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Lecithin's antioxidant properties protect against cyclophosphamide-induced reproductive toxicity and infertility in male rats

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ABSTRACT

Background: Infertility is a prevalent problem that affects 15% of couples globally. Male infertility, which accounts for half of all cases, can be caused by several factors, including aging, drug use, genetic problems, and exposure to environmental toxins. Cyclophosphamide (CP) is a commonly utilized immunosuppressant and anticancer medication that can induce oxidative stress and harm the reproductive system of men. The beneficial effects of antioxidants in mitigating the CP-induced reproductive toxicities are well-documented. *Aim:* The aim of this study was to assess the effects of lecithin (as an antioxidant) against the CP-induced reproductive toxicity in male rats. *Methodology:* Thirty Sprague-Dawley male rats were divided into three groups: (i) the negative control group (that received sterile water via the intragastric route for 13 consecutive days), (ii) the positive control group (that received sterile water via the intragastric route for 5 days and, subsequently, a single, intraperitoneal dose of CP on day 6, followed by sterile water via the intragastric route for the next 7 consecutive days), and (iii) the lecithin-treated group (that received lecithin via the intragastric route for 5 days and, subsequently, a single, intraperitoneal dose of CP on day 6, followed by lecithin via the intragastric route for the next 7 consecutive days). *Results:* Lecithin treatment improved sperm parameters, plasmatic testosterone levels, and glutathione peroxidase, thereby preventing histopathological alterations in the rat testes. *Conclusion:* Lecithin demonstrated its potential protective effects against reproductive impairments and oxidative stress in the CP-treated rat group, and might prove promising for use in clinical practice as a protective agent against chemotherapy-induced male reproductive toxicity and infertility.

KEYWORDS

lecithin, cyclophosphamide, testicular damage, infertility, oxidative stress

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1. INTRODUCTION

Infertility is a prevalent problem that affects 15% of couples globally. Male infertility, which accounts for half of all cases, can be caused by several factors, including aging, drug use, genetic problems, and exposure to environmental toxins [1]. Cancer and its treatments, such as chemotherapy and radiotherapy, can cause infertility in men [2]. Cancer is a deadly illness that exhibits abnormal cell division, altered gene expression, and unchecked proliferation that results in the development of malignant

cells and the beginning of tissue invasion or metastasis. Different therapies are used to treat cancer, including hormonal therapy, immunotherapy, radiotherapy, chemotherapy, and surgery [3]. Young cancer survivors often experience azoospermia or prolonged and potentially permanent reductions in sperm counts, leading to infertility. Chemotherapeutic drugs can affect testicular tissues and growing spermatogonia that are mitotically active. The death of these cells leads to the depletion of the later stages of germ cells and to a decrease in sperm counts [4]. Cyclophosphamide (CP) is the most used immunosuppressant and anticancer drug in chronic and acute leukemia, lymphomas, multiple myeloma, bone marrow transplantation, rheumatic arthritis, and some immune-related diseases. Through the cytochrome P450 in the hepatocytes, CP metabolism generates active alkylating compounds such as 4-hydroxycyclophosphamide, aldophosphamide, phosphoramide mustard, andacrolein. Phosphoramide-mustard alkylates DNA, leading to DNA synthesis inhibition, preventing cell division, and inhibiting tumor cell growth. Many studies have shown that CP causes oxidative stress in the male reproductive system [5]. The toxic effects of CP are associated with increased oxidative stress and its interaction with DNA, leading to DNA defects, abnormal cell functions, and cell death [6]. CP toxicity is not specific to cancer cells and will affect all dividing cells, including those in the immune and reproductive systems. In fact, CP interferes with sperm differentiation in the testicles and may cause infertility in males. Studies have shown that CP treatment in male rats causes significant increases in oxidative stress and abnormal sperms and decreases the sperm concentration and motility, the testosterone levels, and the testicular antioxidant capacity. However, due to reactive oxygen species (ROS) and nitric oxide production, acrolein exerts toxic effects on the body's healthy cells; it leads to peroxynitrite production, thereby destroying intracellular lipids, proteins, and DNA. More than 50% of male patients receiving chemotherapy for various solid tumors suffer severe and irreversible seminiferous epithelial damage. Since many cancer patients are treated with CP before and during their reproductive years and cure rates for several types of cancer are high, infertility caused by CP is a very significant concern.

In an attempt to overcome the toxic side effects of anti-cancer drugs, some antioxidant agents were considered beneficial in decreasing oxidative stress. Antioxidants can reduce oxidative stress and protect against DNA damage, abnormal cell function, and cell death caused by CP. Soybean lecithin is a by-product from the processing of soybean oil that, apart from being a source of energy, also serves as an emulsifier and has the potential to facilitate fat absorption. Lecithin is also a polyunsaturated phosphatidylcholine (PPC), which is present in all biological membranes' energy, functional, and structural elements. Moreover, lecithin is a naturally occurring mixture of diglycerides of stearic, palmitic, linoleic, and oleic acids that links to the choline ester of phosphoric acid [7] (Figure 1). Notably, PPC plays a rate-limiting role in the activation of numerous membrane-located enzymes, including superoxide dismutase (SOD), which protect the cell membrane from damage by ROS. ROS-induced damage to mitochondrial DNA may also lead to reduced mitochondrial function. Lecithin can protect mitochondrial DNA, thereby preserving the age-related decline in mitochondrial membrane potentials and their activity [8].

Lipids play a crucial role in the structural and functional aspects of sperm, while physiological processes and diet are known to influence their composition. The human body cannot produce the n-6 and n-3 polyunsaturated fatty acids (PUFA) alone, because they lack the necessary fatty acid desaturase enzymes. Therefore, the n-6 PUFA linoleic acid and the n-3 PUFA alpha-linolenic acid must be obtained through diet. These fatty acids are essential for various bodily processes such as growth, reproduction, vision, and brain development. There is considerable evidence that PUFA supplementation can influence biosynthetic pathways involved in prostaglandin synthesis and steroidogenesis, which play multiple roles in regulating reproductive function [9].

This study aimed at investigating the potential protective effects of lecithin against CP-induced male infertility in rats, by examining alterations in sperm parameters and testicular histology in order to determine their efficacy in mitigating adverse reproductive outcomes.

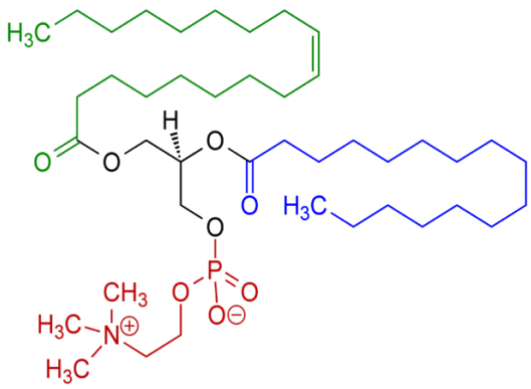

Figure 1. The chemical structure of lecithin.

2. METHODOLOGY

2.1. Chemicals and drugs used

The CP (CAS NO. 6055-19-2) and lecithin (CAS N0. 8002-43-5) were sourced from Hangzhou Jinlan Pharmaceutical Technology (China), and prepared as a solution before each use. Testosterone and malondialdehyde (MDA) were obtained from the Cloud-Clone Corp. (USA), while glutathione peroxidase (GPx) was procured from Elabscience (USA).

2.2. Animals used

Thirty, previously untreated, male Sprague-Dawley rats (weighing 280-350 g; aged six-weeks-old) were obtained from the Al-Nahrain University College of Pharmacy. The rats were certified as pathogen-free and were maintained under specific pathogen-free conditions. The animals were not genetically modified, nor had undergone any previous experimental procedures prior to this study. The rats were housed in experimental cages measuring 20×25×35 cm, with the bedding being replaced twice a week using wood chips. The rats were given free access to food and water. The animal housing facility at the Biotechnology Centre of the University of Al-Nahrain maintained a 12-h light-dark cycle with proper ventilation and a temperature of 23°C (± 2°C). Before the initiation of this study, all rats were given one week to acclimate to their new environment. The study was approved by the Research Ethical Committee of the College of Medicine of the Al-Nahrain University (approval number: UNCOMIRB20240509; date: 17-Sep-2023).

2.2.1. Inclusion and exclusion criteria for the male rats

The fertility of the male rats was evaluated by introducing them to untreated females. Healthy rats have a well-groomed appearance, bright eyes, and smooth hair coat. Male rats should have a scrotal sac, and their penis should have a long, cylindrical body and a bulbous glans penis. Rats displaying signs of illness or pain (such as reduced alertness, ruffled hair, hunched posture, ocular or nasal discharge, abnormal feces, decreased activity, or mucoid discharge) were not used in the study. Rats displaying abnormal or aggressive behavior, those medicated, and those injured were also excluded from the study.

2.2.2. Randomization and blinding of the used animals

The allocation of the rats to the experimental groups involved randomization [10]. A computer-based random number generator was utilized in order to create the randomization sequence. Each experimental unit (male rat) was assigned a number and then randomly arranged to determine group allocation. This method ensured the unbiased assignment of experimental units to each group. The study employed several strategies so as to prevent potential confounding factors. These included randomized treatment orders and measurements, rotating cage positions weekly so as to avoid the effects of any environmental conditions, and blinding the researchers to the group allocations. The individual generating randomization sequences was aware of the allocations but had no other role in the experiment. Researchers conducted treatments without knowing the assigned groups, and assessors used the same coded identifiers throughout the experiment. Data sets were labeled with codes and only decoded after analysis. These measures were implemented in order to minimize potential bias and ensure accurate results.

2.3. Experimental design, process, and sampling

The 30 male rats were randomly allocated into three groups (n=10 in each): (i) the negative control group in which the rats received sterile water via the intragastric route for 13 consecutive days, (ii) the positive control group in which the rats received sterile water via the intragastric route for 5 days and, subsequently, a single, intraperitoneal dose of CP (200 mg/kg) [11] on day 6, followed by sterile water via the intragastric route for the next 7 consecutive days, and (iii) the lecithin-treated group in which the rats received lecithin (300 mg/kg) [12] via the intragastric route for 5 days and, subsequently, a single, intraperitoneal dose of CP (200 mg/kg) on day 6, followed by lecithin (300 mg/kg) via the intragastric route for the next 7 consecutive days). On day 14 of the trial, the animals were anesthetized with a ketamine (10%) and xylazine (10%) mixture [13,14]. Blood samples were collected from a cardiac puncture with 5 mL syringes for the hormonal assay. The serum samples were obtained by using a centrifuge at 3,000 rpm for 5 min, and hormonal tests were conducted as previously described [15]. Moreover, a testis was taken for histopathologic assessment (maintained in formalin 10%), and the other was collected

for the undertaking of quantitative measurements of oxidative and antioxidant markers by using ELISA assays (Elabscience, USA).

2.4. Testicular homogenization and ELISA assays

Each testicular sample weighed 100 mg and was homogenized in 900 µL of 0.1 M PBS at a pH of 7.4. The samples were then centrifuged at 4,500 rpm, for 15 min, at 4°C. The resultant liquid fraction was then gathered [16]. The levels of GPx and MDA in the homogenates were measured spectrophotometrically through ELISA assays (Elabscience, USA) with the use of an ELISA reader (Diagnostic Automation / Cortez Diagnostics®, California, USA).

2.5. Sperm analysis

The cauda epididymis of each rat was carefully isolated and cleaned so as to remove the fat pad. It was then finely chopped in a Petri dish with prewarmed (37°C) liquid paraffin and 1 mL of flushing solution. The dish was incubated at 37°C for 5 min in order to allow for the dispersion of spermatozoa. The WHO guidelines were used for the sperm analysis. Sperm counts were determined by loading approximately 10 µL of caudal epididymis fluid onto a cover slide in a hemocytometer for 7 min, evaluating different fields of counting chambers. Sperm concentration was expressed as millions per mL of liquid and was measured using a light microscope at a magnification of 400x [17]. Sperm motility was assessed by placing 10 µL of caudal epididymis fluid onto a clean slide covered with a coverslip and by manually evaluating it [17]. The percentage of motility incidence was then calculated [18]. Finally, sperm viability, the percentage of dead/live sperm, was determined by using the eosin-nigrosin staining technique [19].

2.6. Histopathological studies

The testicular tissues were fixed, dehydrated, and embedded in paraffin, following dissolution in a 10% formalin solution. Subsequently, 5-µm-thick sections were obtained and stained with hematoxylin and eosin. The evaluation of the spermatogenesis efficacy was performed by using the Johnsen score. The testicular injury was graded on a scale of 1 to 10, with “1” indicating the absence of seminiferous epithelium and “10” indicating complete spermatogenesis and intact tubules [20]. All assessments were conducted blindly with regard to the treatment group to which each sample belonged.

2.7. Statistical analysis

The data entry and analysis were conducted by using SPSS version 26. Normality tests revealed that the data were not normally distributed. Therefore, the Mann-Whitney test was utilized (instead of the independent *t*-test) in order to assess the mean differences between groups. Results were deemed statistically significant at a $p<0.05$.

3. RESULTS

Table 1 shows that the rats of the CP-treated (positive control) group displayed a significant increase in sluggish sperm motility, immotile sperm, and dead sperm percentages, and a significant decrease in sperm concentration, progressive sperm motility percentage, and viable sperm percentage, when compared to those of the negative control group. Moreover, the lecithin-treated group rats exhibited a significant reduction in sluggish sperm motility, immotile sperm, and dead sperm percentages, and a significant increase in sperm concentration, progressive sperm motility percentage, and viable sperm percentage, when compared with those of the positive control (CP-treated) group (Table 1).

Table 2 shows that the rats of the CP-treated (positive control) group exhibited a significant increase in the levels of MDA and a significant decrease in the levels of testosterone and GPx, when compared with those of the negative control group. On the other hand, the lecithin-treated group rats revealed a significant increase in the levels of testosterone and GPx, and a significant decrease in the levels of MDA when compared to those of the positive control (CP-treated) group (Table 2).

Finally, Table 3 shows that the rats of the positive control (CP-treated) group exhibited a significant decrease in the histopathology score (Johnsen score) when compared with that of the negative control group, while the lecithin-treated group rats displayed a significant increase in the same score when compared to that of the CP-treated (positive control) group rats. The histological examination of the negative control group revealed that the rat testicular tissues had a normal appearance, possessing intact spermatogenic cells, seminiferous tube characteristics, and epithelial layers (Figures 2.A1 and 2.A2). The CP-treated (positive control) group samples exhibited a substantial loss of Leydig cells, resulting in the inadequate development of spermatids and mature sperms, as illustrated in Figures 2.B1 and 2.B2. On the other

hand, rats treated with lecithin demonstrated a protection against the CP-induced testicular tissue injury, with the seminiferous tube gradually returning to its predicted shape (Figures 2.C1 and 2.C2).

Table 1. Comparison of the seminal fluid analysis findings among the studied groups. Notes: [a], statistically significant difference (p<0.05) from the negative control group; [b], statistically significant difference (p<0.05) from the negative control group; SD: standard deviation.

		Negative control group (mean ± SD)	**Positive control group** (mean ± SD)	**Lecithin-treated group** (mean ± SD)
Sperm concentration (×10⁶/mL)		66.14 ± 24.10	18.00 ± 12.22 [a]	64.57 ± 15.4 [b]
Sperm motility (%)	**Progressive** (%)	96.00 ± 1.15	7.00 ± 2.83 [a]	87.71 ± 3.59 [b]
	Sluggish (%)	0.57 ± 0.53	58.43 ± 8.70 [a]	3.29 ± 3.3 [b]
	Immotile (%)	3.43 ± 0.79	33.14 ± 7.90 [a]	8.29 ± 3.2 [b]
Sperm viability (%)	**Viable** (%)	96.00 ± 1.29	28.00 ± 8.41 [a]	94.43 ± 1.72 [b]
	Dead (%)	4.00 ± 1.29	72.00 ± 8.41 [a]	5.57 ± 1.72 [b]

Table 2. Comparison of gonadotropin and antioxidant levels among the studied groups. Notes: [a], statistically significant difference (p<0.05) from the negative control group; [b], statistically significant difference (p<0.05) from the negative control group; GPx, Glutathione peroxidase; MDA, malondialdehyde; SD: standard deviation.

		Negative control group (mean ± SD)	**Positive control group** (mean ± SD)	**Lecithin-treated group** (mean ± SD)
Gonadotropins	**Testosterone** (pg/mL)	147.93 ± 8.04	11.09 ± 1.93 [a]	42.04 ± 7.08 [a,b]
Antioxidants	**MDA** (ng/mL)	8.80 ± 0.52	123.23 ± 7.88 [a]	22.32 ± 1.39 [a,b]
	GPx (pg/mL)	967.68 ± 16.77	322.89 ± 16.42 [a]	410.77 ± 18.31 [a,b]

Table 3. Comparison of testicular histopathology (Johnsen) scores among the studied groups. Notes: [a], statistically significant difference (p<0.05) from the negative control group; [b], statistically significant difference (p<0.05) from the negative control group; SD: standard deviation.

	Negative control group (mean ± SD)	**Positive control group** (mean ± SD)	**Lecithin-treated group** (mean ± SD)
Johnsen score	10.00 ± 0.00	8.00 ± 0.82 [a]	10.00 ± 0.00 [b]

4. DISCUSSION

The discovery of successful chemotherapy regimens for patients with acute leukemia, malignant lymphoma, testicular cancer, and other neoplastic disorders has led to a range of new concerns regarding the long-term adverse effects of cancer treatment [2]. Since many patients with potentially curable cancers are under 35 years of age when diagnosed, the impact of cytotoxic chemotherapy on male fertility has become significant. Various strategies have been explored in order to protect male fertility from the harmful effects of cytotoxic drugs. It is recommended that before patients undergo chemotherapy, germ cell cryopreservation (or, in some cases, testicular sperm extraction) should be considered [21]. Ongoing research aims to find natural or synthetic compounds that can help maintain male fertility without reducing the effectiveness of chemotherapy [21]. Various factors influence the lack of a standardized treatment for male infertility caused by cytotoxic drugs. Research shows that the impact of cytotoxic therapy on male fertility is complex [22-25].

In the current study, hormonal and oxidative biomarkers were assessed because of their significant roles in evaluating and diagnosing reproductive issues. The chemical compound CP induces male infertility through various mechanisms. Research has indicated that CP leads to testicular damage by causing oxidative stress, lipid peroxidation, and apoptosis [23,24]. It disrupts normal spermatogenic structure, diminishes sperm count and motility, and increases sperm abnormalities

[25,26]. Additionally, CP affects testicular antioxidant enzymes, serum testosterone levels, and histological structures in the testes, ultimately resulting in infertility [27]. The drug's impact on rapidly dividing cells, including those in the testes, contributes to its infertility-inducing effects. Furthermore, CP's ability to alter hormonal balance also plays a role. In summary, CP's complex actions on testicular function and sperm parameters underscore its role in inducing male infertility.

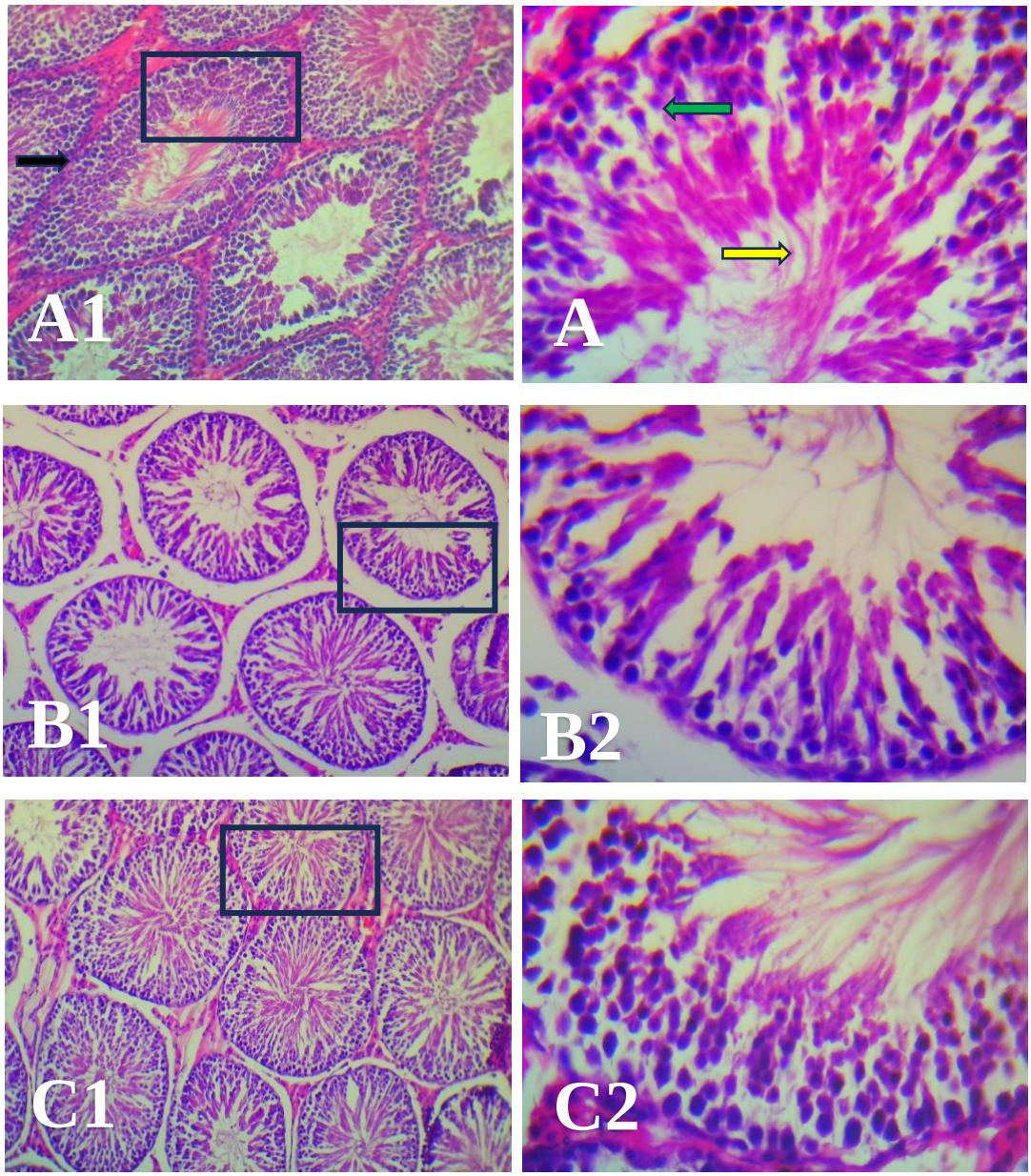

Figure 2. Representative histological sections of rat testicular tissues stained with hematoxylin and eosin; microphotographs A1 and A2 represent the negative control group; microphotographs B1 and B2 represent the positive control group; microphotographs C1 and C2 represent the lecithin-treated group. The black arrow indicates a seminiferous tubule, the green arrow highlights a Sertoli cell, while the yellow arrow shows a sperm cell. Magnification: 10x (for A1, B1, and C1) and 40x (for A2, B2, and C2).

According to the WHO guidelines, male infertility can be predicted through seminal fluid analysis by assessing sperm parameters, which depend on sperm concentration, motility, and viability [28]. Our study has found that CP significantly decreased sperm concentration, hindered sperm motility, and reduced sperm viability. Testicular damage caused by CP treatment is mainly due to increased generation of ROS: the elevated ROS levels can cause harm to the sperm plasma membrane, leading to lipid peroxidation, DNA fragmentation, and the depletion of mitochondrial ATP. These abnormalities in the sperm membrane disrupt membrane fluidity and impair sperm count, motility, and viability [29,30]. Similar observations were made by Elangovan *et al.* [31] and Kanno *et al.* [32], both of whom have shown that CP can reduce the sperm concentration and lead to the production of atypical sperm in rats. Furthermore, the CP-induced mitochondrial impairment in the sperm can damage the Na^+,K^+-ATPase and inhibit ATP synthesis, impair the sperm's flagellar motility, and reduce the ability of the sperm cells to fertilize [33].

The group receiving lecithin showed a significant increase in sperm concentration, progressive sperm motility, and viable sperm count, along with a notable decrease in sluggish sperm motility, immotile sperm, and dead sperm percentages, when compared to those of the positive control group. Lecithin, as a source of choline, is believed to indirectly impact spermatogenesis by influencing various metabolic pathways and cellular functions. The presence of choline in lecithin is essential for the cellular membrane structure and function, and it may support spermatogenesis by contributing to the formation and function of sperm cells. Additionally, choline's role in liver and brain function as well as its antioxidant activity suggest its broader impact on physiological functions, potentially also extending to reproductive processes like spermatogenesis [34].

Testosterone levels are considered an essential measure of spermatogenic activity and can be used to assess male reproductive health. Testosterone serum levels are predictive indicators of the testicular function and can help identify male infertility. In this study, the intraperitoneal delivery of CP was used in order to induce infertility, resulting in a substantial reduction in testosterone levels due to increased oxidative stress [23]. However, the group treated with lecithin exhibited a significant increase in testosterone levels when compared to the CP-treated (positive control) group; this can be attributed to the antioxidant effects of lecithin. Previous research suggests that antioxidant therapies can lead to an increase in testosterone levels [35].

Oxidative stress occurs when there is an imbalance between free radicals and antioxidants, leading to male infertility. SOD and GPx are essential markers of the body's ability to eliminate free radicals. The majority of the testicular GPx activity is attributed to Leydig cells. In contrast, the testicular SOD activity is linked to the seminiferous tubules, and is critical in the steroidogenic pathway of Leydig cells [36]. In this study, the treatment with CP resulted in a significant decrease in testicular GPx levels. CP can increase the generation of ROS, particularly superoxide radicals, while simultaneously reducing glucose-6-phosphate dehydrogenase (G6PD), glutathione reductase, GPx, vitamin C, and vitamin E levels [37]. As a result, lipid peroxidation increases, as evidenced by the elevated MDA levels. The suppression of the antioxidant system leads to an increase in hydrogen peroxide levels, which triggers the Fenton reaction and the production of hydroxyl radicals. These radicals have the potential to damage proteins, induce lipid peroxidation, and cause DNA damage, ultimately leading to the degeneration of the testicular tissues [37,38]. In sperm, GPx plays a protective role in maintaining the integrity of lipids within the sperm membrane; therefore, it may help protect the sperm from any damage caused by ROS and oxidative stress.

Interestingly, the administration of lecithin in our study led to a significant increase in GPx levels and a substantial decrease in MDA levels (as compared to those of the CP-treated / positive control group). These findings align with those of previous research indicating that lecithin can mitigate oxidative stress markers, including SOD. A study has also shown that lecithin can significantly increase the SOD activity in ethanol-induced immunomodulatory settings, highlighting its potential as an antioxidant [38].

Chemotherapy can exert detrimental effects on spermatozoa and the seminiferous tubules. The damage includes reduced tube layer thickness, significant loss of Leydig cells, and impaired spermatid production, leading to testicular atrophy [39]. Additionally, the same study [39] has reported suppressed serum testosterone levels, disrupted spermatogenesis, and the presence of oligozoospermia, along with a reduced Johnsen score. These findings are in line with the outcomes of Kohsaka *et al.* [40] and Yucel *et al.* [41], which have also demonstrated a decline in the Johnsen score and changes in the testicular tissue architecture following a CP injection in rats. In contrast, the experimental group treated with lecithin in our

study has exhibited a notable restoration of its testicular histomorphologic integrity and Johnsen score. Prior research has also shown the protective effects of other antioxidants against the CP-induced histopathological abnormalities in various organs [42-44].

Our study's limitations include difficulties in obtaining laboratory animals of the desired weight and age due to previous medications or interventions by other researchers. In order to address this, researchers could consider establishing selective breeding programs. Limited purchasing options have made the prompt acquisition of experimental materials, kits, and specialized chemical compounds challenging. Creating a university site in order to facilitate the researchers' access to resources and the ordering of inaccessible drugs from reliable sources could be beneficial. Furthermore, the limited funding available for the undertaking of research activities has posed challenges, but sharing expenses and resources could mitigate the need for new equipment or kits.

5. CONCLUSION

CP administration results in significant oxidative stress, leading to substantial damage to the testicular function and integrity of rats. Our study suggests that lecithin can protect against the CP-induced toxicity. Our findings suggest that lecithin might prove promising for use in clinical practice as a protective agent against chemotherapy-induced male reproductive toxicity and infertility.

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CONFLICT OF INTEREST STATEMENT
The authors declare no conflicts of interest.

REFERENCES

1. Vander Borght M., Wyns C.: Fertility and infertility: definition and epidemiology. *Clin. Biochem.* 62: 2-10 (2018).
DOI: 10.1016/j.clinbiochem.2018.03.012

2. Al-Rashidi R. R., Noraldeen S. A. M., Kareem A. K., Mahmoud A. K., Kadhum W. R., Ramírez-Coronel A. A., *et al.*: Malignant function of nuclear factor-kappaB axis in prostate cancer: molecular interactions and regulation by non-coding RNAs. *Pharmacol. Res.* 194: 106775 (2023).
DOI: 10.1016/j.phrs.2023.106775

3. Tarin D.: Causes of cancer and mechanisms of carcinogenesis. In: Tarin D.: Understanding Cancer: The Molecular Mechanisms, Biology, Pathology and Clinical Implications of Malignant Neoplasia. Cham: *Springer*, 229-280 (2023).
DOI: 10.1007/978-3-030-97393-3_9

4. Ghafouri-Fard S., Shoorei H., Abak A., Seify M., Mohaqiq M., Keshmir F., *et al.*: Effects of chemotherapeutic agents on male germ cells and possible ameliorating impact of antioxidants. *Biomed. Pharmacother.* 142: 112040 (2021).
DOI: 10.1016/j.biopha.2021.112040

5. Ebokaiwe A. P., Obasi D. O., Njoku R. C., Osawe S.: Cyclophosphamide-induced testicular oxidative-inflammatory injury is accompanied by altered immunosuppressive indoleamine 2,3-dioxygenase in Wister rats: influence of dietary quercetin. *Andrologia* 54(3): e14341 (2022).
DOI: 10.1111/and.14341

6. Mustafa Y. F.: Harmful free radicals in aging: a narrative review of their detrimental effects on health. *Indian J. Clin. Biochem.* 39(2): 154-167 (2024).
DOI: 10.1007/s12291-023-01147-y

7. Wendel A.: Lecithin. In: Kirk-Othmer (editor): Kirk-Othmer Encyclopedia of Chemical Technology - Fourth Edition. 15: 192-210 (2000).
DOI: 10.1002/0471238961.1205030923051404.a01

8. Ezzat S. M., Salem M. A., El Mahdy N. M., Mahfouz M. M.: Lecithin. In: Nabavi S. M., Silva A. S. (editors): Antioxidants Effects in Health: The Bright and the Dark Side. Amsterdam: *Elsevier*, 375-386 (2022).
DOI: 10.1016/B978-0-12-819096-8.00060-4

9. Burke J. M., Carroll D. J., Rowe K. E., Thatcher W. W., Stormshak F.: Intravascular infusion of lipid into ewes stimulates production of progesterone and prostaglandin. *Biol. Reprod.* 55(1): 169-175 (1996).
DOI: 10.1095/biolreprod55.1.169

10. Tveden-Nyborg P., Bergmann T. K., Jessen N., Simonsen U., Lykkesfeldt J.: BCPT policy for experimental and clinical studies. *Basic Clin. Pharmacol. Toxicol.* 128(1): 4-8 (2021).
DOI: 10.1111/bcpt.13492

11. Kaya C., Barbaros Baseskioglu A., Yigitaslan S., Yasemin Ozatik F., Ozatik O., Uslu S.: The therapeutic potential of amifostine on cyclophosphamide-induced testicular dysfunction in rats: an experimental study. *Int. J. Reprod. Biomed.* 17(4): 245-252 (2019).

DOI: 10.18502/ijrm.v17i4.4549

12. Li H., Wang X., Li X., Zhou X., Wang X., Li T., *et al.*: Role of soy lecithin combined with soy isoflavone on cerebral blood flow in rats of cognitive impairment and the primary screening of its optimum combination. *Nutr. Res. Pract.* 17(2): 371-385 (2023).
DOI: 10.4162/nrp.2023.17.2.371

13. Cooney K., Kipperman B.: Ethical and practical considerations associated with companion animal euthanasia. *Animals (Basel)* 13(3): 430 (2023).
DOI: 10.3390/ani13030430

14. Pierozan P., Jernerén F., Ransome Y., Karlsson O.: The choice of euthanasia method affects metabolic serum biomarkers. *Basic Clin. Pharmacol. Toxicol.* 121(2): 113-118 (2017).
DOI: 10.1111/bcpt.12774

15. Mnati M. A. A., Mshimesh B. A. R., Ibraheem M. M., Jasim S. Y.: Ameliorative effect of thiamine pyrophosphate against cisplatin-induced reproductive system damage within male rats. *Syst. Rev. Pharm.* 11(11): 396-403 (2020).

16. Mesbahzadeh B., Hassanzadeh-Taheri M., Aliparast M. S., Baniasadi P., Hosseini M.: The protective effect of crocin on cisplatin-induced testicular impairment in rats. *BMC Urol.* 21(1): 117 (2021).
DOI: 10.1186/s12894-021-00889-2

17. Raeeszadeh M., Karimfar B., Amiri A. A., Akbari A.: Protective effect of nano-vitamin C on infertility due to oxidative stress induced by lead and arsenic in male rats. *J. Chem.* 2021: 9589345 (2021).
DOI: 10.1155/2021/9589345

18. Cao X. W., Lin K., Li C. Y., Yuan C. W.: A review of WHO Laboratory Manual for the Examination and Processing of Human Semen (5th edition). *Zhonghua Nan Ke Xue* 17(12): 1059-1063 (2011).

19. Kanna S., Shetty A.: Eosin nigrosin staining technique in assessing sperm vitality in medical laboratories – a snippet from our experience implementing the staining, interpretation and quality control procedures. *Indian J. Obstet. Gynecol. Res.* 10(2): 227-229 (2023).
DOI: 10.18231/j.ijogr.2023.048

20. Roshankhah S., Gholami M. R., Salahshoor M. R.: Evaluation of male infertility treatment following *Rhus coriaria* extract administration on rats exposed to morphine. *Mol. Biol. Rep.* 47(8): 6073-6081 (2020).
DOI: 10.1007/s11033-020-05682-2

21. Rabaça A., Sousa M., Alves M. G., Oliveira P. F., Sá R.: Novel drug therapies for fertility preservation in men undergoing chemotherapy: clinical relevance of protector agents. *Curr. Med. Chem.* 22(29): 3347-3369 (2015).
DOI: 10.2174/0929867322666150821094334

22. Meistrich M. L.: Restoration of spermatogenesis by hormone treatment after cytotoxic therapy. *Acta Paediatr. Suppl.* 88(433): 19-22 (1999).
DOI: 10.1111/j.1651-2227.1999.tb14398.x

23. DeSantis M., Albrecht W., Höltl W., Pont J.: Impact of cytotoxic treatment on long-term fertility in patients with germ-cell cancer. *Int. J. Cancer* 83(6): 864-865 (1999).
DOI: 10.1002/(sici)1097-0215(19991210)83:6<864::aid-ijc33>3.0.co;2-e

24. Yamaguchi K., Fujisawa M.: Anticancer chemotherapeutic agents and testicular dysfunction. *Reprod. Med. Biol.* 10(2): 81-87 (2011).
DOI: 10.1007/s12522-011-0080-y

25. Blumenfeld Z., Haim N.: Prevention of gonadal damage during cytotoxic therapy. *Ann. Med.* 29(3): 199-206 (1997).
DOI: 10.3109/07853899708999337

26. Yadav S. K., Pandey A., Kumar L., Devi A., Kushwaha B., Vishvkarma R., *et al.*: The thermosensitive gene expression signatures of spermatogenesis. *Reprod. Biol. Endocrinol.* 16(1): 56 (2018).
DOI: 10.1186/s12958-018-0372-8

27. Mohammadi F., Nikzad H., Taghizadeh M., Taherian A., Azami-Tameh A., Hosseini S. M., *et al.*: Protective effect of *Zingiber officinale* extract on rat testis after cyclophosphamide treatment. *Andrologia* 46(6): 680-686 (2014).
DOI: 10.1111/and.12135

28. Xu Y., Lu H., Wang Y., Zhang Z., Wu Q.: Comprehensive metabolic profiles of seminal plasma with different forms of male infertility and their correlation with sperm parameters. *J. Pharm. Biomed. Anal.* 177: 112888 (2020).
DOI: 10.1016/j.jpba.2019.112888

29. Ibrahim D., Abozied N., Abdel Maboud S., Alzamami A., Alturki N. A., Jaremko M., *et al.*:

Therapeutic potential of bone marrow mesenchymal stem cells in cyclophosphamide-induced infertility. *Front. Pharmacol.* 14: 1122175 (2023).
DOI: 10.3389/fphar.2023.1122175

30. Klees C., Manzano N., Demeestere I., Lybaert P.: Impact of chemotherapy exposure using 4-hydroperoxycyclophosphamide on a male germ cell line (GC1-spg) and testes from the prepubertal mouse. *Hum. Reprod.* 38(s1): dead093.778 (2023).
DOI: 10.1093/humrep/dead093.778

31. Elangovan N., Chiou T. J., Tzeng W. F., Chu S. T.: Cyclophosphamide treatment causes impairment of sperm and its fertilizing ability in mice. *Toxicology* 222(1-2): 60-70 (2006).
DOI: 10.1016/j.tox.2006.01.027

32. Kanno T. Y. N., Sensiate L. A., de Paula N. A., Salles M. J. S.: Toxic effects of different doses of cyclophosphamide on the reproductive parameters of male mice. *Braz. J. Pharm. Sci.* 2009; 45(2): 313-319 (2009).
DOI: 10.1590/S1984-82502009000200017

33. Crouch M. L., Knowels G., Stuppard R., Ericson N. G., Bielas J. H., Marcinek D. J., *et al.*: Cyclophosphamide leads to persistent deficits in physical performance and *in vivo* mitochondria function in a mouse model of chemotherapy late effects. *PLoS One* 12(7): e0181086 (2017).
DOI: 10.1371/journal.pone.0181086

34. Marchesini G., Segato S., Stefani A. L., Tenti S., Dorigo M., Gerardi G., *et al.*: Lecithin: a by-product of biodiesel production and a source of choline for dairy cows. *Ital. J. Anim. Sci.* 11(2): e37 (2012).
DOI: 10.4081/ijas.2012.e37

35. Al-Rubiey F. K.: Effect of L-carnitine and meloxicam treatment on testicular Leydig cell numbers of varicocelized rats. *Middle East Fertil. Soc. J.* 17(1): 47-53 (2012).
DOI: 10.1016/j.mefs.2011.08.009

36. Pei J., Pan X., Wei G., Hua Y.: Research progress of glutathione peroxidase family (GPX) in redoxidation. *Front. Pharmacol.* 14: 1147414 (2023).
DOI: 10.3389/fphar.2023.1147414

37. Yahya R. A. M., Azab A. E., Shkal K. E., Attia A. M., Yehia M. A.: 5-Fluorouracil improves the testicular antioxidant status and alleviates oxidative stress in male albino rats treated with cyclophosphamide. *World J. Cancer Oncol. Res.* 1(1): 29-38 (2022).
DOI: 10.31586/wjcor.2022.492

38. Hernández-Montiel H. L., Vásquez López C. M., González-Loyola J. G., Vega-Anaya G. C., Villagrán-Herrera M. E., Gallegos-Corona M. A., *et al.*: Chronic administration of thiamine pyrophosphate decreases age-related histological atrophic testicular changes and improves sexual behavior in male Wistar rats. *Histol. Histopathol.* 29(6): 785-795 (2014).
DOI: 10.14670/HH-29.785

39. Sherif I. O., Sarhan O. M.: Candesartan in a rat model of testicular toxicity: new insight on its protective mechanism. *Exp. Biol. Med. (Maywood)* 244(7): 593-601 (2019).
DOI: 10.1177/1535370219842149

40. Kohsaka T., Minagawa I., Morimoto M., Yoshida T., Sasanami T., Yoneda Y., *et al.*: Efficacy of relaxin for cisplatin-induced testicular dysfunction and epididymal spermatotoxicity. *Basic Clin. Androl.* 30: 3 (2020).
DOI: 10.1186/s12610-020-0101-y

41. Yucel C., Arslan F. D., Ekmekci S., Ulker V., Kisa E., Erdogan Yucel E., *et al.*: Protective effect of all-trans retinoic acid in cisplatin-induced testicular damage in rats. *World J. Mens Health* 37(2): 249-256 (2019).
DOI: 10.5534/wjmh.180105

42. Elsayed F. F., Elshenawy W. M., Khalifa E. M., Rizq M. R., Abdelaziz R. R.: Ameliorative effect of flavocoxid on cyclophosphamide-induced cardio and neurotoxicity via targeting the GM-CSF/NF-κB signaling pathway. *Environ. Sci. Pollut. Res. Int.* 29(46): 69635-69651 (2022).
DOI: 10.1007/s11356-022-20441-5

43. Attia A. A., Sorour J. M., Mohamed N. A., Mansour T. T., Al-Eisa R. A., El-Shenawy N. S.: Biochemical, histological, and ultrastructural studies of the protective role of vitamin E on cyclophosphamide-induced cardiotoxicity in male rats. *Biomedicines* 11(2): 390 (2023).
DOI: 10.3390/biomedicines11020390

44. Salah H. M., Aboul-Ela E. I., Swelim H. H.: Ameliorative effect of peach seed extract on cyclophosphamide-induced cytogenetic and histological effects in mice. *Int. J. Genet. Genomics* 7(4): 136-147 (2019).
DOI: 10.11648/j.ijgg.20190704.19