Original Article

The Prophylactic Effect of Royal Jelly in Amelioration of 6-Mercaptopurine - Induced Testicular Damage in Albino Rats (Histological and Immunohistochemical study)

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ABSTRACT

Background: Testicular toxicity is a well-documented adverse impact of a large number of chemotherapeutic drugs. 6-mercaptopurine (6MP) is an anti-cancer agent with strong anti-tumor activity. Despite this, it has a detrimental impact on the male reproductive system. Royal jelly (RJ) is a nutrient-dense substance that may also include antibacterial, antioxidant, and anti-inflammatory characteristics. These qualities may account for the majority of royal jelly's health claims. This study aimed to investigate the protective effect of RJ on 6MP-induced testicular damage in adult albino rats.

Materials and Methods: Forty male adult albino rats were categorized into four equivalent groups (n= 10): control, RJ group (200 mg/kg RJ for 30 days), 6MP group (5 mg/kg 6MP for 20 days), and RJ+6MP group (200 mg/kg RJ for 10 days alone then concomitant with 5 mg/kg 6MP for 20 days). Histological and immunohistochemical examinations of the testes were performed.

Results: The histological results revealed that oral administration of 6MP triggers a substantial reduction in testis weights, count, sperm motility %, and viability. 6MP also caused severe testicular degeneration with weak immunoreactivity to Bcl-2and N-Cadherin. RJ attenuated testicular degeneration and upregulated the expression of Bcl-2 and N-Cadherin.

Conclusion: RJ reduced all testicular changes induced by 6MP. Thus, RJ may be a viable medication for infertility problems associated with testicular injuries.

Key Words: 6MP, Bcl-2, N-Cadherin, Royal Jelly, testis.

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INTRODUCTION

6-Mercaptopurine (6MP) was the first purine analog to show promise in the treatment of neoplastic disorders[1]. It is used in cancer therapy to keep acute lymphoblastic leukemia in remission[2]. Inflammatory bowel disorder, organ transplant failure, systemic lupus erythematosus, and rheumatoid arthritis are all treated with 6MP[3].

6MP has many adverse impacts on various organs involving the liver[4], bone marrow and reproductive organs[5]. Atrophy of the testes, failure of the Leydig cells, and impairment of spermatogenesis are all signs of 6MP-induced male reproductive organ injury[6]. Testicular inflammation induces damage to the seminiferous epithelium and promotes spermatogenic cell death, making spermatogenesis extremely vulnerable[7].

Natural products are being promoted as a way to save the body from the side impacts of various chemotherapy medications in recent trends in disease treatment[8]. Royal Jelly (RJ) is made of water (50–60%), proteins (18%), lipids (3–6%), mineral salts (1.5%), vitamins, and carbohydrates (15%), and is produced by the hypopharyngeal glands of worker honeybees. Elements of RJ are required for cell development and tissue repair[9]. When administered as an adjuvant to certain medicines, RJ has been shown to be a strong anti-inflammatory and antioxidant[10,11]. RJ enhanced spermatogenesis and mitigated several drugs' negative impacts on the reproductive organs[12,13].

The blood-testis barrier (BTB) is a physical barrier present in the testis of mammals that blocks the diffusion of many exogenous and endogenous toxic substances. It serves as a structural barrier between the interior of the seminiferous tubules and the testicular fenestrated capillaries[14]. Exogenous substances have the potential to damage Sertoli cell intercellular connections, disintegrate and destroy BTB's structure and function, almost definitely causing reproductive harm[15]. Claudin, cadherin, and catenin are all related proteins that may play critical roles in the control of the BTB during spermatogenesis[16]. The BTB will change if certain protein expressions and (or) assembly are altered, and toxins will enter the seminiferous epithelium more readily[17].

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In addition, Royal jelly can modulate apoptosis via Bcl-2 expression regulation, which is found in cellular or animal models of many diseases[18, 19, 20]. The mechanism by which RJ protects versus 6MP-induced testicular damage is unknown. Based on the aforementioned, this study aims to investigate the possible protective effect of RJ on 6MP induced testicular damage in male albino rats, as well as the mechanistic pathway of protection by assessing histological and immunohistochemical changes in the testis.

MATERIALS AND METHODS

Drugs and Chemicals:

1- 6-mercaptopurine (5gm packet) was obtained from Sigma–Aldrich Company in the United States of America. The required dose is 5 mg/kg[21]. The powdered 6MP was weighed using a digital scale, dissolved in physiological saline (0.9 percent NaCl) and given in a volume of 1ml/ dose via oral gavage daily for twenty days for each animal.

2- Royal jelly was provided by GlaxoSmithKline, based in Egypt. The required dose is 200 mg/kg[22]. We opened 1 g of RJ capsules and dissolved them in physiological saline (0.9 percent NaCl) and given in a volume of 1ml/dose via oral gavages daily for 30 days for each animal.

Experimental animals

Forty adult male albino rats, 8 weeks old and weighing a mean of 180-200 g, were acclimated two weeks before the start of the experiment in the animal house of the Faculty of Science, Beni-Suef University. The rats were housed under typical laboratory conditions, which included a 12 hour light/dark cycle and a constant temperature of 22-24°C. Each group was housed in a separate cage with free access to food and was fed a conventional commercial pellet diet. All procedures involving the management of agents, blood sampling, and tissue collection followed the National Institutes of Health's (NIH) guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978), as well as the eligibility requirements established by the investigations and Ethics Committee of the Community Laws governing the use of experimental animals at Beni-Suef University.

Experimental design:

Rats (n = 10) were randomly allocated to one of four groups following adaption. As illustrated in Figure 1, the groups were as follows:

- **Group I (Control group):** rats were given saline (0.9 percent NaCl) orally via gastric gavage tube for 30 days.
- **Group II (RJ group):** rats were given RJ (200 mg/kg /day) in the volume of 1ml/dose via oral gavages for 30 days[22].
- **Group III (6MP group)**: rats were given saline (0.9 percent NaCl) for ten consecutive days followed by 6MP (5 mg/kg) in the volume of 1ml/dose via oral gavages daily for twenty days[21].
- **Group IV (RJ + 6MP)**: Rats were given RJ (200 mg/kg B.W.) in the volume of 1 ml/dose via oral gavage daily for 30 days, On the tenth day, rats were administered 6MP (5 mg/kg) in the volume of 1 ml/dose via oral gavages for 20 days[21, 22]. To ensure the correctness of the findings, all measurable parameters were examined blindly.

Tissue collection:

On the last day of the experiment, the animals were sacrificed under deep anesthesia by intraperitoneal injection of phenobarbitone (60 mg/kg). The testes were removed and weighed. For histological and immunohistochemical analysis, they were promptly fixed in Bouin's fluid.

Weighting testes and Semen evaluation:

The testes were removed and weighted separately after scarifying the rats. Additionally, the testes were submerged in 5 mL of normal saline and their volumes were measured. The supernatant was then mixed in a proportionate (1:100) fashion to a solution comprising 1 ml formalin (35%) in 100 ml water, 25 mg Eosin, and 5 gm $NaHCO_3$[23]. To count sperms, ten milliliters of the created mixture was transferred to the counting chamber of a hemocytometer (Sanli, China Mainland) coupled with a light microscope, where it was necessary to remain for five minutes before counting sperms at a magnification of forty times. To determine sperm viability, equal parts of sperm solution and Eosin-Nigrosin stain were combined, a thin layer of the mixture was spread on a clean slide, and the specimen was examined under a light microscope for the existence of dead (pink) and living (unstained) sperms. The ratio of live sperms in the specimen was then determined[24]. Sperm motility was determined by adding semen solution to Tris-buffer and counting motile sperms at a multiplicative factor of 40x of the total sperm count. Additionally, abnormal forms were assessed and quantified at a magnification of 40 x[25].

Histopathological examination:

Testes were preserved in Bouin's fluid and processed for paraffin sections of 5 µm and the following studies were carried out:

A) Histological Study: Hematoxylin and eosin (H&E)[26].

C) Immunohistochemical Study:

Testis tissue specimens were routinely evaluated for deparaffinization, hydration, antigen recovery, and phosphate-buffered saline washing (PBS). After blocking with normal goat serum, Bcl-2 rabbit polyclonal antibodies (anti-Bcl-2 antibody, Cat #PA1-30411, Thermo Scientific, USA), and N-cadherin mouse monoclonal antibodies (anti-N-cadherin antibody, Cat #33-3900, Thermo Scientific, USA) was administered. The slices were rewarmed at 37°C for 45 minutes after overnight incubation at 4°C. Following that, three times in PBS for five minutes each, the parts were washed. The tissue was treated for 30 minutes at 37°C with a secondary goat anti-mouse antibody coupled to horseradish peroxidase. 3,3′-diaminobenzidine was generated for 5 minutes. The tissue was rinsed in PBS for 5 minutes, counterstained for 2 minutes with hematoxylin, and then differentiated with hydrochloric acid and ethanol. The tissue was rinsed in PBS for ten minutes before cover-slipping, dehydrated, and cleaned again. The slices were incubated at 180°C for 15 minutes in a sodium citrate solution (H 3300, pH 6.0). The slides were cleaned twice with distilled water after 20 minutes at room temperature. The histological changes were then analyzed using a light microscope. Brown particles in the cytoplasm were identified as immunopositive cells[27, 28].

Morphometric study:

The data were collected in the Department of Histology at Kasr Al-Aini, Cairo University, using a computer system equipped with a "Leica Qwin-C 500" image analyzer (England). We used a video camera to take real-time photos of sections viewed under a light microscope (Olympus BX-40, Olympus Optical Co. Ltd., Japan) (Panasonic color CCTV camera, Matsushita Communication Industrial Co. Ltd., Japan). Digitization of the video images was accomplished through the use of an IBM PC hard drive attached to the microscope and managed by the "Leica Qwin-C 500" software. On each slide for each group, ten non-overlapping fields were evaluated to estimate the mean area ratio of the Bcl-2 antibody and N-cadherin immunopositivity.

Statistical Analysis:

Data were collated and statistical analysis was conducted utilizing the Statistical Package for Social Science (SPSS) version 17 software to assess group differences. The mean and standard deviation were used as descriptive statistics. The one-way analysis of variance (one-way ANOVA) was utilized to contrast the means of each group to the means of the other groups, accompanied by the post hoc "Tukey" test. A significant variation is one with a P value less than 0.05[29].

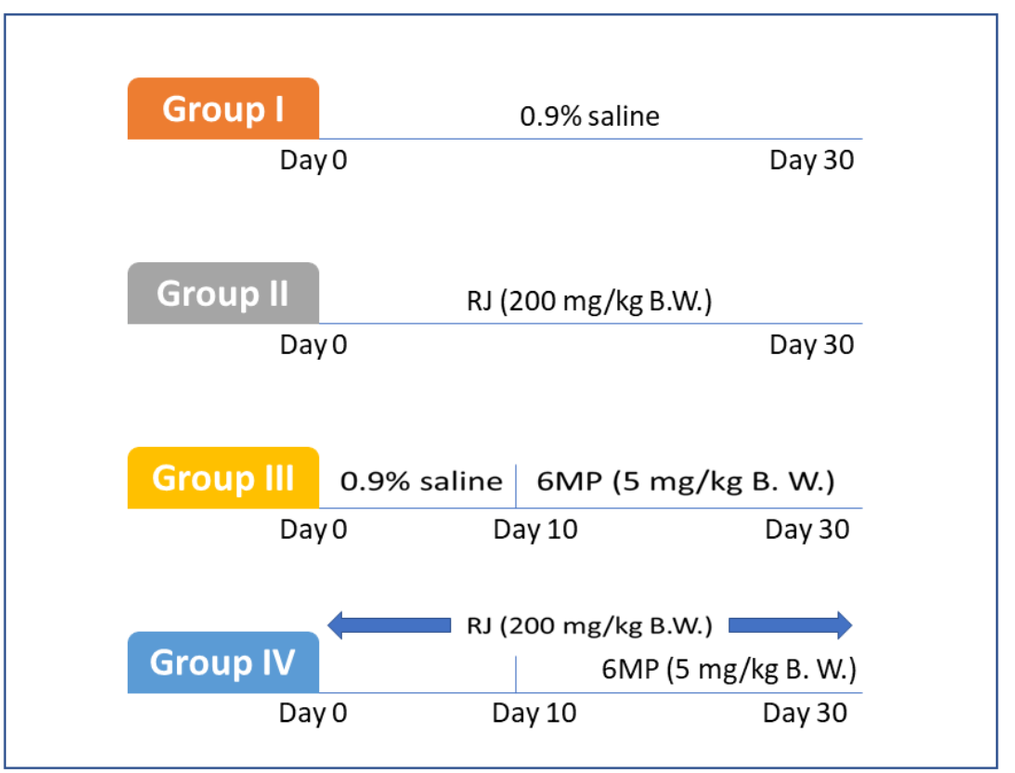


Fig. 1: Experimental Design outlining the 6-Mercaptopurine (6MP)-induced testicular damage model and Royal Jelly (RJ) treatment protocol. In the control group (n=10); rats received saline orally by gastric gavage tube for 30 days. RJ group (n=10); rats received RJ (200 mg/kg B.W.) for 30 days. 6MP group (n=10); rats received saline (0.9% NaCl) for 10 successive days followed by 6MP (5 mg/kg B. W.) daily for 20 days. RJ + 6MP group (n=10): Rats received RJ (200 mg/kg B.W.) daily for 30 days, at the 10th-day rats were administered 6MP (5 mg/kg B. W.) for 20 days. All measured parameters were blindly examined to confirm the accuracy of the results.

RESULTS

No deaths were observed in all rats.

Testicular weights

RJ achieves the same safety level as the control group with no statistically substantial differences from the control group. We studied the impacts of 6MP on the testicular weight in rats whether used alone or in conjunction with RJ pretreatment. At *P-value <0.05*, As demonstrated in Table 1, a significant decline in testis weights was observed in the 6MP treated group against the control group. Previous administration of RJ drastically decreased this drop in the RJ+6MP group contrasted to the 6MP group at *P-value <0.05*.

Sperm parameters:

6MP treatment significantly reduced sperm motility %, sperm count, and sperm viability % when compared to the control group. As indicated in (Table 2), 6MP led to a dramatic elevation in the percent of sperm abnormalities as compared to the control group, (*P<0.05*). When compared to the 6MP group, RJ significantly increased sperm count, sperm motility, and sperm viability, while concurrently decreasing the percentage of sperm abnormalities. At *P-value< 0.05*, sperm testing showed no substantial difference between the RJ and control groups.

Histological results (Hematoxylin and eosin):

Histological examinations of rat testis from the control group (group I) displayed the normal histological structure of testis with densely packed normal seminiferous tubules. The seminiferous tubules were lined by Sertoli cells and spermatogenic cells associated with interstitial Leydig cells (Fig.2 a, b). Testicular tissue from the RJ group (group II) had more or less typical seminiferous tubules with full spermatogenesis (Fig.2c). The 6MP group (group III) was found to have severe testicular deterioration presented by the loss of normal testicular architecture with irregular seminiferous tubules, exfoliated cells, and absence of sperms in the lumen. The interstitial areas were congested and filled with hemorrhagic exudate. Moreover, separation of basal and adluminal cellular compartments with vacuolated cytoplasm of spermatogonia were observed (Fig. 2d, e, f, g). Histologically, the testicular tissue of the RJ+6MP group (group IV) improved to a degree similar to that of the control group except for congestion of interstitial blood vessels and hemorrhagic interstitial exudation (Fig. 2h).

Immunohistochemical results:

Immunohistochemical results for Bcl-2: (Fig.3)

Testis of the control group (Group I), RJ group (Group II) and RJ+6MP group (Group IV) displayed strong positive Bcl2 immunoreaction in the cytoplasm of germinal cells (Fig.3 a, b, d). In contrast, Testis of the 6MP group (Group III) showed a weak positive Bcl2 reaction (Fig.3c). Bcl-2 expression was substantially lower in Group III than in Group I and significantly greater in Group IV than in Group III (*P <0.05*).

Immunohistochemical results for N-cadherin: (Fig.4)

Testis of the control group (Group I), RJ group (Group II) and RJ+6MP group (Group IV) have strong positive N-cadherin immunoreaction in the germinal cells (Fig.4a, b, d). In contrast, Testis of the 6MP group (Group III) showed a weak positive N-cadherin reaction (Fig.4c). Group III had considerably lower N-cadherin expression than Group I, while Group IV had significantly higher N-cadherin expression than Group III (*P <0.05*).

Morphometric results:

When compared to the control group (group I), the mean area percent of Bcl-2 immunopositive cells in the 6MP group (group III) decreased significantly (*P<0.05*). When compared to group III, the mean area percent of Bcl-2 immunopositive cells in the RJ+6MP group (group IV) increased significantly (*P<0.05*). Furthermore, there was no significant difference between the RJ group (group II) and the control group (group I) in the mean area percent of Bcl-2 immunopositive cells (Histogram 1) (Table 3).

When compared to the control group (group I), the mean area percent of N-cadherin immunopositive cells in the 6MP group (group III) decreased significantly (*P<0.05*). When compared to group III, the mean area percent of N-cadherin immunopositive cells in the RJ+6MP group (group IV) increased significantly (*P<0.05*). Furthermore, there was no significant difference between the RJ group (group II) and the control group (group I) in the mean area percent of N-cadherin immunopositive cells (Histogram 2) (Table 3).

Table 1: The weights and volumes of testes of different treated groups.

Groups	Testicular Weight (g)	Testicular Volume (cm3)
Control group	5.2±0.1	4.76 ± 0.01
RJ group	4.9±0.01	4.5±0.06
6MP group	2.01±0.04[a]	2.12± 0.02[a]
RJ+6MP group	4.2±0.01[b]	4.3±0.02[b]

The results were expressed as means ± SE n = 10. The data were analyzed by using one way ANOVA test. a: indicates a significant difference compared to control at level *p<0.05*. b: indicates a significant difference compared to 6MP at *P<0.05*.

Table 2: The changes in sperm motility, viability, abnormality percentage and sperm count in all examined rats

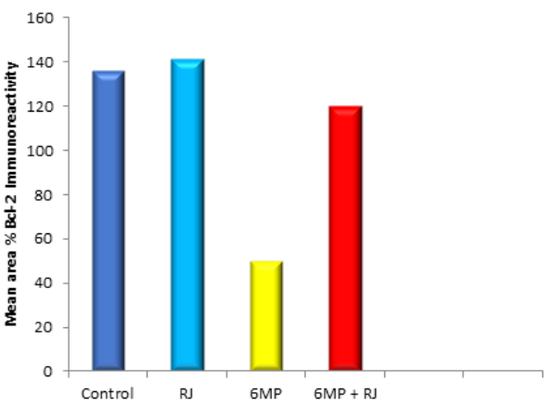
Groups	Motility %	Sperm cell count. (X 10^6)	Viability (%)	Abnormalities (%)
Control group	81.6±1.02	30±2	90.2±1.09	15.9±0.9
RJ group	77.8±1.01	31±0.1	88.2±0.1	16.3±0.3
6MP group	40.2 ± 0.5[a]	15.9±0.4[a]	50.5± 1[a]	42.1± 0.5[a]
RJ+6MP group	79.9±1[b]	29.32±1[b]	88.12±1.2[b]	16.5± 1[b]

The results were analyzed by using one way ANOVA test and expressed as means ± SE, n = 10. The data. a: indicates a significant difference compared to control at level $p<0.05$. b: indicates a significant difference compared to 6MP at $P<0.05$.

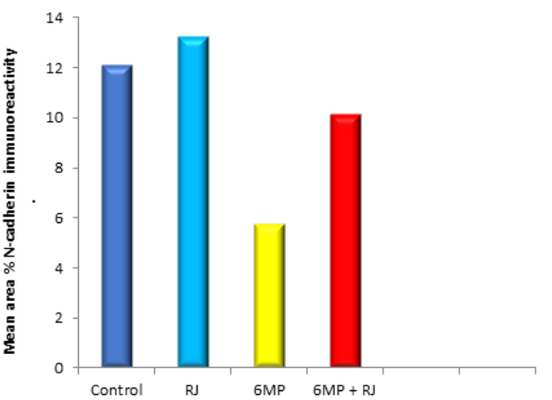
Table 3: The area percent of immunoreactivity of Bcl-2 and N-Cadherin in all groups

Groups	Bcl2 area %	N-Cadherin area %
Control group	136 ± 16	12.115 ± 0.528
RJ group	141 ± 8	13. 232 ± 0.382
6MP group	50 ± 5.5[a]	5.762 ± 0.478[a]
RJ+6MP group	120 ± 18[b]	10.120 ± 0.349[b]

The results were analyzed by using one way ANOVA test and expressed as means ± SE, n = 10. The data. a: indicates a significant difference compared to control at level $p<0.05$. b: indicates a significant difference compared to 6MP at $P<0.05$.



Histogram 1: Mean area % of Bcl-2 immunoreactivity



Histogram 2: Mean area % of N-cadherin immunoreactivity

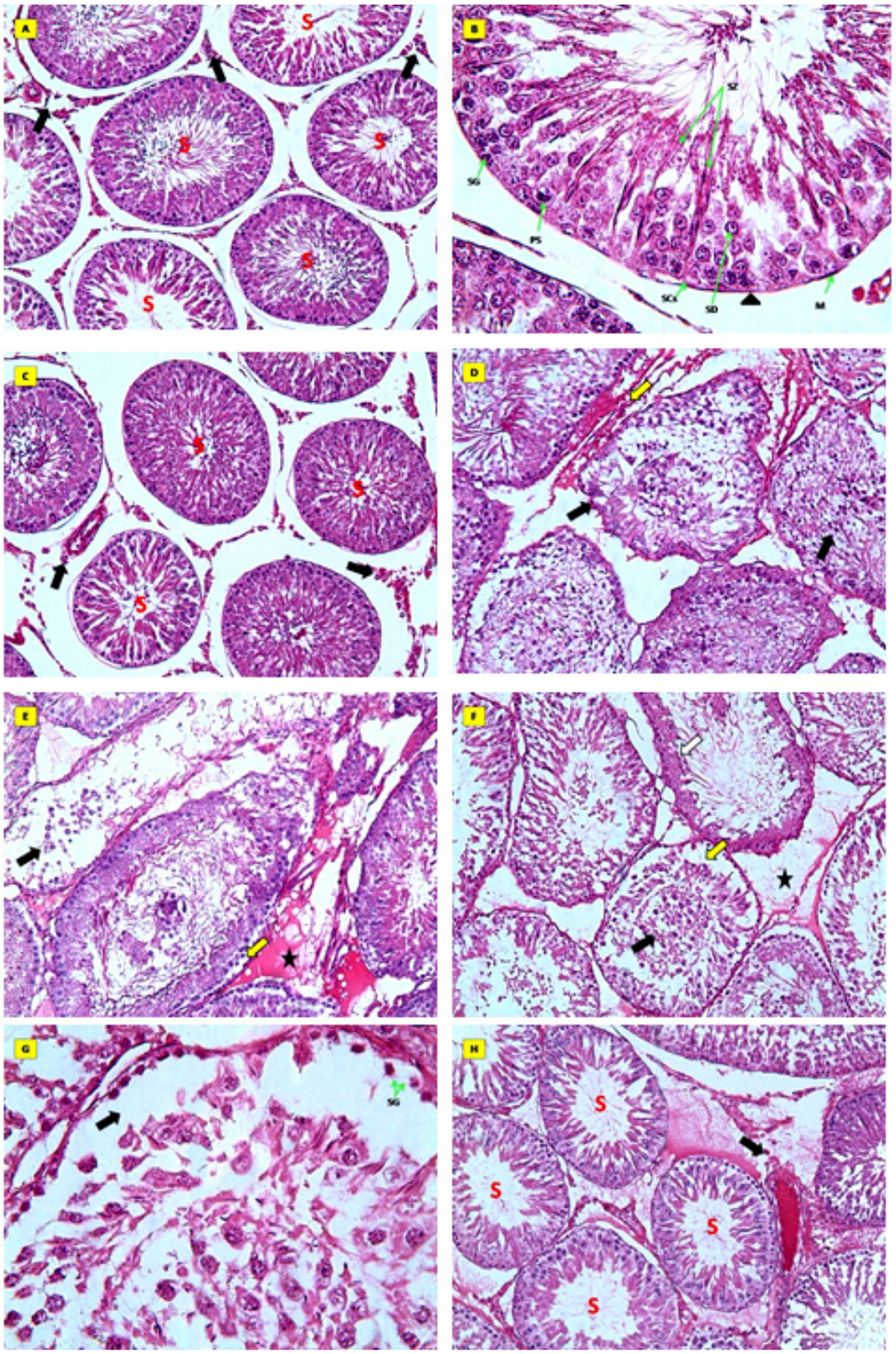


Fig. 2: Histological structure of rat testis as assessed in paraffin sections. Hematoxylin and eosin staining. A. Control rat. The normal shape of closely packed seminiferous tubules (S) separated by interstitial cells of Leydig and blood capillaries (black arrows) (H&E_200). B. normal arrangement of spermatogonia (SG) and Sertoli cells (SCs) resting on intact basement membrane surrounded by myoid cells (M). Typical structure of primary spermatocytes (Ps), spermatids (SD) and late-stage spermatozoa (SZ) at the apices of SCs (H&E_400). C. Testis of Royal jelly group showing normal seminiferous tubules with interstitial cells (black arrow) (H&E_200). D, E, F, G. Testis of 6-mercaptopurine group showing D: irregular seminiferous tubules with exfoliated cells and no sperms in the lumen (black arrow) surrounded with congested interstitial blood vessels (yellow arrow). E & F degenerated seminiferous tubule with few exfoliated cells (black arrow) and separation of basal and adluminal cellular compartments (yellow arrow) with hemorrhagic interstitial exudates (Astrix). F. Marked reduction of germinal epithelium (White arrow) (H&E_ 200). G. separation of basal and adluminal cellular compartments (black arrow) vacuolated cytoplasm of spermatogonia (Sg) (H&E_ 400). H. Testis of 6-mercaptopurine + Royal jelly group showing nearly normal seminiferous tubules (S) surrounded with congested interstitial blood vessels and hemorrhagic interstitial exudation (Black arrow) (H&E X 200).

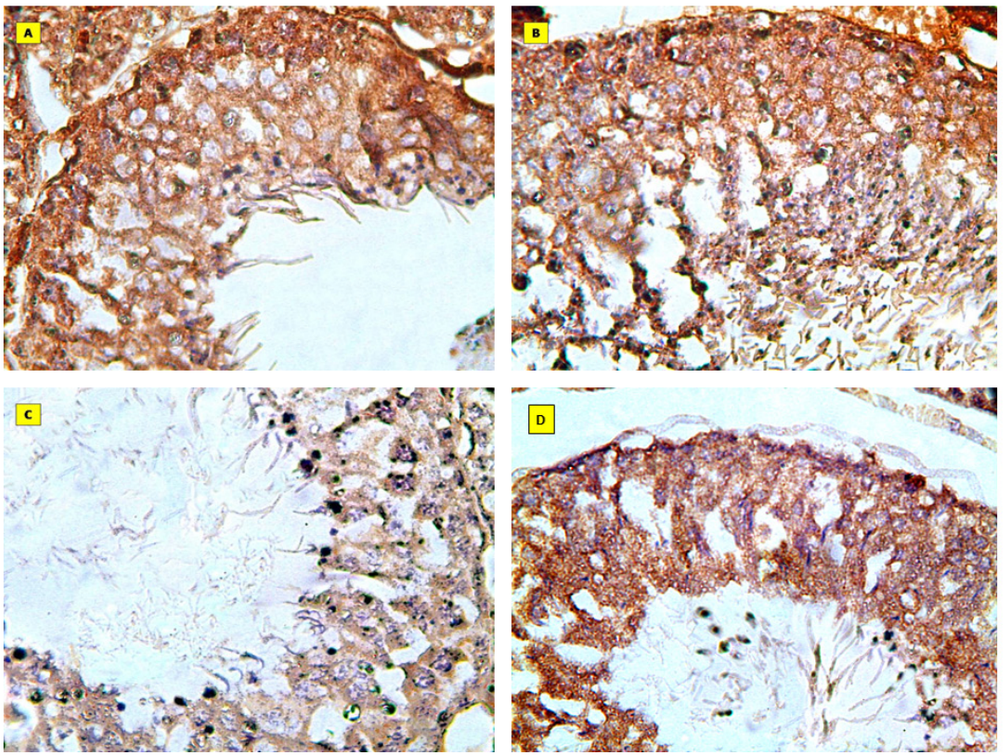


Fig. 3: A photomicrograph of a paraffin section in testis (Bcl2 X400). A & B: Testis of Control and royal jelly groups showing strong positive Bcl2 reaction in the cytoplasm of germinal cells. C: Testis of 6-mercaptopurine treated group showing weak positive Bcl2 reaction in the cytoplasm of germinal cells. D: Testis of 6-mercaptopurine + royal jelly treated group showing strong positive Bcl2 reaction in the cytoplasm of germinal cells.

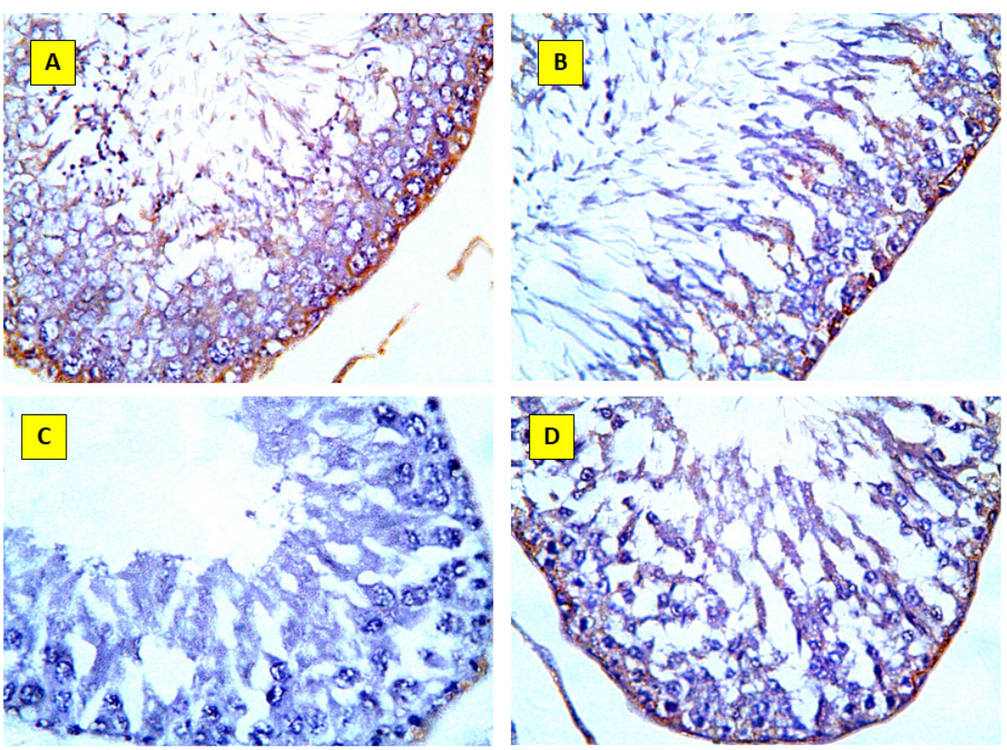
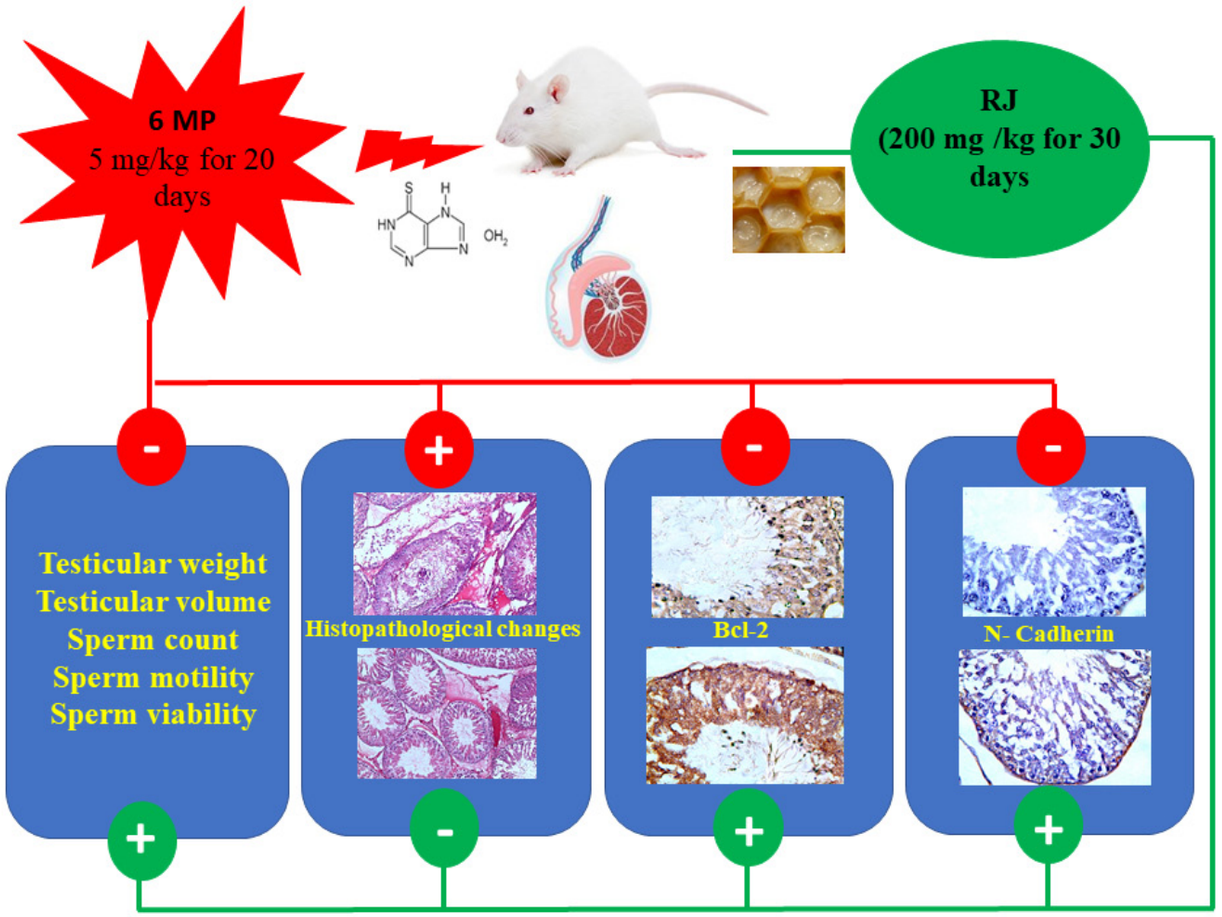


Fig. 4: A photomicrograph of a paraffin section in testis (N-cadherin X400). A & B: Testis of Control and royal jelly groups showing strong positive N-cadherin reaction in the germinal cells. C: Testis of 6-mercaptopurine treated group showing negative N-cadherin reaction in germinal cells. D: Testis of 6-mercaptopurine + royal jelly treated group showing strong positive N-cadherin reaction in the germinal cells.



DISCUSSION

6MP is an active azathioprine metabolite. It is employed in the clinic for immunosuppression and chemotherapy. Regardless of its therapeutic efficacy, 6MP is toxic to normal human tissues[30,31]. Our research examined alterations in sperm parameters and seminiferous tubules to better understand 6MP gonadal toxicity, infertility, and the protective impact of RJ. The active metabolites of 6MP have been shown to be harmful to rapidly proliferating cells, particularly those seen in adult reproductive organs[32].

In the current study, light microscopy of testis from albino rats in the control group (group I) and RJ group (group II) revealed no significant differences and their results were identical to the normal structure. They showed a normal histological structure of testis with densely packed normal seminiferous tubules. The seminiferous tubules were lined by Sertoli cells and spermatogenic cells associated with interstitial Leydig cells.

Meanwhile, light microscopic analysis of H&E-stained testicular samples from the 6MP group (group III) indicated significant histological alterations. They showed loss of normal testicular architecture with irregular seminiferous tubules, exfoliated cells and absence of sperms in the lumen. The interstitial areas were congested and filled with hemorrhagic exudate. Moreover, separation of basal and adluminal cellular compartments with vacuolated cytoplasm of spermatogonia were displayed. Karawya *et al*. (2006) reported that azathioprine produced testicular shrinkage, histopathological distortion, and decreased spermatogenesis in male albino rats[33], which is similar to our findings.

Multiple investigations found that the rate of mercaptopurine-induced mutagenicity and mortality was dosage-dependent[34]. 6-MP treatment at doses as low as 0.05 mM has been indicated to induce sperm toxicity[35]. In both rats and mice, 150 mg/kg of 6-MP is considered fatal and mutagenic[36]. In this study, statistical results of sperm parameters in rats treated with 6MP showed a substantial diminish in sperm count, motility, and viability, and also an elevation in aberrant spermatozoa. 6MP's propensity to disrupt nucleic acid synthesis in quickly splitting somatic and germ cells, culminating with germ cell death and mutations, may account for this effect[37].

6MP has been identified as a cytotoxic drug capable of producing reactive oxygen species (ROS), particularly H2O2[38]. Because spermatozoa contain a high concentration of polyunsaturated fatty acid, they are particularly vulnerable to oxidative stress and lipid peroxidation[39].

In this study, we demonstrated RJ's potential to promote spermatogenesis in RJ-pretreated rats and its ability to alleviate 6MP-induced testicular dysfunction. RJ+6MP animals showed a substantial elevation in testicular weight, motility, sperm count, and viability, and also a decrease in aberrant sperm forms, reversing the damage seen in 6MP treated rats. The potential of RJ to heal and protect against tissue injury was indicated by the enhancement in histomorphological data of rats treated with RJ. In accordance with our results, Temamoğullar *et al*. (2018) indicate that RJ protects mice against testicular degeneration and spermiotoxicity caused by flunixin[40].

In comparison to the 6MP group (group III), our histological findings of RJ+6MP (group IV) demonstrated a significant improvement in the appearance of seminiferous tubules. The interstitial hemorrhagic exudate was still seen.

RJ possesses anti-inflammatory properties in addition to its antioxidant properties. Kohno *et al*. (2004) indicated that RJ embedded the pro-inflammatory cytokines generation by stimulating macrophages[41], whereas Aslan *et al*. (2015) showed that RJ protected the kidneys against ethylene glycol-induced inflammation[42].

According to immunohistochemical results, testis from the control and RJ groups displayed strong Bcl-2 immunoreactivity. Testis of the 6MP group (group III) displayed weak positive Bcl-2 immunoreactivity. RJ+6MP group (group IV) displayed strong positive Bcl-2 immunoreactivity. Our findings were confirmed by morphometrical and statistical results; there was a significant increase in the area percent of Bcl-2 immunoreactivity in the RJ+6MP group (group IV) in comparison to the 6MP group (group III)

Bcl-2 expression was determined to get insight into testicular apoptosis triggered by 6MP. Bcl-2 is a multidomain prosurvival protein that inhibits the mitochondrial release of proapoptogenic chemicals (e.g., cytochrome c) and subsequent caspase stimulation. The poor Our results were consistent with the results of Schaalan *et al*. (2018), who discovered that Bcl-2 levels were significantly lower in 6MP-treated rat testes homogenate than in control rats[43]. One of the mechanisms by which 6MP impairs testicular function is by dysregulation of the fine-tuned apoptotic pathway, which may account for reduced sperm viability and mobility. According to Kanemitsu *et al*. (2009), 6MP induces apoptosis via the p53, caspase-3 pathway[44], which is compatible with our findings.

In the current study, N-cadherin expression was considerably lower in the 6MP group (group III) than in the control group (group I). N-cadherin is a cell surface protein that facilitates Ca2+-dependent intercellular adhesion and is thought to be required for the maintenance of the multicellular structure. N-cadherin expression on germinal and Sertoli cells makes more sense because it is known to enhance homophilic cell-cell contact. Thus, N-cadherin is one of the substances that assist in Sertoli cell differentiation from germinal cells and regulates crucial adhesion forces that promote the formation of more stable cell junctions[45]. Decreased N-cadherin staining indicates that spermatogenic cells and Sertoli cells interact less. As a consequence of disrupting the blood-testis barrier, 6MP may induce the cessation of spermatogenesis.

Furthermore, N-cadherin overexpression activates an anti-apoptotic pathway (or suppresses apoptosis) in cancer cells by increasing the decoy receptor-2 (DCR2), which inhibits apoptosis, decreasing the death receptor-5 (DR5), which regulates apoptosis and interfering with nuclear factor kappa-light-chain-enhancer of activated B cells/p65 (NF-kB/p65) and mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) signaling[46].

Additionally, it was observed that N-cadherin promotes the expression of Bcl2, a gene encoding an anti-apoptotic factor, in human prostate cancer cells. It promotes Akt (protein kinase B) via phosphatidylinositol-3 kinase (PI3K), thus, N-cadherin promotes cell survival by activating the Akt signaling system; thus, N-cadherin prevents apoptosis via the PI3K/Akt signaling pathway[47]. Moreover, N-cadherin has been shown to bind with and stabilize the fibroblast growth factor (FGF) receptor 1, hence preventing apoptosis[48].

Based on the results of this study, administration of RJ was found to increase Bcl-2 and N-cadherin immunoreactivity across the group treated with it. A statistically significant increase was seen when injury groups were compared with treatment groups ($p < 0.05$).

The anti-apoptotic effect of RJ is detected by increased expression of Bcl2 which is corroborated by a study performed by Karadeniz *et al*. (2011), who declare that RJ acts as an antioxidant and anti-apoptotic drug[49]. RJ also promotes apoptosis, as Amiri *et al*. (2015) reported[50].

In accordance with the present results, Lin *et al*. (2020) reported that 10-Hydroxy-2-decanoic acid (10-HDA) (royal jelly acid) may be a potential therapy for human lung cancer as it inhibited cell migration by regulating transforming growth factor-beta 1 (TGF-β1), E-cadherin, N-cadherin, and vimentin[51].

Finally, our work demonstrated that using Royal Jelly is a safe product for albino rats and that it can be utilized in conjunction with 6MP to decrease 6MP toxicity in testicular tissues as revealed by the histological results except for the interstitial hemorrhagic exudation. The protective effect of RJ occurs via up-regulation of Bcl-2 and N-Cadherin expression.

CONCLUSION

RJ rescues testicular tissues from 6MP-induced toxicity by increasing Bcl-2 and N-Cadherin expression. As a result, RJ may be a viable therapy option for infertility illnesses associated with testicular injury. Further research should be conducted to elucidate a variety of other pathways by which RJ safeguards against 6MP-induced testicular injury.

CONFLICT OF INTEREST

There are no conflicts of interest.

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