RESEARCH ARTICLE

Attenuation potentials of royal jelly against hydroxyurea-induced infertility through inhibiting oxidation and release of pro-inflammatory cytokines in male rats

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Abstract

Hydroxyurea (HDU), a class of antineoplastic drugs, has a powerful efficacy in the treatment of several types of malignancies. However, it has multiple adverse effects including reduced fertility, especially in males. Thus, 60 male albino rats were used to investigate the chemoprotective potentials of royal jelly on HDU-induced testicular damage. Animals were gastro-gavaged with HDU (225 or 450 mg kg^{-1} bw day^{-1}) before royal jelly (100 mg kg^{-1} bw day^{-1}) for 60 days. Blood samples and testicles were collected, and spermatozoon was obtained. In a dose-dependent manner, the sperm count, motility and liveability, and testosterone, GSH, and catalase concentrations were decreased in HDU groups, whereas MDA, FSH, LH, IL-6, and IFN-γ expression levels were increased. Germinal epithelium degeneration, germ cell sloughing, reduction in the number of luminal spermatozoa, interstitial congestion, and severe leukocyte infiltration besides no glandular secretion in most of the acini were identified. However, royal jelly intake in HDU-treated rats successfully improved sperm quality, hormonal and antioxidant status, and reproductive organ histoarchitecture. Thus, it could be concluded that royal jelly is endowed with antioxidative and anti-inflammatory activities and could be, therefore, used as an adjuvant remedy to improve HDU-induced male subfertility.

Keywords Hydroxyurea · Royal jelly · Male infertility · Oxidative damage · Cytokines · Histopathology

Introduction

Hydroxyurea (HDU) is one of the antineoplastic drugs which are widely used in the treatment of neoplasm (as melanoma, prostatic carcinoma, ovarian cancer, transitional carcinoma of the urogenital tract, cervical cancer, and squamous neoplasm of head and neck), sickle cell disease, and thalassemia. Its action depends on inhibition of DNA production (Liebelt et al. 2007) through the inhibition of ribonucleotide reductase enzyme and decreasing deoxynucleotide triphosphates (especially dATP) pool (Charache et al. 1995). The side effects of HDU may include myelosuppression (anemia, leukopenia, and thrombocytopenia), genotoxicity, and cytotoxicity (Ahmad et al. 2018; Friedrisch et al. 2008). Also, spermatogenic arrest, decreased sperm production, and decreased male infertility have been reported in experimental animals (Gu et al. 2013; Shin et al. 1999; Wiger et al. 1995) and human (Berthaut et al. 2017; Berthaut et al. 2008; Grigg 2007). Cytotoxic effect of HDU may be attributed to the production of carbamoyl nitroso as a metabolite. Carbamoyl nitroso may be implicated in the reactive oxygen species (ROS) formation, thus oxidative stress induction (Cokic et al. 2003). Excessive production of ROS has a deleterious effect on spermatozoa, which associated with male sub-fertility. The plasma membrane of spermatozoa contains a high concentration of unsaturated fatty acids, which made it more vulnerable to

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oxidative injury (Gharagozloo and Aitken 2011). However, research studies regarding the impact of HDU on the male reproductive endpoints and fertility are still partial.

Royal jelly (RJ) is a creamy material secreted by the hypopharyngeal glands of worker honeybees, its ingredients consist of a mixture of glucose, lipids (fatty acids and sterols), protein, vitamins, and minerals. Royal jelly has several beneficial properties including antineoplastic, antioxidant, anti-inflammatory, anti-allergic, hypotensive, and immune-stimulatory potencies (Fujiwara et al. 1990; Guo et al. 2008; Hattori et al. 2007; Sver et al. 1996). Furthermore, several studies have proved that RJ has positive potentials on male reproductive organs and fertility besides its role against induced testicular oxidative damage (Amirshahi et al. 2014; Najafi et al. 2014; Shalizar Jalali et al. 2015; Zahmatkesh et al. 2014). Thus, this study was resumed to assess the adverse and/or toxic potentials of HDU on male reproductive organs and fertility and the probable protective role of RJ in diminishing its injurious effects in albino rats.

Materials and methods

Chemicals and reagents

Hydroxyurea was obtained as hard capsules (Hydrea®) containing 500 mg of hydroxycarbamide as the active ingredient (E.R. Squibb & Sons Ltd., England). Royal jelly was also obtained in the form of hard capsules (Royal Jelly®) containing 340 mg of lyophilized royal jelly (equivalent to 1000-mg-crude royal jelly) (Pharco Pharmaceuticals Company, Cairo, Egypt). HDU and RJ were dissolved in purified water immediately before administration.

Animals and experimental protocol

Sixty adults male Wistar albino rats (11-week old, weighing 190 ± 10 g) were utilized, which obtained from the closed random bred colony at the Alexandria Medical Research Institute, Egypt. They were raised in plastic cages, fed standard balanced diet, and allowed feed and water ad libitum. The Research Ethics Committee of Alexandria University as the instructions of "Care and Use of Laboratory Animals" prepared by the Faculty of Veterinary Medicine, Alexandria University, approved experimental procedures. The experimental rats were acclimatized for 2 weeks prior to experimentation and then randomly allocated into six groups ($n = 10$ per each) as follows:

Group 1: (control; CTR): administered 2 ml saline solution/ rat day^{-1}.
Group 2: (RJ) received 100-mg royal jelly kg^{-1} bw day^{-1} (Amirshahi et al. 2014).

Group 3: (HDU) received therapeutic dose 225 mg HDU kg^{-1} bw day^{-1} (Nair and Jacob 2016).
Group 4: (2HDU) received double therapeutic dose 450 mg HDU kg^{-1} bw day^{-1}.
Group 5: (RJ + HDU) received 100-mg royal jelly plus 225 mg HDU kg^{-1} bw day^{-1}.
Group 6: (RJ + 2HDU) received 100-mg royal jelly plus 450 mg HDU kg^{-1} bw day^{-1}.

The dosages were given orally using a stomach tube for 60 successive days taking in mind the period required to ensure a spermatogenic cycle. The following procedures were performed after 24 h of the last drug dose administration.

Determination of serum FSH, LH, and testosterone levels

Under the effect of ketamine (7.5:10 mg/kg) and xylazine (1 mg/kg i.p.) anesthesia, blood was collected from inner canthus of the eyes of all anesthetized rats. Blood samples were left to clot, then centrifuged to separate and collect serum aliquots, which were kept at -4 °C. Levels of serum testosterone, FSH, and LH hormones were measured using rats' highly sensitive ELISA Kits (Elabscience and Mybiosource, Texas, USA).

Determination of epididymal sperm count

Immediately after euthanization and abdominal opening, the right cauda epididymis was carefully dissected using anatomical scissors and pressed by fine surgical forceps in a small petri dish containing 1-ml phosphate buffer saline (PBS; pH 7.4 and 280 mOsm kg^{-1}) to obtain the maximum amount of semen. Then, a part of the seminal suspension was diluted using formalin buffer saline and the sperm heads were calculated using a hemocytometer under a light microscope at × 200 magnification (Ciftci et al. 2012a).

Evaluation of sperm motility

A drop of semen suspension was diluted with warm (37 °C) sodium citrate solution 2.9% on a glass side and covered with a cover slide. The percentage of motile sperms in a forward progressive manner was recorded using a light microscope with a heated stage (37 °C) at × 400 magnification (Ciftci et al. 2012b). For accuracy, motility evaluation was assessed in three random fields in each sample and their mean was considered the result of the motility score.

Determination of epididymal alive sperm percent

To detect viability percentage of the sperms, 20 μl of epididymal semen suspension was mixed with 60 μl warm Eosin

and Nigrosin stain (Nigrosin 5% and Eosin-Y 4% at ratio 3:1, 37 °C) for 30 s. Thereafter, smears were made and left to dry at room temperature and examined under a light microscope at × 400 magnification. Black-headed sperms were considered dead sperms (Bjorndahl et al. 2003).

Antioxidant/oxidant capacity

Later after euthanization, one testicle of each rat was rapidly excised, weighted, washed in warm normal saline, and preserved at − 80 °C for assessment of malondialdehyde (MDA) (Ohkawa et al. 1979), reduced glutathione (GSH) (Richardson and Murphy 1975), and catalase (CAT) (Aebi 1984) contents in the testicular homogenates. The homogenates were prepared by addition of 1 ml PBS for each 1 g of tissues and 9-ml ice-cold Tris–HCl buffer (10 mM, pH 7.4) with the aid of a tissue homogenizer (Glas-Col Tissues Homogenizer, Shanghai Konmix Mechanical & Electrical Equipment Co., Ltd., China) at 13,500 rpm for 3 min. The obtained homogenates were then centrifuged at 6000 rpm for 60 min at 4 °C. The supernatant fluids were carefully collected and stored at − 20 °C until the analysis time using commercially available kits (Biodiagnostic, Cairo, Egypt). The protein content of the obtained homogenate was detected using Bradford's method.

Assessment of testicular pro-inflammatory biomarkers

As well, the testicular levels of interleukin-6 (IL-6) and interferon-γ (IFN-γ) were detected in the testicular homogenates by a quantitative sandwich enzyme immunoassay method using Rat High Sensitivity ELISA kits (Sigma-Aldrich, St Louis, MO, USA).

Histopathological examination

One testicle, epididymis, prostate, and seminal vesicle of each rat were excised and quickly fixed in 10% buffered formalin (24 h each). The samples were processed through the paraffin-embedding technique and cut into 4–5-μm-thick sections. The sections were deparaffinized with xylene and stained with hematoxylin-eosin for studying the pathological histoarchitecture.

Statistical analyses

The obtained data were performed by one-way ANOVA followed by post hoc multiple comparisons Duncan's test using the SPSS statistical package v22.0 for Windows (IBM, Armonk, NY, USA). p values less than 0.05 were regarded as statistically significant differences. The analyzed data are presented as the mean ± standard error of mean (SEM).

Results

Hormonal changes

As shown in Table 1, comparatively with control rats, treatment with RJ alone did not show a significant effect ($p > 0.05$) on the testosterone and FSH and LH hormonal levels. However, serum concentration of testosterone showed a significant reduction ($p < 0.05$) in HDU-treated rats, which came parallel with significant enhancement ($p < 0.05$) in FSH and LH levels. These hormonal disturbances were much greater in the 2HDU-treated group. Fortunately, they were significantly attenuated ($p < 0.05$) with RJ co-treatment compared with HDU-treated groups.

Sperm parameters

Compared with control rats, the animals that were given RJ showed normal sperm motility, sperm count, and a significantly improved ($p < 0.05$) sperm liveability. However, these parameters were significantly reduced ($p < 0.05$) in HDU-treated rats, with a higher reduction in the 2HDU-treated group. The RJ co-treatment was significantly modified ($p < 0.05$) HDU-induced impairment in sperm parameters, compared with HDU-treated rats (Table 2).

Testicular oxidative stress parameters

Data recorded in Table 3 illustrated that the levels of MDA were significantly elevated ($p < 0.05$) in testicular tissues in HDU-treated groups. Meanwhile, the activity of testicular CAT enzyme and content of GSH were significantly decreased ($p < 0.05$) compared with controls that were more severe in 2HDU-treated rats. These alterations were remarkably reversed when RJ was administrated with HDU. RJ alone

Table 1 Potential effects of royal jelly (RJ, 100 mg kg^{-1} bw day^{-1}) on serum concentrations of testosterone and FSH and LH hormones in male Wistar albino rats administered therapeutic (HDU, 225 mg kg^{-1} bw day^{-1}) or double therapeutic (2HDU, 450 mg kg^{-1} bw day^{-1}) doses of hydroxyurea

Groups	Testosterone (ng/ml)	FSH (ng/ml)	LH (ng/ml)
CTR	3.88 ± 0.07[a]	7.33 ± 1.01[d]	0.63 ± 0.02[d]
RJ	3.96 ± 0.05[a]	7.33 ± 0.67[d]	0.62 ± 0.03[d]
HDU	2.11 ± 0.08[c]	16.83 ± 1.17[b]	1.04 ± 0.04[b]
2HDU	1.09 ± 0.04[d]	24.17 ± 1.88[a]	1.85 ± 0.09[a]
RJ + HDU	2.87 ± 0.05[b]	11.17 ± 1.01[c]	0.84 ± 0.02[c]
RJ + 2HDU	1.94 ± 0.03[c]	16.50 ± 1.32[b]	1.11 ± 0.11[b]

All values are expressed as mean ± standard error. Means within the same column of different superscript letters are significantly different at ($p < 0.05$). *FSH*, follicular stimulating hormone; *LH*, luteinizing hormone

Table 2 Potential effects of royal jelly (RJ, 100 mg kg^{-1} bw day^{-1}) on sperm motility, count, and liveability in male Wistar albino rats administered therapeutic (HDU, 225 mg kg^{-1} bw day^{-1}) or double therapeutic (2HDU, 450 mg kg^{-1} bw day^{-1}) doses of hydroxyurea (HDU)

Groups	Sperm count ($\times 10^6$/ml)	Sperm motility (%)	Sperms liveability (%)
CTR	269.33 ± 7.51[a]	82.67 ± 3.18[a]	84.33 ± 2.91[b]
RJ	270.67 ± 6.17[a]	83.33 ± 2.33[a]	87.67 ± 2.33[a]
HDU	184.00 ± 8.19[c]	64.67 ± 2.33[c]	70.00 ± 1.73[d]
2HDU	114.00 ± 9.45[d]	52.33 ± 1.86[d]	54.67 ± 2.60[e]
RJ + HDU	226.67 ± 4.81[b]	68.00 ± 3.06[b]	76.33 ± 7.51[c]
RJ + 2HDU	181.33 ± 7.69[c]	60.67 ± 1.20[c]	65.33 ± 2.73[d]

All values are expressed as mean ± standard error. Means within the same column of different superscript letters are significantly different at ($p < 0.05$)

significantly enhanced ($p < 0.05$) antioxidant status of testicular tissues—GSH concentration and CAT activity were significantly increased ($p < 0.05$) and the MDA level recorded a significant decline ($p < 0.05$) in testicular tissues compared with control.

Testicular inflammatory cytokines level

The level of pro-inflammatory cytokine IL-6 in testicular homogenates of HDU-treated rats recorded a significant increment ($p < 0.05$), but the magnitude of this increase was higher in 2HDU-treated animals as compared with controls. Combined treatment with RJ significantly decreased ($p < 0.05$) the testicular level of IL-6 when compared with HDU-treated rats. Also, the treatment with HDU significantly increased ($p < 0.05$) the level of IFN-γ nearly by the same extent; administration of RJ with HDU significantly augmented ($p < 0.05$) the increase in the testicular IFN-γ level compared with HDU-treated groups. The sole treatment with RJ did not seem to have any effect on the levels of these pro-inflammatory cytokines comparatively with control values (Table 3).

Histological findings

Testis Histopathological examinations of the testicles of control and RJ-received rats had normal histoarchitecture, dis-

playing complete spermatogenesis in well-organized seminiferous tubules and interstitium connective tissue (Fig. 1a). The seminiferous tubules had stratified germinal epithelium of spermatogenic and Sertoli cells, and their lumina were loaded with matured spermatozoa. The primary spermatocytes were markedly observed with their prominent nuclei having dark colored–stained chromatin material; meanwhile, subsidiary spermatocytes were rarely observed on the grounds. Spermatids in both round and elongated stages were observed. The testicles of HDU-treated rats showed that the lumina of some seminiferous tubules contained sloughed germinal epithelial (Fig. 1b), with depletion of germinal cells and coagulative necrosis with hyalinized luminal contents (Fig. 1c) and giant cell formations (Fig. 1d). On the other hand, interstitium exhibited edema that appeared as faint eosinophilic material and congestion of the interstitial blood vessels (Fig. 1e). In HDU + RJ rats, the presence of spermatids and spermatozoa in most of the seminiferous tubules declared amelioration of spermatogenesis (Fig. 1f). Histopathological alterations of 2HDU-treated rats composed of disorganized, shrunken seminiferous tubules with buckled and irregular basement membrane (Fig. 2a) besides clear arresting of spermatogenesis. Majorities of seminiferous tubules were lined with single or double cell layers, which are devoid of spermatids and spermatozoa (Fig. 2b) with giant cells (Fig. 2c). Some seminiferous tubules exhibit coagulative necrosis with hyalinization of the luminal contents. Additionally, there was

Table 3 Potential effects of royal jelly (RJ, 100 mg kg^{-1} bw day^{-1}) on testicular level of MDA, GSH, CAT, IL-6, and INF-γ in male Wistar albino rats administered therapeutic (HDU, 225 mg kg^{-1} bw day^{-1}) and double therapeutic (2HDU, 450 mg kg^{-1} bw day^{-1}) doses of hydroxyurea (HDU)

Groups	MDA (μmol/mg protein)	GSH (mmol/mg protein)	CAT (IU/mg protein)	IL-6 (pg/ml)	IFN-γ (pg/ml)
CTR	14.00 ± 2.08[c]	0.38 ± 0.04[b]	0.42 ± 0.05[b]	82.83 ± 7.70[e]	40.60 ± 5.72[c]
RJ	7.17 ± 1.01[e]	0.43 ± 0.03[a]	0.48 ± 0.01[a]	73.27 ± 9.32[e]	42.67 ± 4.81[c]
HDU	42.67 ± 2.91[b]	0.23 ± 0.02[d]	0.27 ± 0.03[d]	423.00 ± 21.28[c]	75.00 ± 4.04[b]
2HDU	74.33 ± 5.24[a]	0.15 ± 0.04[e]	0.13 ± 0.02[e]	818.33 ± 22.05[a]	77.67 ± 6.96[b]
RJ + HDU	18.67 ± 2.33[d]	0.29 ± 0.05[c]	0.34 ± 0.03[c]	253.67 ± 16.90[d]	150.00 ± 8.66[a]
RJ + 2HDU	38.33 ± 2.91[b]	0.22 ± 0.01[d]	0.21 ± 0.04[d]	670.67 ± 63.94[b]	145.33 ± 6.49[a]

All values are expressed as mean ± standard error. Means within the same column of different superscript letters are significantly different at ($p < 0.05$). *MDA*, malondialdehyde; *GSH*, glutathione; *CAT*, catalase; *IL-6*, interlukin-6; *IFN-γ*, interferon-gamma

Fig. 1 Photomicrograph of rat testes stained with HE × 400, scale bar = 50 μm). **a** Normal testes histoarchitecture of control rats. **b**, **c**, **d**, **e** Therapeutic HDU rats showing sloughing of the germinal epithelium in the lumen of seminiferous tubules (arrowheads), depletion of germinal cells and hyalinization of the luminal contents (stars) with single or double cell layers (arrows) beside giant cell formations in the lumen of seminiferous tubules (red arrowhead) and interstitial edema which represented by faint eosinophilic albuminous material (asterisks). **f** Therapeutic HDU + RJ rats showing normal histoarchitecture of seminiferous tubules
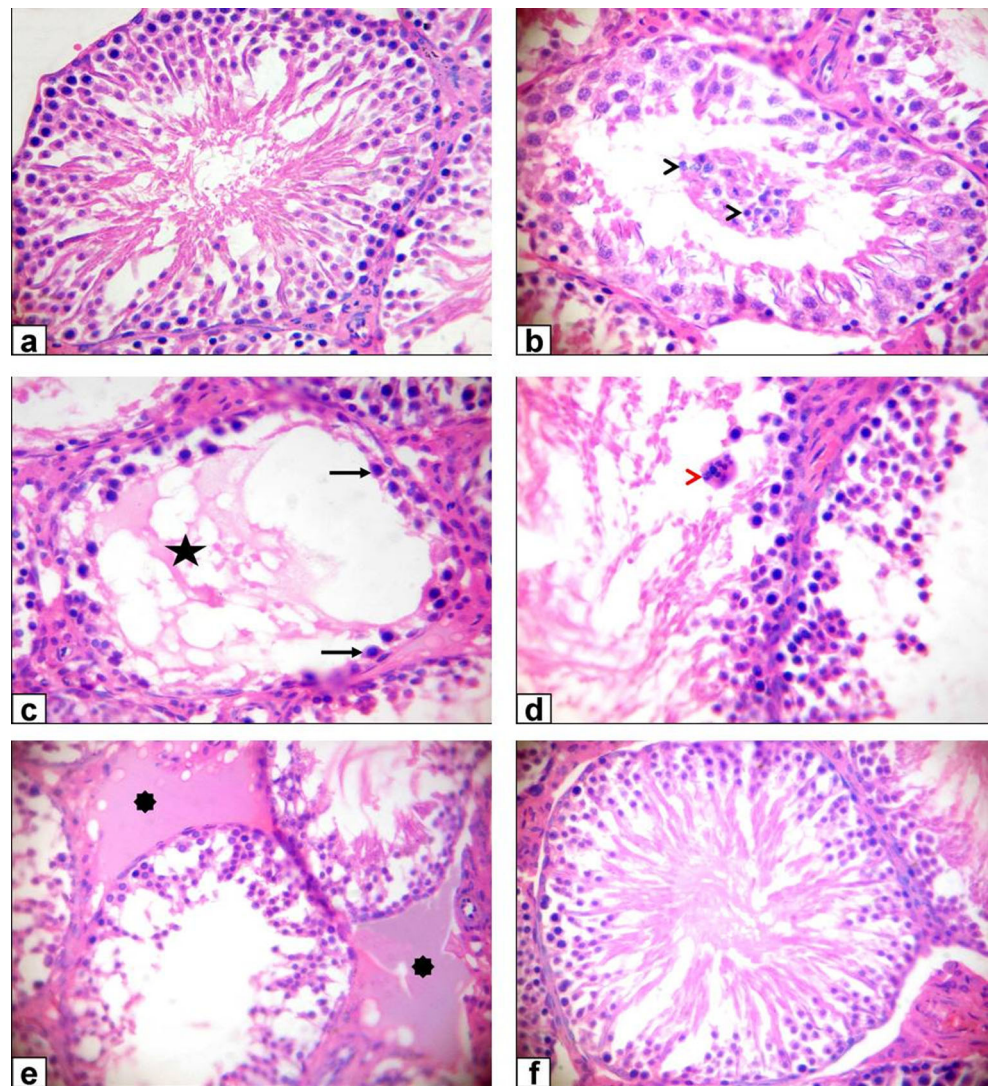

an obvious thickening of interstitium by congestion of the blood vessels, fibroplasia, hyperplasia of Leydig cells (Fig. 2d), and faint eosinophilic albuminous material (Fig. 2e). On the contrary, interstitium of 2HDU + RJ-treated rats showed congestion of the blood vessels and mildly edematous. Some seminiferous tubules contained sloughed necrotic germinal epithelium, while the majority had complete spermatogenesis (Fig. 2f).

Epididymis Epididymal sections of the control and RJ-treated rats showed a normal histoarchitecture with normal sperm density (Fig. 3a). Epididymis of HDU rats exhibited sloughed germ cells in some cauda epididymal ductules (Fig. 3b). In HDU + RJ-treated rats, cleared improvement in sperm density and epididymal picture (Fig. 3c) was noticed. Epididymis of 2HDU-treated rats showed sloughing of lining epithelial cells, vacuolation of few germinal epithelial cells (Fig. 3d), and some of the epididymal ductules had no or low numbers of spermatozoa in their lumina beside mild mononuclear cell

infiltration in interstitial connective tissue (Fig. 3e). Also, 2HDU + RJ rat's epididymis showed a normal histoarchitecture with normal sperm density in the majority of epididymal ductules (Fig. 3f).

Prostate gland The prostate gland sections of the control and RJ-treated rats had no obvious histopathological alterations (Fig. 4a). Interstitial congestion of blood vessel and moderate leukocyte infiltration including neutrophils, lymphocytes, and plasma cells besides no luminal secretions in most of the glandular acini (Fig. 4b) were the commonly observed lesions in HDU rats while co-treatment with RJ showed only interstitial congestion of blood vessel and moderate glandular secretions (Fig. 4c). The histoarchitecture of prostatitis was obvious in 2HDU-treated rats, which composed of interstitial congestion and severe leukocyte infiltration besides no glandular secretion in most of the acini (Fig. 4d and e) while co-treatment with RJ showed mild leukocyte infiltration with an adequate quantity of glandular secretions (Fig. 4f).

Fig. 2 Photomicrograph of rat testes stained with HE (× 200, scale bar = 100 μm). **a**, **b**, **c**, **d**, **e** Double therapeutic HDU rats showing shrunken, buckled, and disorganized seminiferous tubules (black arrowheads), the majority of seminiferous tubules had single or double cell layers (black arrows), giant cell formations in the lumen of seminiferous tubules (red arrowheads), coagulative necrosis of seminiferous tubule, depletion of germinal cells, and hyalinization of the luminal contents (stars) with hyperplasia of interstitial endocrine cells (short black arrows), congestion of the interstitial blood vessels (white arrow), and interstitial edema (asterisks). **f** Double therapeutic HDU + RJ rats showing normal histoarchitecture of seminiferous tubules with presence of spermatids and spermatozoa in their lumen
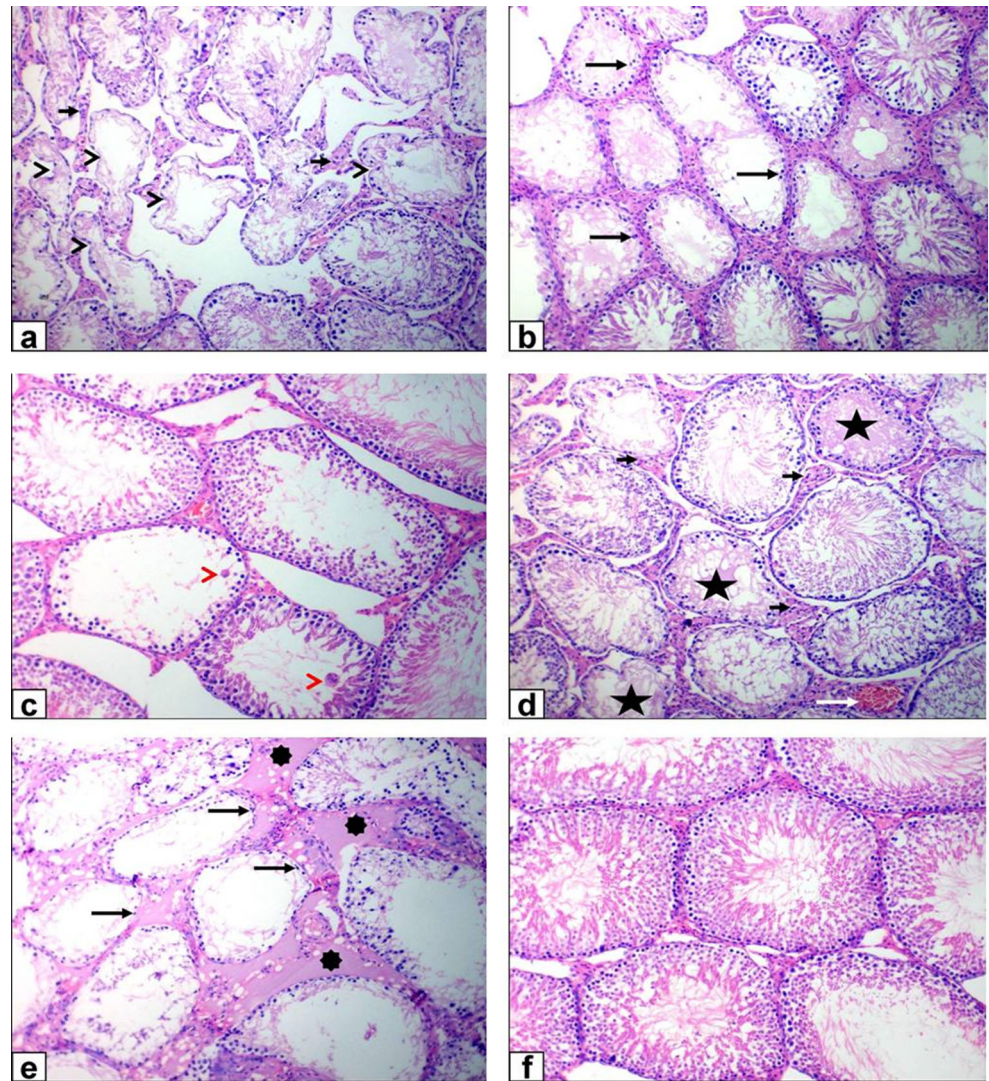
Seminal vesicle The seminal vesicle sections of the control and RJ-treated rats were histologically normal (Fig. 5a). The histoarchitecture of seminal vesiculitis was marked in all HDU-treated rats, with higher severity in 2HDU-treated rats, which represented by interstitial congestion of blood vessel and WBC infiltrations mainly neutrophils, lymphocytes, and plasma cells (Fig. 5b and d). A smaller number of epithelium folds with pyknosis of the epithelium nuclei were noticed. The RJ + HDU rats exhibited normal histological structure (Fig. 5c). Meanwhile, in 2HDU-treated rats, there were mild WBC infiltrations (Fig. 5e) and most of seminal vesicle sections had normal histology that was roughly like the controls (Fig. 5f).

Discussion

Chemotherapy-induced testicular damage is an emerging reason for azoospermia and disrupted hormonal status in males.

HDU is widely used as an effective chemotherapeutic agent to manage cancerous and non-cancerous diseases. It is considered the first-choice antineoplastic medication for sickle cell anemia (Agrawal et al. 2014) and another neoplasm (Liebelt et al. 2007). Nevertheless, its clinical applications were currently restricted due to its declared cytotoxic adverse effects including testicular derangement (Perreault et al. 2008). Herein, HDU treatment induced a significant decline in the blood testosterone level which may be explained based on testicular Leydig cell dysfunction or oxidative damage. Primarily, it has been shown that HDU suppresses testosterone synthesis and release (Jones et al. 2009) through the suppressive effect of nitric oxide, HDU metabolite (Huang et al. 2006) on the testosterone production by Leydig cells (Lamanna et al. 2007). Moreover, HDU is proven to have the capability to generate ROS and free radicals (Cokic et al. 2003), which expressed through an increased MDA (the end product of lipid peroxidation) (Ahmad et al. 2018) with lowered GSH and CAT contents in testicular tissues (El-Maddawy and El-

Fig. 3 Photomicrograph of rat epididymis stained with HE (× 200, scale bar = 100 μm). **a** Normal histological structure with normal sperm density of cauda epididymis of control rats. **b** Therapeutic HDU rats showing some cauda epididymal ductules contained sloughed germ cells (black arrows). **c** Therapeutic HDU + RJ rats showing normal histological structure with normal sperm density of cauda epididymis. **d**, **e** Double therapeutic HDU rats showing sloughed germ cells (black arrows), vacuolation of few lining germinal epithelial cells (black arrowhead), and mild epididymitis with inflammatory cell infiltration (asterisks). **f** Double therapeutic HDU + RJ rats showing normal histological structure with normal sperm density

Sayed 2018; El-Neweshy et al. 2013). Besides HDU, its substrate is proven as metal chelators and inhibitors of CAT activity (Italia et al. 2013; Juul et al. 2010). The significant increase in the serum FSH and LH levels following HDU treatment may be due to a lack of negative feedback mechanism of testosterone on GnRH production that stimulates the pituitary gland to secrete FSH and LH to enhance (Corradi et al. 2016) and thus impairing testosterone production and spermatogenesis process. Co-treatment with RJ significantly ameliorated the hormonal imbalance and this may be attributed to its antioxidant properties that was derived from the short-chain peptides (Guo et al. 2008), phenolic compounds (flavonoids and cinnamic acid derivatives) (Kolayli et al. 2016), some vitamins (A, E, and C), minerals (Fe, Zn, and Cu), and fatty acids (*trans*-10-hydroxy-2-decenoic acid) (Xue et al. 2017) in the rebalancing of oxidant-antioxidant status (Ghanbari et al. 2015; Zahmatkesh et al. 2014), as reflected on the MDA and GSH concentrations and CAT activity in testicular tissues.

Sperms have an abundance of polyunsaturated fatty acids in their membrane and lack enzymatic-linked antioxidant defensive systems; this collectively makes them more sensitive to oxidative damage (Hsu et al. 1998). This could provide an acceptable explanation for decreased sperm motility, count, and liveability in HDU-treated (Grigg 2007), which is primarily mediated through oxidative stress induced by HDU-reactive metabolites, carbamoyl nitroso that involved in ROS generation (Cokic et al. 2003). The reduced number of spermatozoa in HDU-treated rats may be also attributed to the direct effect of HDU on spermatogenesis, which can arrest mitotic division of spermatogonia through inhibition of DNA synthesis (Florensa et al. 2003) or its apoptotic effect upon germ cell (Zhou et al. 2015). It has been described that treatment with HDU may cause a significant reduction in total sperm count (Berthaut et al. 2017; Berthaut et al. 2008), which can reach to azoospermia (Garozzo et al. 2000) and deterioration of sperm motility and morphology (Berthaut et al. 2008; Grigg 2007) and sperm livability (Grigg 2007) in human patients. Treatment of HDU-treated rats with RJ significantly

Fig. 4 Photomicrograph of rat prostate stained with HE (× 200, scale bar = 100 μm). **a** Normal histological structure of control rats' prostate. **b** Therapeutic HDU rats showing interstitial congestion of blood vessel (black arrow) and moderate leukocytes infiltration (asterisks). **c** Therapeutic HDU plus royal jelly rats showing roughly equal normal histological structure with mild interstitial congestion of blood vessel (black arrow). **d**, **e** Double therapeutic HDU rats showing interstitial congestion of blood vessel (black arrow) and severe leukocytes infiltration (asterisks) **f** double therapeutic HDU plus royal jelly rats showing mild interstitial leukocytes infiltration (asterisks)
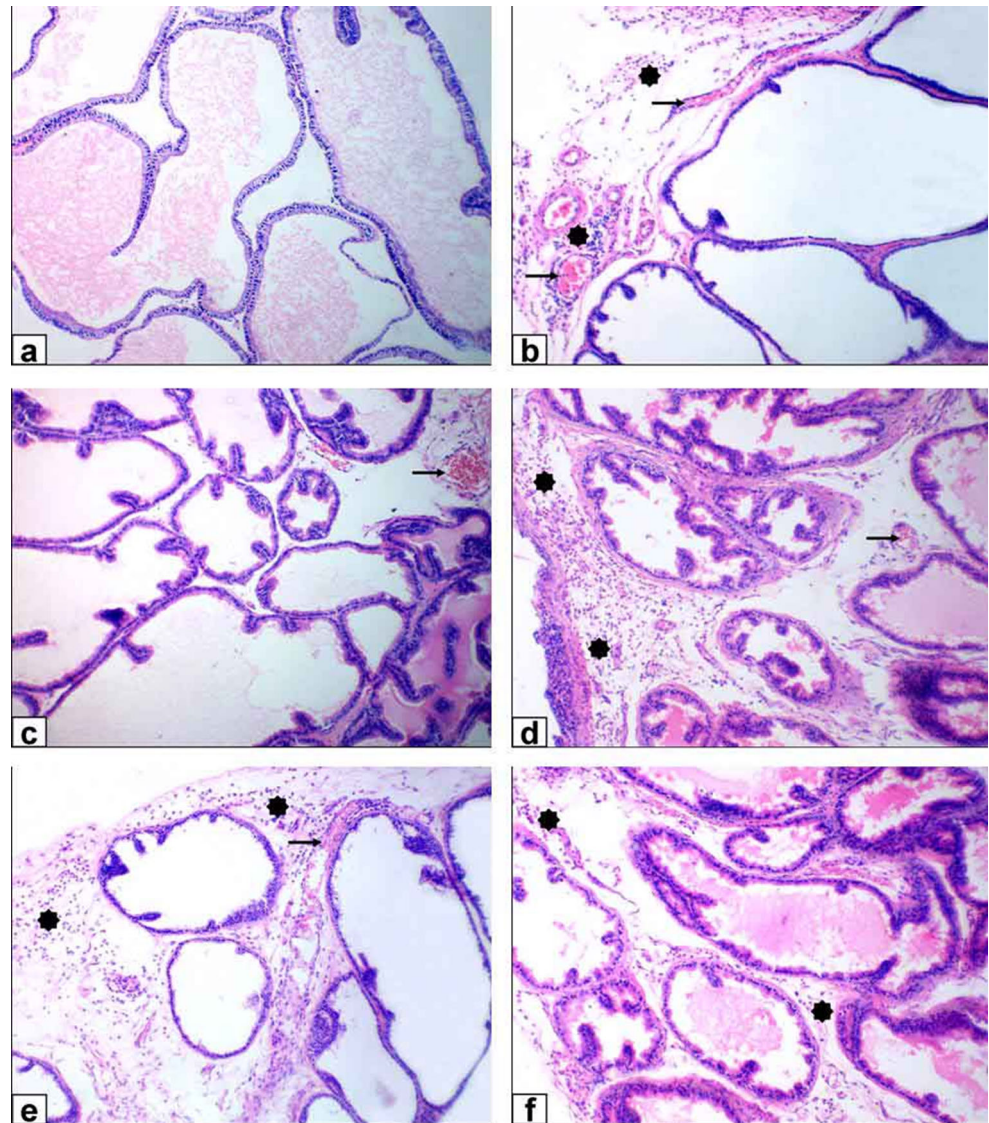
improved sperm motility, count, and liveability, which might be mediated through its reputable antioxidant activity that remarkably protects sperms against oxidative damage (Ghanbari et al. 2015; Shalizar Jalali et al. 2015). Testicular inflammatory state related to oxidative stress in HDU-treated rats was clearly highlighted by the increment in pro-inflammatory cytokine IL-6, the major inflammatory mediator (Hodge et al. 2005) and IFN-γ, and has several anti-inflammatory properties that modify and control the degree of inflammatory conditions (Muhl and Pfeilschifter 2003). This inflammatory state was approximately alleviated upon administration of RJ, indicating that it has anti-inflammatory properties and an inhibitory effect on the release of pro-inflammatory cytokines (Pasupuleti et al. 2017), which might be related to its component of *trans*-10-hydroxy-2-decenoic acid, 10-hydroxydecanoic acid, and sebacic acid (Chen et al. 2016).

The HDU induced degenerated, irregular, buckled, thick, and hyalinized basement membrane of seminiferous tubules. Vacuolization of the germinal and Sertoli cells may be attributed to smooth endoplasmic reticulum dilatation that possibly signifies cellular permeability changes (Creasy and Chapin 2013). Sertoli cells which provide an abundance of factors required for spermatogenesis are the supportive cells within the seminiferous tubule (O'Donnell et al. 2011). Consequently, the detachment of germinal cells was induced as a result of severe Sertoli cell impairment due to microtubule morbidness (Kumar et al. 2006) in seminiferous tubules' lumina. The incomplete spermatogenesis with many seminiferous tubules having single or double cell layers may be referred to reduce testosterone levels upon HDU treatment, which is critical for the attachment of various generations of germ cells. Thus, a low level of intra-testicular testosterone may initiate sloughing of germ cells from seminiferous epithelium and may induce cell apoptosis and thereby male infertility (Blanco-Rodriguez and Martinez-Garcia 1998). The marked thickening of the

Fig. 5 Photomicrograph of rat seminal vesicles stained with HE (× 200, scale bar = 100 μm). **a** Normal histological structure of control rats seminal vesicles. **b** Therapeutic HDU rats showing interstitial congestion of blood vessel (black arrows) and moderate leukocytes infiltration (asterisks). **c** Therapeutic HDU + RJ rats showing normal histological structure. **d** Double therapeutic HDU rats showing severe interstitial congestion of blood vessel (black arrows) and severe leukocytes infiltration (asterisks). **e**, **f** Double therapeutic HDU + RJ rats showing mild interstitial congestion of blood vessel (black arrows), mild interstitial leukocytes infiltration (asterisk), and normal histoarchitecture
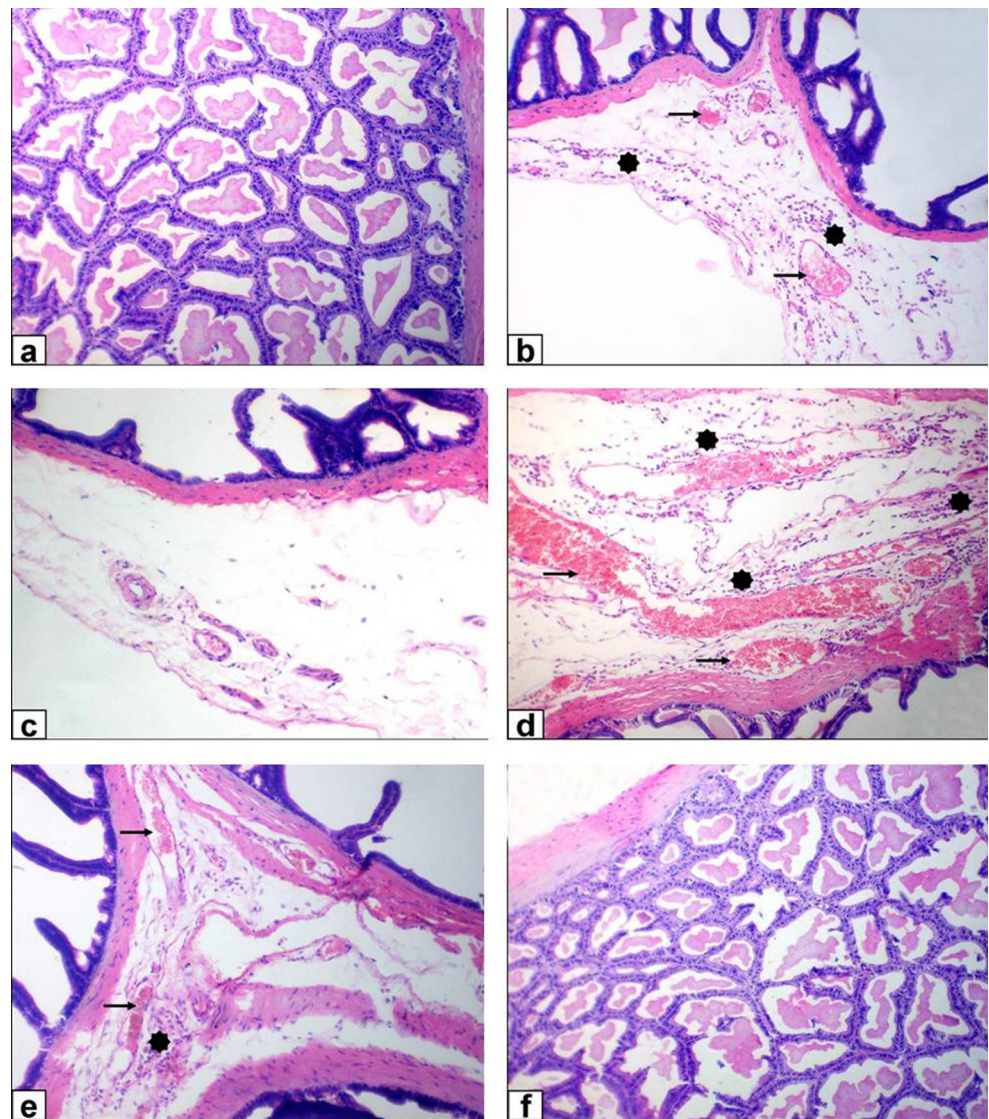
interstitium was seen particularly in 2HDU because HDU may cause androgen receptor (AR) antagonist. Accordingly, there is generally congestion of the interstitial blood vessels, edema, fibroplasia, and Leydig cell hyperplasia. Increased LH secretion from the anterior pituitary stimulates the Leydig cells, increasing testosterone production to maintain androgenic homeostasis (Jones et al. 2009; O'Connor et al. 2002), which are essential for differentiation, development, and maintenance of epithelial cell of prostate glands and seminal vesicles (Ono et al. 2004). The epididymal histoarchitecture reflected the arrest of spermatogenesis, where there is no or low number of spermatozoa in most of the epididymal ducts. Degenerating detached germ cells in the lumina of the epididymal tubule signifies testicular dysfunction (El-Neweshy et al. 2013; Roy et al. 2001). Our result reveals HDU treatment decreased the serum testosterone level leading to hyposecretion of both glands beside prostatitis and seminal vesiculitis. Co-treatment with RJ ameliorated the pathological changes of testicles, epididymis, and accessory sex glands, which might be mediated through scavenging generated ROS and protecting against inflammatory reactions.

Conclusion

Taken together, these findings provide additional information that royal jelly (RJ) prevented testicular injury, oxidative stress, and inflammation associated with HDU antineoplastic medication. Hence, RJ can be used as an adjuvant remedy to improve HDU-induced toxicity and male subfertility.

Compliance with ethical standards

Competing interests The authors declare that there are no conflicts of interest.

Abbreviations AR, androgen receptor; CAT, catalase; CTR, control; GSH, reduced glutathione; HDU, hydroxyurea; HE, hematoxylin and eosin; IL-6, interleukin-6; IFN-γ, interferon-γ; FSH, follicular stimulating hormone; LH, luteinizing hormone; MDA, malondialdehyde; PBS, phosphate buffer saline; RJ, Royal jelly; ROS, reactive oxygen species; SEM, standard error of mean

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