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Impact of scutellarin on cyclophosphamide-induced testicular damage in Sprague Dawley male rats

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ABSTRACT

Cyclophosphamide is one of the most invasive chemotherapeutic drugs, which is commonly used despite its adverse effects and toxicities. Scutellarin is an herbal flavonoid with multiple pharmacological activities and clinical applications. The goal of the current study was to investigate whether scutellarin protected the gonads of male rats exposed to a single dose of cyclophosphamide. In the experiment, 21 male Sprague-Dawley male rats were divided into three groups (n=7/group). Control group; received normal saline for 12 days by mouth; Induction group; received normal saline for 12 days by mouth and a single dose of cyclophosphamide (100 mg/kg) intraperitoneally on day 6; and Scutellarin group; received intragastric scutellarin (100mg/kg) for 12 days and a single dose of cyclophosphamide (100mg/kg) intraperitoneally on day 6. At the end of treatment, the samples were collected, the gonadosomatic index (GSI) was calculated, sperm parameters, serum testosterone, testicular malondialdehyde (MDA), and glutathione peroxidase (GPx) levels were measured, and histopathological alternation was evaluated. The results showed that GSI, sperm parameters, testosterone, and GPx levels were notably declined, while the level of MDA was significantly higher in the induction group compared to the control group. Interestingly, these measures markedly improved in scutellarin treated group. Additionally, histopathological analysis revealed a substantial change in the testicular tissue structure and a decrease in Jonson's score in the induction group, although scutellarin co-treatment considerably attenuated and even reversed these changes. In conclusion, the outcomes demonstrated that scutellarin may act as a protective agent that alleviates rats' testicular toxicity caused by cyclophosphamide therapy. Further, the proposed process may be attributed to its potent antioxidant properties.

INTRODUCTION

Cancer continues to be a global burden and the increasing incidence of cancer is a complex and multifaceted issue influenced by a range of factors, including demographic shifts, environmental exposures, lifestyle changes, and advancements in medical detection and diagnosis [1]. Chemotherapy is a key component of cancer treatment, but it often leads to a number of negative side effects, among which are long-term consequences for male infertility. Chemotherapeutic agents, aggressive in their ability to kill cancer cells due to their rapid growth, also destroy healthy cells in the body, including the reproductive system [2]. This produces a defect in spermatogenesis, which ends up causing temporary or permanent infertility in the male [2]. This lightweight silhouette affects young adult males, a portion of the cancer pool that is widely deemed cured. Being in their most productive years of potential childbearing is heartbreaking and emotional baggage for them, who are already fighting for survival. It is, moreover, an urgent, and mandatory issue concerning the epidemiology of chemotherapy-induced infertility among these groups of patients [3].

Cyclophosphamide (CP), one of the chemotherapeutic agents, has negative effects on many organ systems, including the testes, liver, kidneys, and bladder, despite its

widespread use in the treatment of various malignancies [4, 5]. Although the exact mechanism by which CP produces toxicity in the testis and other organs is still unclear, evidence suggests that CP may disrupt tissue redox equilibrium, which in turn may lead to oxidative stress and subsequent tissue damage [6].

Nevertheless, the input on medicinal plant research has increased due to the presence of bioactive compounds with demonstrated chemo-preventive potential, the absence of adverse effects, and a lower level of toxicity [7]. As a result, a screening process for therapeutic compounds in plants that exhibit beneficial interactions with biological molecules has been developed. Scutellarin, a natural flavonoid extracted from *Erigeron breviscapus*, possesses potent antioxidant properties due to its capability of scavenging reactive oxygen species (ROS), enhancing the effectiveness of detoxification enzymes, and improving the immune system [8]. Results from preclinical studies demonstrated that scutellarin exerts protective effects against liver injury [9], decreases cardiomyocyte death [10], and attenuates neuroinflammation in cerebral ischemia/reperfusion injury [11].

Many diabetes-related disorders have benefited from scutellarin's extensive use due to its efficacy, safety, and tolerance [12]. Therefore, this study aimed to determine the therapeutic effect of scutellarin on CP-induced testicular toxicity in male rats.

MATERIALS AND METHODS

Chemicals and reagents

Cyclophosphamide and scutellarin were purchased from Hangzhou Jinlan pharma-drugs technology (China). Ketamine 10% (Alfasan, Holland), xylazine 10% (Bimeda, Canada), normal saline 0.9% (Pioneer, Iraq), phosphate buffered saline (PBS) (Chemical Point, Germany), flushing medium with phenol red and gentamicin (Fertipro, Belgium), heavy liquid paraffin (Alpha Chemika, India), eosin 1% (Thomas Baker/India), nigrosine 10% (Alpha chemika, India), and buffered formalin 10% solution (Edutek, India) were also purchased. Enzyme-Linked Immunosorbent Assay (ELISA) kit for testosterone and malondialdehyde (MDA) from Cloud-Clone Corp., USA and glutathione peroxidase (GPx) ELISA kit from Elabscience, USA were used, unless otherwise mentioned.

Animals

Twenty-one apparently healthy Sprague-Dawley male rats weighing (250-360 gm) were purchased and housed in wood chip-bedded plastic cages (20x25x35 cm) in the animal house of the Biotechnology Centre/AL-Nahrain University, where this *in vivo* study was conducted, in a well- ventilated, non-pathogenic environment with adequate water and food supply with a 12-hour light-dark cycle, and temperature adjusted to 22°C±3° throughout the study. One-week acclimatization for the new environment before starting the work was provided. All procedures performed on animals Lab. such as housing and care, and experimental protocols, were approved by the Institute Review Board (IRB) for revision of research ethics approval by the College of Medicine at Al-Nahrain University (No. 20240517) on September 17, 2023 and were conducted according to the guidelines from the National Institutes of Health and the American Veterinarian Medical Association (AVMA) guidelines (2020) [13].

Experimental design

The animals were randomly divided into three groups of seven rats each (*n*=7) **(**Figure 1). Rats in Group 1 served as the control group and received normal saline (1 mL, p.o.) once daily for twelve days. Group 2: Induction group: given normal saline (1 mL, p.o.) once daily for 5 days (1–5), followed by a single dose of CP (100 mg/kg) intraperitoneally (IP) on day 6, then return to normal saline (1 mL, p.o.) for seven days (6–12). Group 3: scutellarin group; given scutellarin solution once daily at a 100mg/kg dose for 5 days (1–5), followed by a single dose of CP (100 mg/kg) IP on day 6, then again given scutellarin (100 mg/kg, p.o.) for seven sequential days (6–12)**.**

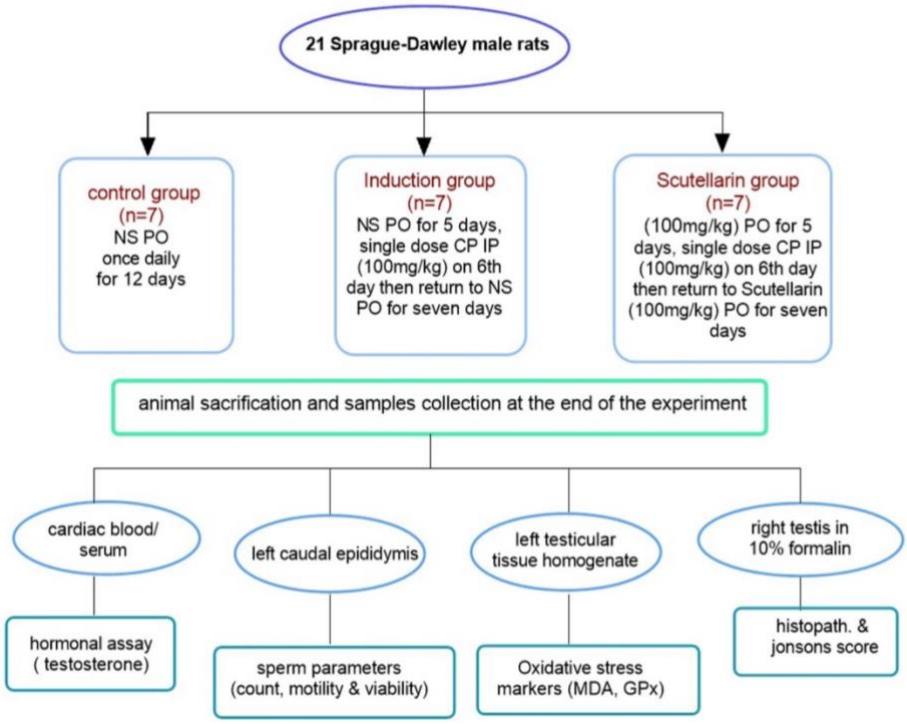

Figure 1. A schematic illustration of the experimental design for the study of testicular damage-induced by Cyclophosphamide. NS = normal saline, CP = cyclophosphamide, IP = intraperitoneal, PO = by mouth, MDA = malondialdehyde, GPx = glutathione peroxidase.

Analysis of blood and tissue samples

At the end of the experiment (on day 13), the animals were anesthetized by intramuscular injection of a mixture of ketamine 10% and xylazine 10% (10 mg/kg) (0.1 ml of mixture for each 100 gm). The weights of the animals in each group were measured. The blood samples were obtained via cardiac puncture using a 5 ml syringe and transferred to gel/serum separating tubes to clot. Then, they were centrifuged at 3000 rpm for 10 minutes and frozen in 1.8 ml Eppendorf tubes at -20°C until the assessment of the testosterone (Cloud-Clone Corp., USA) level as described previously [14].

The rat testes and cauda were quickly removed from the body after laparotomy. The weight of cauda was used to measure the gonadosomatic index (GSI) according to the following equation as described [15]:

$$GSI = \frac{gonadal\ weight\ (g)}{(total\ bidy\ weight\ (g)} \times 100$$

The right testis was cleansed of adherent connective tissue and immersed in a formalin-phosphate buffered saline (PBS) solution at a ratio of 1:9 for histopathological and Jonson's score evaluations. Testis was cut into two parts to ensure a good fixation process, followed by dehydration, cleaning, wax impregnation, sectioning, 5 μm thick, staining with hematoxylin (Riedel-de Haen/Germany) and eosin (Thomas baker, India), then dewaxing in an oven at 55°C for 20 minutes, and finally, rehydration was done. The serial histological sections were examined microscopically using an eyepiece micrometer (Olympus optical, Japan) [16]. The testicular damage was categorized using Jonson's ten-grade scoring system, where a score of 1 denotes the total lack of germ cells and a score of 10 represents the maximum amount of spermatogenesis activity.

The testicular tissue homogenization was adapted from Swayeh (2014) [17]. The homogenate was used to estimate the levels of oxidative stress biomarkers, including MDA (Cloud-Clone Corp., USA) and GPx (Elabscience, USA) using the Sandwich ELISA technique following manufacturing instructions. Each plate was pre-covered with a specific antibody, and the plates were filled with a homogenate sample so that a specific antigen-antibody reaction would be performed (incubation with enzyme-conjugated antibody), followed by a washing-out process to remove the unbound antibodies. Finally, the substrate is added to produce a calorimetric signal that can be read by the plate reader (Huma Reader HS, Japan).

Measurement of sperm parameters

The left part cauda of each epididymis was harvested immediately after sacrifice and minced in a petri dish containing prewarmed 37°C 1ml of flushing media. They were incubated at 37°C for 5 minutes to allow the dispersal of spermatozoa [18]. The sperm analysis was conducted in accordance with the standards set by the World Health Organization (WHO). To determine the sperm count, 10 μL of caudal sperm suspension was placed on a hemocytometer slide. Under a light microscope set at x40 magnification, only sperm cells identified as having a head, middle, and tail were counted. Each sample was counted twice, and the mean was reported. The results were presented as the concentration of sperm per milliliter [19]. In order to assess sperm motility, a single drop of caudal sperm suspension was placed onto a slide that had been pre-warmed. The percentage of motile sperm was then determined by observing their movement under a light microscope with a magnification of ×10. This assessment was conducted in three different fields for each sample. Additionally, the percentage of sperm exhibiting normal and abnormal motility in these fields was recorded. The present study adhered to the guidelines set forth by the WHO in order to evaluate the proportion of sperm cells exhibiting motility, following the enumeration of 200 sperm cells [20]. To evaluate sperm viability, 20 μL of sperm suspension was mixed thoroughly with 20 μL of eosin-nigrosine. A glass slide was used to transfer 10 microliters of the stained sperm mixture, and tress smears were conducted for each rat. The glass transparencies were permitted to dry at room temperature. A bright field microscope was used to observe the coverslips under x100 oil immersion after placing one drop of mounting medium on them. The viable sperm exhibited a whitish or colorless head, while the deceased sperm exhibited a dark coloration of the head. Approximately 200 sperms were observed for the presence of dead and active cells, and the percentage of each were recorded [21].

Statistical analysis

The data was entered and analyzed using Microsoft Excel 365 and SPSS version 26. Using normality tests showed that the data was non-normally distributed; the Mann - Whitney test and Kruskal -Walli's test were used instead of an independent t test and a one-way analysis of variance (ANOVA) for continuous variables to determine mean differences between groups [22]. The results were considered statistically significant (P<0.05) or highly significant (P<0.001).

RESULTS

Effect of scutellarin on gonadosomatic index

By measuring the cauda of all rats in each group, the results showed, a statistically significant reduction (P<0.05) in the GSI (cauda%) in the induced group compared to the control group. On the other hand, the GSI (cauda%) of animals treated with scutellarin was significantly elevated compared to the induced group, with statistical p-value (P<0.05) as shown in Figure 2.

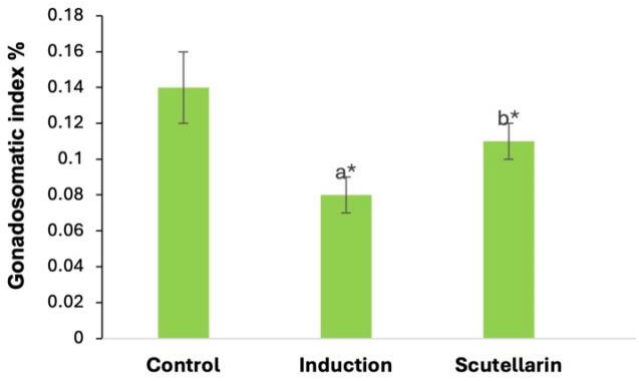

Figure 2. Effect on gonadosomatic index (GSI) among studied groups. Data were expressed as Mean ± SD. a: comparison with Control group; b: comparison with induction group; *P significant at level <0.05.

Effect of scutellarin on sperm parameters

In the current study, the induction group shows significant elevation (P<0.05) in sluggish sperm motility, immotile sperms, and dead sperms. A significant reduction (P<0.05) in sperm concentration, progressive sperm motility, and viable sperms was observed in induction group compared to the control group. Meanwhile, the scutellarin group showed a significant elevation (P<0.05) in sperm concentration, progressive sperm motility, and viable sperms; and a significant reduction (P<0.05) in sluggish sperms motility, immotile sperms, and dead sperms compared to the induction group, as shown in Table 1 and Figure 3.

Table 1. Effect on sperm parameters among studied groups.

Sperm parameters		Control	Induction	Scutellarin
Sperm concentration *10⁶/ml		66.14±24.10	18.00±12.22 a*	45.43±4.43 b*
Sperm motility%	Progressive	96.00±1.15	7.00±2.83 a*	82.71±2.69 b*
	Sluggish	0.57±0.53	58.43±8.70 a*	6.00±2.31 b*
	Immotile	3.43±0.79	33.14±7.90*	11.29±1.38 b*
Sperm viability %	Viable	96.00±1.29	28.00±8.41 a*	93.57±2.15 b*
	Dead	4.00±1.29	72.00±8.41 a*	8.14±2.48 b*

Data were expressed as Mean ± SD. a: comparison with Control group; b: comparison with Induction group; *P significant at level <0.05.

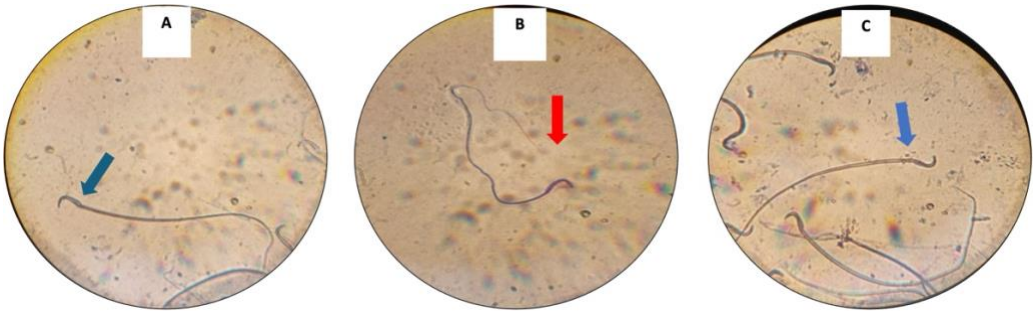

Figure 3. Effect of scutellarin on the sperm's viability**.** A = control group; B = induction group; and C = scutellarin group. Blue arrow denotes viable sperm; red arrows indicate dead sperm; sperms stained with Eosin & Nigrosine; A, B, & C =100X magnification.

Effect of scutellarin on serum testosterone

The current study demonstrated that the serum level of testosterone was significantly reduced (P = 0.002) as compared to the corresponding level in the control group. While all animals treated with scutellarin have shown a significant elevation in the serum level of testosterone (P = 0.002) in comparison with the induction group (Figure 4).

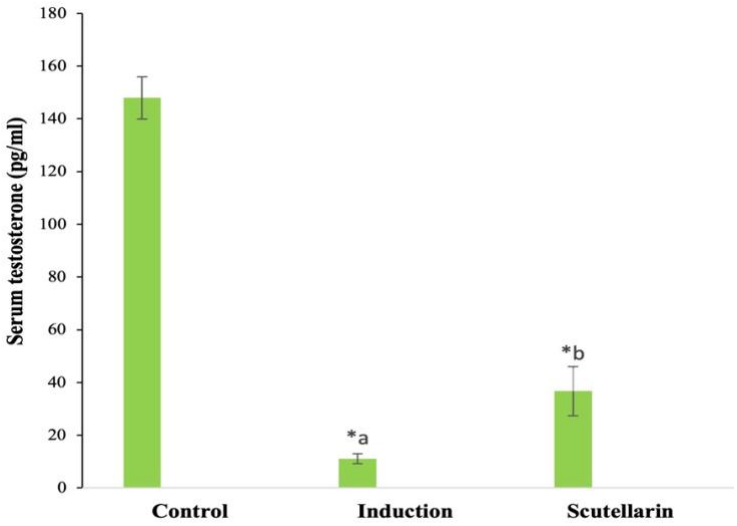

Figure 4. Effect on serum testosterone level among studied groups. Data were expressed as Mean ± SD. a: comparison with Control group; b: comparison with Induction group; *P significant at level <0.05.

Effect of scutellarin on testicular oxidative stress markers

Measurement of biomarkers related to oxidative stress was done in testicular tissue homogenate using the ELISA technique. Selected markers that reflect oxidative stress and antioxidant activity were MDA and GPx, respectively. The result of the analysis demonstrated a significant elevation (P = 0.002) in the oxidative stress biomarker (MDA) and a significant reduction (P = 0.002) in the antioxidant biomarker (GPx) in the induction group compared to the control group. On the other hand, the scutellarin group showed a significantly reduced (P = 0.002) level of oxidative biomarker (MDA) and significantly elevated (P = 0.002) level of antioxidant biomarker (GPx) in testicular tissue homogenate as compared to the induction group (Figures 5A and B).

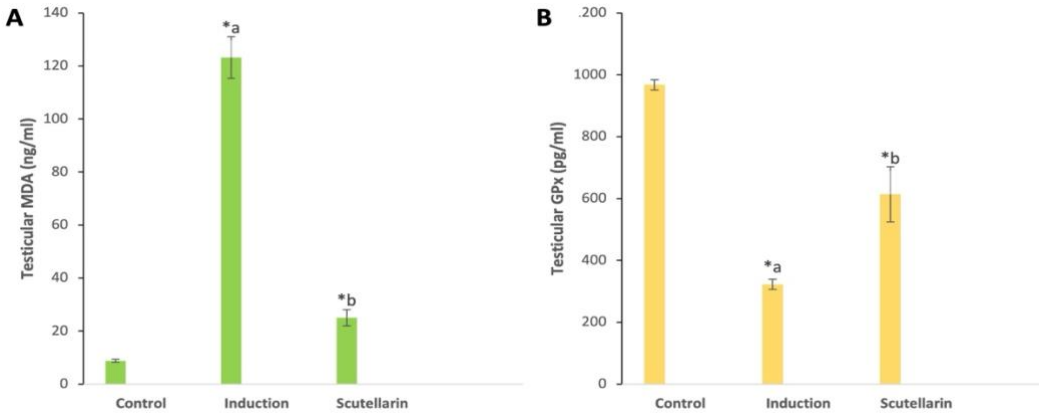

Figure 5. Effect on testicular oxidative stress markers among studied groups. A) Malondialdehyde evaluation and B) Glutathione peroxidase evaluation. Data were expressed as Mean ± SD. a: comparison with Control group; b: comparison with Induction group.

Effect of scutellarin on testicular histology

The histopathological evaluation within the control group exhibited normal testicular architecture, including intact seminiferous tube structure, epithelial layers, and an abundance of germ cells and spermatozoa, as shown in Figure 6A and B. Conversely, the induction group exhibited atrophic seminiferous tubules with few germ cells and abnormal spermatozoa. This is evident in Figure 6C and D. Meanwhile, the scutellarin group possesses the typical histological structure of the testicles, with an increase in the quantity of germ cells and spermatozoa in the majority of seminiferous tubules. The seminiferous tubules appear normal (Figure 6E and F).

In the present study, Jonson's score was used for the detection of graded testicular damage, with a score value as low as testicular damage as high. Rats in the induction group showed a significant reduction ($P = 0.001$) in the mean of Jonson's score compared to the control group. On the other hand, rats in the scutellarin groups showed a significant elevation ($P = 0.001$) in score means compared to the induction group Figure 7.

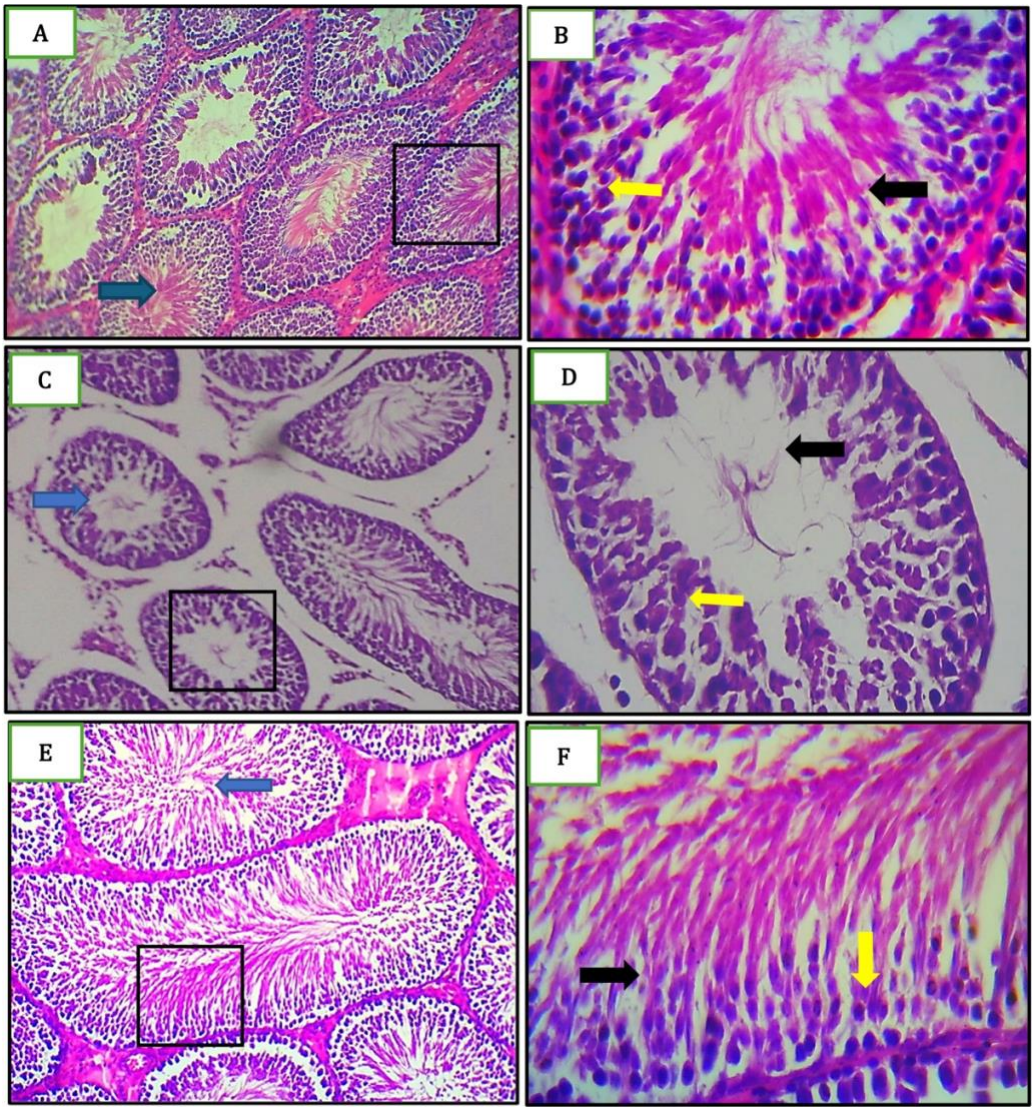

Figure 6. The histological section of testicular tissue in rats stained with H&E. A, B=Control group, C, D=Induction group, and E, F= Scutellarin group. Blue arrows denote the tubular lumen, black arrows the presence of sperm, and yellow arrows the epithelial layers of the lumen. A, C, and E= 10X magnification, B, D and F= 40X magnification.

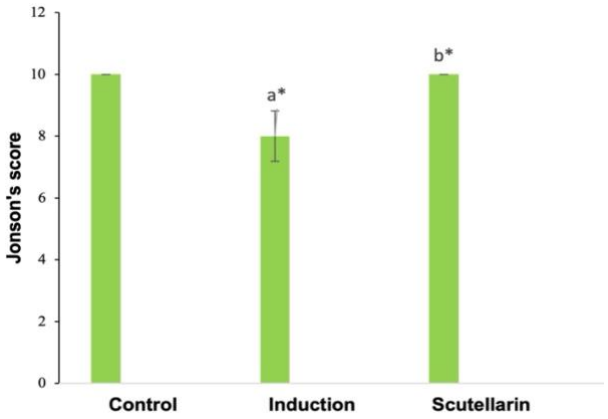

Figure 7. Effect on Jonson's score among studied groups. Data were expressed as Mean ± SD. a: comparison with Control group; b: comparison with Induction group; and *P significant at level <0.05.

DISCUSSION

The GSI was used for monitoring the ratio of gonad size to total body weight. It is used as a tool for measuring sexual maturity and function in correlation to reproductive organs, and it can also reflect gonadal damage [15]. In the current study, administration of CP in a single dose of 100mg/kg IP significantly reduced GSI compared with the control group. These results were consistent with previous studies by Abd El Tawab et al. (2014) and Afkhami-Ardakani et al. (2017), which confirmed that the administration of a single dose of CP to rats can reduce body and gonad weight when compared to the untreated group, thus reducing GSI [23, 24]. According to Kasim et al. (2021), a reduction in testis weight is caused by testicular atrophy and/or shrinkage of the epididymis [25]. The mechanism by which CP and MDA induces organ toxicity was not completely clear, but they thought it was due to oxidative stress and the generation of toxic ROS [26]. Testicular and caudal weight depend mainly on the density of spermatogenic cells, which affects the overall mass of the seminiferous tubule, and its reduction by ROS indicates a severe decrease in sperm production and Leydig cell atrophy [27]. Co-treatment of scutellarin with CP in the current study significantly improves GSI when compared to the induction group. This improvement is supposed to be related to the effect of scutellarin on maintaining body weight and gonads weight by protecting the tissues from the toxic effects of ROS, thus preventing cell atrophy. These findings align with previous studies that observed a reduction in gonad and body weights in diabetic rats following the administration of a flavonoid [28]. The Long et al study (2015), found that scutellarin is a potent agent for the prevention and treatment of diabetes-associated reproductive disorders by reducing Bcl-2/Bax expression and inhibiting cell apoptosis [12].

The recommendations of WHO state that seminal fluid examination can predict male infertility by evaluating sperm parameters such as concentration, motility, and viability [29]. In the current study, administration of CP significantly reduced epididymal sperm concentration, motility, and viability compared with the control group; these results confirm the observation of Razak et al. (2019) study, suggesting CP induces reproductive toxicity, thus decreasing the sperm parameter [18]. Reportedly, CP exposure reduces sperm motility and viability. This is likely because it disrupts the function of the sperm flagellum, which is crucial for sperm cell movement, by rapidly losing intracellular ATP and impaired energy metabolism [30]. According to Freitas et al. (2017), the common source of energy for cellular metabolism is ATP, which is required for both sperm motility and fertilization [31]. The process of oxidative phosphorylation in the mitochondrial membrane produces ATP, which is then transported to the microtubules in order to propel sperm motility [32]. Therefore, decreased sperm motility may result from oxidative damage to mitochondrial DNA. Another thing that can affect sperm movement and survival is when ROS attacks make the sperm membrane less fluid, which can mess up the membrane pump and cellular Ca2+ homeostasis [33].

CP therapy leads to spermatogonial stem cell loss, which in turn causes long-term or permanent azoospermia [34]. Spermatogenesis fails and sperm production drops because these quickly dividing, differentiating spermatogonia are more vulnerable to injury by cytotoxic chemotherapeutic agents than the later-stage germ cells [34]. According to Singh et al. (2015), CP can hinder spermatogenesis by interfering with pituitary luteinizing hormone regulation, which in turn inhibits testosterone production in Leydig cells and causes low blood testosterone levels. Additionally, CP has the potential to directly harm the spermatogenic compartment and change the activity of the antioxidant enzymes in the testes [35]. Low levels of antioxidant enzymes

in semen are essential for the proper differentiation and development of spermatogonia cells into mature spermatozoa, as they protect these cells from ROS-induced damage [36]. Therefore, sperm structural defects and non-viable sperm production can result from inhibiting the activities of these antioxidant enzymes.

Scutellarin administration in the current study significantly attenuated the toxic effect of CP on the studied sperm parameters. These were consistent with the Khamis et al. (2023) study, which showed that the administration of flavonoid glycosides like hesperidin could improve sperm parameters in CP treated rats [37]. The reduction of lipid peroxidation, increased antioxidants in these tissues, and protection of the sperm from the damage caused by CP may be achievable through the protective potential of flavonoids in improving testicular function and maintaining the cellular components of DNA, RNA, nucleic acids, and lipid in the sperm membranes [35] .

Serum testosterone levels can serve as a diagnostic tool for male infertility and a predictive biomarker for testicular function [36]. According to the results of this study, there was a notable and statistically significant decrease in serum testosterone in the group that was treated with CP. This result was in line with, Bakhtiary et al. (2020), who reported that increased oxidative stress results in severe damage at the Leydig cell level, thus reducing their responsiveness to luteinizing hormone and directly inhibiting of testosterone production [38]. Testosterone plays a crucial role in the spermatogenesis process, including the division of germ cells, meiosis, and the formation of the blood-testis barrier [39]. In this study, the administration of 100 mg/kg of scutellarin significantly reversed the effects of CP on the studied testosterone level compared to the induction group. Studies indicate that flavonoid glycosides can reduce the oxidative stress induced by CP, which is a major factor contributing to hormonal imbalance and testicular damage [37]. By scavenging ROS and enhancing antioxidant defenses, flavonoid glycosides help maintain the integrity of Leydig and Sertoli cells, which are crucial for testosterone production and spermatogenesis, respectively [40]. This antioxidant action underscores the therapeutic potential of scutellarin for preserving male reproductive health during chemotherapy treatment.

Compared to histology alterations, biochemical markers of oxidative stress are considerably more sensitive and can be used as early indications of tissue damage [19]. In the present study, exposure to CP significantly raised testicular MDA while significantly reducing testicular GPx concentration in the induction group compared to the control group. The findings were consistent with those of Shabaan et al. (2021), who suggested that CP induced an imbalance in oxidant and antioxidant levels due to excessive ROS generation, resulting in oxidative stress status [41]. This occurs because the level of ROS within cells increases, resulting in the production of free radicals such as H_2O_2, hydroxyl radicals, and singlet oxygen. Two types of antioxidants, enzyme-based and nonenzymatic, are built into cells. They work to stop the growth of ROS by converting free radicals into harmless substances. Oxidative stress occurs when there is an imbalance in these configurations [23, 30].

The testis is particularly susceptible to oxidative stress due to its high polyunsaturated membrane lipid content. MDA is an indicator of lipid peroxidation, which is indicative of tissue injury caused by oxidative stress [42]. GPx functions as a detoxifying agent by engaging in the GSH redox cycle, wherein it transforms H_2O_2 and lipid peroxides into harmless substances. CP can increase the production of lipid peroxides, which can interfere with the activity of testicular GSH-dependent antioxidant enzymes [37], as reflected in the reduced GPx activity in this study.

In the current study, co-administration of scutellarin with CP significantly reduced the testicular concentration of MDA with a significantly elevated level of GPx compared to the induction group. This was in line with Sun et al. (2023), who reported that scutellarin has a protection effect against chemotherapy-induced cardiotoxicity like doxorubicin by reducing oxidative stress [43]. Scutellarin attenuates oxidative stress and neuroinflammation in cerebral ischemia/reperfusion injury through activation of Nrf2- signaling pathways [11]. Scutellarin has free radical-scavenging activity, highly effective antioxidant properties and cell protection from oxidative stress induced in the testicular tissue of male rats by diabetes mellitus [12]. These properties of scutellarin may protect testicular tissue from the toxic levels of ROS induced by CP.

Histopathological findings accompanied by testicular evaluation are an important index in detecting chemotherapy-induced testicular damage [28]. This study established that CP markedly induced damage to seminiferous tubules and spermatozoa, which is characterized by spermatogenic epithelial degradation, developed severe destruction of Leydig cells, and the appearance of multinucleated giant cells, ending with a defect in the formation of spermatids and mature sperms (Figure 6C and D). These facts are also demonstrated in the current study by elevating the testicular oxidative stress biomarker, the MDA level which may affect GSI and cause testicular shrinkage. According to the histologic location, damage to the Leydig cells can be proved by inhibition of the serum testosterone level that is labelled by impaired spermatogenesis and oligozoospermia and a decrease in the testicular Jonson's score, as verified in Figure 7. These findings concur with Hamzeh et al. (2019) and Fani et al. (2024) outcomes, who also showed a major decline in Jonson's score accompanied by an alteration in testicular tissue architectures after CP injection [19, 44].

Consistent with the present study, the co-treatment of scutellarin with CP significantly recovered the histo-morphologic integrity of the testis and Jonson's score within the tested doses, as shown in Figure 6E and F and Figure 7, where the CP-damaging effect on spermatogenic cells (Leydig and Sertoli cells) was reduced, and tubular architecture approximately returned to its normal appearance. Again, these findings support the results that showed improvements in testosterone levels and sperm parameters, as discussed above. Indeed, these data were in line with previous studies that used different agents to ameliorate the histopathological damage induced by CP, as reported by Khamis et al. (2023), who used hesperidin [37].

Despite numerous publications exploring scutellarin as an antioxidant therapy, this is the first study to use scutellarin to counteract cyclophosphamide as a testicular toxicity inducer. This study has several limitations, such as measuring the protective effects of scutellarin against a single dose of cyclophosphamide. For further research, the authors suggest evaluating its effects in a long-term administration of CP and with multiple doses, which may yield different results. Further research is necessary to measure the other sperm parameters, as this study does not show the effects of the studied agents on sperm morphology.

CONCLUSIONS

The present study suggests that the antioxidant activities of scutellarin, which have been shown to contribute to the improvement of testicular dysfunction induced by CP. Results of the present study suggest that scutellarin, an active natural flavonoid, has efficacy for preventive intervention on testicular dysfunction. This problem is critical for preserving fertility during chemotherapy with CP and other alkylating agents in the treatment of a broad spectrum of malignancies and autoimmune diseases. Therefore,

although the therapeutic potential of flavonoids (including scutellarin) appears to be highly promising, this should not lead to premature clinical translations.

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AUTHOR CONTRIBUTIONS

SAH: coordinated study recruitment, implementation and progress of this study and helped with data interpretation and manuscript drafting. FKG: supervised the study and made the final revision of the article.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

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