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Protective effects of saffron (*Crocus sativus* L.) hydro-alcoholic extract against paraquat-induced reproductive toxicity in mice

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Abstract

Objective: Paraquat (PQ), a commonly used herbicide, is known to induce male reproductive toxicity through oxidative stress. This study evaluated the protective effects of saffron hydroalcoholic extract (SFE) against PQ-induced testicular damage in adult male mice.

Materials and Methods: Thirty male BALB/c mice were randomly assigned to six groups (n=5) including control, saffron extract (80 mg/kg/day; SFE), PQ (20 mg/kg/day; PQ20), PQ (40 mg/kg/day; PQ40), PQ20 + SFE80, and PQ40 + SFE80. Treatments were administered orally for 30 days. After the treatment period, the mice were euthanized, and parameters such as testosterone concentration, sperm quality, gonadosomatic index (GSI), antioxidant enzyme activity, and the expression of apoptosis-related genes were analyzed. Additionally, histopathological evaluation of testicular tissue was performed using hematoxylin and eosin (H&E) staining.

Results: PQ significantly reduced body weight, testicular weight, GSI, sperm count, viability, motility, and normal morphology compared to the control group. Furthermore, the PQ20+SFE80 and PQ40 + SFE80 groups exhibited a significant decrease in catalase (CAT) activity, total antioxidant capacity (TAC), serum testosterone levels, and histological indexes. However, co-treatment with SFE significantly mitigated these effects. Compared to the PQ-only groups, mice treated with both PQ and SFE exhibited significant improvements in sperm parameters, restored antioxidant enzyme activities (CAT and TAC), normalized serum testosterone levels, and preserved testicular histology.

Conclusion: Saffron extract exerts protective effects against PQ-induced reproductive toxicity by enhancing antioxidant defenses, hormonal regulation, and preserving testicular integrity and apoptotic status.

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Introduction

Paraquat (PQ) is a widely used non-selective dipyridyl herbicide, raising significant concerns due to its impact on the endocrine system and reproductive health in humans and animals (Soni et al. 2019). It acts as a strong stimulant in forming superoxide anions, highly reactive molecules that can inflict severe damage on various organs including the reproductive system (Ijaz et al. 2023; Pang et al. 2019). Acute exposure to PQ can lead to respiratory failure and damage to vital organs such as the heart, kidney, liver, and nervous system (Chen et al. 2021). Recent research has linked PQ exposure to reproductive toxicity (Bora et al. 2021; Sun et al. 2021). These studies have revealed that PQ can have adverse effects on reproductive function, particularly in males, even at medium to high exposure levels (Edo 2022). Exposure to PQ can lead to decreased organ weight, degeneration of reproductive tissues, abnormal sperm morphology, and hormonal imbalances in rats (Chen et al. 2017). Furthermore, PQ has been implicated in embryonic developmental issues, thus significantly inhibiting normal development, even at minimal exposure levels (Hausburg et al. 2005).

The toxic mechanism of PQ involves a complex interplay of factors. PQ exerts its toxicity through cyclic redox reactions which lead to increased oxidative stress (Teleken et al. 2020) and depletion of cellular components (Bai et al. 2019). This herbicide causes toxicity by interfering with mitochondrial electron transport, which causes the production of oxygen free radicals and lipid peroxidation damage (Wang et al. 2021). The mechanism of PQ toxicity is also associated with the induction of lipid peroxidation, depletion of cellular NADPH, and the involvement of multiple organs like the lungs, kidneys, liver, and heart (Blanco-Ayala et al. 2014; Wang et al. 2016). The toxic mechanism of PQ on reproductive function involves oxidative stress, disruption of redox balance, and modulation of key cellular processes (Cai et al. 2024). PQ induces toxicity by generating reactive oxygen species (ROS) through redox cycling, leading to oxidative damage in reproductive organs (Chen et al. 2012). The induced oxidative stress can impact male reproductive function by causing genotoxic and cytotoxic effects on germ cells, affecting sperm quality and hormone levels (Kamali et al. 2019). Recently, it has been shown that PQ can induce derangement in pituitary-gonadal-hormone synthesis/secretion, leading to histological abnormalities in the testes, such as the presence of multinucleated giant cells in the lumen of testicular tubules (Li et al. 2019; Mustafa et al. 2023). Additionally, PQ exposure has been associated with impaired spermatogenesis in caprines and rats (Li et al. 2019; Rathee et al. 2024).

The use of saffron extract (SFE) as a natural source of antioxidants has emphasized its role as a complementary approach to managing male reproductive system toxicity (Goyal et al. 2024). This extract, derived from the dried stigma of the saffron flower, is rich in bioactive compounds like picrocrocin, safranal, and crocetin, which contribute to its anti-inflammatory and antioxidant properties (Sablania et al. 2022). Recent findings indicated that saffron maintains mitochondrial function, regulates apoptosis by affecting Bax and Bcl-2 levels, and promotes testosterone production through Leydig cell protection. Additionally, saffron reduces inflammation and stabilizes the testicular structure, thereby enhancing sperm quality and hormonal balance in conditions of oxidative damage (Kashani et al. 2022; Hasanpour et al. 2018). Studies have indicated that saffron extract improves sperm count, motility, and vitality in individuals exposed to factors like cadmium, cyclophosphamide, and electromagnetic field-induced testicular damage (Maleki-Saghooni et al. 2018; Ahmad et al. 2018; Asadi et al. 2014).

The present study aimed to investigate the protective role of saffron hydro-alcoholic extract against PQ-induced reproductive toxicity in adult male mice. Specifically, this study sought to evaluate the biochemical, hormonal, histopathological, and gene expression changes associated with PQ exposure and to determine how SFE influences these parameters.

Materials and Methods

Animals and study design

Thirty healthy adult male BALB/c mice, aged 6–8 weeks and weighing 25–30 g, were obtained from a Laboratory Animal House of Urmia University, Urmia, Iran. The mice were housed in a specific pathogen-free environment under standardized conditions, including a temperature of 22.00 ± 2.00°C, relative humidity of 50.00 ± 10.00%, and a 12-hr light/dark cycle. They were provided with a standard pellet diet and had *ad libitum* access to food and water. The experimental protocol and procedures complied with the Guidelines for the Humane Care and Use of Laboratory Animals using the approved protocols of Urmia University (IR-UU-AEC- 3.55). The mice were randomly divided into six groups (n = 5): control, saffron extract (80 mg/kg/day; SFE) (Shoja et al. 2018), paraquat (20 mg/kg/day; PQ20) (Ait-Bali et al. 2016), paraquat (40 mg/kg/day; PQ40) (Onur et al. 2022), PQ20 + SFE80, and PQ40 + SFE80. PQ and SFE were administered orally once daily for 30 days.

Preparation of hydro-alcoholic extract of saffron (SFE)

To prepare the saffron hydroalcoholic extract, 3 g of dried stigma of *Crocus sativus* L. was ground into a fine powder using a laboratory homogenizer. The powder was then macerated with 170 ml of 96% ethanol and placed on a shaker at room temperature for 10 hr. After shaking, the solution was filtered through Whatman No. 1 filter paper, and the solvent was evaporated under reduced pressure using a rotary evaporator at 40°C to yield the dried extract (Jelodar et al. 2018).

Sampling

At the end of the treatment period, mice body weight was recorded using a Sartorius digital scale (accuracy 0.0001 g). Mice were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg; Alfasan, Voorden, The Netherlands) via IP injection, followed by blood collection via cardiac puncture (Feat-Vetel et al. 2024). Blood samples were kept at room temperature for 20 min, centrifuged at 3000× g for 15 min, and serum was stored at -20°C for hormonal analysis. The mice were euthanized by cervical dislocation, and the right and left testes were weighed to calculate the gonadosomatic index (GSI). The epididymis was dissected, placed in human tubal fluid (HTF) medium with bovine serum albumin (BSA), and incubated at 37°C for sperm suspension. The left testis was fixed in 10% buffered formalin for histopathology, and the right testis was snap-frozen in liquid nitrogen for oxidative stress and gene expression analyses.

Evaluation of sperm quality

The Neubauer hemocytometer was used to evaluate the epididymal sperm counts. Sperm samples were diluted 1:20 in distilled water, and 10 µl was loaded into the hemocytometer. Cells were counted under a light microscope (400× magnification), and the results are expressed as sperm/mL (Sadeghi et al. 2020). For each mouse, 200 spermatozoa were analyzed for morphology, motility, and viability. The motility is reported as the total percentage of rapid, slow progressive, and non-progressive sperm, and eosin staining was used to assess sperm viability (Khazaeel et al. 2022).

Histopathological assay

Testis samples fixed in formalin were processed for histopathological analysis.

After fixation, testicular tissues were dehydrated, embedded in paraffin, sectioned into 5 µm slices, and stained with H&E. Histological analysis included Leydig cell quantification and evaluation of the tubular differentiation index (TDI), spermatogenesis index (SPI), meiotic index (MI), and the height of the seminiferous and germinal epithelium, all examined under a light microscope (Sadeghi et al. 2017).

Serological analyses

To evaluate serum testosterone levels, blood samples were collected and centrifuged, and the serum was separated. Testosterone concentrations were measured using an ELISA method with the Accu-Bind Testosterone Test Kit, following the manufacturer's instructions.

RNA extraction

Total RNA isolation and cDNA synthesis

Total RNA was extracted from testicular tissue using RNX™ reagent (SinaClon, Tehran, Iran). RNA concentration was measured at 260 nm with an Eppendorf µCuvette G1.0, and purity (OD 260/230 > 1.8) confirmed for cDNA synthesis. cDNA was synthesized using a YTA kit with random hexamer primers (Yekta Tajhiz, Tehran, Iran). Specific *p-53* gene primers were designed (Pishgam Biotech, Tehran, Iran): Forward (5′-ACCTGGCACCTACAGTGAAAT-3′) and Reverse (3′-AAATGCAGACAGGCTTTGCAG-5′).

Quantitative real-time RT-PCR

The *p-53* gene expression in testicular tissue was quantified via real-time PCR using the LightCycler® System (Roche, USA) and SYBR Green dye. Each 12.5 µl reaction included 6.25 µl SYBR Green Master Mix (Yekta Tajhiz, Iran), 0.25 µl primers (200 nM), 3 µl cDNA (200 ng), and 2.75 µl sterile ddH2O. Thermal cycling involved initial denaturation at 94°C for 5 min, followed by 45 cycles at 94°C for 15 sec and 60°C for 30 sec. Results were analyzed with the ΔΔCt method using LightCycler SW1.1 software. Gene expression was calculated as $2^{-\Delta\Delta Ct}$.

Assessment of oxidative stress markers

Lipid peroxidation in the testes was evaluated by measuring malondialdehyde (MDA) levels using the thiobarbituric acid reactive substances (TBARS) assay as described previously (Sadeghi et al. 2020). Catalase activity was measured by its ability to decompose hydrogen peroxide in homogenized testicular tissue, based on the Aebi method (Aebi 1984). The total antioxidant capacity (TAC) or ferric-reducing antioxidant power was evaluated using the described method of Gupta et al. (Gupta et al. 2021).

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 9.0 (GraphPad Software, Inc., San Diego, CA, USA). Data normality and distribution were verified before analysis. One-way ANOVA followed by Tukey's post hoc test was used to compare group differences. Results are presented as mean ± standard deviation, with statistical significance set at $p<0.05$.

Results

Body and testicular weight

PQ administration (20 and 40 mg/kg) significantly reduced body weight, testicular weight, and GSI compared to the controls ($p<0.05$ for these parameters). Co-administration with SFE improved these metrics versus PQ-only groups ($p<0.05$ for these parameters), but body and testicular weights in PQ20 + SFE and PQ40 + SFE remained lower than the controls ($p<0.05$ for both parameters). No significant GSI difference was noted between the PQ20 + SFE and controls ($p>0.05$; Figure. 1).

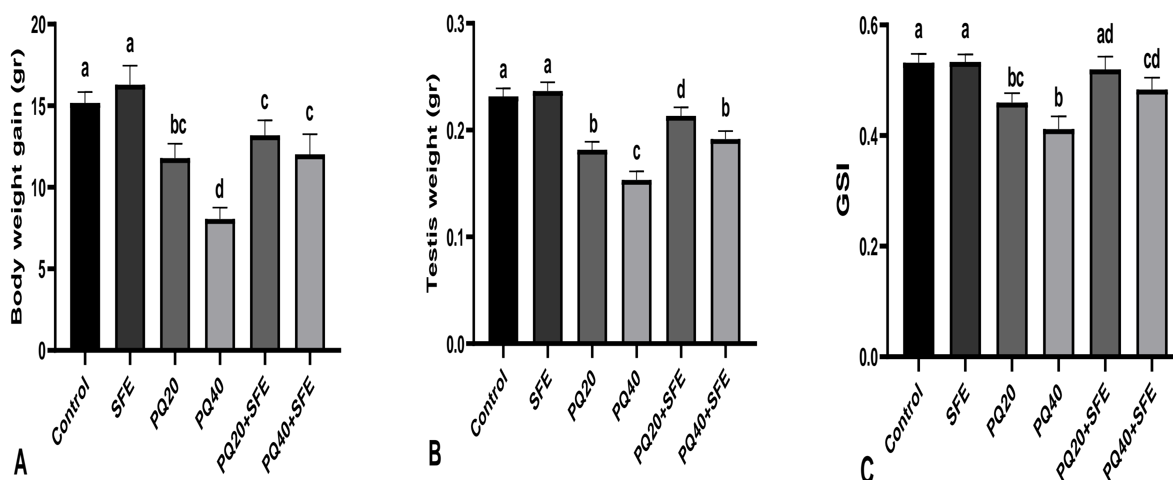

Figure 1. Impact of paraquat (PQ) and saffron extract (SFE) on body and testicular weight, and GSI in male mice (n = 5 mice in each group). Different letters (a, b, c, and d) within each column indicate statistically significant differences between the experimental groups (p<0.05). Groups labeled with different letters exhibit statistically significant differences (p<0.05).

Epididymal sperm parameters

Sperm count analysis showed that PQ at 20 and 40 mg/kg significantly reduced sperm count, viability, motility, and normal morphology (p<0.05 for these parameters). Co-administration of SFE with PQ improved these parameters (p<0.05). The PQ20 + SFE group had a sperm count comparable to the control (p>0.05), though sperm viability, motility, and normal morphology in PQ20 + SFE and PQ40 + SFE groups remained lower than the control (p<0.05 for these parameters; Table 1).

Histopathological changes

As detailed in Table 2, the histological analysis of testicular tissue revealed a significant reduction in TDI, SPI, meiosis index, Leydig cell count, spermatogenic tubule diameter, and germinal epithelium height in the PQ20 and PQ40 groups versus the control and SFE groups (p<0.05 for these parameters). Co-treatment with SFE (PQ20+SFE and PQ40 + SFE) significantly improved these parameters compared to the PQ groups (p<0.05; Table 2 and Figure. 2).

Table 1. Impact of paraquat (PQ) and saffron extract (SFE) on sperm count, as well as the percentages of sperm viability, motility, and normal morphology in different experimental groups (5 mice per group).

Groups	Sperm count (10^6)	Sperm viability (%)	Sperm normal morphology (%)	Sperm motility (%)
Control	21.50 ± 1.12 ^{a}	86.75 ± 1.10 ^{a}	89.34 ± 2.05 ^{a}	86.90 ± 1.82 ^{a}
SFE	22.12 ± 1.13 ^{a}	87.26 ± 1.85 ^{a}	91.33 ± 2.11 ^{a}	88.75 ± 1.49 ^{a}
PQ20	12.50 ± 1.64 ^{b}	53.71 ± 1.10 ^{b}	55.41 ± 2.35 ^{b}	63.75 ± 2.49 ^{b}
PQ40	7.80 ± 1.53 ^{c}	36.25 ± 1.62 ^{c}	41.19 ± 2.57 ^{c}	40.50 ± 2.64 ^{c}
PQ20+SFE	20.71 ± 1.91 ^{a}	77.25 ± 1.10 ^{d}	81.52 ± 2.39 ^{d}	79.87 ± 2.33 ^{d}
PQ40+SFE	14.25 ± 0.85 ^{d}	55.79 ± 4.02 ^{b}	58.83 ± 2.93 ^{b}	66.25 ± 2.85 ^{b}

Different letters (a, b, c, and d) within the same column represent statistically significant differences between groups (p<0.05). Groups labeled with different letters exhibit statistically significant differences (p<0.05).

Table 2. The effect of paraquat (PQ) and saffron extract (SFE) on the number of Leydig cells, the diameter of seminiferous tubules, height of germinal epithelium, and the percentage of the tubular differential index (TDI), spermiogenesis index (SPI), and meiotic index in different experimental groups (5 mice in each group).

Groups	Number of Leydig cells	Diameter of seminiferous tubules (μm)	Height of germinal epithelium (μm)	TDI (%)	SPI (%)	MI (%)
Control	15.50 ± 0.64 [a]	179.50 ± 2.25 [a]	43.50 ± 1.64 [a]	83.50 ± 4.78 [a]	0.80 ± 0.40 [a]	21.25 ± 0.85 [a]
SFE	16.00 ± 0.40 [a]	184.75 ± 2.39 [a]	44.50 ± 1.73 [a]	85.00 ± 5.77 [a]	0.83 ± 0.02 [a]	22.00 ± 0.40 [a]
PQ20	9.50 ± 0.64 [b]	151.00 ± 1.29 [b]	31.50 ± 2.44 [b]	55.00 ± 2.88 [b]	0.37 ± 0.02 [b]	13.50 ± 0.64 [b]
PQ40	6.75 ± 0.47 [c]	135.50 ± 2.72 [c]	28.50 ± 1.06 [c]	37.50 ± 9.57 [c]	0.22 ± 0.04 [c]	11.00 ± 0.40 [c]
PQ20+SFE	13.50 ± 0.64 [d]	161.75 ± 2.65 [d]	37.75 ± 1.10 [d]	73.00 ± 4.08 [d]	0.55 ± 0.06 [d]	17.50 ± 0.94 [d]
PQ40+SFE	10.50 ± 0.64 [b]	158.25 ± 2.75 [d]	32.75 ± 1.84 [b]	62.50 ± 4.78 [e]	0.45 ± 0.64 [e]	16.25 ± 0.87 [d]

Different letters (a, b, c, d, and e) within the same column representative statistically significant differences between groups ($p<0.05$). Groups labeled with different letters exhibit statistically significant differences ($p<0.05$).

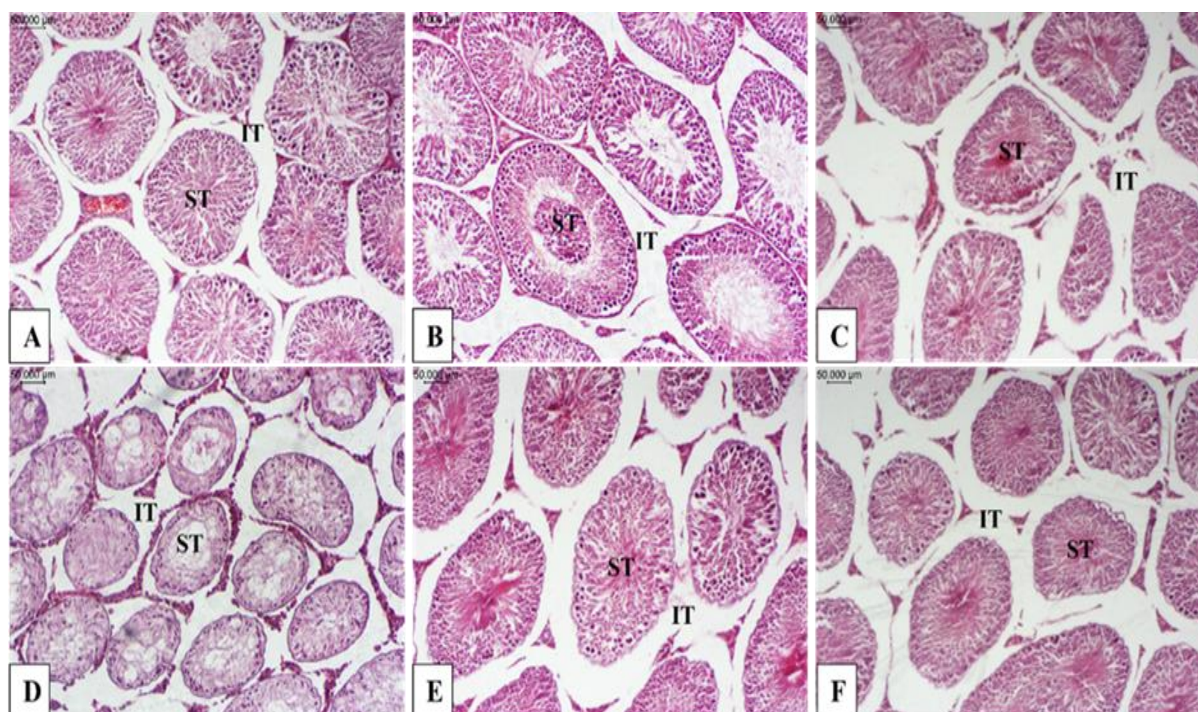

Figure 2. Cross-sections of testicular tissue in different experimental groups (H&E staining, 100x magnification; Scale bar = 50 µm). The control and SFE groups displayed normal histology of testicular interstitial tissue and seminiferous tubules. The PQ-treated groups, especially the PQ40-treated group showed impaired testicular tissue. The co-administration of SFE in the PQ20 + SFE and PQ40 + SFE groups improved the testicular tissue structure. A: Control group, B: SFE group, C: PQ20 group, D: PQ40 group, E: PQ20 + SFE group, and F: PQ40 + SFE.

Serum testosterone levels

Testosterone levels were lower in the PQ20 and PQ40 groups than the controls (p<0.05). The PQ20 + SFE and PQ40 + SFE groups showed higher levels than the PQ-only groups (p<0.05) but remained lower than the controls (p<0.05; Figure. 3).

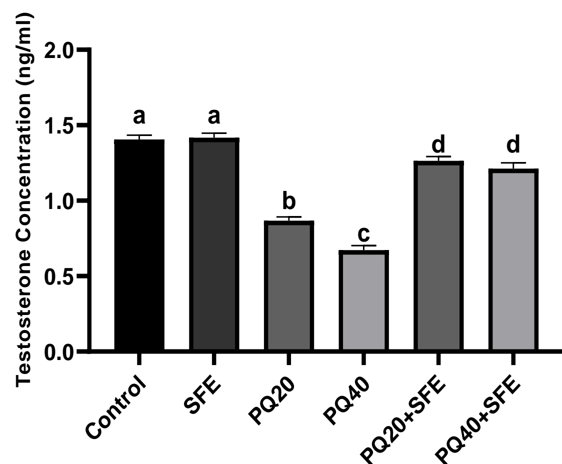

Figure 3. Effect of paraquat (PQ) and saffron extract (SFE) on serum testosterone levels in male mice (n = 5 mice in each group). Different letters (a, b, c, and d) within each column indicate statistically significant differences between the experimental groups (p<0.05). Groups labeled with different letters exhibit statistically significant differences (p<0.05).

Biochemical parameters in testicular tissue

MDA levels were significantly higher in the PQ20 and PQ40 groups compared to the controls (p<0.05), but co-administration of SFE reduced these levels (p<0.05). MDA levels in the PQ20 + SFE and PQ40 + SFE groups remained elevated compared to the controls (p>0.05; Figure. 4 A). Catalase activity and TAC were lower in the PQ-treated mice than the controls (p<0.05 for these parameters). No significant differences in TAC were observed between the PQ20 + SFE group and controls (p>0.05; Figure. 4 B-C).

p-53 gene expression levels

As presented in Figure 5, the expression of the *p-53* gene was significantly higher in the PQ20 and PQ40 groups than in the control (p<0.05). SFE co-administration reduced *p-53* levels in the PQ groups (p<0.05). No significant difference was found between the PQ20 + SFE and control (p>0.05), while the expression of the p-53 gene in the PQ40 + SFE group remained higher than control (p<0.05; Figure. 5).

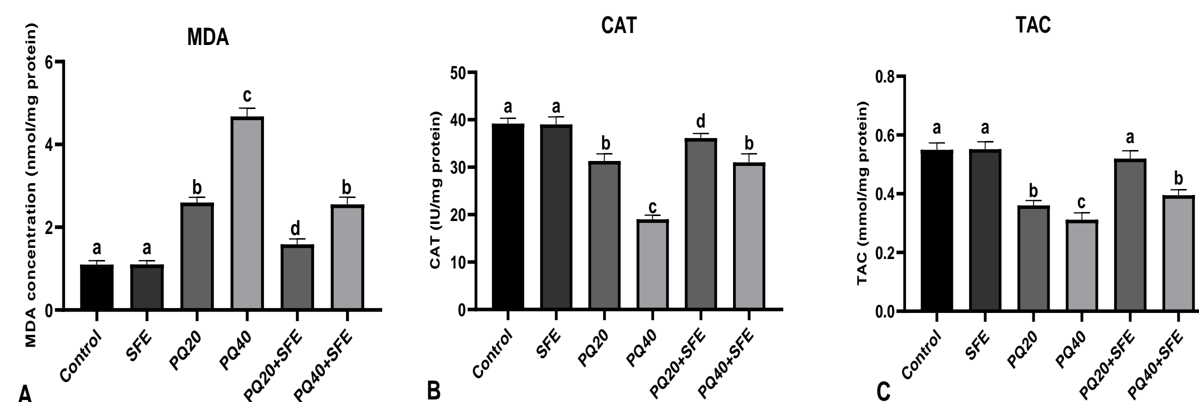

Figure 4. Effect of paraquat (PQ) and saffron extract (SFE) on testicular malondialdehyde (MDA), catalase activity, and total antioxidant capacity (TAC) in male mice (n = 5 mice in each group). Different letters (a, b, c, and d) within each column indicate statistically significant differences between the experimental groups (p<0.05). Groups labeled with different letters exhibit statistically significant differences (p<0.05).

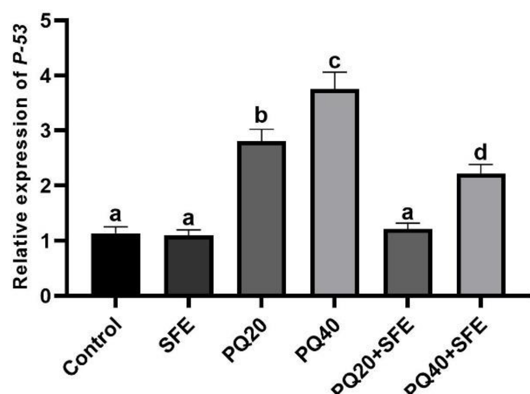

Figure 5. Effect of paraquat (PQ) and saffron extract (SFE) on the expression levels of the *p-53* gene (n = 5 mice in each group). Different letters (a, b, c, and d) within each column indicate statistically significant differences between the experimental groups ($p<0.05$). Groups labeled with different letters exhibit statistically significant differences ($p<0.05$).

Discussion

Emerging research suggests that exposure to herbicides, particularly PQ-based formulations, poses significant risks to the reproductive health of both humans and animals (Sun et al. 2021). These toxic chemicals disrupt the endocrine system, potentially leading to reproductive disorders and infertility (Bhardwaj et al. 2020). PQ is an herbicide that has been found to decrease semen quality and cause cell death in the reproductive system by increasing oxidative stress (Agarwal et al. 2014; Ijaz et al. 2024). Additionally, PQ administration is linked to harmful damage in male reproductive organs (D'Souza et al. 2006).

Plant-based compounds have garnered attention for their therapeutic potential, particularly due to their bioactive phytochemicals (Goyal et al. 2024). Saffron hydro-alcoholic extract (SFE), rich in constituents such as crocin, crocetin, safranal, and picrocrocin, has demonstrated notable antioxidant, anti-inflammatory, and anti-apoptotic properties. These compounds act by neutralizing reactive oxygen species (ROS), enhancing antioxidant enzymes like CAT and superoxide dismutase (SOD), and regulating apoptotic gene expression (Khazdair et al. 2015; Sablania et al. 2022).

The present study evaluated protective role of SFE against PQ-induced testicular damage. PQ doses (20 and 40 mg/kg) impaired reproductive performance in male mice, reducing antioxidant enzyme activity, increasing apoptotic gene expression, damaging spermatogenic tubes, lowering testosterone levels, and deteriorating sperm parameters. PQ also decreased body weight, testicular weight, and GSI. SFE administration significantly mitigated these effects, improving body/testicular weight, GSI, sperm parameters, histopathology, serum testosterone, and reducing oxidative stress.

Herbicide exposure has been associated with reduced fertility, shortened gestation, and disruption of hormonal systems in both males and females (Cremonese et al. 2017; Kongtip et al. 2017; Teleken et al. 2020). Additionally, herbicides have been demonstrated to accumulate in cell membranes and potentially lead to cell disruption, carcinogenic impacts, and neurological effects (Shahabadi et al. 2022; Sabarwal et al. 2018). The mechanisms underlying PQ-induced testicular toxicity are multifaceted, primarily involving oxidative stress, inflammation, and apoptosis, which collectively lead to impaired spermatogenesis and hormonal dysregulation (Ijaz et al. 2024; Mustafa et al. 2023). The observed reproductive toxicity in this study is associated with PQ-induced oxidative stress, as evidenced by increased MDA levels and reduced activities of CAT and TAC in testicular tissues. Recent studies have confirmed that PQ exerts its toxic effects through a complex mechanism involving redox cycling, which leads to the generation of ROS and subsequent oxidative damage to various cellular components, including the reproductive system (Chen et al. 2021; Mustafa et al. 2023). PQ causes oxidative stress in testicular tissues by generating ROS such as superoxide anions and

hydrogen peroxide. This overwhelms the antioxidant defense system, leading to decreased activity of important enzymes like SOD, CAT, and glutathione peroxidase (GPx) (Ijaz et al. 2023). The imbalance between ROS and antioxidants results in lipid peroxidation, which is evidenced by increased MDA levels. Lipid peroxidation compromises the integrity of cellular membranes, particularly in Leydig and Sertoli cells, which are essential for testosterone production and the support of spermatogenesis, respectively (Ijaz et al. 2022). The disruption of these cells can lead to reduced testosterone levels, further impairing reproductive function (Chen et al. 2017). Studies have demonstrated that PQ can impair oocyte maturation in female mice, leading to reduced fertility due to disruptions in spindle assembly and increased oxidative stress, which affects early embryo development (Sun et al. 2021; Zhou et al. 2022). Moreover, evidence suggests that PQ exposure is associated with hormonal disruptions, including decreased testosterone and alterations in the hypothalamic-pituitary-gonadal axis, potentially affecting spermatogenesis and overall reproductive function (Ghosh et al. 2022). This disruption occurs through the inhibition of gonadotropin-releasing hormone (GnRH) from the hypothalamus, leading to decreased secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary gland. Both LH and FSH are essential for stimulating testosterone production in Leydig cells and supporting spermatogenesis in Sertoli cells (Quassinti et al. 2009).

PQ-treated mice exhibited germ cell loss, disrupted seminiferous epithelium, altered seminiferous tubule structure, and increased sperm abnormalities, including reduced motility. These effects, associated to mitochondrial damage and impaired ATP production, indicate significant testicular damage and disrupted spermatogenesis (Chen et al. 2017).

Apoptosis induction is another important consequence of PQ exposure in testis tissue (Kamali et al. 2019; Li et al. 2019). The oxidative stress induced by PQ is characterized by increased lipid peroxidation which compromises cell membrane integrity and activates various signaling pathways associated with apoptosis (See et al. 2022). Oxidative stress can trigger the mitochondrial pathway of apoptosis, resulting in the release of cytochrome c from mitochondria into the cytosol. This process activates caspases, particularly caspase-3, a crucial executioner of apoptosis (Sule et al. 2022). Mechanistically, PQ exposure leads to the upregulation of pro-apoptotic proteins such as p53 and caspase-3, while downregulating anti-apoptotic factors like Bcl-2 (Chen et al. 2012). Additionally, PQ-induced oxidative stress can activate the *p-53* pathway which is a crucial regulator of the cell cycle and apoptosis (Ghasemi et al. 2024). The stabilization and activation of *p-53* lead to the transcription of genes that promote apoptosis, thereby increasing the rate of programmed cell death in testicular cells (Yang and Tiffany-Castiglioni 2008). This shift in the balance between pro- and anti-apoptotic signals results in increased rates of programmed cell death among spermatogenic and Sertoli cells, further diminishing spermatogenic output and compromising testicular structure (Kamali et al. 2019).

Recent research has indicated that saffron may offer protective benefits effects against chemical-induced reproductive damage in males and females (Ranjbar and Ashrafizaveh 2019; Babayev et al. 2022; Goyal et al. 2024). The protective mechanisms of SFE can be attributed to its rich content of bioactive compounds, such as picrocrocin, safranal, and crocetin, which possess potent antioxidant and anti-inflammatory properties (Cerdá-Bernad et al. 2022). These compounds have been shown to modulate lipid oxidation and enhance the activity of antioxidant systems, thereby mitigating oxidative stress-induced

damage to the reproductive organs and improving sperm quality. Also crocin, has been shown to restore mitochondrial membrane integrity, increase testosterone synthesis, and regulate the expression of pro- and anti-apoptotic genes (Rossi et al. 2021; Saadat et al. 2024). Furthermore, saffron has been associated with increased levels of FSH, LH, and testosterone in animal studies, suggesting a positive influence on male sexual hormones (Amini et al. 2023). The improved effects of SFE on PQ-induced reproductive toxicity may be attributed to hormonal regulation. In line with our findings, a study by Ahmad et al. showed that saffron reduced zearalenone-induced changes in reproductive hormone levels in mice. Consumption of saffron resulted in hormone levels similar to the control group, indicating its potential to counteract chemical-induced reproductive damage (Ahmad et al. 2018). The PQ20 + SFE group showed significant improvement in most parameters compared to PQ20 alone, but sperm viability, motility, and normal morphology remained lower than the control group. While saffron enhances reproductive outcomes at lower toxic doses, its effectiveness diminishes with severe high-dose toxicity (Ahmad et al. 2018; Hatziagapiou and Lambrou 2018). These findings suggest that the protective effects of SFE may be dose-dependent and may not be able to completely reverse the severe reproductive damage caused by high-dose PQ exposure.

Despite the promising findings, this study has several limitations. First, the sample size was relatively small, which may limit the statistical power and generalizability of the results. Second, the study was conducted on a rodent model, and while mice are commonly used in toxicological research, the findings may not fully translate to human physiology without further clinical validation. Third, only one dose of saffron extract was used; a dose-response analysis would provide better insight into the optimal therapeutic concentration.

In conclusion, saffron hydro-alcoholic extract (SFE) has shown potential in mitigating PQ-induced reproductive toxicity in adult male mice. Its antioxidant and hormone-regulating properties appear to counteract the oxidative stress and hormonal disruptions caused by PQ exposure. Saffron-based interventions may provide a promising complementary strategy for managing herbicide-induced reproductive health issues. However, further research is needed to fully elucidate the underlying mechanisms and optimize the therapeutic potential of saffron in this context.

Acknowledgment

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Conflicts of interest

There is no conflict of interest.

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Ethical Considerations

This study was approved by the ethics committee of Urmia University (IR-UU-AEC-3.55).

Code of Ethics

IR-UU-AEC-3.55

Authors' Contributions

FF and AS designed the study. SHP performed the experiments and helped in data analysis and manuscript writing. AS helped in data analysis and writing the manuscript. FF supervised the study and helped in manuscript writing. All authors reviewed and approved the final manuscript.

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