Research Article

Effect of *Chelidonium majus* L. Root Extract on Cyclophosphamide-Induced Testicular Toxicity in Rats

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Abstract | Cyclophosphamide (CP) is a chemical drug that can induce infertility and have adverse effects on various organs, including male reproductive system. In the present investigation, we investigated the effect of oral administration of *Chelidonium majus* L. root extract on the testes of rats. Fifty adult male rate were divided into five equal groups, and each group received thirty days of treatment. first group (G1). Gave only distilled water (negative control group). second group (G2). These animals were administered CP at a dose of 14.2 mg/kg/BW (positive control group). third party (G3). Gave only 200 mg/kg/B.W. of Chelidonium majus alcoholic extract and 14.2 mg/kg/B.W. of cyclophosphamide were administered orally via gastric tube. Fourth group (G4): These animals were administered 300 mg/kg/B.W. of *Chelidonium majus* L. alcoholic extract and 14.2 mg/kg/B.W. of cyclophosphamide orally via gastric tube. Five groups (G5) Animals of this group were administered 400 mg/kg/B.W. of *Chelidonium majus* L. alcoholic extract and 14.2 mg/kg/B.W. of cyclophosphamide orally via gastric tube. *Chelidonium majus* L. roots extract significantly altered all tested parameters (sperm count, total testosterone levels, FSH, and LH) compared to the CP group (P 0.05). Root extract of *Chelidonium majus* L. can be administered. Increase in all parameters and decrease in CP's adverse effects on the testis.

Keywords | Herbal medicine, Biochemical indices, *Chelidonium majus* L. roots extract, Sperm count, Testosterone, FSH, LH and Cyclophosphamide

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INTRODUCTION

Cyclophosphamide (CP) has been used to treat breast cancer, ovarian cancer, melanoma, and non-Hodgkin's lymphoma. According to clinical studies (Falzone *et al.*, 2018), in addition to being an effective chemotherapy medicine for a variety of malignancies. it also has significant anti-proliferative effects, has been extensively tested throughout the world and the United States, and is considered a relatively safe drug It is an alkylating nitrogen mustard anticancer agent (Saha *et al.*, 2019). In addition, a prodrug that is transformed into the active metabolite in the liver (Takimoto and Calvo, 2005). It is promptly absorbed after oral administration and then converted to potent metabolites in the liver (cytochrome P450 system) (Petr *et al.*, 2011). A potent CP metabolite. Practically protein-safe and present in all tissues, it can cross the placenta and is considered a breast milk mediator. When

renal impairment is present, medication dosage can be adjusted appropriately because metabolites are primarily excreted unchanged in the urine (Huynh *et al*., 2020). In conjunction with other chemotherapeutic drugs, CP is used to prevent various forms of lymphomas, leukemia, some solid tumors, and brain cancer. It is a chemotherapeutic medication that causes some cells to die (T cells). The dose-dependent nature of CP's organic activities (Nelius, *et al*., 2010).

DNA harm active metabolites of CP include acrolein and phosphoramide mustard, both of which are cytotoxic alkylating agents and, respectively, are responsible for cell cytotoxicity. Phosphoramide mustard results in DNA cross-linking and cell demise. (Groehler *et al*., 2016). possesses potent immunosuppressive properties and is used to treat chronic autoimmune disorders like systemic lupus erythematosus, multiple sclerosis, and rheumatoid arthritis (McGaha *et al*., 2012). CP is a charity that treats malignancies and autoimmune diseases. It is utilised to quickly regulate the disease. Numerous adverse effects, including reproductive toxicity, are associated with CP, which has numerous clinical applications (Kasper *et al*., 2005). CP reduces luteinizing hormones, follicle-stimulating hormones, testosterone, and spermatogenesis, according to studies (Johari *et al*., 2011). The majority of chemotherapy medications harm healthy cells in a variety of ways. Cyclophosphamide possesses powerful anticancer properties. Gonadal toxicity is a prevalent side effect of cyclophosphamide therapy, which is used to treat a variety of diseases (Dollery, 1999). Because oxidative pressure causes biochemical and physiological damage, CP is deleterious to the testis and spermatozoa (Selvakumar *et al*., 2005a). *Chelidonium majus* L. (*Papaveraceae juss*) is a valuable herb that is extensively utilised in North America, Europe, Asia, and Northwest Africa. It is considered a native species in Latvia and is found as isolated specimens and extensive growths throughout the United States. In traditional medicine, C. majus has been used to treat bile and liver disorders, as well as warts, corns, fungal infections, dermatitis, and skin lesions. Additionally, plant latex has been applied topically (Barnes *et al*., 2007; Biswas, 2013). According to Latvian folklore, fresh latex and *Chelidonium majus* L. tea are used to treat diarrhoea, ocular problems, and skin diseases, as well as lichen and warts (Sile *et al*., 2020). In numerous ethnobotanical investigations throughout Europe, the treatment of ophthalmological, gastrointestinal, and skin disorders is mentioned (Menkovic *et al*., 2011; Fortini *et al*., 2016; Kujawska *et al*., 2017). The European Medicines Agency (EMA) has proposed potential therapeutic indications in the monograph for chelidonii herbal for the symptomatic relief of digestive issues such as dyspepsia and flatulence (oral administration) and for the treatment of warts, calluses, and corns (cutaneous application) (European Medicines Agency, 2021). Isoquinoline alkaloids isolated from Chelidonium majus extracts were discovered to be numerous of the most potently active molecules for bacteria, viruses, fungi, and protozoa. This disposition of compounds is trifling customarily flip phenanthridine, protoberberine, and protopine derivatives, roots are richer in these compounds (Zielin'ska *et al*., 2019), however the European pharmacopoeia (European Pharmacopoeia 9th Print Run, 2019) alone refers to the alkaloid qualification of herbs (which endeavour to yell be incomparably yon than zero to 6% and is calculated in phrases of chelidonine content material). In take care of take the approachable pamphlets, tribe is composed of calm down and numerous abbreviated alkaloids, while the aerial portions contain 0.1–1% alkaloids (excluding coptisine, which is abundant in sufficient quantities, particularly in fruits). Due to differences in their molecular structures, C. majus alkaloids exhibited synergy in modifying rudimentary activities (Zieli'nska *et al*., 2018) quaternary nitrogen in molecules, such as chelerythrine, sanguinarine, berberine, or coptisine, has been found to be enthusiastic arranged the check b determine of solution respiratory, to the fullest extent a finally the air of an imine moiety in the systems of sanguinarine and chelerythrine determines the ability of these compounds to inhibit the pastime of proteins and enzymes (Barreto *et al*., 2003). Cyclophosphamide has various adverse effects on different organs including the male reproductive system and can cause infertility. Therefore, it is used *Chelidonium majus L* roots in this to decrease side effects of drug.

MATERIALS AND METHODS

Preparation of *Chelidonium majus* L. roots extract

Chelidonium majus L. root hydroethanol alcohol extract was prepared according to the method described by (Chyad, 2017) using Soxhlet apparatus to heat extract plants at a concentration of 70%. The apparatus consists of several components: A heater, a glass flask for the solvent, an extraction unit set on top of the flask and containing a thimble-shaped cup of cellulose in which the *Chelidonium majus* L. root powder is placed, and a distillation unit. The final distiller passes chilly water through for condensation. The extraction procedure is initiated by heating the solvent to 50 °C in the glass beaker, and the vapour rises through a tube connecting the beaker and the distillation unit. The extraction unit has a cavity, and the solvent and plant materials dissolved in it are withdrawn to the beaker by syphoning. The procedure is repeated until the solvent collected in the extraction unit becomes transparent. Concentrate the extract in a vacuum rotary evaporator at 45°C, and then place the concentrate in Petridish containers to dry the powder. The extract was collected and

stored in a freezer at -20 degrees Celsius until use.

Animals

This study was conducted at the University of Baghdad's Department of Physiology, Biochemistry, and Pharmacology, College of Veterinary Medicine. 30 adult male mice, 3 months of age, evenly weighted between 190 and 220 g, were housed in a temperature range of 22 to 25 degrees celsius in a suitable environmental setting. Seven days prior to the experiment, the rats were confined in plastic cages in an animal house in order to acclimatize them. They had free access to standard pellets and potable water, under standard circumstances and according to university guidelines, all animals in each group were sacrificed and anesthetized according to the ethical guidelines of the Medical Ethical Committee of the National Research Centre in Iraq (IAEC, 2010).

Experimental design

Fifty adult male mice were divided equally into five groups; the period of treatment was thirty days, given orally by stomach tube as follows:

The first group (G1): The animals in this group were intubated with distilled water only (negative control group).

The second group (G2): This group's animals received cyclophosphamide at 14.2 mg/kg/B.W. (positive control group).

The third group (G3): This group's animals received *Chelidonium majus* L. root extract at a dose of 200 mg/kg/B.W. and 14.2 mg/kg/B.W. cyclophosphamide, given orally by stomach tube (Manikowska *et al* 2014).

The fourth group (G4): This group's animals received *Chelidonium majus* **L. root** extract at a dose of 300 mg/kg/B.W. and 14.2 mg/kg/B.W. cyclophosphamide, given orally by stomach tube (Naglaa *et al*., 2022).

The five group (G5): This group's animals received *Chelidonium majus* L. root extract at a dose of 400 mg/kg/B.W. and 14.2 mg/kg/B.W. cyclophosphamide, given orally by stomach tube (Manikowska *et al*., 2014).

The cyclophosphamide was given after 24 hours. *Chelidonium majus* L. root extract was administered once daily for thirty (30) days. At the completion of the treatment, the rats were anaesthetized by inhalation with diethyl ether and sacrificed (Hosseini *et al*., 2010).

Sperm concentration

Sperm counts were performed according to Sakamoto and Hashimoto (1986). Use a hemocytometer (Neubauer type). The side of the hemocytometer is filled with 5 µl of sperm suspension and covered with a cover slip; sperm are counted in 25 small squares of the chamber Sperm estimates are based on the following formula:

Sperm concentration = Number of sperm X 10000.

Blood collection

After the finish of the experiment, ketamine was used to anesthetize of animal. Each anesthetized animal's heart was punctured using a single-use insulin syringe to obtain blood samples. After 15 minutes of centrifugation at 2500 rpm, store serum samples in the refrigerator (-18°C) until use.

Serum testosterone levels

After samples were treated with I^{125} (a labelled testosterone tracer), the hormone testosterone levels were measured using a radioimmunoassay (RIA) kit, and the correlation between I^{125} and testosterone hormone was calculated in ng/mL using a gamma counter.

Serum FSH and LH levels

The determination of circulating serum concentrations of FSH and LH were done in each control and treatment group by the quantitative analysis using an enzyme - linked immunosorbent assay (ELISA Kits).

Statistical analysis

The statistical analysis of the data was performed using SAS (Statistical Analysis System, Version 9.1). Perform an ANOVA and post hoc least significant difference (LSD) test to determine significant differences between means. Results are expressed as mean, standard error, and percentage. P0.05 was considered statistically significant (SAS, 2014).

RESULTS AND DISCUSSION

Effect of *Chelidonium majus* L. Roots extracts on sperm count

It was observed clearly from Table 1 that sperm count was significantly decreased (P 0.05) in the treated group with cyclophosphamide (135124027.23) in comparison with the control negative group (214520038.27), while significantly increased (P 0.05) in the treated group with *Chelidonium majus* L. Roots extracts at doses 200mg (223143016.26), 300 mg (235324038.27), and 400 mg (2734328±12.12) as opposed to the control positive group treated with cyclophosphamide at dose 14.2 mg/kg (135124027.23).

Table 1: Effect *Chelidonium majus* L. Roots extracts on Sperm count, total testosterone levels, FSH and LH.

Group	Sperm count / ml	Total testosterone (ng/ml)	FSH (Iu/L)	LH (Iu/L)
Control (-ve)	2145200±38.27B	0.15± 1.83B	0.70± 13.24B	0.51±1.95B
Control (+ve)	1751240±27.23A	0.22±0.91A	0.47± 8.33A	0.26 ±0.95A
Ch. Majus roots (200mg/B.W.)	2231430±16.26B	0.90±1.79B	0.35±13.58B	0.65±1.84B
Ch. Majus roots (300mg/B.W.)	2353240±38.27B	0.44± 2.11BC	0.52± 15.69C	0.11± 2.33C
Ch. Majus roots (400mg/B.W.)	2734328±12.12C	0.28±3.35C	0.30±16.27D	0.11±2.79CD
LSD	2.6924	1.4506	1.9148	1.2899

Values were expressed as Mean ±SE., N=8,Values at the same column with different letters are significant at P<0.05

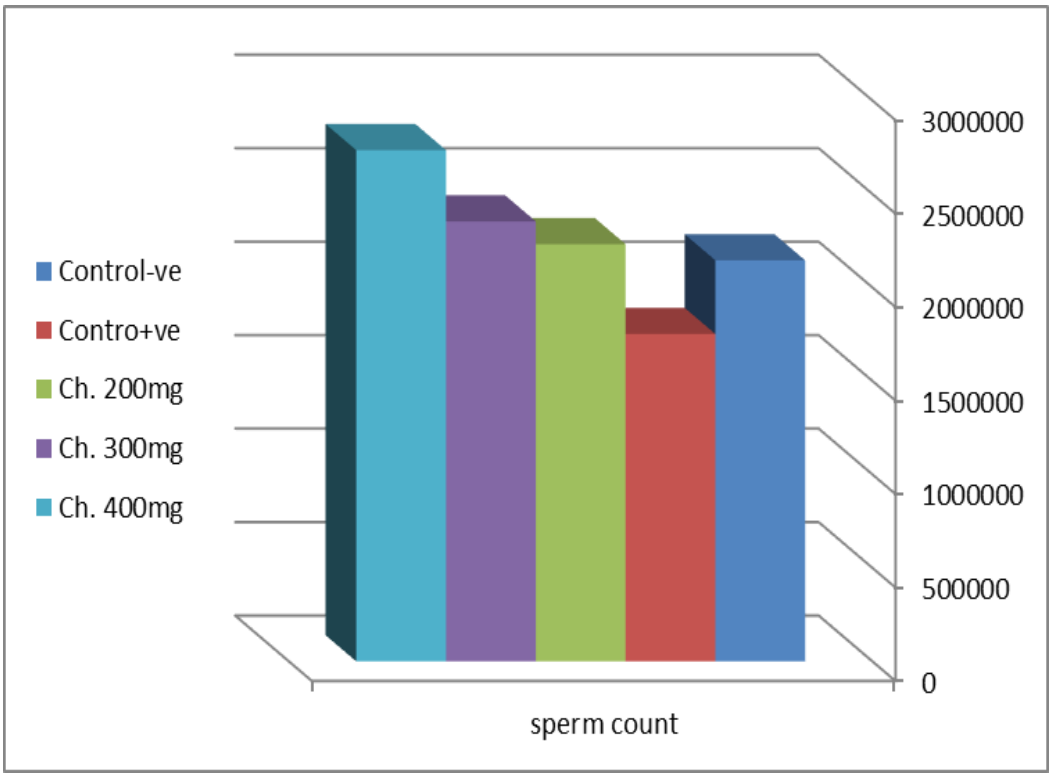

Figure 1: Effect *Chelidonium majus* L. roots extracts on sperm count, total testosterone levels (Mean±SE) (n=5).

Effect *Chelidonium majus* L. roots extracts on FSH

Effect of *Chelidonium majus* L. roots extracts on total testosterone levels

It was observed clearly from Table 1 that testosterone levels (ng/ml) were more effect and decrease (P 0.05) in treated animals with cyclophosphamide (0.91-0.22) in comparison with the control negative group (1.83 0.15) while significantly increasing (P 0.05) in the treated group with *Chelidonium majus* L. Roots extracts at doses of 200mg (1.97-0.90), 300mg (2.11-0.44), and 400 mg (3.35±0.28) as opposed to the control positive group treated with cyclophosphamide at dose 14.2 mg/kg (0.91-0.22).

It was observed clearly from Table 1 that FSH levels (Iu/L) were noticeably decreased (P 0.05) in treated animals with cyclophosphamide (8.33 0.47) in comparison with the control negative group (13.24 0.70), while significantly increased (P 0.05) in the treated group with *Chelidonium majus* L. Roots extracts at doses 200mg (13.58 0.35), 300 mg (15.69 0.52), and 400 mg (16.27 0.30) as opposed to the control positive group treated with cyclophosphamide at dose 14.2 mg/kg (8.33 0.47).

Effect *Chelidonium majus* L. roots extracts on LH

It was observed clearly from Table 1 that LH levels (Iu/L) were noticeably decreased (P 0.05) in treated animals with cyclophosphamide (0.95 0.26) in comparison with the control negative group (1.95 0.51) while significantly increasing (P 0.05) in the treated group with *Chelidonium majus* L. Roots extracts at doses of 200mg (1.84 0.65), 300 mg (2.33 0.11), and 400 mg (2.79 0.11) as opposed to the control positive group treated with cyclophosphamide at dose 14.2 mg/kg (0.95 0.26).

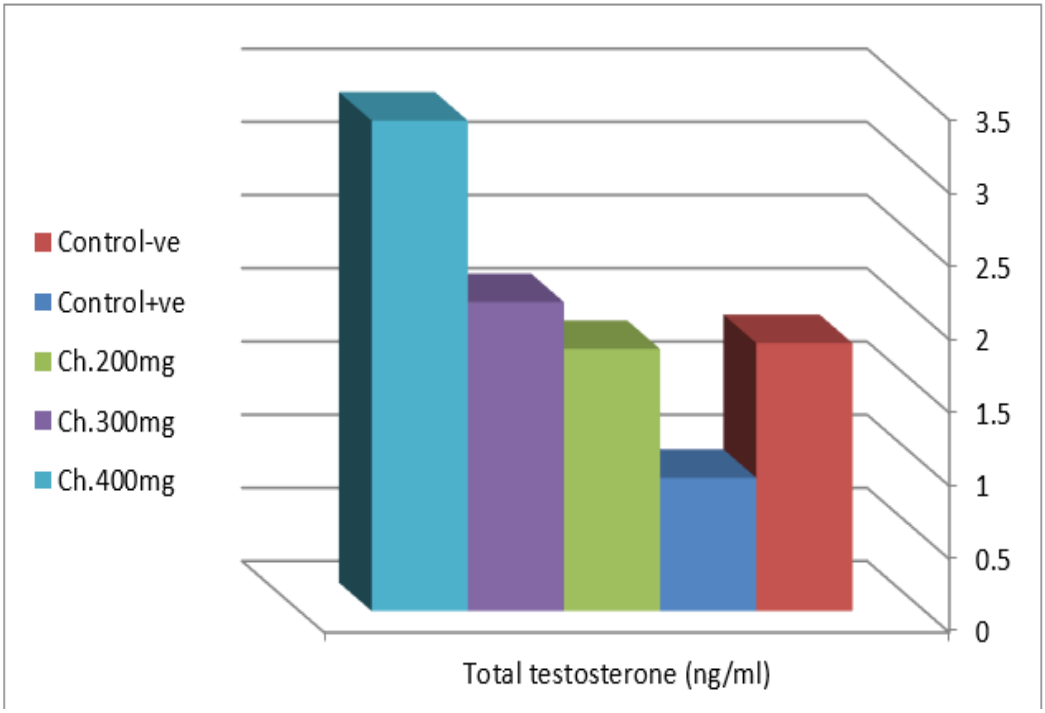

Figure 2: Effect *Chelidonium majus* L. roots extracts on total testosterone levels. (Mean ± SE) (n=5).

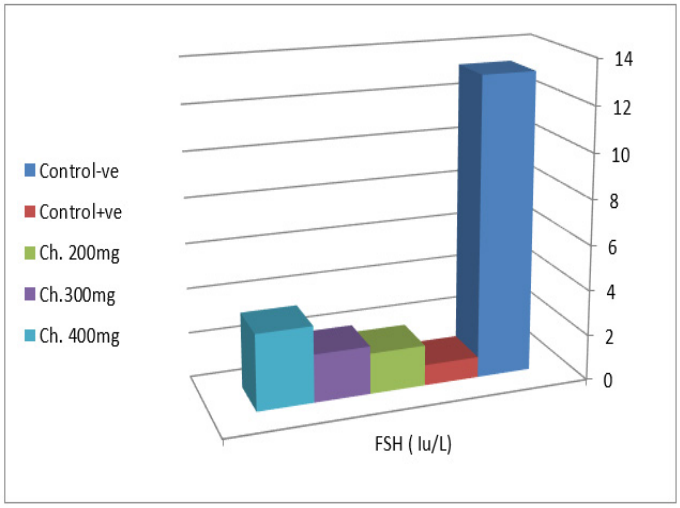

Figure 3: *Chelidonium majus* L. roots extracts on FSH levels. (Mean ± SE) (n=5). (Mean ± SE) (n=5).

Rats administered cyclophosphamide exhibited a significant reduction in sperm count, total testosterone levels, FSH, and LH. These findings are consistent with those reported by Ilbey *et al.* (2009) regarding the use of CP and other chemotherapeutic agents. The adverse effects of cancer treatments limit their effectiveness. Multiple studies have verified that CP's adverse effects include toxicity to reproduction (Mohammadi *et al.*, 2013, 2014). Cyclophosphamide (N, N-bis (2-chloroethyl) tetrahydro-2H-1, 3, 2-oxazaphosphorin-2-amine 2-oxide) is a nitrogenous mustard that belongs to the class of cytotoxic or cytostatic drugs (Aghaei *et al.*, 2014; Yu *et al.*, 1999). Traditional medicine employs CP extensively to treat a variety of diseases, including cancer. Cyclophosphamide (CP), the most extensively used immunosuppressive and anticancer drug, has a number of severe side effects, particularly on the reproductive system. Patients who require CP treatment have decreased fertility or infertility, which may affect their physical and emotional decisions to take the medication (Pavin *et al.*, 2018).

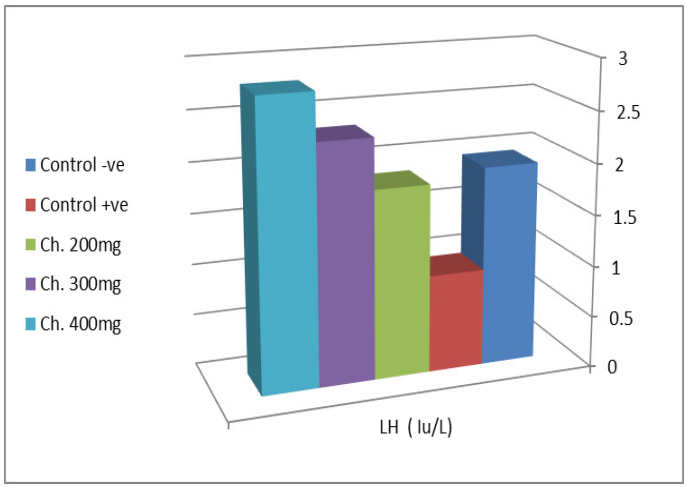

Figure 4: *Chelidonium majus* L. roots extracts on LH levels. (Mean ± SE) (n=5).

Consumers who consume CP may experience numerous side effects, including reproductive interference. Due to the demonstrated testicular toxicity in multiple animal species, CP has a limited therapeutic effect (Elangovan *et al.*, 2006). Oligospermia and azoospermia, as well as biochemical and histological alterations in the human and rat testis and epididymis, are associated with CP treatment

(Masala *et al*., 1997; Kirkland *et al*., 1976). In CP-treated rodents, the toxic effect was manifested by a significant decrease in the number of spermatogonia, spermatocytes, and spermatozoa, and our results support those studies describing irregular and diminished seminiferous tubules with few germ cells in the CP group (Ilbey *et al*., 2009). In addition, patients who received CP exhibited symptoms of gonadotropin secretion problems, testicular injury, and decreased plasma testosterone levels (Hoorweg-Nijman, 1992). Chinese celandine contains numerous compounds with distinct biological properties and chemical structures, including flavonoids, phenolic acids, and alkaloids such as chelidonine, chelerythrine, sanguinarine, berberine, prototropine alkaline, allocryptine, and coptisine, among others. Various pharmacological activities (antioxidative, immunomodulatory, anticancer, hepatoprotective, analgesic, etc.) have been observed in both the crude extract and the purified fraction of *C. majus* (Paul *et al*., 2013) *Chelidonium majus* L. root extract exhibited a considerable increase in all tested parameters (sperm count, total testosterone levels, FSH, and LH) when administered to rats. These results concur with those of Khodabande *et al.* (2017).

Vitamin C, on the other hand, is an antioxidant molecule that is water-soluble. Vitamin C decreases free radicals after tissue injury. It has been demonstrated that vitamin C reduces tissue injury and enhances tissue function (Kabirian *et al*., 2018).

The importance of vitamin C's anti-inflammatory properties cannot be overstated. It serves to reduce the tissue-damaging effects of reactive oxygen species (ROS) due to its inhibition of phospholipase A2 (Mohamed *et al*., 2013). In celandine, the primary secondary metabolites of flavonoids are phenolic compounds. Include antioxidant, anti-inflammatory, hypolipidemic, and anticancer activities (Lotito *et al*., 2006). Antioxidant compounds serve an essential role in protecting sperm in testicular tissue, and a deficiency of these compounds can result in infertility. In sperm and sperm-producing cells, this enzyme inhibits the harmful effects of DNA fragmentation (Milfard *et al*., 2011).

CONCLUSIONS AND RECOMMENDATIONS

In the treatment group, the use of *Chelidonium majus* L. root extract increased the values of the tested parameters, including sperm count, total testosterone levels, FSH, and LH. The group receiving cyclophosphamide had adverse reactions and side effects.

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NOVELTY STATEMENT

The novel study on the effect of *Chelidonium majus* L. Root Extract on cyclophosphamide-induced testicular toxicity in rats is an exciting development in the field of medicine and animal science. The potential of natural plant extracts to mitigate the adverse effects of chemotherapy is a fascinating avenue of research that could lead to improved outcomes for cancer cases.

AUTHRS' CONTRIBUTION

All the authors contributed equally.

Conflict of interest

The authors have declared no conflict of interest.

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