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Original Article

Effects of Hydroalcoholic Extract of *Hypericum Perforatum* on Spermatogenesis of BALB/c Male Mice Treated by Cyclophosphamide

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ABSTRACT

Background: *Hypericum perforatum* is a flowering plant from the Hypericaceae family. This herb has warm and dry nature based on traditional medicine and includes many healing properties like noticeable effects on mild and moderate depression cases. Cyclophosphamide is used to treat various types of cancers and autoimmune diseases with the side effect of fertility reduction. We studied the effects of the hydroalcoholic extract of *Hypericum perforatum* on the spermatogenesis process of cyclophosphamide-treated male mice.

Methods: In this experimental study, 70 adult BALB/c male mice weighing 26-28 g were randomLy divided into 7 groups of 10, including, a control group (no injection), a sham group (normal saline), and three experimental groups treated with 5 mg/kg body weight of cyclophosphamide, and also doses of 50, 100, and 150 mg/kg body weight of hydroalcoholic extract, respectively. In addition, a cyclophosphamide group (5 mg/kg body weight) and an extract group (100 mg/kg body weight) were evaluated. All the injections were performed intraperitoneally for 10 consecutive days. After two weeks, the blood samples were collected for a testosterone level examination. Testes and epididymis were removed for tissue sectioning and were stained with the H&E method. The data were analyzed by using one-way ANOVA and Tukey post-hoc test.

Results: The results showed that taking 5 mg/kg body weight of cyclophosphamide decreased mouse and testis weight and had a detrimental effect on the spermatogenesis process in the way that spermatogenic cell counts, and seminiferous and epididymis tubules diameter were decreased in comparison with the control and sham groups. However, 100 and 150 mg/kg body weight doses of the extract with 5 mg/kg body weight of cyclophosphamide showed a significant increase ($P \leq 0.05$ and $p \leq 0.01$) on the

mentioned parameters compared with the positive control group.
Conclusion: It seems that the main component in this herb such as hypericin could decrease the detrimental effects of cyclophosphamide on mouse fertility.

Keywords: Cyclophosphamide, BALB/c Mice, *Hypericum Perforatum,* Spermatogenesis, Testosterone.

1. Introduction

Herbal remedies have been widely used for treating diseases as most, but their usage was decreased and replaced by chemical drugs for a while. Nowadays, people are more aware of the harm of chemical drugs consequences to the complications of using chemical drugs. Therefore, the application of herbal remedies has been considered again.

One of the most widely used herbs in traditional medicine is *Hypericum perforatum* which is commonly found as an invasive weed in wheat and maize fields. This plant is used to treat tetanus disease, and if the leaf powder is sprayed on deep wounds, it helps the healing process [1]. Especially its oil has anti-ulcer, anti-burn, and anti-inflammatory effects. This herb is particulaly famous for its anti-gout, anti-rheumatic, and anti-depressant properties in traditional Iranian medicine [2-8]. The most crucial active ingredient of this herb is Hypericin, a red substance formed in the tiny black glands in various plant organs [9-11].

Cyclophosphamide is prescribed to treat many cancers like leukemia, lymphoma, bladder, breast cancer, and autoimmune diseases. The mechanism of the cyclophosphamide action is DNA alkylation. Like other alkylating agents, it binds an alkyl group to the DNA molecule and prevents DNA replication [12-14]. Clinical reports have indicated testicular germ cell damage in patients exposed to chemotherapy with cyclophosphamide for a limited period could finally lead to infertility or long-term genetic changes. Cyclophosphamide can cause the oxidative stress by disrupting the oxidation balance**.** Such biochemical stresses cause a disturbance in access to androgens and energy metabolism. On the other hand, stimulating apoptosis and inflammatory reactions can cause male reproductive system poisoning. Cyclophosphamide can reduce fertility and even cause infertility in humans treated with this drug [15-20].

Despite the widespread use of *Hypericum perforatum* in treating some diseases, especially depression, its effects on the male reproductive system have not been studied yet. Therefore, this study aimed to investigate these effects.

2. Materials and Methods

2.1. Hydroalcoholic extract preparation

Hypericum perforatum was prepared by Iran Genetic Resources Centre and dried in the open air under the shade, and then it was powdered by electric milling. After that, 500 g of the powder was dissolved in 70% alcohol, followed by the extraction process by using a Soxhlet extractor. The final extract was kept at 4 °C for the following experiments. 3 g of the extract was obtained from every 100 g of the dried herb powder.

2.2. Animals and experimental design

70 BALB/c male mice weighing 26-28 g were obtained from the Razi Vaccine and Serum Research Institute, Karaj, Iran. Every six animals were housed in a cage and kept in the standard conditions, including 06:00–20:00 h light, 22 ± 2 °C temperature, and 50 ± 5% humidity. The animals had free access to food and

water one week before and during the experiments.

All experiments were conducted with adherence to the National Research Council's Health Guide for the Care and Use of Laboratory Animals (NIH). Intraperitoneal Injection of herb doses was estimated according to the LD50 of *Hypericum perforatum,* which is 1111.47 mg/kg in mice, and the approximate daily drop intake for humans is 30 mL/day [21]. In this experimental study, 70 adult male mice were randomLy divided into 7 groups of 10, including a negative control group (no injection), a positive control group (5 mg/kg body weight cyclophosphamide), a sham group (normal saline), and three experimental groups treated by 5 mg/kg body weight cyclophosphamide and 50, 100, and 150 mg/kg body weight doses of the herb extract, respectively (Experimental 1-3). Likewise, the last group received only 100 mg/kg body weight of herb extract (Experimental 4). All the injections were performed daily for 10 consecutive days. Two weeks after treatments, the animals were weighed and anesthetized by ketamine and xylazine injection, and the blood samples were taken from the heart. Testosterone levels were measured by VIDAS test by using the radioimmunoassay method. The left testes were removed for macroscopic and microscopic studies, and the testes' diameter was measured by using a caliper and weighed with a sensitive scale. Then, the samples were fixed in formaldehyde (10%) for 48 hours and seven-micrometer sections of testes were prepared by using a microtome and stained by the H&E method. Histological assessment was performed through the light microscopy. Five sections were randomLy prepared from each group, and 5 fields were carefully examined in each section. The number of spermatogenic cells was counted and compared between different groups.

All data were analyzed by using SPSS23 software by One-way ANOVA, and Tukey post-hoc test. The significant level was considered as $P \leq 0.05$.

3. Results

In the positive control group and all the experimental groups, we distinguished a significant decrease in mouse body weight, testicular weight, testicular length, and width compared with the control and sham groups ($P \leq 0.05$ and $P \leq 0.001$). However, in comparison between experimental groups 2 and 3 with the positive control group, these groups revealed a significant increase in the mentioned parameters (Table 1). These results indicated that doses of 100 and 150 mg/kg body weight of hydroalcoholic extract could partially prevent the harmful effects of cyclophosphamide.

Examination of testicular tissue cells (Leydig, Sertoli, spermatogonia, primary spermatocyte, and spermatid) and the diameter of seminiferous tubules in the positive control group and experimental groups 1-3 showed a significant decrease compared with the control and sham groups ($P \leq 0.05$ and $P \leq 0.001$), (Table 2). Especially in the positive control group and experimental group 1 (50 mg/kg body weight of herb extract), vacuolation and creation of empty spaces in spermatogenic tubes were observed (Figure 1). However, in comparison between the effects of herb extract with the positive control group, an increase in the number of most cell lines, ($P \leq 0.001$) was detected except for the primary spermatocytes and spermatids. These results indicated that doses of 100 and 150 mg/kg body weight of herb extract had some protective effects against cyclophosphamide side effects as chemotherapy medication. It seemed that less effect of the hydroalcoholic extract on the two cell lines (the primary spermatocyte and spermatid) was due to

insufficient time for new cells proliferation from healthy spermatogonia, and perhaps with increasing the treatment time, the protective effect of herb extract on these lines could be seen. According to Table 1, the injection of the herb extract at the dose of 100 mg/kg body weight combined with 5 mg/kg body weight cyclophosphamide caused a significant increase in testosterone level compared with the sham, negative control, and positive control groups (P≤0.05).

Table 1. Body weight and testis characteristics after treatments

Groups	Negative control	Sham	Positive control	Exp. 1	Exp. 2	Exp. 3	Exp. 4
Body weight (gr)	6.75±1.73	3.50±1.67	0.125±0.93^{aa}	-4.50±1.64^{a}	4.12±2.72$^{aa, bb}$	2.87±0.89^{aa,b}	0.37±0.83^{aa}
Testis weight (gr)	0.087±0.00	0.07±0.011	0.05±0.004^{aa}	0.041±0.004^{aa}	0.061±0.012^{aa,b}	0.056±0.007^{aa,b}	0.066±003^{aa,bb}
Testis length (mm)	0.28±0.02	0.27±0.006	0.21±0.02^{aa}	0.2± 0.03^{aa}	0.24±0.01^{a,b}	0.24±0.005^{a,b}	0.25±0.003^{b}
Testis width (mm)	0.18±0.02	0.19±0.01	0.15±0.01^{aa}	0.15±0.01^{aa}	0.19±0.009^{aa}	0.14±0.008^{aa}	0.153±0.005^{aa}
Epididymis germinal layer (µm)	7±0.75	9±0.75	4±0.75^{aa}	8±0.53^{bb}	8±0.75^{bb}	9±1.06^{bb}	7±0.75^{bb}
Testosterone (ng/mL)	0.51±1.17	0.51±1.17	0.07±0.05	0.017±0.21	0.1±0.00$^{a, b}$	0.1±0.00	0.15±0.09

a: p<0.05 compared with the negative control and sham groups. aa: p<0.01 compared with the negative control and sham groups, b: p<0.05 compared with the positive control group, and bb: p<0.01 compared with the positive control group.

Table 2. Number of spermatogenic cells after treatments

Groups	Negative control	Sham	Positive control	Exp. 1	Exp. 2	Exp. 3	Exp. 4
Spermatogonia	53.8±2.5	54.00±5.0	19.8±5.0^{aa}	27.8±5.8^{aa}	43.2± 11.0^{bb}	45.5±3.8^{bb}	39.2±5.4^{a,bb}
Spermatocytes	42.67±10.3	38.3±8.2	19.3±5.5^{aa}	25.67±4.97^{a}	26.8±8.8^{a}	27.3±6.6^{a}	33.2±9.5
Spermatid	50.8±7.62	63.8±9.7	9.8±4.35^{aa}	13.7±4.36^{aa}	18.3±10.51^{aa}	18.5±2.34^{aa}	13.7±4.65^{aa}
Sertoli	5.2±0.75	6.0±1.3	2.8±0.75^{a}	4.0±0.9^{a}	4.3±0.52^{a}	5.7±0.82^{bb}	5.0±0.82^{b}
Leydig	9.8±0.68	5.8±1.00	4.92±2.17^{aa}	9.2±0.28^{b}	12.0±1.89^{bb}	5.2± 3.15^{a}	5.5±1.64^{a}
Diameter of seminiferous tubes (µm)	21.3±0.81	21.7± 0.51	13.7±0.51^{aa}	16.7±1.36^{aa}	18.7±2.58^{bb}	19.7±0.51^{bb}	21.2±3.1^{bb}

a: p<0.05 compared with the negative control and sham groups. aa: p<0.01 compared with the negative control and sham groups, b: p<0.05 compared with the positive control group, and bb: p<0.01 compared with the positive control group

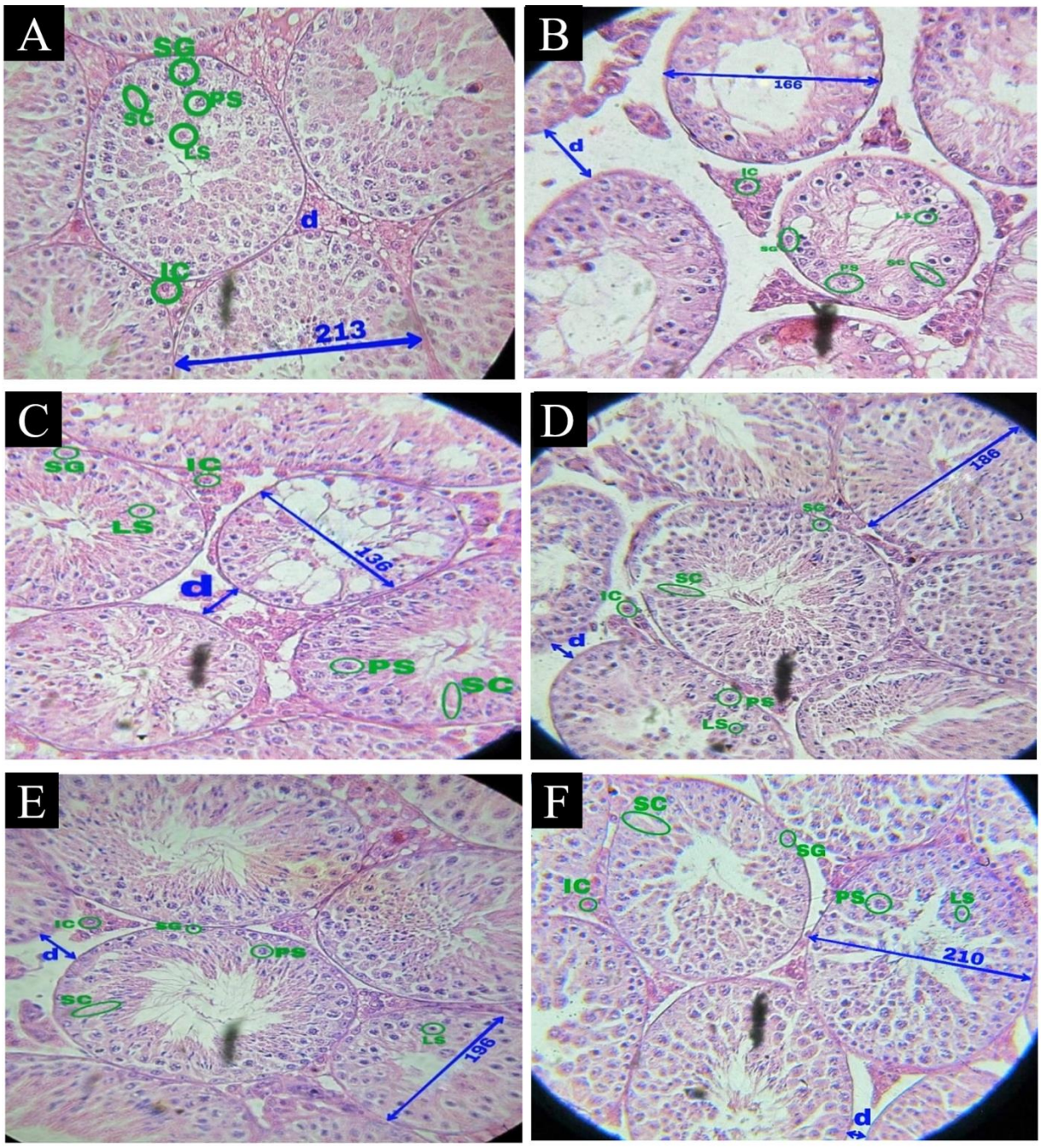

Figure 1. Cross-section of mice testis in different groups, H & E staining, 400 x; (A) control group, (B) positive control group, (C) Exp. 1, (D) Exp. 2, (E) Exp 3, and (F) Exp. 4. Spermatogonia (SG), Primary spermatocyte (PS), Spermatid (LS), Sertoli (SC), Leydig (IC), and Space between seminiferous tubes (d)

4. Discussion

Hypericum perforatum has many healing properties such as anti-inflammation and wounds decrescent, and also some internal diseases healer. Chemotherapy medicines, including cyclophosphamide, have destructive the effects on spermatogenesis and may cause infertility in men. This study revealed that doses of 100 and 150 mg/kg body weight of *Hypericum perforatum* hydroalcoholic extract could partly prevent the spermatogenesis damage

caused by cyclophosphamide in BALB/c male mice.

Doses of 100 and 150 mg/kg body weight of hydroalcoholic extract *Hypericum* per *15 foratum* could increase the body weight, testis weight, testis length, and width parameters compared with the cyclophosphamide-treated group. Likewise, we could see a significant increase in the number of spermatogenic cell lines except for the primary spermatocytes and spermatids in the extract-treated groups compared with the cyclophosphamide-treated group ($P \leq 0.001$). These results showed that doses of 100 and 150 mg/kg body weight of hydroalcoholic extract of *Hypericum perforatum* had some protective effects against cyclophosphamide side effects on the spermatogenesis process. It seemed that the short-length treatment period of *Hypericum perforatum* could be the reason for the lack of proliferation of the primary spermatocytes and spermatids cell lines from spermatogonia, and there was a possibility that a more extended length treatment period could have indicated a positive effect on the number of these cell lines.

In another study, the effect of *Hypericum perforatum* extract on the reduction of lung inflammation caused by cigarette smoke illustrated that this herb has a high resistance to the toxicity of cigarette smoke and can prevent lung damage caused by the smoke [22].

In another study, the effect of 1.457 g/kg *Hypericum perforatum* extract on the pregnancy of BALB/c mice showed that this herb could not cause any damage or fetal growth disorder [23].

In 2017 Hammami *et al.,* evaluated the effect of acute treatment with 200 and 400 mg/kg body weight of an aqueous and methanol extract of *Hypericum himifusum* on the testes and epididymis of Wistar rats. The results showed epididymis damage, emptying of seminiferous tubules, and sperm morphology changes. Furthermore, they reported that suppression of the antioxidant system was the reason for these disorders. However, their results were not compatible with our results which showed the positive effects of herb extract on spermatogenesis. It seems that the most probable reasons were the difference between herbal species and the period length and treatment methods [24].

Hypericum perforatum, as a flavonoid-rich herb, contains high levels of hypericin and hyperforin [25], which can prevent spermatogenesis from the side effects of cyclophosphamide and improve the spermatogenesis parameters compared with the positive control (5 mg/kg body weight cyclophosphamide) group.

In 2014, Haghighat *et al*. studied the hypericin effects on the ovulation of Wistar rats and demonstrated that hypericin had increased the follicular growth, the number of the Graafian follicle, corpus luteum, and improved ovulation. This study confirmed our results that in 100, 150 mg/kg body weight doses of *Hypericum perforatum* extract, spermatogenesis was improved and had positive effects on the reproductive process [26].

Overall, this study showed that *Hypericum perforatum* extract in moderate and high doses could prevent the side effects caused by cyclophosphamide on spermatogenesis of BALB/c male mice, which was probably due to flavonoid components existing in the extract.

Declarations

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The authors declare that there is no conflict of interest.

Authors' contributions

Mina Ramezani and Parvin Torabzadeh designed this study. Diba Zare and Fariba Ghalebi obtained and analyzed the data. Diba Zare, Fariba Ghalebi, Mina Ramezani, and Parvin Torabzadeh proceeded to the quality control and the manuscript drafting. Mina Ramezani revised the final version.

Consent for publications

All authors agree to have read the manuscript and authorize the publication of the final version of the manuscript.

Conflict declaration

The authors declare that there is no conflict.

Conflict of interest

None of the authors has any conflict of interest to declare.

Availability of data and material

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Ethics approval and consent to participate

The ethical approval was certified by Islamic Azad University Karaj Branch (Certificate No: IR.IAU.K.REC.1398.004).

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References

1. Altıparmak M, Eskitaşçıoğlu T. (2018). Comparison of Systemic and Topical Hypericum Perforatum on Diabetic Surgical Wounds. *J Invest Surg.*, 31(1): 29-37. [Crossref], [Google Scholar], [Publisher]
2. Butterweck V. (2003). Mechanism of action of St John's wort in depression: what is known? *CNS Drugs*, 17(8): 539-62. [Crossref], [Google Scholar], [Publisher]
3. Greeson J M, Sanford B, Monti DA.(2001) St. John's wort (Hypericum perforatum): a review of the current pharmacological, toxicological, and clinical literature. *Psychopharmacology* (Berl). 153(4): 402-14. [Crossref], [Google Scholar], [Publisher]
4. Neary J T, Bu Y. (1999). Hypericum LI 160 inhibits uptake of serotonin and norepinephrine in astrocytes. *Brain Res.*, 816(2): 358-63. [Crossref], [Google Scholar], [Publisher]
5. Stojanović N M, Radulović N S, Randjelović P J, Laketić D. (2016). Antinociceptive Properties of St. John's Wort (Hypericum perforatum) and Other Hypericum Species. *Nat Prod Commun*, 11(11): 1741-1747. [Crossref], [Google Scholar], [Publisher]
6. Süntar I P, Akkol E K, Yılmazer D, Baykal T, Kırmızıbekmez H, Alper M, Yeşilada E. (2010). Investigations on the in vivo wound healing potential of Hypericum perforatum L. *J Ethnopharmacol*, 127(2): 468-77.

[Crossref], [Google Scholar], [Publisher]
7. Wurglics M, Schubert-Zsilavecz M. (2006). Hypericum perforatum: a 'modern' herbal antidepressant: pharmacokinetics of active ingredients. *Clin Pharmacokinet*, 45(5): 449-68. [Crossref], [Google Scholar], [Publisher]
8. Zhou W, Yang S, Zhang Q, Xiao R, Li B, Wang D, Niu J, Wang S, Wang Z. (2021) .Functional Characterization of Serotonin N-Acetyltransferase Genes (SNAT1/2) in Melatonin Biosynthesis of Hypericum perforatum. *Front Plant Sci*, 12: 781717. [Crossref], [Google Scholar], [Publisher]
9. Linde K. (2009). St. John's wort - an overview. *Forsch Komplementmed*, 16(3): 146-55. [Crossref], [Google Scholar], [Publisher]
10. Rizzo P, Altschmied L, Stark P, Rutten T, Gündel A, Scharfenberg S, Franke K, Bäumlein H, Wessjohann L, Koch M, Borisjuk L, Sharbel T F. (2019). Discovery of key regulators of dark gland development and hypericin biosynthesis in St. John's Wort (Hypericum perforatum). *Plant Biotechnol J*., 17(12): 2299-2312. [Crossref], [Google Scholar], [Publisher]
11. Revuru B, Bálintová M, Henzelyová J, Čellárová E, Kusari S. (2020). MALDI-HRMS Imaging Maps the Localization of Skyrin, the Precursor of Hypericin, and Pathway Intermediates in Leaves of Hypericum Species. *Molecules*, 25(17): 3964. [Crossref], [Google Scholar], [Publisher]
12. Mills K A, Chess-Williams R, McDermott C. (2019). Novel insights into the mechanism of cyclophosphamide-induced bladder toxicity: chloroacetaldehyde's contribution to urothelial dysfunction in vitro. *Arch Toxicol*., 93(11): 3291-3303. [Crossref], [Google Scholar], [Publisher]
13. Korkmaz A, Topal T, Oter S. (2007). Pathophysiological aspects of cyclophosphamide and ifosfamide induced hemorrhagic cystitis; implication of reactive oxygen and nitrogen species as well as PARP activation. *Cell Biol Toxicol*., 23(5): 303-12 [Crossref], [Google Scholar], [Publisher]
14. Ogino M H, Tadi P. (2022). Cyclophosphamide, StatPearls Publishing, Treasure Island (FL). [Google Scholar], [Publisher]
15. Meistrich M L, Wilson G, Brown B W, Da Cunha M F, Lipshultz L I. (1992). Impact of cyclophosphamide on long-term reduction in sperm count in men treated with combination chemotherapy for Ewing and soft tissue sarcomas. *Cancer,*. 70(11): 2703-12. [Crossref], [Google Scholar], [Publisher]
16. Hao X, Anastácio A, Viñals-Ribé L, Lacuesta A S, Diakaki C, Alonso de Mena S, Liu K, Kenny A. (2021). Rodriguez-Wallbergcorresponding author. Follicle Rescue From Prepubertal Ovaries After Recent Treatment With Cyclophosphamide-An Experimental Culture System Using Mice to Achieve Mature Oocytes for Fertility Preservation. *Front Oncol,*. 11: 682470 [Crossref], [Google Scholar], [Publisher]
17. Özbilgin M. K, Demirören S, Üçöz M, Oztatlici M (2021). Cyclophosphamide suppresses spermatogenesis in the testis of mice through downregulation of miR-34b and miR-34c. *Andrologia,*. 53(7): e14071. [Crossref], [Google Scholar], [Publisher]
18. Liu X, Li Q, Wang Z, Liu F. (2021). Identification of abnormal protein expressions associated with mouse spermatogenesis induced by cyclophosphamide. *J Cell Mol Med*., 25(3): 1624-1632. [Crossref], [Google Scholar], [Publisher]

19. Abdul Razak R N H, Ismail F, Lokman M, Abdul Wahab A Y, Muhammad H, Ramli R, Raja Ismail R A S. (2019) .Ameliorative Effects of Aquilaria malaccensis Leaves Aqueous Extract on Reproductive Toxicity Induced by Cyclophosphamide in Male Rats. *Malays J Med Sci*, 26(1): 44-57. [Crossref], [Google Scholar], [Publisher]
20. Sadeghzadeh F, Sadeghzadeh A, Changizi-Ashtiyani S, Bakhshi S, Jalali Mashayekhi F, Mashayekhi M, Poorcheraghi H, Zarei A, Jafari M. (2020). The effect of hydro-alcoholic extract of Ceratonia Silique L. on spermatogenesis index in rats treated with cyclophosphamide: An experimental study. *Int J Reprod Biomed.*, 18(4): 295-306. [Crossref], [Google Scholar], [Publisher]
21. Mahmoudi M, Semnani Morteza K, Saeedi M, Javanmardi A. (2006). Anti-Inflammatory, Analgesic Activity, Acute Toxicity And Hypericins Content Of Iranian Hypericum Perforatum. *J. Babol. Univ. Med. Sci.*, 8(4): 7-14. [Google Scholar], [Publisher]
22. Kaplan H M, Doran F. (2017). Hypericum Perforatum Extract inhibits Cigarette Smoke Induced Lung Inflammation. *Biological and Chemical Research*, 35-43. [Google Scholar], [Publisher]
23. Mohseni Kouchesfahani H, Parivar K, Hayati Roudbari N. (2006). Surveying The Effects of Hypericum Perforatum on Balb/C Rats' Pregnancy. *Med. Sci. J. Islam. Azad Univ.*, 16(2): 79-83. [Google Scholar], [Publisher]
24. Hammami I, Ali R B, Nahdi A, Kallech-Ziri O, Boussada M, El May A, El Maya M V. (2017). Chronic consumption of Hypericum humifusum leaf extracts impairs epididymis spermatozoa characters in association with oxidative stress in adult male Wistar rats. *Biomed Pharmacother*, 93: 616-625. [Crossref], [Google Scholar], [Publisher]
25. Alahmad A, Alghoraibi I, Zein R, Kraft S, Dräger G, Walter J G, Scheper T. (2022). Identification of Major Constituents of Hypericum perforatum L. Extracts in Syria by Development of a Rapid, Simple, and Reproducible HPLC-ESI-Q-TOF MS Analysis and Their Antioxidant Activities. *ACS Omega*, 7(16): 13475-13493. [Crossref], [Google Scholar], [Publisher]
26. Najafzadeh H, Erfanimajd N, Gouraninejad S, Haghighat F. (2014) Evalution of Hypericin Effect on Rat Ovulation. *IJMAPR*, 29(4): 721-731. [Crossref], [Google Scholar], [Publisher]

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