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Provitamin A carotenoid (β-cryptoxanthin) ameliorated testicular ischemia-reperfusion injury in mature rats

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Abstract

Buildup of reactive oxygen species during testicular torsion causes oxidative stress and ischemia-reperfusion (I/R) injury in testis. The purpose of this study was to investigate influence of β-cryptoxanthin (BCX) on I/R injury in testicular torsion/detorsion in mature rats. Thirty mature male Wistar rats were divided into five groups of six animals each, including sham group: In this group, midline incision of the scrotum was performed and the testicles were taken out for 2 hr with a 720-degree rotation, I/R group: In this group, midline incision of the scrotum was performed and the testicles were taken out and undergone ischemia for 2 hr with a 720-degree rotation, I/R/Oil group: In this group, a midline scrotum cut was performed, the testicles were taken out, ischemia was created for 2 hr with a 720-degree rotation, and at the end of ischemia 100 µL of corn oil (BCX solvent) was injected intraperitoneally, I/R/BCX10 group: The same as I/R/Oil group, as well as intraperitoneal administration of 100 µL of BCX (10.00 µg kg^{-1}) at the end of ischemia, and I/R/BCX40: The same as I/R/Oil group, as well as intraperitoneal administration of 100 µL of BCX (40.00 µg kg^{-1}) at the end of ischemia. Evaluations were based on histopathological and spermatological parameters and oxidative stress assessments. Histopathological spermatological and oxidative stress parameters values obtained from I/R/BCX40 were significantly different from those of other groups ($p < 0.05$). It could be concluded that BCX could ameliorate testicular injuries in acute testicular torsion/detorsion in mature rats.

Introduction

Delayed diagnosis and treatment of surgical emergencies, like urological ones, can end up to tissue necrosis and atrophy. Testicular torsion is the most common cause of testicular loss in newborns, children, and adolescent boys.[1,2] The extent and duration of torsion are critical in testicular injury determination. Well-timed treatment of the damage offers a significant chance to save testicles; so, with that the survival rate can fall in a range of 90.00 - 100%.[3] Urgent surgery is mandatory to restore blood flow; however, severe testicular damage is the consequent to reperfusion of the ischemic tissue.[4]

Long-lasting testicular degeneration even after the initial ischemic damage occurs following ischemia-reperfusion (I/R).[5,6] Over-production of pro-inflammatory cytokines and increased leukocyte migration to the testicular tissue take place within I/R phases, activating the assembly of reactive oxygen species (ROS) by neutrophils causing oxidative damage to testicular cells.[7-9] Lipid peroxidation, excessive pro-inflammatory cytokines, and intra-cellular calcium release can result in infertility.[10] Oxidative stress ruins the capacity of biological systems to detoxify reactive mediators,[11] and plays a role in the development and progression of various diseases.[12]

The efficacy of antioxidants with free radical scavenging properties in reducing I/R damage in different organs, including testis, has already been reported.[13] Antioxidants prevent the oxidation of molecules and buildup of free radicals that can trigger harmful chain reactions.[14] Numerous enzymes, chemical medicines, and herbal remedies have been used to support tissue repair and prevent post-I/R testicular damage.[15]

The β-cryptoxanthin (BCX) is an oxygenated carotenoid with a chemical structure similar to, but more polar than, β-carotene. Although β-carotene is present in large amounts in numerous fruits and vegetables, BCX is found at high concentrations in only a small number of

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foods. The BCX can accept energy from singlet oxygen, and the evidence that it is an antioxidant *in vitro* at most physiological concentrations is persuasive.[16]

To the best knowledge of authors, the literature is poor regarding the effect of intra-peritoneal (IP) administration of BCX on testicular I/R injury. Therefore, the present study was designed to determine whether BCX could in fact help protect I/R-induced testicular damage in an animal model.

Materials and Methods

Chemicals. All chemical substances were purchased from Sigma (St. Louis, USA) unless otherwise stated.

Animals and ethical considerations. The study involved thirty mature male Wistar rats, weighing between 200 and 250 g, and aged 6 to 8 weeks, obtained from the Animal Resource Center of Urmia University, Urmia, Iran. The rats were maintained and observed in a controlled environment that was carefully regulated to maintain a stable temperature range of 20.00 to 22.00 ˚C, sufficient ventilation for optimal air quality, a precisely regulated natural humidity, and natural light-dark cycle. They had unrestricted access to food and water and were acclimatized for one week before the experiment. The procedures were approved by the Institutional Animal Care and Use Committee of the University under the Code Number of IR-UU-AEC-3/13, dated 14/05/2024. All procedures were performed under conditions to minimize any potential suffering of the animals. Ketamine (500 mg kg^{-1}; Alfasan, Woerden, The Netherlands) and xylazine (50.00 mg kg^{-1}; Alfasan) were administered intra-peritoneally (IP) to euthanize the animals.[17]

Experimental protocol. Following 1 week of acclimatization, 30 mature male Wistar rats were divided into five groups of six animals each, including sham group: In this group, midline incision of the scrotum was performed and the testicles were manipulated without rotation, I/R group: In this group, midline incision of the scrotum was performed and the testicles were taken out and undergone ischemia for 2 hr with a clockwise 720-degree rotation, I/R/Oil group: In this group, a midline scrotum cut was performed, the testicles were taken out, ischemia was created for 2 hr with a 720-degree rotation, and at the end of ischemia 100 µL of corn oil (BCX solvent) was injected IP, I/R/BCX10 group: The same as I/R/Oil group, as well as IP administration of 100 µL of BCX (10.00 µg kg^{-1}) at the end of ischemia, and I/R/BCX40 group: The same as I/R/Oil group, as well as IP administration of 100 µL of BCX (40.00 µg kg^{-1}) at the end of ischemia.

Surgical procedure. The surgical procedure was performed under sterile conditions. The rats were anesthetized using ketamine (80.00 mg kg^{-1}; IP; Alfasan) xylazine (10.00 mg kg^{-1}; IP; Alfasan). Following the surgical preparation of the testicular region, shaving, and cleaning with a 10.00% povidone-iodine solution, the testes were exposed through testicular approach and rotated clockwise 720 degrees. The testis was then fixed in the torsion position and detorsion was performed after 60 days. The incision was then closed with a simple running suture technique using 4/0 nylon (Supa, Tehran, Iran). In each rat, the left testis was dissected out and removed after 24 hr for enzymatic antioxidant activity assessments and right testis was dissected out and removed after 60 days for histo-pathological analyses and sperm parameters evaluations.

Enzymatic antioxidant activity assessment. To assess enzymatic antioxidant activity, 20.00 - 30.00 mg of testicular tissue was homogenized in 1,000 µL of lysis buffer and centrifuged at 9,000 rpm for 15 min, and the supernatant was collected for biochemical analyses and subsequently stored at a temperature of – 20.00 ˚C until the tests were performed.[18]

Total antioxidant capacity (TAC) determination. The TAC levels in the testis were quantified using a TAC assay kit (Naxifer; Navand Salamat, Urmia, Iran). For the testicular TAC assay, the reaction was conducted in the 1,000 µL of reaction buffer, containing 100 µL of supernatant, 400 µL of distilled water, and 500 µL of (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid; $ABTS^{+}$) buffer (containing 100 µL of $ABTS^{+}$ and 800 µL of distilled water, with 100 µL of potassium persulfate [10×]). The absorbance was 1.14. Following a 5-min incubation period at the room temperature, the absorbance was monitored at 414 nm using ultraviolet-visible spectrophotometer (CamSpec M330; Camspec Ltd., Sawston, UK). The results were reported as nmol mg^{-1} protein.[19]

Malondialdehyde (MDA) level quantification. The MDA test kit (Nalondi™ Lipid Peroxidation Assay Kit; Navand Salamat) was used to measure oxidative stress. The MDA concentration was determined at a wavelength of 523 nm using a spectrophotometer (Thermo Fisher Scientific, Waltham, USA) and a standard curve, and expressed as nmol mg^{-1} protein.[19]

Testicular histopathology and histomorphometry. The right testes of rats were preserved in a 10.00% formalin solution. They were dehydrated with a series of ethanol and then embedded in paraffin. Thin sections with a thickness of 7.00 µm were prepared using a microtome. The resulting sections were then stained by Hematoxylin and Eosin staining.[20] The stained sections were examined under a light microscope (Olympus, Tokyo, Japan). Johnsen's score was used to assess spermatogenesis in seminiferous tubules in each cross-section (Table 1).[18] To monitor spermatogenesis, 200 seminiferous tubules were examined under a light microscope (Olympus). To determine the seminiferous tubules diameter (STsD), 200 randomly selected round or nearly round cross-sections of seminiferous tubules (100 from each testis) were examined.

Table 1. Johnsen's scoring system used for testicular damage evaluation.[18]

Johnsen's score	Description of histological criteria
10	Full spermatogenesis
9	Slightly impaired spermatogenesis, many late spermatids, and disorganized epithelium
8	Less than five spermatozoa *per* tubule, and few late spermatids
7	No spermatozoa, no late spermatids, and many early spermatids
6	No spermatozoa, no late spermatids, and few early spermatids
5	No spermatozoa or spermatids, and many spermatocytes
4	No spermatozoa or spermatids, and few spermatocytes
3	Spermatogonia only
2	No germinal cells, and Sertoli cells only
1	No germinal epithelium

Two diameters of each seminiferous tubule cross-section were measured with the light microscopic eye micrometer and their average values were calculated. The Sertoli cell index (SCI), repopulation index (RI), and mitotic index (MI) were calculated by randomly selecting sixty seminiferous tubules *per* group. The SCI is the ratio of Sertoli cells with a distinct nucleus and nucleolus being present in seminiferous tubules, to the number of germ cells. The RI calculates the proportion of tubules populated with germ cells that have reached at least the middle spermatogonial stage or later.[21] To determine the proportion of cells lost during cell division, the MI (number of round spermatids for each pachytene primary spermatocyte) was used. The Leydig cell nuclear diameter (LCND) was determined using a calibrated ocular micrometer. Two hundred transverse sections of the seminiferous tubules of each animal (100 *per* testis) were randomly examined to determine the tubular differentiation index (TDI) and spermiogenesis index (SPI). The TDI refers to the proportion of seminiferous tubules having at least three fully developed germ cells; while, the SPI calculates the proportion of seminiferous tubules normally containing sperm cells. The degree of testicular injury was assessed using the Cosentino scoring system.[18] This system divides the testicle into four grades. Grade one represents normal testicular architecture, grade two indicates less ordered, non-cohesive germ cells and closely packed seminiferous tubules, grade three represents disordered, sloughed germ cells with shrunken, pyknotic nuclei and less distinct seminiferous tubule borders, and grade four shows seminiferous tubules being densely packed along with germ cell coagulative necrosis.[22]

Epididymal sperm evaluations. The caudal epididymis was quickly cut into small pieces and placed in a Petri dish, containing 1.00 mL of human tubal fluid medium (2.00 mM $CaCl_2$-$2H_2O$, 2.50 mM glucose, 5.00 mM KCl, 0.40 mM KH_2PO_4, 0.20 mM $MgSO_4$-$7H_2O$, 100mM NaCl, 25.00 mM $NaCHO_3$, 18.50 mM sodium lactate, 0.30 mM sodium pyruvate, 0.20 mM penicillin G sodium salt, 0.30 mM streptomycin sulfate, 4.00 g L^{-1} bovine serum albumin, and 2.00 mg L^{-1} phenol red) for 30 min at 37.00 ˚C and 5.00% CO_2.[20] For epididymal sperm count, the sperm cells were diluted in distilled water at a ratio of 1:5, devitalized and then, counted using a Neubauer hemocytometer (HBG Henneberg-Sander GmbH, Giessen, Germany). Using a digital laboratory precision balance (Sartorius, Göttingen, Germany) with a capacity of 0.0001 g, epididymal tissue pieces were carefully collected and weighed. Finally, the average number of epididymal sperm *per* g of epididymal tissue was determined.[23] The motility analyses were performed at a room temperature using a computer-assisted semen analysis system (Test Sperm 3.2; Videotest, Saint Petersburg, Russia). The system measured total motility (%), progressive motility (%), curvilinear velocity (VCL; μm sec^{-1}), straight-line velocity (VSL; μm *per* sec), average path velocity (VAP; μm sec^{-1}), straightness (STR; %), linearity (LIN; %), amplitude of lateral head displacement (ALH; μm sec^{-1}), and beat-cross frequency (BCF; Hz).[23] To examine the sperm, an aliquot of sperm suspension of approximately 10.00 μL was loaded onto a microscopic slide and observed using the phase-contrast field microscopy. At least 500 spermatozoa were observed in five microscopic fields.[19] To evaluate sperm vitality and morphology, the Eosin-Nigrosin staining method was used according to the World Health Organization protocol.[23] Eosin and Nigrosin dyes (Merck, Darmstadt, Germany) were mixed in distilled water. The combination of one volume of semen with two volumes of 1.00% Eosin was analyzed using the light microscope (CHT; Olympus) at a magnification of 400 ×. The non-viable sperm appeared red due to the Eosin staining, while the viable sperm remained colorless, allowing viability assessment. Additionally, Eosin-Nigrosin staining was used to determine the percentage of abnormal sperms. Sperms with discolored parts of the head, neck, or tail were considered dead. Sperms with obvious abnormalities, such as cytoplasmic remnants, were classified as abnormal. From each sample, 200 sperms were examined at 400 × magnification, and the results were presented as a percentage.[19] Acridine orange staining was used to detect denatured, native, and double-stranded DNA in sperm chromatin under low pH challenge. The denatured DNA showed the strongest fluorescence. Concentrated smears were soaked in a mixture of acetic acid (1:3) and Carnoy's fixative for 2 hr and then, dried outdoors for 5 min. They

were then added to a stock solution, consisting of 1,000 mL purified water and one mg Acridine orange. The mixture was kept in a dark room at 4.00 ˚C for 5 min. After staining, the spermatozoa were examined under a fluorescence microscope (GS7; Nikon, Tokyo, Japan) at a magnification of 400 ×. Spermatozoa appearing red or yellow were considered damaged or aberrant.[19]

The hypo-osmotic swelling test identified sperms with sperm plasma membrane functionality (PMF). To carry out the test, 100 µL of a hypo-osmotic solution, consisting of fructose (1.351 g L^{-1}) and sodium citrate (0.735 g L^{-1}), was mixed with 10.00 µL of sperm sample and then, incubated at 37.00 ˚C for 1 hr. The sperm PMF was evaluated using the phase contrast microscope at a magnification of 400×. The main indicators of an effective PMF were identified as coiled or swollen tails.[19]

Statistical analysis. The study data were analyzed using SPSS Software (version 26.0; IBM Corp., Armonk, USA). A one-way ANOVA was performed to determine significant differences between the groups. Tukey's post hoc analysis was used to identify specific groups that differed significantly. A *p*-value of ≤ 0.05 was considered statistically significant.

Results

Testicular oxidant/antioxidant status. Tissue TAC values were significantly higher in the I/R/BCX40 group compared to others ($p < 0.05$). The MDA levels in the I/R/BCX40 group were significantly lower in comparison with other groups ($p < 0.05$; Table 2).

Body and reproductive organs weights. Table 3 shows the weights of the testis and epididymis. A significant increase in the testis-to-body weight ratio was found in the I/R/BCX40 group in comparison with other groups ($p < 0.05$).

Histopathological findings. The testis underwent significant morphological changes due to the testicular torsion (Table 3). In the sham group, the structure of the spermatogenic epithelium of the seminiferous tubules and the process of spermatogenesis in these tubules were normal. In the I/R and I/R/Oil groups, degenerative changes in seminiferous tubules, including disintegration and severe reduction of spermatogenic epithelium layers, germ cells shedding, and oligospermia were observed. Hypoplastic changes caused extreme hypo-cellularity in the seminiferous tubules in the I/R and I/R/Oil groups. The BCX, depending on the dose, caused the reconstruction of the cyto-structure of the seminiferous tubules and also improved the process of spermatogenesis (Fig. 1). Johnsen's score determination also confirmed germ cell degeneration and cyto-architectural dis-organization in torsed testes. The mean Cosentino's score showed that the I/R/BCX40 group had significantly the least histopathological changes in comparison with other experimental groups ($p < 0.05$). The I/R/BCX40 group also showed a significant increase in SCI, RI, SPI, and MI compared to the other groups ($p < 0.05$). Moreover, analysis of histological parameters revealed a significant rise in LCND and STsD in the I/R/BCX40 group compared to the other groups ($p < 0.05$; Table 4).

Epididymal sperms characteristics. The I/R/BCX40 group had significantly higher sperm concentration compared to the other groups ($p < 0.05$). Additionally, the I/R/BCX40 group showed significantly higher total and progressive sperm motilities in comparison with other groups ($p < 0.05$). The VAP, VCL, VSL, LIN, ALH, STR, and BCF values were also significantly increased in the I/R/BCX40 group in comparison with other groups ($p < 0.05$; Table 5). Furthermore, sperms PMF and viability were significantly increased in the I/R/BCX40 group in comparison with other groups ($p \leq 0.05$). Significant decreases in sperm DNA damage and abnormal morphology were also found in the I/R/BCX40 group in comparison with other groups ($p \leq 0.05$; Table 6).

Table 2. Biochemical findings in different experimental groups. Values are expressed as mean ± SEM.

Parameters	Sham	I/R	I/R/Oil	I/R/BCX10	I/R/BCX40
TAC (mmol *per* mg protein)	14.29 ± 0.32[a]	2.83 ± 0.27[d]	2.86 ± 0.25[d]	7.96 ± 0.28[c]	10.58 ± 0.49[b]
MDA (µmol *per* g protein)	268.15 ± 19.84[e]	559.40 ± 29.33[a]	560.91 ± 25.49[a]	440.64 ± 30.86[c]	304.25 ± 24.63[d]

TAC: Total antioxidant capacity; MDA: Malondialdehyde; I/R: Ischemia-reperfusion; Oil: Corn oil; BCX: β-cryptoxanthin. I/R: ischemia-reperfusion, I/R/Oil: ischemia-reperfusion+corn oil, I/R/BCX10: ischemia-reperfusion+10.00 µg kg^{-1} β-cryptoxanthin; I/R/BCX40: ischemia-reperfusion+40.00 µg kg^{-1} β-cryptoxanthin.

[a-e] Different superscripts within the same row demonstrate significant differences ($p < 0.05$).

Table 3. Body and reproductive organs weights in different experimental groups. Values are expressed as mean ± SEM.

Parameters	Sham	I/R	I/R/Oil	I/R/BCX10	I/R/BCX40
Testis weight (g)	0.52 ± 0.06[a]	0.09 ± 0.04[e]	0.09 ± 0.06[e]	0.20 ± 0.04[c]	0.37 ± 0.03[b]
Epididymis weight (g)	0.28 ± 0.04[a]	0.05 ± 0.05[d]	0.05 ± 0.02[d]	0.10 ± 0.03[c]	0.21 ± 0.02[b]
Testis/body weight (%)	1.75 ± 0.21[a]	0.28 ± 0.11[e]	0.29 ± 0.13[e]	0.58 ± 0.25[c]	1.46 ± 0.20[b]

I/R: ischemia-reperfusion, I/R/Oil: ischemia-reperfusion+corn oil, I/R/BCX10: ischemia-reperfusion+10.00 µg kg^{-1} β-cryptoxanthin; I/R/BCX40: ischemia-reperfusion+40.00 µg kg^{-1} β-cryptoxanthin.

[a-e] Different superscripts within the same row demonstrate significant differences ($p < 0.05$).

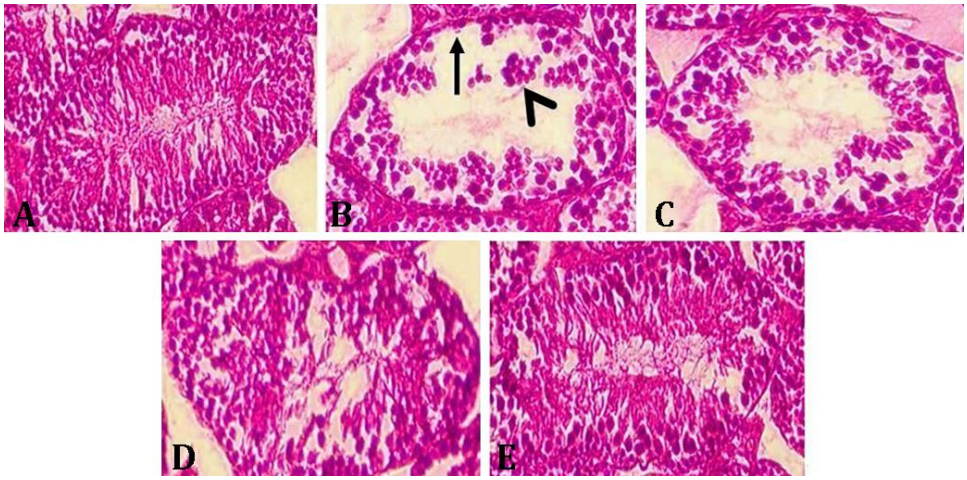

Fig. 1. Photomicrographs of testicular tissue in different experimental groups. **A)** Sham, **B)** Ischemia-reperfusion (I/R), **C)** I/R/Oil, **D)** I/R/β-cryptoxanthin (BCX) 10, and **E)** I/R/BCX40. Arrow shows the severe reduction of the layers of the spermatogenic epithelium and arrowhead shows the germ cells exfoliation into the lumen of the seminiferous tubule of the testis (Hematoxylin and Eosin staining, 400×).

Table 4. Histopathological parameters in different experimental groups. Values are expressed as mean ± SEM.

Parameters	Sham	I/R	I/R/Oil	I/R/BCX10	I/R/BCX40
Johnsen's score	8.39 ± 0.19^{a}	3.58 ± 0.36^{e}	3.55 ± 0.29^{e}	6.62 ± 0.63^{c}	7.65 ± 0.98^{b}
Cosentino's score	1.07 ± 0.03^{d}	3.53 ± 0.19^{a}	3.56 ± 0.31^{a}	2.31± 0.05^{c}	1.60 ± 0.04^{d}
Seminiferous tubule diameter (µm)	304.41 ± 13.49^{a}	176.49 ± 17.61^{d}	185.83 ± 13.46^{d}	233.34 ± 17.49^{c}	270.26 ± 15.41^{b}
Sertoli cell index	13.31 ± 1.36^{a}	7.09 ± 0.07^{e}	7.06 ± 0.09^{e}	9.73 ± 0.30^{c}	11.95 ± 0.79^{b}
Repopulation index (%)	79.21 ± 2.18^{a}	35.93 ± 1.44^{e}	35.89 ± 1.61^{e}	50.89 ± 1.63^{c}	72.09 ± 2.30^{b}
Miotic index	3.04 ± 0.43^{a}	1.01 ± 0.25^{d}	1.09 ± 0.49^{d}	1.93 ± 0.06^{c}	2.58 ± 0.04^{b}
Leydig cell nuclear diameter (µm)	7.76 ± 0.31^{a}	3.88 ± 0.15^{d}	3.85 ± 0.46^{d}	6.64 ± 0.59^{c}	8.23 ± 0.43^{b}
Tubular differentiation index (%)	80.05 ± 2.69^{a}	45.78 ± 1.63^{e}	47.50 ± 1.39^{e}	63.85 ± 1.43^{c}	71.95 ± 2.69^{b}
Spermiogenesis index (%)	75.13 ± 3.84^{a}	44.21 ± 1.40^{e}	44.04 ± 1.60^{e}	61.82 ± 1.30^{c}	69.23 ± 1.83^{b}

I/R: ischemia-reperfusion, I/R/Oil: ischemia-reperfusion+corn oil, I/R/BCX10: ischemia-reperfusion+10.00 µg kg^{-1} β-cryptoxanthin; I/R/BCX40: ischemia-reperfusion+40.00 µg kg^{-1} β-cryptoxanthin.
$^{a-e}$ Different superscripts within the same row demonstrate significant differences ($p < 0.05$).

Table 5. Epididymal sperm motility. Values are expressed as mean ± SEM.

Parameters	Sham	I/R	I/R/Oil	I/R/BCX10	I/R/BCX40
Epididymal sperm concentration (10^{6} mL^{-1})	66.19 ± 24.01^{a}	24.71±19.39^{de}	23.18 ± 21.35^{e}	33.62 ± 20.54^{c}	59.88 ± 23.82^{b}
Total motility (%)	82.59 ± 3.96^{a}	45.37±2.51^{e}	44.35 ± 1.29^{e}	54.83 ± 1.62^{c}	73.70 ± 3.66^{b}
Progressive motility (%)	45.23 ± 1.51^{a}	13.95±1.38^{d}	14.20 ± 1.27^{d}	24.37 ± 1.01^{c}	37.49 ± 1.47^{b}
VAP (µm sec^{-1})	36.84 ± 1.33^{a}	16.51±1.93^{d}	16.59 ± 1.38^{d}	25.48 ± 1.37^{c}	32.57 ± 1.20^{b}
VCL (µm sec^{-1})	93.49 ± 3.75^{a}	46.19±1.18^{d}	46.83 ± 1.70^{d}	58.11 ± 1.65^{c}	84.34 ± 2.52^{b}
VSL (µm sec^{-1})	16.63 ± 1.20^{a}	5.29±0.63^{e}	5.25 ± 0.47^{e}	10.52 ± 0.93^{c}	14.96 ± 0.87^{b}
LIN (%)	18.25 ± 0.22^{a}	17.12±0.19^{a}	17.31 ± 0.13^{a}	17.61 ± 0.15^{a}	18.21 ± 0.21^{a}
ALH (µm sec^{-1})	8.31 ± 0.47^{a}	2.17±0.52^{d}	2.52 ± 0.84^{d}	6.28 ± 0.79^{c}	8.32 ± 0.63^{b}
STR (%)	49.07 ± 0.29^{a}	48.23 ± 0.20^{a}	48.99 ± 0.23^{a}	49.03 ± 0.21^{a}	49.11 ± 0.24^{a}
BCF (Hz)	12.43 ± 0.36^{a}	4.75 ± 0.61^{d}	4.48 ± 0.75^{d}	8.26 ± 0.76^{c}	12.15 ± 0.27^{b}

VAP: Average path velocity; VCL: Curvilinear velocity; VSL: Straight line velocity; LIN: Linearity; ALH: Amplitude of lateral head displacement; STR: Straightness; BCF: Beat-cross frequency. I/R: ischemia-reperfusion, I/R/Oil: ischemia-reperfusion+corn oil, I/R/BCX10: ischemia-reperfusion+10.00 µg kg^{-1} β-cryptoxanthin; I/R/BCX40: ischemia-reperfusion+40.00 µg kg^{-1} β-cryptoxanthin.
$^{a-e}$ Different superscripts within the same row demonstrate significant differences ($p < 0.05$).

Table 6. Epididymal sperm. Values are expressed as mean ± SEM.

Parameters	Sham	I/R	I/R/Oil	I/R/BCX10	I/R/BCX40
Sperm plasma membrane functionality (%)	88.86 ± 2.04^{a}	48.62 ± 1.19^{e}	48.48 ± 1.75^{e}	58.99 ± 1.75^{c}	76.11 ± 2.26^{b}
Sperm viability (%)	89.77 ± 2.59^{a}	52.21 ± 1.40^{d}	53.38 ± 1.61^{d}	60.13 ± 1.37^{c}	79.53 ± 3.53^{b}
Sperm DNA damage (%)	4.09 ± 0.42^{d}	45.52 ± 1.81^{a}	45.86 ± 1.93^{a}	32.27 ± 1.42^{b}	10.49 ± 0.69^{c}
Sperm abnormal morphology (%)	10.36 ± 0.37^{e}	46.86 ± 1.29^{a}	46.79 ± 1.49^{a}	28.62 ± 1.67^{c}	14.84 ± 0.91^{d}

I/R: ischemia-reperfusion, I/R/Oil: ischemia-reperfusion+corn oil, I/R/BCX10: ischemia-reperfusion+10.00 µg kg^{-1} β-cryptoxanthin; I/R/BCX40: ischemia-reperfusion+40.00 µg kg^{-1} β-cryptoxanthin.
$^{a-e}$ Different superscripts within the same row demonstrate significant differences ($p < 0.05$).

Discussion

In the present study, we investigated whether IP administration of various concentrations of BCX were useful in the prevention of testicular damage in I/R conditions in rat testes and it was found that the concentration of 40.00 μg kg^{-1} showed the most beneficial effects. Testicular oxidant/antioxidant status, and histopathological and spermatological findings indicated that BCX at the concentration of 40.00 μg kg^{-1} could significantly improve damages induced by ischemia.

Delays in detorsion result in germ cell death because reperfusion is insufficient to maintain tissue function, which is the main cause of the pathophysiological effects of testicular torsion. In addition, the accumulation of toxic substances, such as ROS, and low oxygen levels during ischemia contribute to germ cell death.[24] To help protect rats from testicular torsion, this study showed how well BCX acted. It was found in this study that BCX enhanced TAC in the testicular tissue and reduced MDA levels. It has been shown that BCX is an antioxidant *in vitro*, highlighting its ability to scavenge ROS.[25] Accordingly, it has been revealed that testicular I/R increases oxidative stress while reducing antioxidant enzyme concentrations.[13] The simplest method to demonstrate that tissue lipid peroxide concentration is an important predictor of I/R is to measure MDA levels. Correspondingly, it has been reported that MDA levels in the testicular tissue are increased significantly with increasing testicular ischemia duration, leading to oxidative damage.[26] In consistent with the present study, lycopene, a precursor of vitamin A, has been reported to decrease MDA, which is known to be correlated with oxidative damage and is the final product of oxidation of fatty acids, and to increase endogen antioxidants, like superoxide dismutase and glutathione peroxidase.[27-31]

Testicular I/R injury can significantly deteriorate spermatogenesis and may cause infertility. Spermatogenesis was evaluated by the Johnsen score and STsD measurements in all experimental groups and found to be significantly damaged in the I/R group. However, the Johnsen score and STsD were significantly higher in the I/R/BCX40 group in comparison with other treatment groups. This finding indicated that BCX administration (40.00 μg kg^{-1}) before detorsion decreased spermatogenesis damage and also could prevent testicular I/R injury induced infertility.

Changes in morphological and histopathological parameters (testis weight, epididymis weight, testis/body weight, Johnsen score, Cosentino score,[18] STsD, SCI, RI, MI, LCND, TDI, and SPI) have been used in the evaluation of the cellular condition.[32] In consistent with the findings of the present study, it was reported that BCX alleviated myocardial I/R in rats.[33] In a rat model of hepatic I/R injury, supplementation with β-carotene has been shown to increase the antioxidants level.[34] Moreover, β-carotene was demonstrated to protect the gastric mucosa against I/R injury in rats by inhibiting the peroxidation of lipids, improving the activity of antioxidant enzymes, and preventing the infiltration of neutrophils.[35] The results of a study on lycopene used as a therapeutic agent for testicular I/R injury showed impairments, like desquamation of epithelial cells in the lumen, disorder of seminiferous tubule germinal epithelium, multi-nucleated giant cells formation, and necrosis of some seminiferous tubules; however, lycopene was not potent enough to improve the tissue injury.[35]

In the present study, it was found that BCX administration considerably improved epididymal sperms concentration, total motility, and progressive motility. The sperm VAP, VCL, VSL, LIN, ALH, STR, BCF, PMF, viability, DNA damage, and abnormal morphology were also improved by BCX administration. Although in the present study the outcomes were promising, extensive molecular assessments are required to evaluate the outcomes of IP administration of BCX in testicular I/R injury that remained unknown. These could be regarded as limitations of our study.

In conclusion, findings obtained from all the experimental groups indicated that IP administration of BCX at the concentration of 40.00 μg kg^{-1} could be helpful in minimizing I/R injury in testicular tissue exposed to ischemia. Some works were completed in the present study; however, the exact underlying mechanisms of BCX in improving testicular function might be more complicated than our findings.

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Conflict of interest

None.

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