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Baicalin Mitigates Propiconazole-Induced Ovarian Toxicity in Female Albino Rats: Focus on Steroidogenesis, Oxidative Stress, and Apoptosis

Nahla Mohammed Ibrahim Hassan[1], Eman Mohamed Faruk[2], Wesam Barakat[3], Basma A. Ibrahim[4], Dalia Ahmed Selim Ali Abdou[5], Nehal Fahmy Shaheen[6], Ahmed Mohammed Zaazaa[7], Mahmoud M. Hassan[8], Mustafa Mohammed Zaazaa[9], Esraa Mohamed Ali[10], Mohamed Ghazy Attia Hablas[11], Omneya Kamel[12], Mona Atef[13]

[1] Forensic Medicine and Toxicology Department, Faculty of Medicine, Zagazig University, Egypt, , drnahlaibrahimforensic@gmail.com, ORCID: 0009-0004-7238-0812

[2] Department of Anatomy, Faculty of Medicine, Umm Al-Qura University, Makkah P.O. Box 7607, Saudi Arabia, faruk_eman@yahoo.com

[3] Forensic Medicine and Clinical Toxicology Department, Faculty of Medicine, Zagazig University, Egypt, , wesamassy@gmail.com, ORCID: 0009-0004-9654-8579

[4] Medical Biochemistry and Molecular Biology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt, BAIbrahim@medicine.zu.edu.eg, ORCID: 0000-0002-5282-6213

[5] Forensic Medicine and Clinical Toxicology Department, Faculty of Medicine, Zagazig University, Egypt, , Dr_das2006@yahoo.com, ORCID: 0009-0003-4201-3774

[6] Anatomy and Embryology Department, Faculty of Medicine, Benha University, Egypt, nihal.yassine@fmed.bu.edu.eg, ORCID: 0000-0002-8014

[7] Student, Faculty of Medicine, Benha National University, Benha Colleges in Cairo, Egypt

[8] Physiology Department, Faculty of Medicine, Benha University, Benha 13518, Egypt; Physiology Department, College of Medicine, Al-Rayan National Colleges, Al Madinah, Saudi Arabia

[9] Student, Faculty of Medicine, Benha National University, Benha Colleges in Cairo, Egypt

[10] Lecturer of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Ain Shams University, esraaelzwawy@med.asu.edu.eg

[11] Department of Histology and Cell Biology, Faculty of Medicine, Suez University, Suez, Egypt

[12] Department of Physiology, School of Medicine, NewGiza University, Cairo, Egypt, Omneya.kamel@ngu.edu.eg

[13] Forensic Medicine and Clinical Toxicology, Zagazig Faculty of Medicine, Egypt, , monaatefme985@gmail.com, ORCID: 0000-0002-4994-7516

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ABSTRACT:

Background: Propiconazole has potential toxic health effects on humans, which makes it an issue of concern to public health. Baicalin is a natural flavonoid that has antioxidant capabilities.

Aim: The purpose of this research was to look into the mitigating role of Baicalin against the toxic effects of Propiconazole in the ovarian tissues of female albino rats.

Methods: The research has been performed on thirty-five female rats that were equally assigned into five groups, seven animals each. Negative control group: Each rat was given just a regular diet and water; Positive control (DMSO) (vehicle for both Baicalin and Propiconazole); Baicalin group: (50 mg/kg/day); Propiconazole group: (75 mg/kg/day); Propiconazole + Baicalin group: Each rat received Propiconazole and Baicalin at the same mentioned doses. All therapies were administered by oral gavage once per day for four weeks. Upon completion of the experiment, the following markers were measured: levels of 17β-estradiol (E2) and progesterone (P4) in serum; oxidative stress biomarkers: ovarian tissue malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPx); serum inflammatory indicators: tumor necrosis factor-alpha (TNF-α) and nuclear factor-kappa B (NF-κB); and gene expression of newborn ovary homeobox (NOBOX), steroidogenic acute regulatory (StAR), Caspase-3, and B-cell leukemia/lymphoma 2 (Bcl-2) in the ovarian tissues. A histopathological study by H&E, as well as immunohistochemical staining for detection of proliferating cell nuclear antigen (PCNA) and tumor protein p53 (P53), was done.

Results: Propiconazole exposure resulted in hormonal and structural disruption of ovarian tissue, redox imbalance, inflammation, down-regulation of NOBOX, StAR, and Bcl-2, and up-regulation of Caspase-3, besides weak immunoreactivity of PCNA and strong immunoreactivity of p53. Baicalin co-administration led to structural and hormonal improvement, mitigation of redox imbalance, anti-inflammatory effects, up-regulation of NOBOX and StAR, and anti-apoptotic effects. **Conclusion:** Administration of Baicalin produced partial improvement of ovarian function and structure through anti-inflammatory, anti-apoptotic, and antioxidant capabilities against Propiconazole-prompted ovarian toxicity.

1-INTRODUCTION

Normal reproduction is essential for an individual's life, as it is biologically important to create new individual organisms for the species' development [1]. The physiological distinctions between males and females, in addition to their morphological development, are controlled by the reproductive system, which also determines the organism's behavior [2].

In the last few decades, a dramatic increase in human reproduction impairment has been observed which has, consequently, raised concerns about the commonly found chemicals in our environment and their impact on reproductive health [3]. This has attracted more scientific attention, especially with advances in health knowledge regarding their biological toxicity, particularly on the reproductive function [4].

Among these chemicals is a group of fungicides (conazoles), which have extensive pharmaceutical and agricultural applications, being used as agrichemicals for the preservation of several crops (e.g., cereals, fruits, and vegetables) [5]. Conazoles (triazole- and imidazole-antifungal agents) can be used locally or systemically for therapeutic purposes to treat mycotic and yeast infections.

Propiconazole (PRO) has a valuable effect in treating fungal infections by preventing the biosynthesis of ergosterol in fungi through inhibiting its metabolism, subsequently leading to impairment of cell wall synthesis. However, it could cause harmful

effects in mammals with irrational usage [6]. Due to its easy diffusion in the environment as well as long half-life, it can lead to neurobehavioral toxicity as well as disruption of the endocrine system and reproductive function in mammals [7]. In non-lethal doses, it can cause various biochemical effects including damage to cells, genes, and the environment [8].

Studies performed on rats and mice showed that PRO has multiple toxic effects, including hepatotoxicity, neurotoxicity, and reproductive toxicity via endocrine-disrupting effects. It was suggested that this multiorgan toxic effect of PRO may be due to increased levels of radical oxygen species (ROS) that result in oxidative damage to biological membranes[9].

These ROS cause inhibition of the antioxidant systems of the ovaries as well as mitochondrial injury and lipid peroxidation, resulting in ovarian follicle apoptosis and caspase activation [10].

Alternative medicine (herbs) has increasingly become popular therapeutic agents for reducing chemotherapy and toxicant side effects [11]. For example, Baicalin is a natural flavonoid that has been widely used since prehistoric times in China. It is extracted from the roots of the medicinal herb Scutellaria baicalensis. It has many useful human health actions, including strong anti-inflammatory, antibacterial, antioxidant, and anticancer abilities [12].In view of prior research on the role of baicalin, the current experiment was performed to assess baicalin's potential contribution to reducing redox imbalance, inflammation, and apoptosis brought on by PRO in adult female albino rats' ovaries.

2-MATERIAL AND METHODS

2.1.Chemicals

-Baicalin is in the form of yellow powder, the molecular weight is 446.36 g/mol, 95% purity and the CAS No. is 21967-41-9 (Product code: 572667).

-Propiconazole is in the form of a yellow liquid, molecular weight is 342.22 g/mol and the CAS No. is 60207-90-1 (Product code: 45642).

Both Baicalin and propiconazole were acquired from Sigma-Egypt (Eltayaran St., Nasr City ,Cairo) and produced by Sigma-Aldrich Chemical Company, USA.

2.2. Animals

This experiment was conducted in the Physiology Department, Faculty of Medicine, Zagazig University. The study protocol was performed according to guidelines established by Zagazig University's Institutional Animal Care and Use Committee (ZU-IACUC/3/F/277/2023).

Thirty-five female rats, weighing between 200 and 220g, 7 weeks old, and exhibiting a normal estrous cycle, were acquired from the Animal House at the Faculty of Medicine, Zagazig University. Steel wire cages were utilized to house the rats (50 × 30 × 20 cm).

They were kept under standard experimental conditions (with a temperature of 25 ± 2°C, humidity of 50% ± 5%, and a 12-hour light-dark cycle). Before the experiment began, the rats were allowed to adapt for two weeks and had unrestricted access to standard food and water.

The rats were equally distributed into five groups (7 rats in each) as follows:

- **Negative control group**: For four weeks, each rat was given regular food and water.
- **Positive control group:** Each rat received 1 ml of Dimethyl Sulfoxide (DMSO) (vehicle of baicalin and propiconazole) by oral gavage for 4 weeks.
- **Baicalin group**: Each rat was orally gavaged baicalin (50 mg/kg body weight) dissolved in 1 mL of DMSO once daily for 4 weeks [11].
- **Propiconazole group**: Each rat was orally gavaged with Propiconazole (75 mg/kg body weight), dissolved in 1 mL of DMSO, for 4 weeks [12].
- **Propiconazole + Baicalin group**: Each rat received both Propiconazole and Baicalin at the previously mentioned doses.

2.3. Experimental Procedures

The study monitored the stages of the estrous cycle by analyzing the relative amounts of leukocytes, cornified cells, and epithelial cells in vaginal smears. Every day between 8:00 and 9:00 AM, vaginal washing with saline was used to obtain vaginal smears. In accordance with established protocols, fresh, unstained samples were analyzed under a microscope to identify the estrous cycle phase. According to Ajayi and Akhigbe (2020)[13], regular cycles were classified as lasting four to five days, and the phases of the estrous cycle were determined:

• **Proestrus:** Smooth-bordered nucleated epithelial cells predominate.

• **Estrus:** Large, irregularly margined, non-nucleated cornified (keratinized) cells are present.

• **Metestrus:** A mixture of leukocytes and cornified cells.

• **Diestrus:** Small leukocytes are present, but cornified cells are absent.

Anovulation, the formation of follicular cysts, and persistent estrus were all indicated by the presence of cornified cells in smears for a minimum of ten consecutive days. Rats were weighed at the end of the study, and the **Bipembi** et al. (2015) [14] algorithm was used to determine their body mass index (BMI).

2.4. Biochemical analysis

The study was performed in the Zagazig Faculty of Medicine's Biochemistry Laboratory. Rats received thiopental anesthesia at a dosage of 50 mg/kg, followed by blood sample collection from the tail vein. The blood was centrifuged for 15 minutes at 3000 rpm after being allowed to clot for 30 minutes at room temperature. The serum was stored at -20°C before being examined. The rats were put to death via cervical decapitation after the blood was collected. Both ovaries were removed and washed with ice-cold saline after the abdomens were cut lengthwise. After homogenizing the ovarian tissue, it was centrifuged at 3000 rpm for 20 minutes at 4°C. For further biochemical investigation, the supernatant was gathered and kept in Eppendorf tubes at -20°C.

2.4.1. Assessment of Hormonal Levels

Levels of 17β-estradiol (E2) and progesterone (P4) in serum were quantified using commercial ELISA kits (Rat Estradiol ELISA Kit, Cat. No. MBS702969; Rat Progesterone ELISA Kit, Cat. No. MBS265343, MyBioSource, USA) following the manufacturers' protocols.

2.4.2. Assessment of Oxidative/Antioxidative Parameters

The supernatants of ovarian homogenates were analyzed colorimetrically for oxidative stress markers using commercial kits (Biodiagnostic, Egypt): malondialdehyde (MDA) (Cat. No. MD2529), superoxide dismutase (SOD) (Cat. No. SD2521), and glutathione peroxidase (GPx) activities (Cat. No. GP2524) according to the manufacturer's instructions.

2.4.3. Assessment of Proinflammatory Parameters

Nuclear factor-kappa B (NF-ϰB) and tumor necrosis factor-alpha (TNF-α) were measured in serum using quantitative ELISA kits (MyBioSource, USA) with Cat Nos. MBS2507393 and MBS453975. Every procedure followed the instructions provided by the manufacturers.

2.5. Gene Expression Assay

2.5.1. Total RNA Isolation

Ovarian tissue samples were homogenized in TRIzol Reagent (50–100 mg tissue/mL TRIzol) utilizing a Polytron power homogenizer. Total RNA was extracted with TRIzol™ reagent (Cat. No. 15596026, Invitrogen, USA) in accordance with the manufacturer's instructions and measured at 260 nm with a spectrophotometer.

2.5.2. Complementary DNA (cDNA) Synthesis

Complementary DNA (cDNA) for mRNA analysis was synthesized in accordance with the manufacturer's instructions, using the SensiFast™ cDNA Synthesis Kit (Bio-65053, Bioline, UK).

2.5.3. Quantitative Real -Time PCR (qRT-PCR)

Using the Maxima SYBR Green/ROX qPCR Master Mix (2x) (#K0221, Thermo Scientific, USA) and a Rotor-Gene Q 2 plex Real-Time PCR system (Qiagen, Germany), quantitative reverse transcription polymerase chain reaction (qRT-PCR) was carried out. GAPDH was used to standardize the examined genes' expression levels. The primer sequences used for the quantitative reverse transcription polymerase chain reaction (qRT-PCR) are shown in Table 1. The 2^-ΔΔCt technique was used to calculate the relative expression.

GAPDH, glyceraldehyde phosphate dehydrogenase; Bcl-2, B-cell lymphoma 2; NOBOX, newborn ovarian homeobox gene; and StAR, steroidogenic acute regulatory protein.

2.6. Ovarian histopathology analysis

2.6.1. Preparation of Specimens:

- The right ovarian sample was stored in 10% buffered formalin at ambient temperature.
- Paraffin sections (5 μm thick) were prepared and mounted on:
- Balsam-coated slides suitable for H&E staining procedures.
- Poly-L-lysine coated slides with a positive charge for immunohistochemical staining.

2.6.2. Histological and Immunohistochemical Staining:

2.6.2.1. Hematoxylin and Eosin (H&E): pplied in general histological analysis according to **Bancroft and Layton (2018) [15]**.

2.6.2.2. Immunohistochemical Staining:

Staining was conducted to identify proliferating cell nuclear antigen (PCNA) and p53. The avidin-biotin complex method was utilized. Following an overnight incubation at 4°C, the sections underwent deparaffinization and rehydration through a series of graded alcohols. To retrieve the antigen, it was boiled in 0.1 M sodium citrate and then submerged in 3% hydrogen peroxide to prevent the action of endogenous peroxidase. Nonspecific binding was blocked using 10% normal goat serum at room temperature for one hour.

- Primary antibodies were utilized:
- PCNA stands for rabbit monoclonal antibody (RM-9106-R7, Lab Vision Corporation, USA).
- p53: Rabbit polyclonal IgG (Abcam, Cat. #ab8105), diluted 1:80 in phosphate-buffered saline (PBS).

For thirty minutes at room temperature, sections were treated with a biotinylated anti-rabbit secondary antibody (Vector Laboratories, Burlingame, CA, USA). Sections treated without the main antibody served as negative controls. A streptavidin-biotin–peroxidase kit from Vector Laboratories was used to visualize the antigen-antibody complex, and 3,3-diaminobenzidine substrate (DAB) from the same company was used to generate it. Mayer's hematoxylin served as the counterstain.

2.7. Morphometric assessment:

Morphometric studies were conducted in the Anatomy Department at Zagazig Faculty of Medicine using the ImageJ software plugin and the Leica Qwin 500 The image analysis unit of the Human Anatomy and Embryology Department uses the Image Analyzer Computer System (England).

2.7.1. Procedures:

2.7.1.1. Follicle Counting:

Sections stained with hematoxylin and eosin (H&E) were examined at 100× magnification. Five serial sections per group were used to count the number of follicles in randomly selected microscopic areas. A mean of 10 readings was calculated. Every twenty sections of the ovary were stained with H&E to prevent duplicate counting of the same follicle, with a spacing of 50–60 μm between analyzed sections [16]. Only ovarian follicles containing oocytes were included in the analysis.

2.7.1.2. Immunohistochemical Analysis:

- The percentage of the area showing positive PCNA and p53 immunoreactivity was quantified in ovarian sections viewed at 400× magnification.
- Eight non-overlapping fields per section were analyzed for each slide.

2.8. Statistical Design

3. Using one-way ANOVA and Tukey's post-hoc test for pairwise comparisons, the data were examined using GraphPad Prism 8.0 software. The findings were presented as mean ± standard deviation (SD). Statistical significance was deemed significant for p-values less than 0.05 and extremely significant for p-values less than 0.001.

4. RESULTS

The current results revealed no statistically significant difference between negative and positive control (DMSO) groups in any of the biochemical parameters investigated, as well as histological and immunohistochemical analyses. Thus, the negative control was used as reference value for statistical comparison with the other groups.

Biochemical results:

Baicalin reversing propiconazole-induced hormonal suppression

Propiconazole exposure severely disrupted sex hormone production, reducing serum E2 levels by 63.3% and P4 levels by 67.8% in comparison with the negative control group ($p<0.001$). However, supplementation with baicalin partially restored hormone secretion, elevating E2 and P4 levels in comparison with propiconazole alone ($p<0.001$). These findings indicate that baicalin provides critical protective support for steroidogenic function, partially reversing propiconazole-induced hormonal suppression (Table 2).

Baicalin decreased the Propiconazole-induced oxidative stress:

Propiconazole exposure induced significant redox imbalance, as seen by a 2.8-fold rise in MDA levels relative to controls ($p<0.001$), indicating severe lipid peroxidation. This oxidative damage was accompanied by substantial impairment of antioxidant defenses, with SOD and GPx activities reduced by 59.8% and 63.2%, respectively ($p<0.001$ vs. control), confirming compromised ovarian antioxidant capacity against reactive oxygen species (ROS). Co-treatment with baicalin significantly attenuated these effects, reducing MDA levels by 53.9% ($p<0.001$) in association with the propiconazole-only group, suggesting effective but incomplete protection against oxidative damage. While SOD and GPx activities remained below control levels, they showed significant improvement ($p<0.001$) relative to propiconazole-treated animals, demonstrating partial restoration of antioxidant defenses. These findings highlight baicalin's dual protective mechanism: direct free radical scavenging (as shown by MDA reduction) and enhancement of endogenous antioxidant enzyme activity (evidenced by SOD and GPx recovery), collectively mitigating propiconazole-induced oxidative stress (Table 3).

Baicalin act as a flavonoid inhibitor of NF-ϰB signaling, ,disrupt the cytokine cascade ,and mitigate propiconazole-induced inflammatory damage

Propiconazole exposure triggered a pronounced pro-inflammatory response, marked by 3.5-fold higher serum TNF-α levels and 2.9-fold elevated NF-ϰB levels compared to controls ($p<0.001$), indicating systemic oxidative stress and NF-ϰB pathway activation. Co-treatment with baicalin significantly attenuated this inflammation, showing 49.2% lower TNF-α and 45.5% reduced NF-ϰB levels relative to propiconazole-alone treatment ($p<0.001$). This anti-inflammatory effect confirms baicalin's role as a flavonoid inhibitor of NF-ϰB signaling, demonstrating its capacity to disrupt the cytokine cascade and mitigate propiconazole-induced inflammatory damage (Table 3).

Baicalin maintaining folliculogenesis through NOBOX/StAR preservation and inhibiting apoptosis via Bcl-2 upregulation and Caspase-3 suppression

Propiconazole exposure significantly suppressed ovarian gene expression, reducing NOBOX mRNA to 0.45±0.07 (54% of control; $p<0.001$) and StAR mRNA to 0.41±0.04 (57.3% of control; $p<0.001$) compared to control values (0.98±0.09 and 0.95±0.08, respectively), indicating impaired oocyte development and steroidogenesis. The treatment also dramatically altered

apoptotic markers, increasing Caspase-3 mRNA 2.5-fold (2.28±0.19 vs. control 1.03±0.17; $p<0.001$) while reducing Bcl-2 expression to 55.4% of controls (0.56±0.02 vs. 1.01±0.12; $p<0.001$). Baicalin co-treatment partially reversed these effects, restoring NOBOX to 0.85±0.09 (85% of control) and StAR to 0.88±0.06 (90% of control) (both $p<0.001$ vs. propiconazole alone). Apoptotic markers were similarly normalized, with Caspase-3 decreasing to 1.25±0.16 (45% of propiconazole levels; $p<0.001$) and Bcl-2 increasing 1.6-fold to 0.91±0.05 (vs. 0.56±0.02 in the propiconazole group; $p<0.001$). These results demonstrate baicalin's dual protective mechanisms: maintaining folliculogenesis through NOBOX/StAR preservation and inhibiting apoptosis via Bcl-2 upregulation and Caspase-3 suppression (Figure 1).

Baicalin reversing propiconazole-induced morphological changes in ovarian tissue

The cortex and medulla were included in the ovarian region of control rats. The primordial, primary, secondary, Graafian, and corpus luteum stages of ovarian follicles make up the cortex. The follicular cells had pale-looking nuclei. There are two types of primary follicles: unilaminar and multilaminar follicles. The zona pellucida surrounds the primary oocyte in both types of follicles, which are lined by follicular cells. The Graafian follicle is made up of granulosa, theca interna, and theca externa cells lining the antrum (Figure 2a).

In Baicalin-treated albino rats, the ovaries exhibited corpora lutea, Graafian follicles, and ovarian follicles. The zona pellucida and corona radiata encase the granulosa cells and secondary oocyte that make up the Graafian follicle. It is supported by a base made up of the theca externa, theca interna, and cumulus oophorus, as well as an antrum (Figure 2b).

The cortex, which included the corpus luteum, several atretic and cystic follicles (Figure 2c), and a medulla full of clogged blood vessels, constituted the ovarian portion of the Propiconazole group. The atretic follicle showed theca externa and interna, as well as a lining of several layers of vacuolated granulosa. The cystic follicles were found next to blood vessels that were clogged (Figure 2d).

The cortex and medulla of the ovarian section of albino rats given Baicalin and Propiconazole included the corpus luteum, Graafian follicle, primary follicles, atretic follicles, and cystic follicles (Figure 2e). The cortex exhibited numerous primary and secondary follicles, accompanied by a limited number of congested blood vessels. Granulosa cells, theca interna cells, and theca externa cells lined the Graafian follicle, which included an antrum (Figure 2f).

Ovarian tissue immunostaining for PCNA of the control group's ovaries showed a high concentration of PCNA-positive cells in the theca interna and externa cells, as well as in all granulosa cells (Figure 3a). When ovarian tissue was treated with Baicalin, all granulosa cells showed a notable number of PCNA-positive cells; theca interna and theca externa cells showed a lower quantity (Figure 3d).

All granulosa, theca interna, and theca externa cells showed a significant decrease in PCNA-positive cells in the Propiconazole group (Figure 3b). Ovarian specimens treated with Baicalin and Propiconazole showed a moderate immunological response for PCNA in all granulosa cells, with theca interna and theca externa cells showing decreased expression (Figure 3c).

All granulosa cells, theca interna, theca externa, and stromal cells showed a modest positive P53 reaction when ovarian immunostaining was performed on the control and baicalin rat sections (Figures 4a and b). All granulosa cells, theca interna, theca externa, and stromal cells of the ovary in the propiconazole group showed an increase in positive P53 reactivity (Figure 4c). All granulosa cells showed modest positivity in the reaction, while theca interna and externa cells in PCOS animals given baicalin and propiconazole showed decreased positivity (Figure 4d).

Baicalin restore folliculogenesis and reverse the propiconazole-induced apoptosis in ovarian tissue

Follicle counts at every stage of folliculogenesis were analyzed, and the results showed a considerable reduction in Graafian follicles and a notable increase in atretic follicles within the Propiconazole group compared to controls. The count of Graafian follicles increased, whereas the count of atretic follicles decreased significantly in the combined group (Table 4). In ovarian sections from distinct groups, morphometric analysis of the area % of different immunological stains revealed a significantly reduced expression of PCNA in the Propiconazole group compared to controls. The Baicalin/Propiconazole-treated group exhibited a significant increase compared to the Propiconazole group; however, levels continued to be considerably lower than those in the control group. The area percent of P53 immunoreaction in the Propiconazole group exhibited a significant increase relative to controls. The percentage in the Baicalin/Propiconazole group decreased

significantly compared to propiconazole alone, but continued to be noticeably greater than that of the control group (Table 5).

4.DISCUSSION

Farmers all across the world use pesticides, including fungicides, to lessen damage from unwanted organisms and boost crop productivity and quality. A systemic fungicide from the triazole class, propiconazole is used to treat a variety of illnesses. According to Samani and Kabiri [17], this poison damages animals' cells, genes, and metabolism.

Propiconazole causes toxicity in animals and types of tissues, leaves a wide range of biochemical effects in non-lethal doses, and can cause cellular, genetic and environmental damage. The impact of propiconazole on the ovary has not been extensively studied.

The results of the experiment show a range of harmful consequences on organisms, such as endocrine disruption and oxidative stress. The potential toxicity of fungicides in different body organs is therefore an important area for research [18]. A few earlier research have examined the risks of propiconazole on the ovary, according to the literature. Accordingly, the current study was intended to assess the toxicity of Propiconazole on the ovary of rats in addition to the possible protective effects of baicalin.

Due to their remarkable pharmacological characteristics, natural plant-based flavonoids are becoming more widely available. Hepatoprotective, anti-inflammatory, anti-allergic, anticancer, antibacterial, and antioxidant qualities are exhibited by flavonoids. Baicalin is a well-known flavonoid that is derived from the roots of Scutellaria radix. According to Ekalu and Habila [19], it possesses a number of pharmacological characteristics that help treat conditions like inflammation, infection, oxidative stress, and immunological dysfunction.

Histopathological changes observed in ovarian specimens of Propiconazole treated rats confirmed the biochemical changes. Our histopathology and IHC results revealed that Propiconazole has damaging effect on the ovary in the form of reduced ovarian follicles and increased atretic follicles.

Maretta et al. [20] sated that exposure to Although the effects seemed to be transient or reversible, female rats' reproductive development was negatively impacted by high concentrations of propiconazole.

Propiconazole also reduced oestrous cyclicity, according to Taxvig et al. [21] increasing gestational age, virilizing female pups, and altering steroid hormone levels in fetuses and/or females are common characteristics of azole fungicides. These outcomes strongly imply that the disruption of important enzymes involved in the manufacture of steroid hormones is one of the main underlying mechanisms of the endocrine-disrupting effects of azole fungicides, as this investigation demonstrated.

In this study, we found highest positive score of PCNA in the Propiconazole exposed group compared to others. Yang et al.[22] reported in concordance with our study that PCNA is indicative of proliferative ability as marker of cell stress and oxidative damage.

Additionally, Jeong et al. [23] demonstrated that propiconazole's cytotoxicity in bovine mammary epithelial cells was linked to an increase in autophagy and apoptosis. Both the intrinsic mitochondrial system and the extrinsic receptor-mediated mechanism can cause apoptosis, a sort of regulated cell death.

Furthermore, the development and repair of tissue damage are influenced by the tumor suppressor p53. P53 inhibition is one potential treatment for acute injuries and their development. Samarakoon [24] asserts that p53 is a profibrotic initiator required for the activation of profibrotic genes in the near future.

In this study, the combination of baicalin decreased p-53 expression in ovarian tissue, promoted the development of damaged follicles, and enabled us to see a regression of tissue fibrosis. Numerous studies have demonstrated that baicalin can shield mammalian cells from oxidative stress and DNA damage caused by alpha fetoprotein by modulating various signaling pathways, such as the P53-mediated ferroptosis pathway. These results offer a viable approach to treating liver injury and offer new insights into the mechanism by which natural products like baicalin exert their protective benefits [25].

The findings of this study demonstrate that propiconazole exposure induces significant ovarian dysfunction in adult female albino rats through multiple interconnected mechanisms. Our results show that propiconazole treatment led to marked reductions in serum estradiol and progesterone levels, consistent with previous reports of triazole fungicides disrupting steroidogenesis [26]. StAR mRNA expression was significantly downregulated in conjunction with this hormonal disturbance, suggesting impaired cholesterol transport into mitochondria - the rate-limiting step in steroid hormone biosynthesis. These effects likely contribute to the observed decline in ovarian function, as adequate steroid hormone production is essential for normal follicular development and ovulation [27].

The oxidative stress parameters measured in this study provide compelling evidence for another key mechanism of propiconazole's ovarian toxicity. We observed an increase in MDA levels, indicating substantial lipid peroxidation and cellular membrane damage. This was coupled with significant reductions in SOD and GPx activities, demonstrating impairment of the ovarian antioxidant defense system. These findings align with previous studies showing that triazole fungicides can generate reactive oxygen species and deplete antioxidant reserves [28]. The resulting oxidative stress may contribute to the observed hormonal dysregulation, as oxidative damage to ovarian steroidogenic enzymes has been shown to impair hormone production [29].

Our results also reveal significant pro-inflammatory effects of propiconazole exposure, with increasing serum TNF-α and NF-ϰB levels compared to controls. This inflammatory response likely exacerbates ovarian dysfunction, as chronic inflammation has been associated with impaired folliculogenesis and reduced ovarian reserve [30]. The activation of NF-ϰB signaling may be particularly significant, as this transcription factor regulates numerous genes involved in inflammation and apoptosis [31]. These inflammatory changes were accompanied by marked alterations in apoptotic markers, including an increase in Caspase-3 mRNA and a reduction in Bcl-2 expression, suggesting increased susceptibility to programmed cell death in ovarian follicles.

The significant downregulation of NOBOX mRNA observed in our study provides important insights into propiconazole's potential effects on ovarian reserve. As a critical oocyte-specific transcription factor, NOBOX plays essential roles in follicular development and maintenance of primordial follicles [32] . Its suppression may indicate impaired folliculogenesis and reduced reproductive lifespan, consistent with findings from other studies of environmental toxicants on ovarian function [33].

Notably, our results demonstrate that baicalin co-treatment provides substantial protection against these propiconazole-induced alterations. The restoration of StAR mRNA expression near to the control values likely underlies the observed improvements in E2 and P4 levels. Baicalin's antioxidant properties were evident in the reduction of MDA levels and the restoration of SOD and GPx activities near to the controls. These findings are consistent with previous reports of baicalin's free radical scavenging and antioxidant enzyme-preserving effects [34]. The compound's anti-inflammatory action was demonstrated by significant reductions in TNF-α and NF-ϰB levels compared to propiconazole alone, supporting its known role as an NF-ϰB pathway inhibitor [35]. The protective effects of baicalin extended to the regulation of apoptotic and folliculogenic genes. The increase in Bcl-2 expression and the reduction in Caspase-3 mRNA suggest that baicalin helps maintain follicular survival by inhibiting apoptosis, consistent with its known flavonoid-mediated suppression of pro-apoptotic pathways [36]. Similarly, the restoration of NOBOX expression indicates preservation of oocyte developmental competence, aligning with studies demonstrating that antioxidants can protect oocyte-specific transcription factors from oxidative damage [37]. These findings are particularly significant given the growing evidence linking environmental toxicant exposure (e.g., triazole fungicides) to diminished ovarian reserve and premature ovarian failure [30]. While these results are promising, certain limitations should be acknowledged. The study focused on mRNA expression levels, and future work should include protein-level analyses to confirm these findings. Additionally, longer-term studies assessing effects on fertility outcomes would help establish the clinical relevance of these observations. The dose-response relationship for baicalin's protective effects also warrants further investigation to determine optimal therapeutic concentrations.

Conclusion and recommendations

This study demonstrates that propiconazole disrupts ovarian function and structure through multiple pathways, including steroidogenic suppression, impaired folliculogenesis, oxidative stress, inflammation, and apoptotic effects. So, propiconazole use is warranted in female workers occupationally exposed to propiconazole and suffering from infertility. The protective

effects of baicalin against These changes demonstrate its potential as a medicinal treatment for ovarian dysfunction brought on by pesticides.

DECLARATIONS AND STATEMENTS:

ETHICAL APPROVAL

Consent from the Zagazig University IACUC Committee in Egypt and in compliance with international rules for animal research (ZU-IACUC/3/F/149/2023).

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AUTHORS CONTRIBUTIONS

All authors contributed equally to the conception, design, data collection, analysis, and writing of the manuscript. All authors have read and approved the final version.

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[38] Table 1. Rat Oligonucleotide Primers' Sequences (5'-3') in qRT-PCR.

Table 2. Effects of Baicalin and Propiconazole on serum sex hormones and inflammatory markers.

Hormones/ groups	Control	Baicalin	Propiconazole	Propiconazole + Baicalin	p-value (ANOVA)
Estradiol (E2) (pg/mL)	60.21 ± 2.16	60.27 ± 2.35	22.06 ± 1.48**	51.41 ± 1.37##	<0.001
Progesterone (P4) (ng/mL)	11.41 ± 0.45	11.47 ± 0.75	3.67 ± 0.36**	8.92 ± 0.42##	<0.001

Values presented as mean ± SD (n=10). **(p<0.001) vs. Control; ##(p<0.001) vs. Propiconazole.

Table 3. Effects of baicalin and propiconazole on oxidative and inflammatory markers.

Parameters/ Groups	Negative Control	Baicalin	Propiconazole	Propiconazole + Baicalin	p-value (ANOVA)
MDA (nmol/g)	2.40 ± 0.22	2.19 ± 0.31	6.57 ± 0.27**	3.03 ± 0.48##	<0.001

SOD (U/g)	32.87±1.99	33.67± 1.64	13.23±0.75**	28.53±1.81##	<0.001
GPx (U/g)	24.28±1.39	25.01± 1.59	8.94±0.71**	18.72±0.54##	<0.001
TNF-α (pg/mL)	17.55±1.58	17.23± 1.40	61.38±3.04**	31.19±1.35##	<0.001
NF-κB (ng/mL)	5.51±0.70	5.08±0.79	16.21±0.90**	8.83±0.50##	<0.001

Values presented as mean ± SD (n=10). **(p<0.001) vs. Control; ##(p<0.001) vs. Propiconazole.

Table 4. Mean number of various follicles in all groups.

Follicles / Group	Control	Baicalin	Propiconazole	Propiconazole + Baicalin	p-value (ANOVA)
The mean number of intact primordial follicle count	14.80± 0.87	13.7±78	9.3±0.64**	12±0.77##	<0.001
The mean number of intact primary follicle count	6.1±2.3	7±3.4	4.3±2.53**	6.9±2.1##	<0.001
The mean number of intact secondary follicle count	5.8±1.03	5.7±1.9	2.6±0.9**	5.1±2.21##	<0.001
The mean number of intact mature graffian follicle count	0.7±0.64	0.5±0.5	0.2±0.4**	06±0.48##	<0.001
The mean number of atretic follicle count	1.7±1.0	1.4± 1.01	8.2±3.2**	1.2±0.6##	<0.001
The mean number of corpus leutum count	0.6±0.66	0.4± 0.48	0.1±0.3**	0.5±0.5##	<0.001

Values presented as mean ± SD (n=10). **(p<0.001) vs. Control; ##(p<0.001) vs. Propiconazole.

Table 5. Area percentage of PCNA and P53 immune expression in different groups.

Parameter/groups	Control	Baicalin	Propiconazole	Propiconazole + Baicalin	p-value (ANOVA)
PCNA	21.645±1.77	20.547±5.81	8.485±0.53**	19.024±3.59##	<0.001
P53	2.501±0.61	2.99±0.53	21.731±2.16**	8.318±3.09##	<0.001

Values presented as mean ± SD (n=10). **(p<0.001) vs. Control; ##(p<0.001) vs. Propiconazole.

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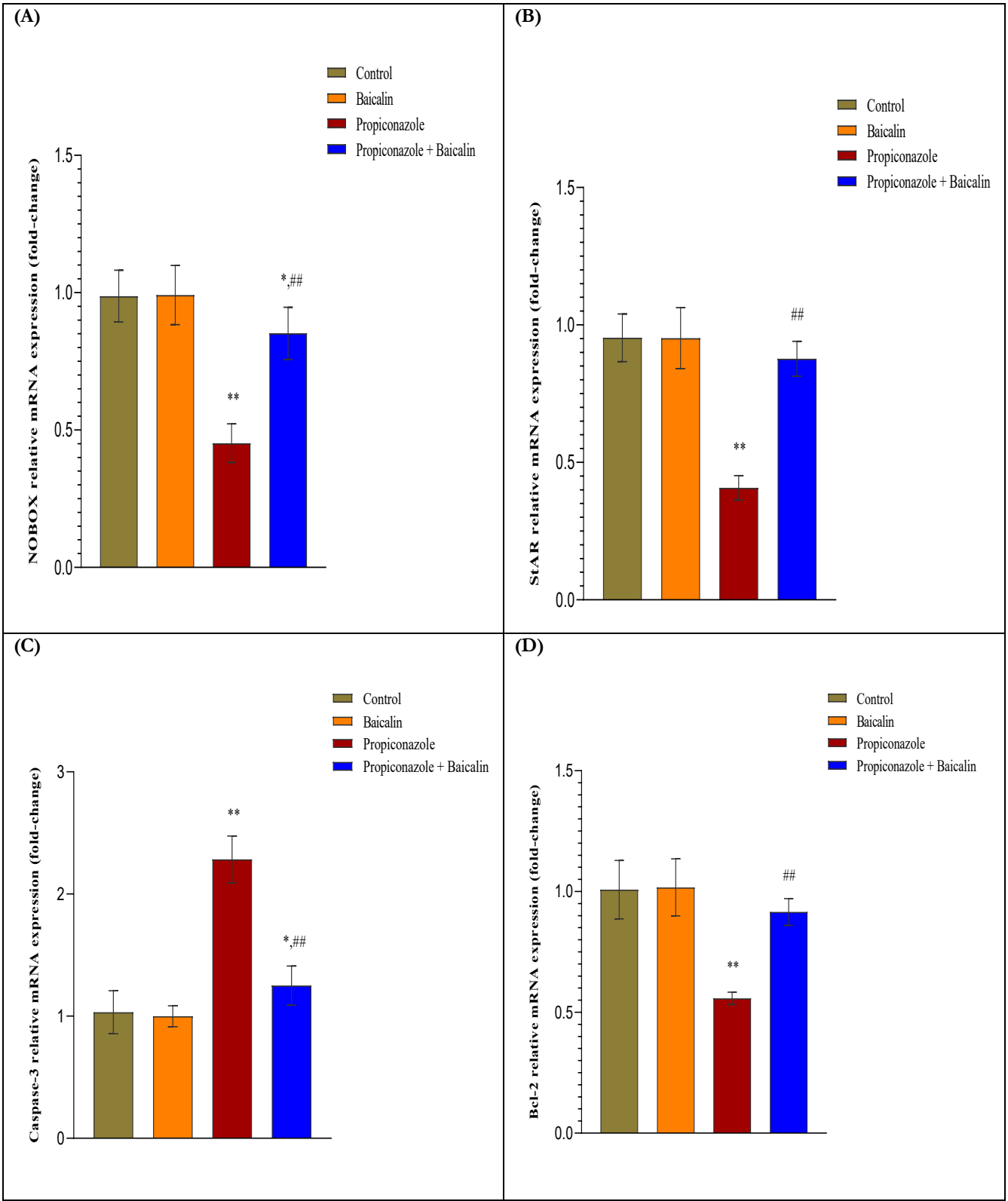


Figure 1. The effect of Baicalin and Propiconazole on ovarian NOBOX **(A)**, StAR **(B)**, Caspase-3 **(C)**, and Bcl-2 **(D)** relative mRNA expressions in adult female albino rats. Results expressed as mean ± SD (n=10). *($p<0.05$), **($p<0.001$) vs. the controls; ##($p<0.001$) vs. Propiconazole-treated group.

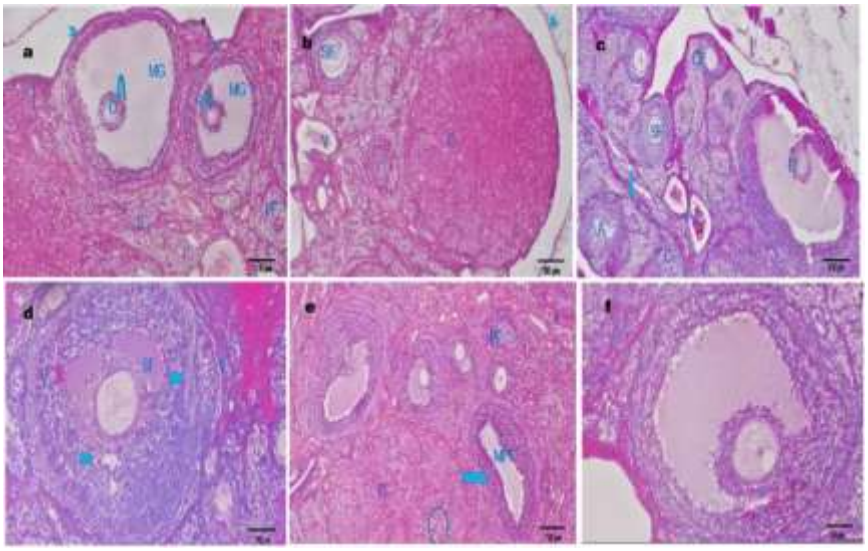


Figure 2: H and E-stained photomicrographs obtained from the studied groups. Control group (a) showing normal ovarian microstructure covered by a germinal epithelium (arrowheads) with an underlying tunica albuginea (arrow). A mature follicle (MF) with intact oocyte (O), healthy zona pellucida (thick arrow) and closely related normal granulosa cells is seen. Apparently normal spindle-shaped interstitial cells (S) were also observed. Notice that the cortex is formed from multiple stages of ovarian follicles, primordial follicle (P) and primary follicle (Pf) (H&E, x200 µm). Baicalin group (b) showing that ovarian section is formed from the cortex and medulla, covered by a germinal epithelium (arrowheads) with corpus luteum (C), and secondary follicles (SF) with few blood vessels (V). (H&E, x200µm). **Propiconazole** group (c&d) showing atretic secondary (SF) and mature follicles (MF) with degenerated oocyte (O) and lined by loose vacuolated granulosa cells(arrow head). Also, congested blood vessels were seen(V). Interstitial cells show vacuolar changes (arrow). (H&E, x200 µm). Propiconazole albino rat group(d) showing degenerated atretic primordial (PF) and multilaminar primary follicles (MPF) with degenerated oocyte and loose vacuolated granulosa cell lining (arrow). Corpus luteum (C) shows focal degenerated areas (outlined by a circle). (H&E, x200µm).(e&f) Baicalin Propiconazole-treated group showing obvious improvement in the ovarian histomorphology with almost normal folliculogenesis. (H&E, x400µm).

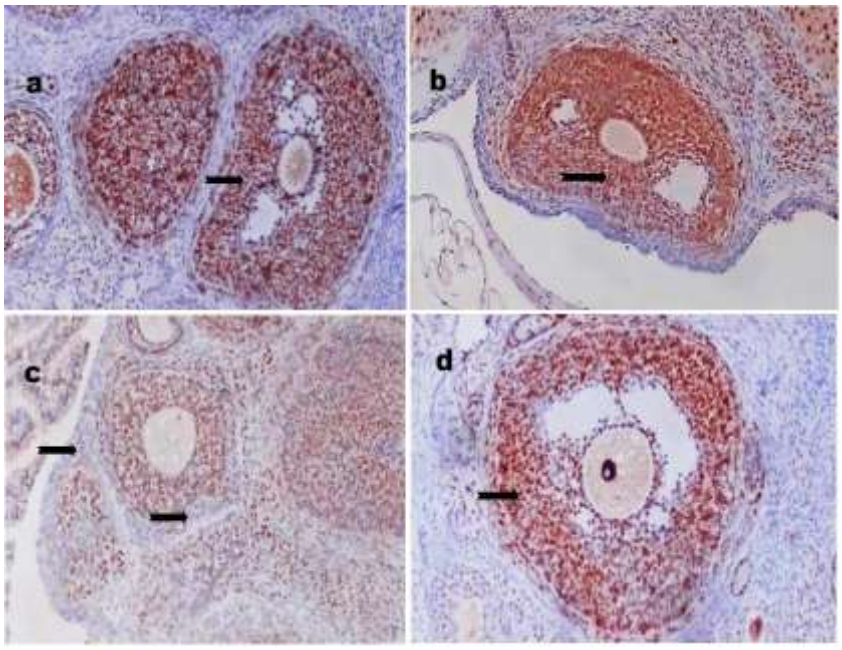


Figure 3: Ovarian Immunostaining for PCNA. Control group (a) Strong PKNA positive cells (arrow) in all granulosa cells, theca interna and theca externa cells. Baicalin group (b) An apparent PCNA positive cells (arrow) in all granulosa cells, theca interna and theca externa cells. Propiconazole group (c) decease immune reaction for PCNA (arrow) in all granulosa cells, less in theca interna and theca externa cells. Propiconazole + Baicalin group (d) Strong presentation of PCNA positive cells (arrow) in all granulosa cells, fewer in theca interna and theca externa cells (magnification ×400).

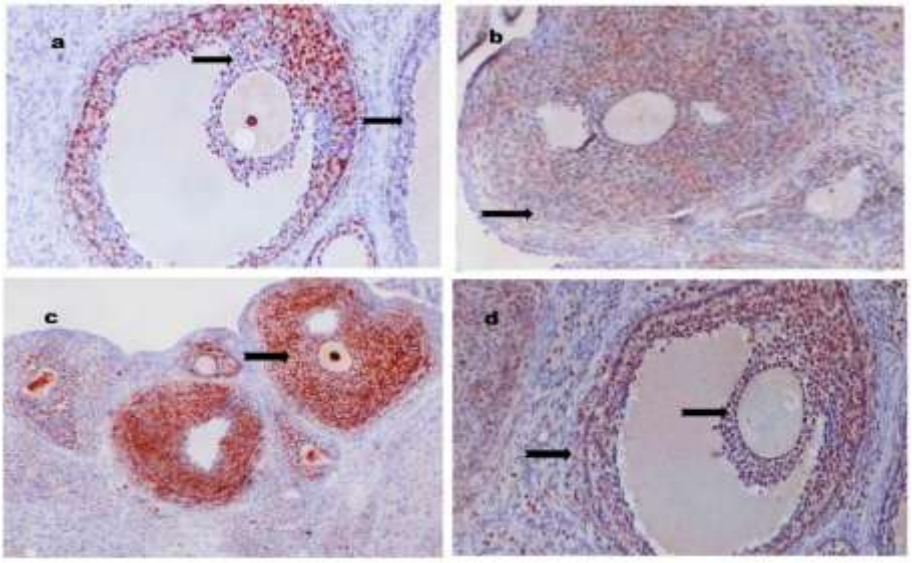


Figure 4: Ovarian immunostaining for P53: control group (a)Very weak positive cytoplasmic P53 reaction in all granulosa cells, theca interna, theca externa cells and stromal cells, with observable reaction in their nuclei (arrow). Baicalin group (b)weak in cytoplasmic positive caspase-3 reaction in all granulosa cells, theca interna, theca externa cells and stromal cells (arrows) and their nuclei. Propiconazole group (c)Moderate to severe positive cytoplasmic P53 reaction in of in all granulosa cells, less in theca interna and theca externa cells and their nuclei (arrow). Propiconazole + Baicalin group (d)Mild presentation of cytoplasmic positive P53 reaction in all granulosa cells, theca interna, theca externa cells and stromal cells and their nuclei (arrow) (magnification ×400).