight, food intake, testicular weight and volume, LH and testosterone concentrations, production of sperm indices, and sperm motility considering the vehicle group. Tamoxifen-induced seminiferous tubular atrophy and testicular damages. Meanwhile, tamoxifen upregulated markers of apoptotic marker genes.

Conclusion: In sum, the PRP supplement is potentially an impressive method for modifying production of sperm dysfunction via tamoxifen damages.

Keywords: PRP, Reproductive health, Spermatogenesis indices, Tamoxifen, Testicular dysfunction

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INTRODUCTION

Chemotherapy can damage sperm-forming cells (germ cells) and male fertility. Moreover, some chemotherapeutic drugs, especially with drugs called alkylating agents, can have negative adverse effects on the reproduction system have been considered by various authors, both in humans [1, 2] and non-human species [3-5].

Tamoxifen is a major anticancer agent that is used in breast cancer. Also, tamoxifen is the most generally used endocrine agent for blocking the growth of cancer cells with interfering with estrogen signaling [6, 7].

Platelet-rich plasma (PRP) has been extensively applied in reproductive medicine and tissue engineering. Recently, using PRP for tissue differentiation and stimulating cell proliferation has emerged as an effective approach in regenerative medicine fields [8, 9], like male infertility [10-12]. PRP can potentially enhance cellular healing and tissue regeneration through the delivery of various growth factors and protein contents [13]. PRP offers a promising method to safeguard reproduction system health by enhancing antioxidant defense systems that lead to reducing oxidative

damage [14, 15]. PRP contains a diverse array of fundamental proteins that support the viability of spermatogonial stem cells (SSCs) [16]. Samy et al. [17] in the Albino rat model on reperfusion injury following experimental detorsion of testis well reported that the PRP method by inhibiting apoptosis, cell migration, and controlling inflammation could have a significant effect on the recovery of the spermatogenesis.

The current study was designed to investigate the effect of the anti-oxidant content of PRP on the recovery of production of sperm in a tamoxifen-treated rat's model with sperm quality, and sex hormone levels, histopathology of testis, and expression of apoptosis-related genes assessment

METHODS

Animals and ethics

Sprague Dawley rats (205 \pm 10 g; 8-week-old) purchased from the Pasteur Institute of Iran (Karaj, Iran) were housed in light- and temperature-controlled situations. The study protocol was approved by the Ethics Review Committee of Tabriz University, Iran (approval no.: 0532.1.25/15).

Treatments

Twenty-four adult male rats were divided into four groups:

*Correspondence to: Dr. Hossein Hamali, Faculty of Veterinary Medicine, Tabriz University, Tabriz, Iran.
Email: hamali@tabrizu.ac.ir, Tel: +989141145575

animals at 48 days' post- normal saline treatment and intra-testicular injection of normal saline (0.2 ml, left testis) as a single dose per week for two weeks. Infertility was induced by the oral administration of tamoxifen [0.6 mg/kg b.wt/day, 8], and was then allocated into three groups: tamoxifen: oral administration of tamoxifen for 14 consecutive days, and intra-testicular injection normal saline; PRP: intra-testicular of 10 µl PRP, single dose; and tamoxifen + PRP.

Sampling

Animals were euthanized, weighed on a 1-mg digital assay balance; testes were processed for volume and weight, apoptosis genes expression, and histopathology; blood serum samples were processed for assessing sexual hormone levels.

Platelet-rich plasma preparation

The PRP was prepared using cardiac puncture from a donor rat. Five ml of whole blood was collected in tubes containing anticoagulant and gently mixed to prevent clotting and mixed with 1 ml of sodium citrate (3.8%). Plasma platelets were separated from red blood cells through centrifugation at 3000 rpm for 5 min at 4°C. To activate the PRP, followed by incubation at 37°C for 15 min, 23 microliters of calcium chloride (10%) were added. Then, he tube was centrifuged for 10 min at 4000 rpm to obtain activated PRP. Finally, 10 µl of PRP suspension containing 2000×10⁹ platelets per microliter was injected into the left testis using a Hamilton microsyringe (Hamilton Co., Bonaduz AG, Switzerland) [18].

Testis and sperm analysis

At first, the whole epididymis and testis were separated and the length, height and width of the testis were obtained by caliper and the whole of the testis was fixed in 10% buffered neutral formalin. The sperm indices were measured as Olfati et al. methods [8].

Hormonal assays

Blood serum was separated (4000 g for 10 min), stored at -20 °C, and evaluated by radioimmunoassay (kit of Monobind Inc., RIA-1000, Technicon)

Histopathological studies

Hematoxylin-eosin (H&E) staining was used to evaluate the degree of testicular tissue damage in each group with following these steps: fixation, dehydration and paraffin inclusion, performing slices by microtome (4 µm). Images of testicular structures were observed and acquired under an inverted fluorescence microscope (Japan's Nikon, Nikon Eclipse Ti-SR).

QRT-PCR

The testes samples were pooled using Eppendorf tubes containing ethylene-diamine-tetra-acetic acid (EDTA), then centrifuged, and their plasmas were stored at -80°C until analysis. Total RNA was extracted from plasma samples using Trizol (Takara Bio, Japan), and alcoholic sediment RNA samples were treated with DNase I (Fermentase, USA) to remove contaminating genomic DNA. The quality of RNA was determined using a Nano-drop 2000 spectrophotometer (Thermo Fisher, USA). Specific primers were provided by Invitrogen, USA (Table 1, housekeeping gene β-actin). Real-time quantitative polymerase chain reaction (QRT-PCR) was performed in triplicate reaction using both forward and reverse primers, cDNA, SYBR Green (Takara, Japan). The

QRT-PCR was performed according to Olfati et al. [8].

Statistical analysis

The results were analyzed using the SPSS 17.0 software. ANOVA and post-hoc Duncan's new multiple range test was used to test differences between treatments. The results were expressed as the mean ± standard deviation (SD). Differences with values of $P < 0.05$ were considered statistically significant.

RESULTS

Before sampling, their body weights were recorded and are detailed in Table 2. Notably, the PRP and tamoxifen + PRP groups exhibited higher body weight compared to the vehicle and tamoxifen groups. The tamoxifen group displayed the lowest weight in the left epididymis, with the PRP group exhibiting the least weight overall. Conversely, the right epididymis in the PRP and tamoxifen + PRP groups showed higher weights compared to the others. Examination of Table 2 revealed that the total weight of the left and right testicles in the PRP group surpassed that of the other groups. This increase in weight observed in the third and fourth groups may be attributed to prolonged storage time.

Morphometry of the testicular components and sexual hormone levels of rats in treated and control groups was shown in Table 3. The administration of tamoxifen decreased testicular weight and diameter. There was a significant variation in the testicular weight and volume of the testis in the right and left testis.

Tamoxifen-injured animals indicated significantly lower testosterone and LH levels. PRP exposure led to an important increase in sex hormone levels. This suggests a direct impact of PRP on Leydig cells, resulting in heightened testosterone levels.

heightened testosterone levels.

After tamoxifen exposure, an important change was observed in the sperm quality (Table 4): a significant decrease in their number and index in all tamoxifen exposure groups, then important increases in sperm parameters were observed in the PRP treatment groups.

Table 2. Sub-Chronic Toxicity Study; Safety profile of *Albizia lebbek* and *Curcuma longa* extracts on Bodyweight of rats.

Group	Body Weight (g)
Control	250 ± 20
Positive Control (Ranitidine)	240 ± 19
<i>Albizia lebbek</i> Aqueous (100mg/kg)	245 ± 20
<i>Albizia lebbek</i> Ethyl Acetate (100mg/kg)	240 ± 19
<i>Curcuma longa</i> Curcuminoid-Rich (100mg/kg)	245 ± 20
<i>Curcuma longa</i> Essential Oil (100mg/kg)	240 ± 19
Combination Therapy	250 ± 20
High-Dose <i>Albizia lebbek</i> Aqueous (400mg/kg)	230 ± 18
High -Dose <i>Curcuma longa</i> Curcuminoid-Rich (400mg/kg)	235 ± 19
Vehicle Control	245 ± 20

*Significant decrease ($p < 0.05$) compared to control group.

TDI was observed to be lower in the second group, with similar levels reported in the other groups. Additionally, SPI measurements indicated a slight increase in the third and fourth groups compared to the tamoxifen group. However, the RI index exhibited a more pronounced increase only in the fourth group compared to the other groups.

In no-tamoxifen groups, histopathological sections were normal (Figure 1). Meanwhile, tamoxifen caused testicular damage and seminiferous tubular atrophy. PRP treatments promoted a repair of the testicular tissue.

Tamoxifen-treated rats showed significantly lower Bcl-2 expression (existence gene) in testis tissue, while ameliorating caspase 3 and iNOS (death gene) levels in this tissue (Figure 2).

DISCUSSION

In the current study in all animal exposed to tamoxifen, the sperm production rate was importantly decreased. The

reasons behind these abnormalities may include the production of reactive oxygen species (ROS), oxidative stress marker, supposed to cause damage to sperm DNA [19-22].

Our results indicated that tamoxifen administration in rats can cause significant changes in the sperm production (Table 4). At any stage of cell differentiation, disruption of spermatogenesis may result in a decreased total spermatogenic cell count [23-25].

Sperm production processes are regulated by a master switch (GnRH pulse generator), under control of which the two separate feedback systems provide independent control of androgen (LH–testosterone) and sperm production (FSH–inhibin) [26]. The decreases in circulating testosterone and LH after tamoxifen exposure may have also been related to the main mechanisms: chemotherapeutic drugs can affect the hypothalamic-pituitary-gonadal axis. In the development and regulation of the male reproductive system, the

Table 2. Comparison of body weight (g, at the time of sampling), epididymis weight, relative weight of gonads (%), and total weight of left and right testes in the studied animals (mean ± SD)

Groups	Body weight	Cumulative weight of both testes	Relative weight of gonads	Relative weight of epididymis	Epididymis weight	
					Left	Right
Control	185.90±2.77 ^a	2.16±0.03 ^{ab}	1.16±0.02	0.41±0.03	0.42±0.03 ^a	0.35±0.06 ^a
Tamoxifen	193.50±8.05 ^a	2.11±0.15 ^a	1.08±0.04	0.42±0.01	0.39±0.03 ^a	0.43±0.03 ^{ab}
PRP	228.50±5.48 ^b	2.71±0.16 ^b	1.19±0.08	0.52±0.02	0.54±0.02 ^b	0.64±0.03 ^c
Tamoxifen+PRP	239.00±6.35 ^b	2.51±0.12 ^{ab}	1.05±0.07	0.49±0.05	0.53±0.03 ^b	0.63±0.07 ^{bc}

Superscripts (a-c) show significant differences in each column ($p < 0.05$).

Table 3. Morphometry and stereology of the testicular components and male sexual hormone levels of rats in treated and control groups (mean ± SD)

Groups	Right testis				Left testis				Hormone level		
	Height (cm)	Width (cm)	Length (cm)	Weight (g)	Height (cm)	Width (cm)	Length (cm)	Weight (g)	Testosterone (nmol/L)	LH (IU/mL)	FSH (IU/mL)
Control	1.06±0.02 ^a	1.11±0.02	1.76±0.04 ^a	1.06±0.03 ^a	1.1±0.03	1.08±0.02	1.88±0.03 ^{ab}	1.1±0.01	1.43±0.16 ^a	1.95±0.13 ^a	1.54±0.18
Tamoxifen	1.04±0.04 ^a	1.04±0.05	1.75±0.06 ^a	1.00±0.06 ^a	1.09±0.04	1.06±0.03	1.80±0.07 ^a	1.1±0.1	0.41±0.07 ^b	1.22±0.08 ^d	1.36±0.03
PRP	1.26±0.03 ^b	1.16±0.02	2.04±0.04 ^c	1.34±0.07 ^b	1.2±0.02	1.13±0.03	2.10±0.08 ^b	1.37±0.09	1.55±0.09 ^a	1.90±0.22 ^{ab}	1.63±0.12
Tamoxifen+PRP	1.12±0.03 ^{ab}	1.1±0.02	1.96±0.04 ^{bc}	1.24±0.07 ^{ab}	1.16±0.02	1.1±0.02	1.94±0.04 ^{ab}	1.26±0.05	1.33±0.18 ^a	1.56±0.06 ^{bc}	1.53±0.09

Superscripts (a-c) show significant differences in each column ($p < 0.05$).

Table 4. Effect of intra-testicular administrated PRP on the sperm quality (%) and histomorphometrical analysis in a tamoxifen-injured rat model (mean ± SD)

Groups	Spermatozoa ($\times 10^6$)	SSI	Motility	Aggressive motility	Normal morphology	TDI	SPI	RI
Control	36.5±0.65 ^a	77.2±1.11 ^a	71.73±0.44 ^a	50.75±1.25 ^a	71.75±0.63 ^a	85±1.85 ^a	75.25±0.63 ^a	80.25±0.48 ^a
Tamoxifen	27.1±0.58 ^b	59.2±2.01 ^b	28.30±0.34 ^b	39.33±2.66 ^b	51.02±0.66 ^b	71±2.01 ^b	62.11±1.11 ^b	68.11±1.01 ^b
PRP	33.2±0.12 ^a	66.8±2.45 ^a	73.14±0.39 ^a	48.12±1.02 ^a	66.74±0.91 ^a	79±3.12 ^a	70.12±2.41 ^a	75.12±2.41 ^a
Tamoxifen+PRP	37.1±0.51 ^a	70.9±1.05 ^a	64.20±0.63 ^a	51.17±3.01 ^a	70.12±0.11 ^a	82±2.22 ^a	71.55±0.04 ^a	78.33±3.02 ^a

SSI: Sperm Survival Index. Superscripts (a-b) show significant differences in each column ($p < 0.05$).

hypothalamic-pituitary-gonadal axis plays a critical role [27].

It is established that dietary consumption of antioxidants contributes to an increase in the conception probability in sub-fertile couples [23, 28, 29]. The results of antioxidant rescue experiments suggest that oxidative stress is a significant factor in male infertility [30, 31]. Our results revealed that intra-testicular injection of PRP improved the sperm production (Tables 4 and 5). Future experiments might focus on explaining how PRP exerts its protective activity when supplemented orally to lab rodents. It may act by increasing the presence of specific amino acids or peptides in the blood, or by the activity of whole polypeptide chains that might cross the gut-blood barrier.

Recently, Salama et al. [32] indicated that the positive protective actions of PRP on cryopreserved buffalo bull's

semen seem to be closely involved in increasing antioxidant capacity activities and suppressing plasma lipid peroxidation. In the present study, the recovery of sexual hormone levels, as well as germinal cell count after PRP administration may be attributed to the potent-free radical scavenger effects of PRP as a novel antioxidant (Table 3).

Apoptosis plays a pivotal role in maintaining tissue homeostasis in organisms. Previous studies have shown that PRP treatment can effectively restore ovarian function [33, 34], granulosa cells damaged with Cyclophosphamide [35], and human endometrial stromal cells [36, 37], which favor cell proliferation and migration by inhibiting apoptosis. Tamoxifen is commonly used to treat patients with ESR/ER-positive breast cancer in Iran. The tamoxifen-induced impairment of the apoptotic flux could be a kind of

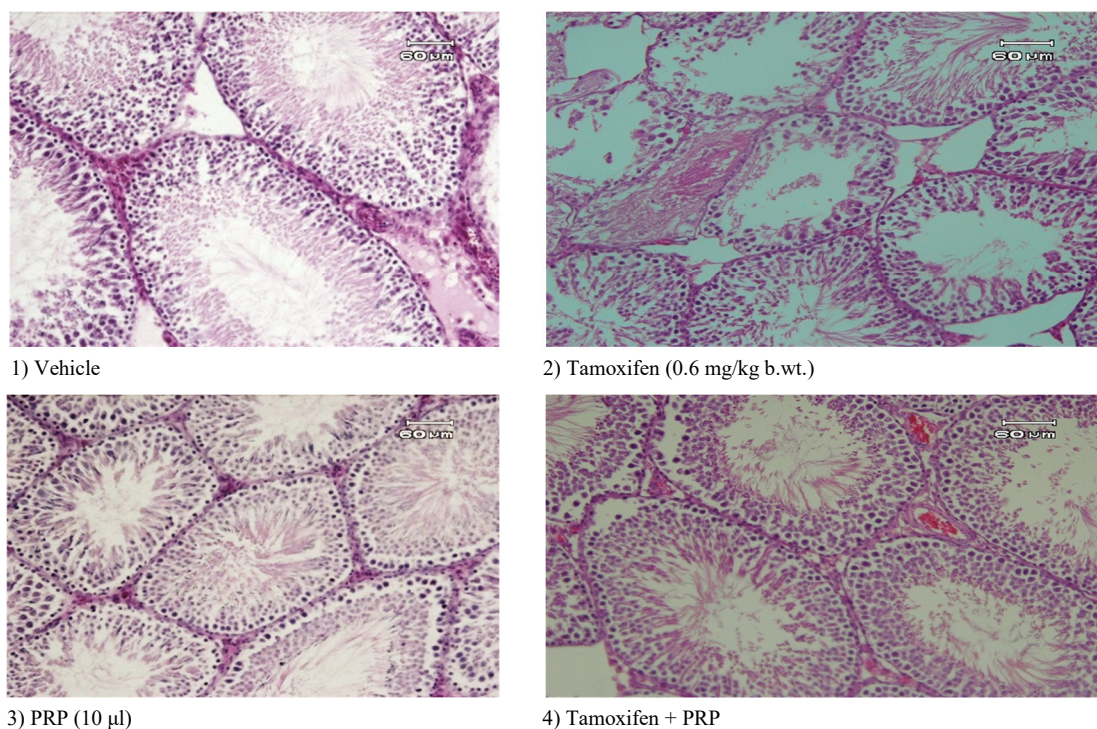


Figure 1. Testicular histopathological structure, and regeneration of the germinal epithelium in rats after 14 days of simultaneous treatments

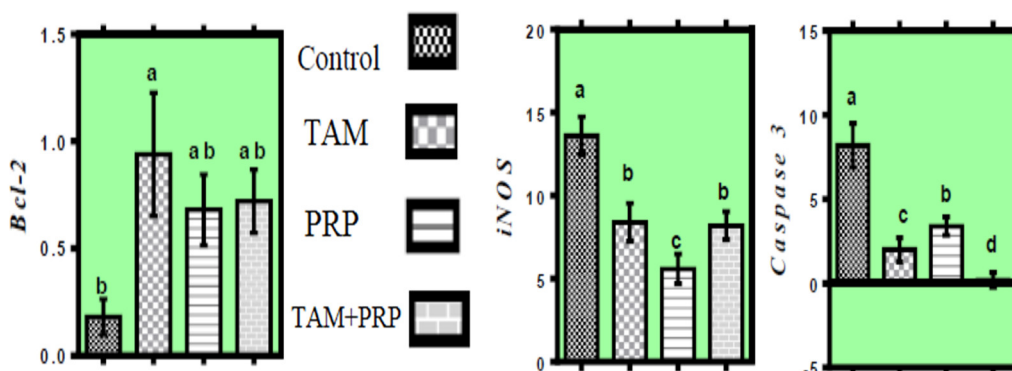


Figure 2. Relative expression of genes related to apoptosis in the rat testis after tamoxifen and PRP treatments (Graphs show mean± S.D.; Letters indicate that treatments differ p<0.05 within each group)

embodiment of tamoxifen toxicity (Figure 2). Promising early results suggest that PRP supplementation exhibited a protective effect on the spermatogenic activity in a tamoxifen treated damages rat model, showing promise as a potential therapeutic agent.

CONCLUSION

In sum, the PRP supplement is potentially an impressive method for modifying production of sperm dysfunction via tamoxifen damages.

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