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Original Article

Silymarin Mitigates Favipiravir Reproductive Toxicity

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ABSTRACT

Background: Silymarin, a flavonoid derived from milk thistle seeds and fruits, has been shown to have cytoprotective and hepatoprotective effects. Using a rat model, the study examines the protective or mitigating effect of silymarin against the adverse effects of favipiravir. This antiviral drug inhibits the RNA-dependent RNA polymerase (RdRp), including those responsible for SARS and COVID-19. The study focuses on the adverse effects of favipiravir, especially on the female reproductive system, and the role of silymarin in protection.

Methods: Fifteen adults female Wistar rats weighing 200–250) grams each were used in the investigation. The experiment spanned 14 days. Three groups of five rats each were randomly selected. Favipiravir was given intraperitoneally (IP) to the G1 group at a dose of 1800 mg/kg on the first day and 800 mg/kg for the next thirteen days. Second Group (G2): This group was given 50 mg/kg of silymarin intraperitoneally (IP) in addition to the same dosage as the G1 group. Normal saline was used to maintain the control group. On the last day, all of the animals were sacrificed, and the rats' ovaries and uteri were taken to be studied using immunohistochemical staining.

Results: The results highlight the harmful effects of Favipiravir on the ovary and uterus of the treatment group G1 by A high positive reaction of samples with the BCL2 antibody and a weak positive reaction with the Ki67 antibody while the mitigating role of silymarin in reducing these harmful effects is find in the G2 as indicated by the results of BCl2 and Ki67 Markers compared to the control group of the study.

Conclusions: Silymarin can mitigate the effect of favipiravir toxicity on reproductive tissue.

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Keywords: Silymarin, Favipiravir, Antiviral drug, BCL2, Ki67.

1 Introduction

Silymarin is a flavonolignan derived from the seeds and fruit of Silybum marianum [1] It possesses significant cytoprotective properties. Silybinin, the active fraction, exhibits antioxidant properties by scavenging free radicals and modulating the enzymatic system. Additional advantageous mechanisms encompass stabilization of cell membranes, stimulation of protein synthesis, and anti-inflammatory effects [2] Silymarin, also known as milk thistle, has been used for more than 2,000 years, mostly to treat liver conditions. With an emphasis on its anti-inflammatory and antioxidant qualities as well as its capacity to alter cellular signaling pathways, recent research has broadened its use to include a number of additional diseases. About 190 investigations on silymarin were published in 2018, demonstrating its considerable importance to the scientific community. Silymarin supplements are commercially available and have a good safety profile, but considering the need for customized dosage adjustments based on patient genotypes and particular diseases, as well as the lack of thorough clinical data on their effectiveness across various conditions, caution is advised when utilizing them [3].

A purine nucleoside analogue, favipiravir, is an antiviral drug that works effectively against a variety of RNA viruses, including influenza, Zika, Ebola, norovirus, and SARS-CoV-2 [4]. It does this by blocking RNA-dependent RNA polymerase (RdRp) [5]. The process involves the transformation into an active favipiravir-ribofuranosyl-5'-triphosphate form, which RdRp recognizes and incorporates into the elongating RNA strand. By acting as a false substrate, this form prevents viral RNA synthesis [6].

The pharmacological profile of favipiravir is favorable; however, it reveals toxicity in the liver and kidneys [7]. Cardiovascular system, gastrointestinal tract, and retina [8], among other organ systems. Teratogenic effects are also induced by it. Hematopoietic tissue disruption, shortened prothrombin time,

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cytoplasmic deficiency, and darkening in renal tubules [7], increased blood urea nitrogen levels, aberrant sperm morphology, and lower weights of testicular tissue [9]. Ovarian tissues are among the negative effects, according to animal research.

Throughout the reproductive cycle, the ovarian epithelium experiences dynamic morphological and molecular changes that are closely controlled by cellular homeostasis systems and hormonal signals. Diseases like endometrial hyperplasia, ovarian neoplasms, and endometrial cancer are characterized by a disruption of this balance, especially between cell proliferation and death.

BCL2, an anti-apoptotic protein encoded by the BCL2 gene, and Ki-67, a nuclear protein linked to cell proliferation, are two important biomarkers that represent these biological dynamics. Ki-67 is a good measure of mitotic activity since it is expressed in all active stages of the cell cycle (G1, S, G2, and mitosis) but not in resting cells (G0). BCL2, on the other hand, promotes cell survival despite environmental or genetic stresses by blocking programmed cell death [10-12].

The purpose of this study was to examine how favipiravir affects the reproductive system and how silymarin can help. Since the co-administration of silymarin with favipiravir, which targets ovarian or uterine protection, has not yet been the subject of any published trials.

2 Materials and methods

2.1 Drugs used.

Favipiravir (Fabiflu) is manufactured by Glenmark Pharmaceutical- India. Favipiravir is an antiviral medication that powerfully and selectively inhibits the RNA-dependent RNA polymerase (RdRp) of RNA viruses. It is useful against a variety of influenza virus types and subtypes, including strains that are resistant to existing antiviral medications [5].
Silymarin 50mg/mL. produced by Melanotan Express Company / Florida. USA

2.2 study animal

In this experiment, 15 adults female Wistar rats weighing (200–250) grams were employed. The rats were bought from the College of Pharmacy at Hawler Medical University's animal house laboratory. Rats were housed for ten days in clean polypropylene cages at 22–25 degrees Celsius with a 12-hour light/dark cycle, following normal standards for the care and management of laboratory animals. Following the ethics committee's approval of the study protocol, they were given unrestricted access to drinking water and were fed a consistent diet of a suitable laboratory formula.

2.3 Design of the study

Rats were randomly divided into three groups with five rats in each group. Rats in the control group (G3) were kept on normal saline. G1 group (Favipiravir group) provided the first day with Favipiravir 1800mg/kg once daily (OD) and the last thirteen days with Favipiravir 800mg/kg once daily (OD) intraperitoneal (IP) dose. G2 (Silymarin) was treated with the same dose of Favipiravir plus 50mg/Kg of silymarin Intraperitoneally (IP).

The experiment spanned 14 days, and on the last day, all the animals were sacrificed.

With 80 mg/kg of ketamine and 20 mg/kg of xylazine, the rats were anesthetized. Five rats from each group were dissected, and their ovaries and uteri were preserved in formal saline 10%. Blocks were formed using the formalin-fixed paraffin-embedded (FFPE) technique. Serial sections of 6 µm thickness were cut with a microtome and then stained for histological analysis using Ki67 and BCL2 antibodies, which are important indicators for comprehending ovarian and uterine cellular proliferation and apoptosis. The antibodies were used in compliance with the manufacturer's protocol instructions.

2.4 Ethical Considerations

The study was approved by the scientific committee of the department to which the author belongs, and then the study got approval from the head of the department and the ethical committee of the college deanship with reference number: HMUD/2526020- date of approval 26/10/2025.

3 Results

The results of the study show different expressions of these antibodies in the study group samples. The control group of the study reacts negatively to BCL2, as shown in Figure 1.

The control group of the study also reacted negatively to the ICH-KI67, as it appears in Figure 2.

A high positive reaction with the BCL2 antibody in the cytoplasm of luteal cells in the ovary, which are visible as golden-brown granules, indicates higher expression of the BCL2 protein, which is thought to be crucial for preventing apoptosis (programmed cell death), as demonstrated by the G1 study group's results (See Figure 3).

Also, the uterus of this group showed a strong positive reaction with the BCL2 antibody in the cytoplasm of the uterine glands, which appear as golden-brown granules (See Figure 4).

Immunohistochemistry of the ovarian section of experimental group G1 showed golden-brown patches, which were evidence of a significant positive reaction with the Ki67 antibody in the nuclei of developing ovarian follicles (See Figure 5).

On the other hand, poor proliferative activity seen in the G1 group's uterine glandular cells is indicated by a weak positive reaction with the Ki67 antibody within the nuclei, which appears

as golden-brown patches in immunohistochemistry (See Figure 6).

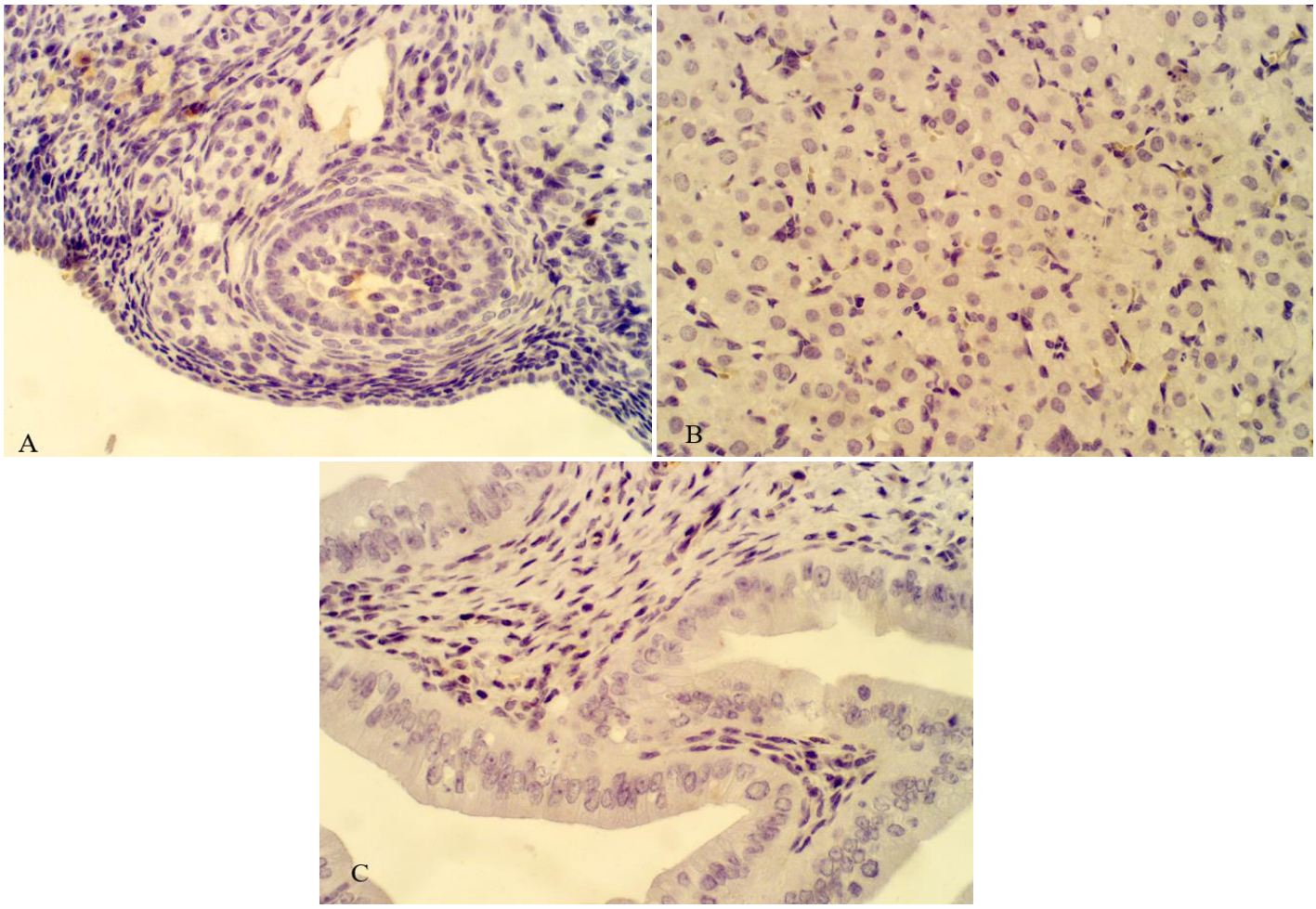

Figure 1: Shows a negative reaction of control group samples with *BCL2* antibody A- in the ovarian tissue and growing follicles. B -in the luteal cells. C-in the uterine glands and endometrium layer. IHC-BCL2-DAB stain. 400x.

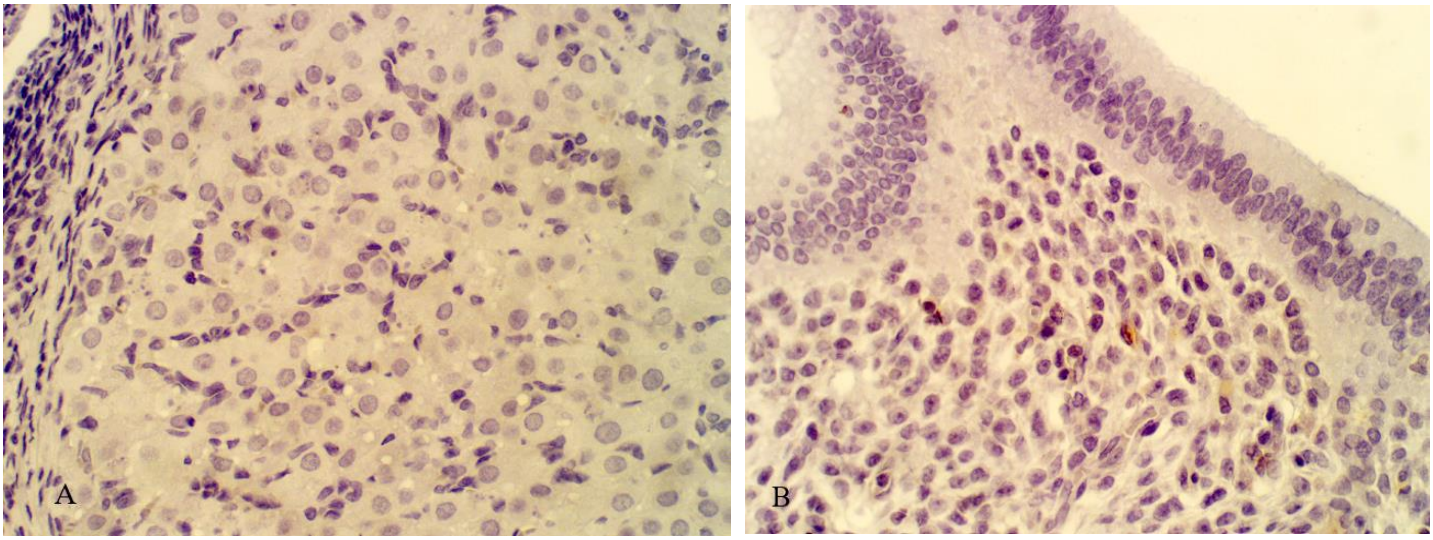

Figure 2: Shows a negative reaction of the control group samples with IHC-Ki67-DAB stain. A-in the luteal cells. B-in the epithelial cells of the uterine tissue.

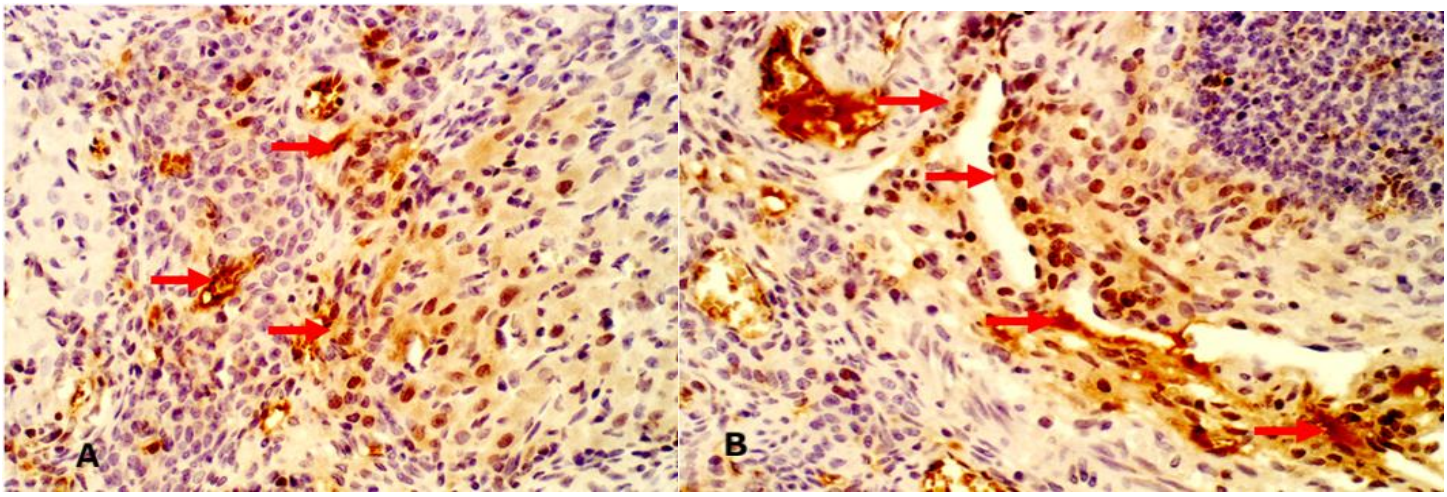

Figure 3: A &B showed a strong positive reaction with the BCL2 antibody in the cytoplasm of the luteal cells, which appear as golden-brown granules (arrow). IHC-BCL2-DAB stain (400x).

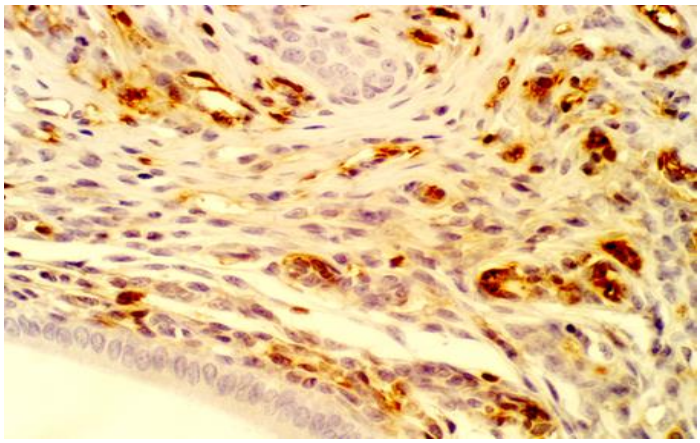

Figure 4: Uterus, slide of G1 group. Showed a strong positive reaction with the BCL2 antibody in the cytoplasm of the uterine glands, which appear as golden-brown granules. IHC-BCL2-DAB stain (400x).

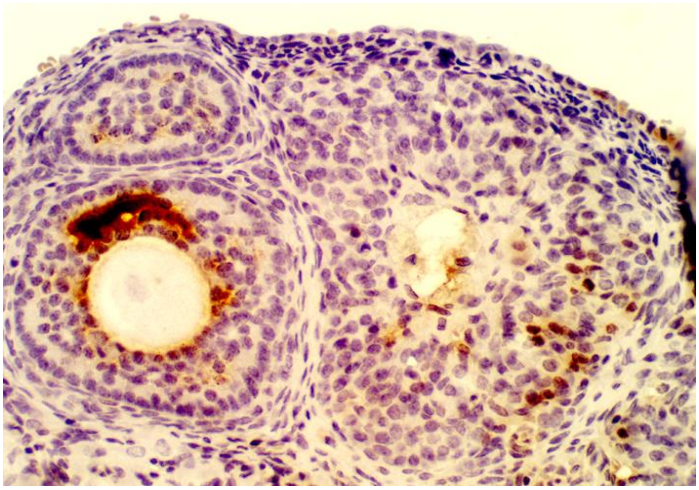

Figure 5: Ovary, G1 group. Showed a strong positive reaction with the Ki67 antibody in the nucleus of the growing follicles, which appear as golden-brown patches. IHC-Ki67-DAB stain (400x).

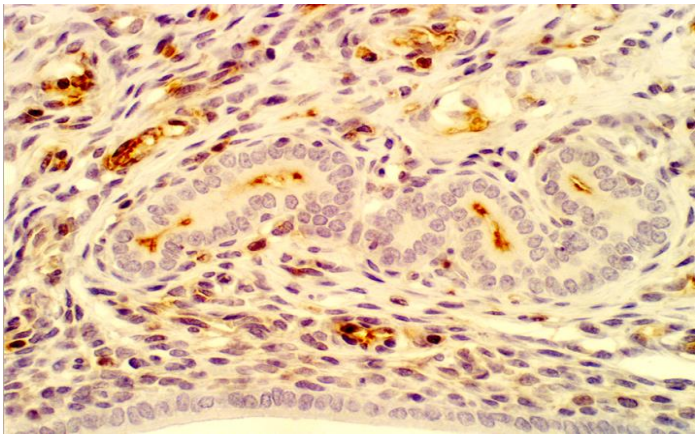

Figure 6: Uterus, G1group. Showed a weak positive reaction with the *Ki67* antibody in the nucleus of the uterine glands, which appears as golden-brown patches. IHC-*Ki67*-DAB stain (400x).

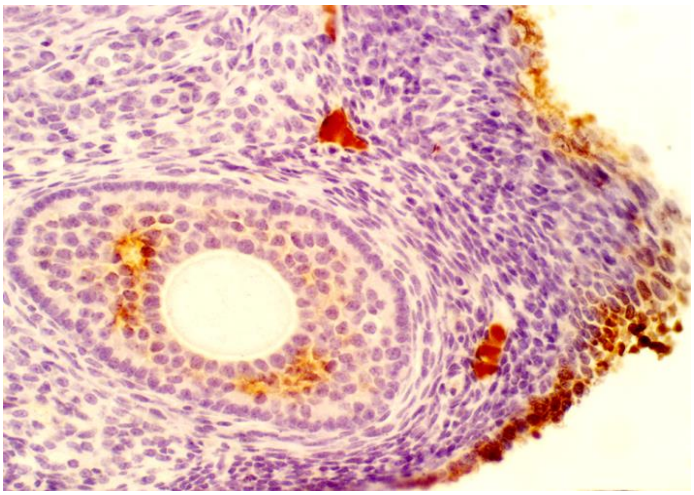

Figure 7: Ovary, G2 group. Showed a weak positive reaction with the *BCL2* antibody in the ovarian tissue and growing follicle as golden-brown cytoplasmic batches. IHC-*BCL2*-DAB stain (400x).

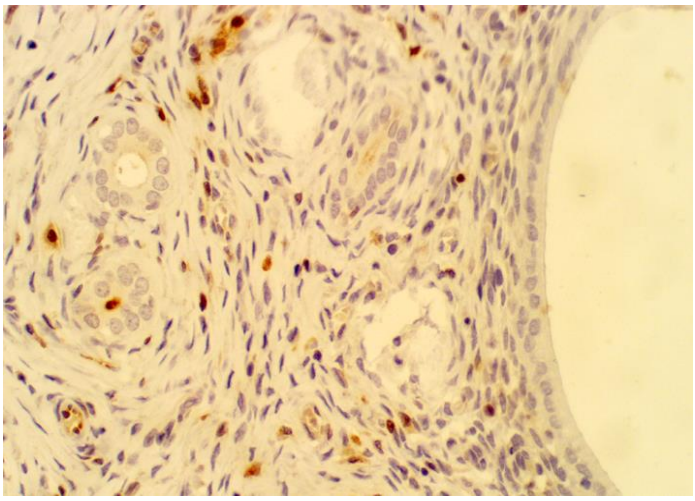

Figure 8: Uterus, G2 group. Showed a weak positive reaction with the BCL2 antibody in the cytoplasm of uterine glands as golden-brown batches. IHC-*BCL2*-DAB stain (400x).

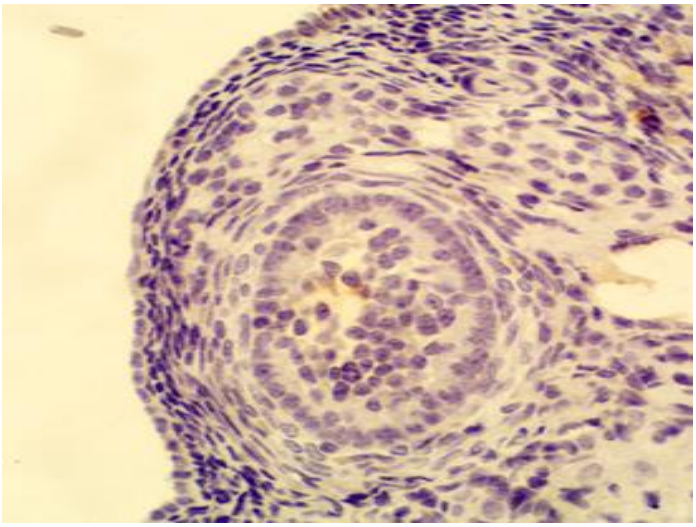

Figure 9: Ovary, G2 group. Showed negative reaction with *Ki67* antibody in the luteal cells. IHC-*Ki67*-DAB stain (400x).

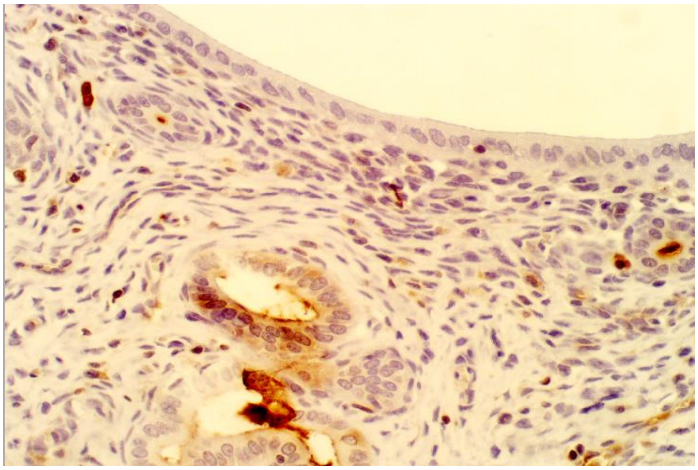

Figure 10: Uterus, G2 group. Showed a negative reaction with the Ki67 antibody in the epithelial cells of the renal tubules. IHC-*Ki67*-DAB stain (400x).

The BCl2 outcomes of the G2 group, which received 50 mg/kg of silymarin in addition to the same dosage of favipiravir as the G1 group, Figures (7 and 8), reveal the improved or reduced effect of Favipiravir on the ovary and uterine sections.

Figures 9 and 10 illustrate the mitigating function of silymarin in protecting the female reproductive system from the toxicity of favipiravir. The ovarian and uterine sections of the G2 group's results, stained with Ki67, showed a negative reaction.

4 Discussion

In this study, female rats' ovarian and uterine tissues were examined for the potentially harmful effects of favipiravir and the potential protective function of silymarin against such damage. Immunohistochemical examination of these reproductive tissues employing BCL2 and Ki67 markers revealed information about cellular proliferation and death.

The treatment of favipiravir (G1 group) produced a high positive BCL2 expression in the uterine glands and ovarian luteal cells, suggesting increased anti-apoptotic action. By blocking the release of cytochrome c from mitochondria and enhancing cell survival, BCL2 is a crucial modulator of the natural apoptotic process [13]. The elevated expression suggests that favipiravir can induce cellular stress, leading to increased BCL2 to prevent apoptosis [14]. In hepatotoxic and gonadotoxic models, where cells try to combat oxidative or metabolic stress, similar drug-induced modification of BCL2 expression has been seen [15] .

Furthermore, Ki67 staining revealed high nuclear positivity in developing ovarian follicles, indicating increased proliferative activity. This increased proliferation could be a regenerative response carried on by stress after cellular injury [16,17]. However, the same group's uterine glandular cells limited Ki67 expression points to a tissue-specific favipiravir sensitivity. Antiviral medications like favipiravir have been shown in earlier research to change ovarian function and lower fertility by causing oxidative stress and mitochondrial dysfunction [18].

BCL2 expression was lower in the uterine and ovarian tissues of the silymarin co-treated group (G2) than in the favipiravir-only group. This attenuation suggests that silymarin efficiently reduces the stress caused by favipiravir, hence lowering the demand for anti-apoptotic compensation, and this result aligns with the result of [19]. The study discovered that silymarin affects ovarian tissue's apoptotic markers and oxidative stress. And highlights silymarin's capacity to control stress-responsive pathways, which obliquely supports its function in reducing apoptotic compensation, even if BCL2 was not the main emphasis. Silymarin, a polyphenolic flavonoid derived from Silybum marianum, has potent antioxidant and membrane-stabilizing qualities that can combat reactive oxygen species (ROS) produced by toxins. This group's BCL2 downregulation is consistent with research showing silymarin can restore apoptotic equilibrium by lowering inflammation and oxidative damage [20].

The protective theory is further supported by the negative Ki-67 staining observed in the G2 group's uterine and ovarian sections. Silymarin mitigated the proliferative stress induced by favipiravir, as evidenced by reduced proliferation in this group, suggesting a return to normal tissue homeostasis. According to similar findings, silymarin reduced drug-induced reproductive toxicity by modifying indicators of cellular proliferation and death [21].

Together, the patterns of BCL2 and Ki67 expression indicate that favipiravir affects reproductive tissues in two ways: it increases oxidative stress, triggering proliferative and anti-apoptotic responses, especially in the ovary. Silymarin's medicinal potential in reducing antiviral-induced reproductive damage is suggested by the protective modulation observed with co-administration. These results are consistent with other research showing silymarin's cytoprotective properties against the toxicity of antiviral and chemotherapeutic drugs [22,23].

Conclusion

By increasing BCL2 expression and Ki67 activity, favipiravir therapy significantly altered the histology of the uterus and ovaries, suggesting higher proliferative and anti-apoptotic responses. Silymarin's ability to preserve cellular integrity and decrease stress on reproductive tissue was demonstrated by the co-treatment that reduced these effects. Therefore, during favipiravir therapy, silymarin may be used as a supplement to reduce reproductive damage.

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Statement regarding Competing Interests

I declare that there are no competing interests. This research was conducted independently, and there are no financial, personal, or institutional conflicts that could influence the results or interpretations presented in this manuscript.

Author Contributions

Conceptualization, Data Curation, Project Administration, Financing of the study, Methodology, Investigation of the study, Software, Supervision, Visualization, Data Analysis, Writing - Original Draft, Review & Editing: all done by Tuqa Yousif Sharef.

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Resources

None.

Validation

Hawler Medical University/ Ethical Committee.

Funding Declaration

The author confirms that no private or external funding was used to carry out this investigation. The author covered every expense related to the study.

Author Declaration

I, the undersigned, hereby declare that the research work entitled "Mitigating Reproductive Toxicity: Silymarin's Protective Effects on Favipiravir-Induced Ovarian and Uterine Alterations in Female Rats" was conducted entirely by myself without any external assistance. All aspects of the study—including conceptualization, experimental design, data collection, analysis, interpretation, and manuscript preparation—were solely undertaken by me.

I affirm that the manuscript is original, has not been published previously, and is not under consideration for publication elsewhere. I have complied with all applicable ethical standards and reporting guidelines.

Data Availability Statement

Not Applicable.

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