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# Histological and Biochemical Changes in Ovaries of Adult Wistar Rats Following Exposure to Risperidone

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**Abstract**
Risperidone is an antipsychotic drug used in the management of certain borderline personality and conduct disorder. *This study evaluated the histomorphological and biochemical changes in ovaries of adult Wistar rats following the administration of risperidone.* Fifteen adult Wistar rats weighing between 130 - 180g were independently assigned into three groups. Group A was the control given unlimited access to food and water. Risperidone was administered as follows to Groups B and C (2.5 mg/kg and 5 mg/kg) per body weight, respectively. After the experimental period, the ovaries were harvested and processed for biochemical and histological examination. One-way analysis of variance (ANOVA) was used to evaluate differences in mean, with a significance level set at $p < 0.05$. Group B rats (2.5 mg/kg of risperidone) showed normal ovarian microstructure, with follicles at various stages of maturity. Group C (5 mg/kg of risperidone) also showed similar tissue composition but with mild to moderate changes notably evidence of cellular injury, including congested blood vessels, white blood cell infiltration, and lymphatics. Biochemical assay of antioxidant activity showed significant decrease in superoxide dismutase (SOD) with proportionate increase in malondialdehyde (MDA) concentration at higher doses, indicating oxidative stress. Risperidone administration could be deleterious to the ovaries as it might induce cellular damage via oxidative stress.

**Keywords:** *Risperidone, Ovaries, Rat*

## Introduction

Risperidone is a second-generation antipsychotic (SGAs) and serotonin-dopamine antagonists (SDAs), commonly prescribed in the management of some mental health disorders. They are often used to treat agitation and other related disorders like dementia, anxiety disorders, autism spectrum disorders, and obsessive-compulsive disorder (Miyake *et al*., 2012). It is approved for various psychiatric conditions, including schizophrenia, a chronic mental disorder characterized by distorted thoughts, hallucinations, and delusions. A useful regimen for acute manic or mixed episodes associated with bipolar disorder, often prescribed to manage irritability in children and adolescents with autism spectrum disorders (Sadock, 2015). In comparison to other atypical antipsychotics, risperidone appears to generate slightly higher extrapyramidal side effects and obviously more prolactin elevation. In the incidence of additional negative effects such as weight gain, metabolic issues, cardiac effects, drowsiness, and seizures, it may differ from other substances (Komossa *et al.,* 2011).

Risperidone primarily blocks serotonin receptors, reducing the likelihood of extrapyramidal symptoms (EPS) and enhancing antidepressant effects (Meltzer and Gadaleta, 2021). Its therapeutic effects are primarily due to its loose binding to D2 receptors and quick dissociation from the receptor. The antidepressant effects are believed to improve positive symptoms by blocking D2 receptors, particularly those in the mesolimbic pathway (Meltzer and Gadaleta,

2021). The precise mechanism of action of risperidone is not fully understood, but it is believed to temporarily inhibit D2 dopaminergic receptors, reducing neurotransmission. The risk of extrapyramidal symptoms increases with a larger D2 receptor occupancy rate, making it advisable to avoid it (Urichuk *et al*., 2008; Kemp *et al*., 2009). It has also been reported to block the action of histamine (H1), alpha-1, and alpha-2 receptors. Risperidone frequently elevates serum prolactin levels through the antagonism of dopamine D2 receptors located on lactotrophes within the pituitary (Green and Brown, 1988, Ereshefsky and Lacombe, 1993) and has been found to be a factor in menstrual dysfunction known to occur in women treated with these medications (Meltzer, 1985). Data regarding the relationship between antipsychotic-induced hyperprolactinemia and ovarian function in women with schizophrenia are limited. Most studies of women treated with typical antipsychotics have reported high rates of menstrual abnormalities (50–75%) (Ghadirian *et al.,* 1982, Sullivan and Lukoff, 1990). However, very few studies have examined the relationship of prolactin and gonadal steroid levels in this population. Although a recent study of acutely psychotic female patients found no correlation between prolactin and estradiol levels, and no difference in the estradiol levels of patients on and off antipsychotic medications (Huber *et al*., 2001). Another study of outpatients with schizophrenia found an inverse relationship between prolactin and estrogen levels (Smith *et al.,* 2002).

The liver substantially metabolizes risperidone, with renal clearance in healthy older individuals being lower and elimination half-lives longer than in younger, healthy subjects (Yamanouchi *et al.,* 2003). Excess intake could result in lethargy, dystonia/spasm, tachycardia, bradycardia, and seizures (de Leon *et al.,* 2010; Bardgett *et al.,* 2013).

Risperidone intake has been associated with weight gain and increased appetite in adult female rats (Lian *et al*., 2015; Downnen *et al*., 2020). The study assessed the effect of risperidone on the histology and oxidative stress parameters of the ovaries.

## Materials and Methods

### *Ethical Consideration*

Ethical clearance for this study was obtained from the Department of Human Anatomy and Cell Biology, Faculty of Basic Medical Science, College of Health Science, Delta State University, Abraka, with the number DELSU/CHS/ANA/2023/13. Ethical guidelines concerning the use of laboratory animals for scientific research were followed strictly.

### Animals care and grouping

Fifteen (15) adult Wistar rats were bought from the College of Health Sciences, Animal House, Delta State University, Abraka. Before the medication was given, the rats were allowed to acclimate for seven (7) days in an iron cage that had compartments. The cages were kept in controlled climatic conditions with a 12-hour light/dark cycle, temperature of 25±5°C, relative humidity of 50±5°C, and well-ventilated, shaded sunlight that was not regulated. Every day, they received regular grower's mash (feed) and unrestricted access to water. The experimental animals were handled according to protocol which is approved by the institutional animals' ethics committee (IAEC) as adopted by the Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria.

### *Experimental Design*

The animals were categorized into three groups: A, B and C, each consisting of six (5) rats.

***Group A*** *(n - 5)* – Control which were fed with only standard grower's mash (feed) and water

***Group B*** *(n – 5) – Received 2.5 mg/kg body weight of* risperidone *for 42 days.*

**Group C** (n – 5) – Received 5 mg/kg body weight of risperidone for 42 days.

### Sample Collection

At the end of the treatment period, the rats were sacrificed via cervical dislocation, and the ovaries harvested (Gao *et al.*,2021). In order to homogenize the tissues for oxidative stress study, the ovaries were removed and examined for any morphological or histological abnormalities. They were then preserved in 10% formal saline (Weydert and Cullen, 2010).

**Tissue Processing and Photomicrography**
Ovarian tissues were fixed and processed following standard histological protocol as described by Drury and Wallington (1980). The fixed tissues were passed through various stages including sectioning and staining. Photomicrographs were taken using a USB 2.0 computer connected to a Digital Microscope Eyepiece "SCOPETEK" DCM 500, 5.0 megapixel.

**Oxidative Stress Parameters**
**Determination of Superoxide Dismutase (SOD) Activity**
The activity of SOD in the tissue homogenates was estimated spectrophotometrically following the method of Misra and Fredorich as adopted by Weydert and Cullen (2010).

**Determination of Reduced Glutathione**
The reduced glutathione was estimated in serum and tissue homogenates using the method of Ellman (1959) as adopted by Hassoun and Cearfoss (2011).

**Determination of MDA Activity**
A breakdown product of lipid peroxidation thiobarbituric acid reactive substance (TBARS) was measured in the tissue homogenates by the method of Gutteridge and Wilkins (1982).

**Statistical Analysis**
Data were analyzed using descriptive statistics, and the results were expressed as the mean and standard error of the mean. Differences in mean values were assessed using one-way analysis of variance (ANOVA) and graph pad prism, with a significance level set at $p<0.05$.

**Results**
The Rats in the control group showed the ovarian tissue composed of the outer cortex and inner medulla. The cortical region contained follicles at varying degrees of maturity, with Graafian follicles lined by cuboidal epithelium, mature oocytes, and well-defined structures. The medulla contained blood vessels and lymphatics, with the corpus luteum appearing unremarkable. This section is consistent with normal ovarian histoarchitecture (Figure 1a & 1b). The ovaries of rats treated with 2.5mg/kg of Risperidone showed ovarian tissue similar to the control group with normal histological features (Plate 1c & 1d). In the high dose group (5mg/kg Risperidone), the ovarian tissue exhibited features of cellular injury. The medulla contained congested blood vessels and white blood cell infiltration, suggesting a cellular reaction to injury (Figure 1d & 1e).

The antioxidant results were presented as graphs comparing the mean serum levels of antioxidant enzymes Catalase (CAT), Glutathione peroxidase (GPX), Superoxide dismutase (SOD), and the end product of lipid peroxidation, Malondialdehyde (MDA) in adult rats treated with Risperidone at graded doses of 2.5mg/kg per body weight and 5mg/kg per body weight, compared to the control group. The results showed no significant change in the mean serum concentration of CAT ($p=0.08$) and GPX ($p=0.11$) in rats that received 2.5 mg/kg (CAT: 25.18 unit/mg ± 4.808; GPX: 61.35 unit/mg ± 12.580) and 5 mg/kg (CAT: 15.98 unit/mg ± 4.808; GPX: 32.41 unit/mg ± 12.580) body weight of Risperidone compared to the control group (CAT: 17.82 unit/mg ± 4.088; GPX: 49.37 unit/mg ± 12.580). However, there was a significant decrease ($p=0.005$) in the mean SOD level of rats treated with 5mg/kg body weight of Risperidone (30.51 unit/mg ± 7.052) compared to the control group (58.85 unit/mg ± 7.052) (Figure 2.1). Furthermore, there was a significant increase in the mean concentration of MDA ($p=0.01$) in rats that received 5mg/kg body weight of Risperidone (2.83 mmol/g ± 0.237) compared to the control group (2.21 mmol/g ± 0.237) (Figure 2.2).

These results suggest that Risperidone administration, particularly at the higher dose of 5mg/kg body weight, may have significant effects on oxidative stress parameters in the ovaries of adult Wistar rats. Specifically, it led to a decrease in SOD levels and an increase in MDA levels, indicating potential oxidative stress in the ovarian tissue. However, there were no significant changes in CAT and GPX levels at either dose levels.

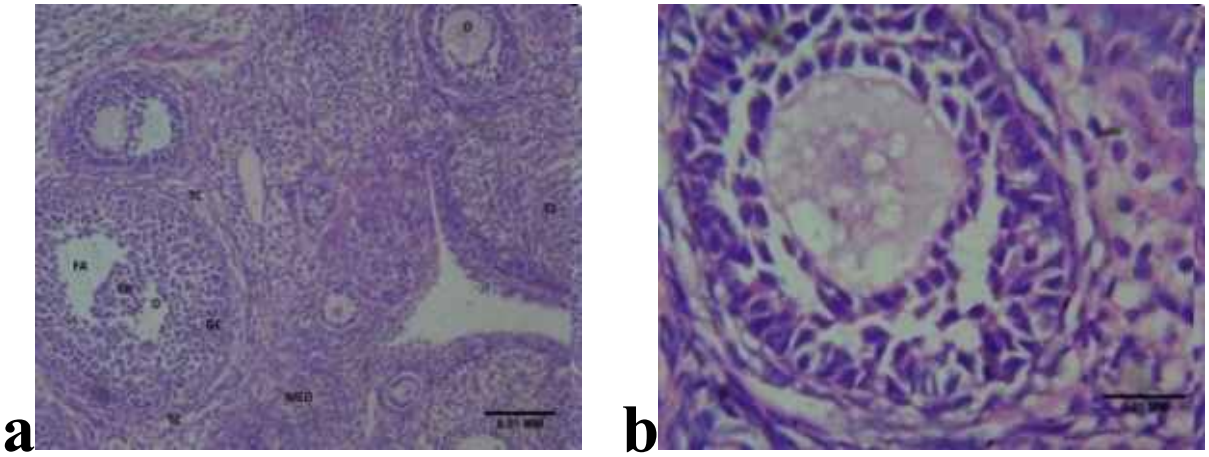


***Figure 1a & 1b - Photomicrograph of the Ovaries of Adult Wistar Rat of the Control Group (H and E X100 & X400)***

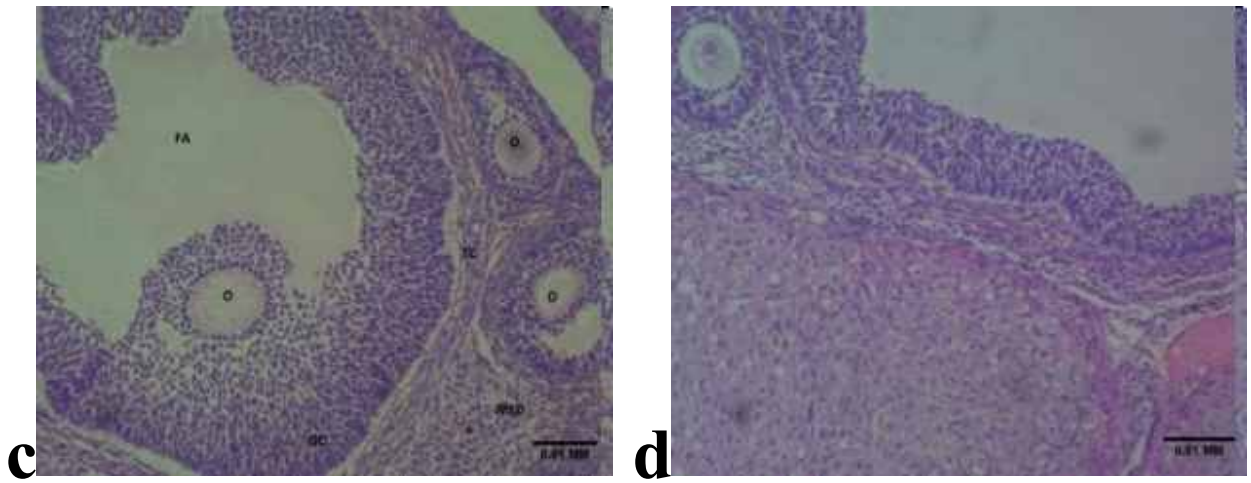


***Figure 1c& 1d - Photomicrograph of the Ovaries of Adult Wistar Rat given 2.5 mg/kg of Risperidone (H and E X100 & X400)***

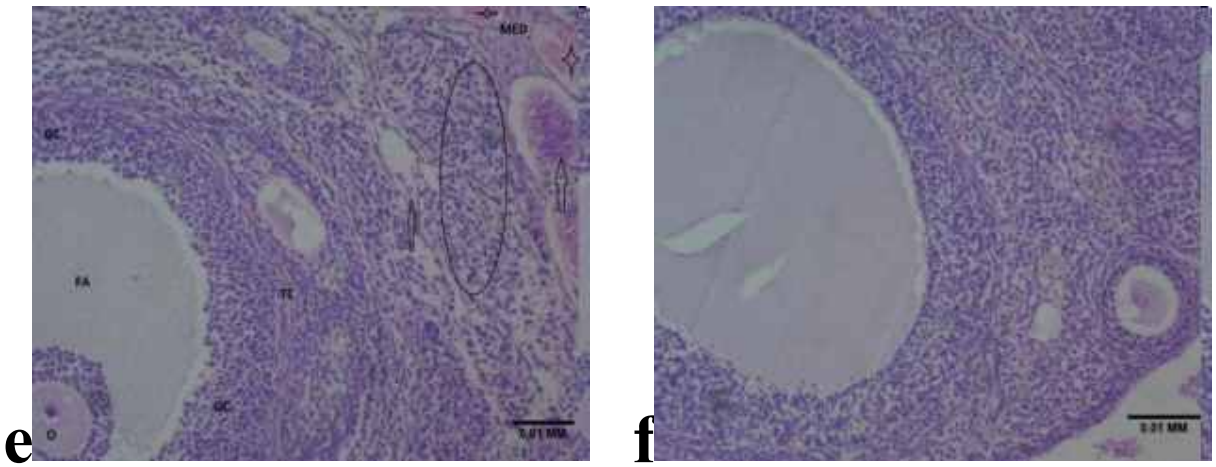


***Figure 1e& 1f - Photomicrograph of the Ovaries of Adult Wistar Rat given 5 mg/kg of Risperidone (H and E X100 & X400)***

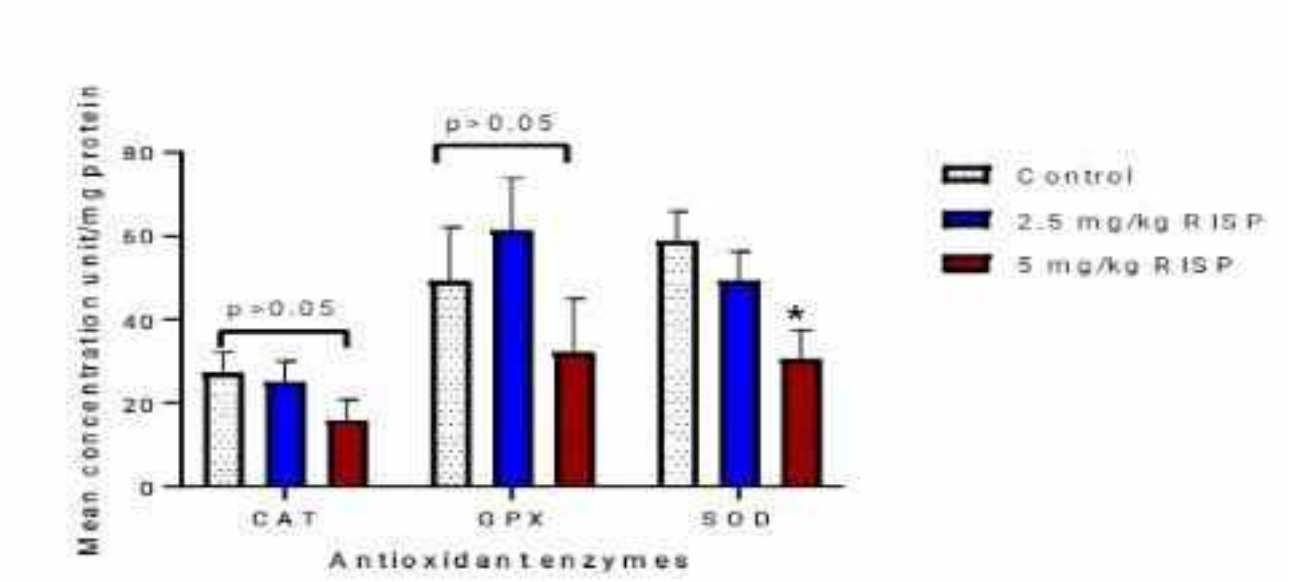


**Figure 2.1: Mean concentration of antioxidant enzymes.**
*Values are presented in graph as mean ± SEM for each group; n=5/group, *p<0.05 denotes*

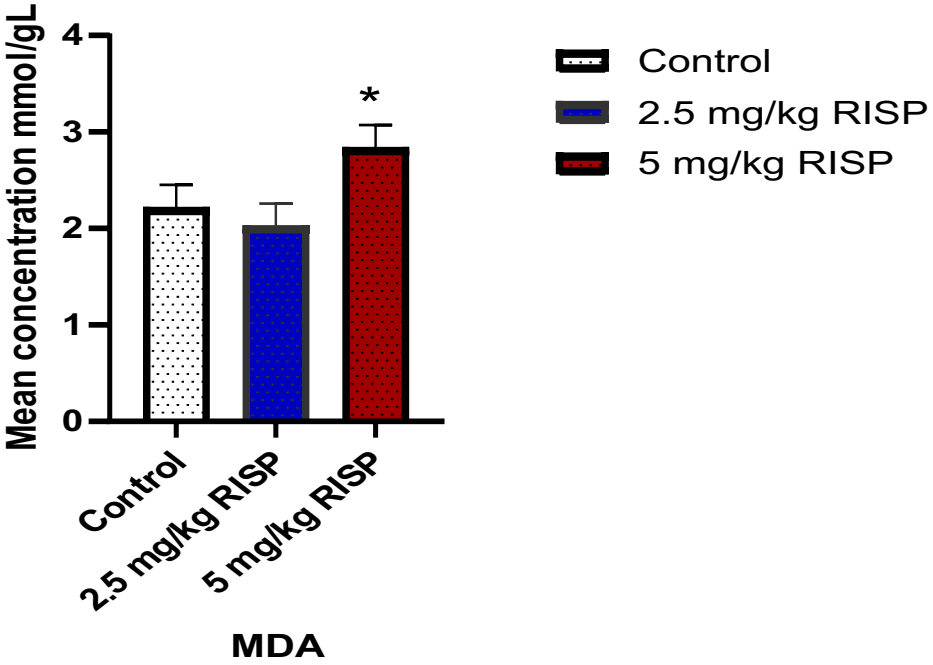


**Figure 2.2: Mean concentration of Malondialdehyde (MDA)**
*Values are presented in graph as mean ± SEM for each group; n=5 /group, *p<0.05 denotes significant difference compared to control.*

**Discussion**

Risperidone is a second-generation antipsychotic drug useful in the management of various mental health disorders. Previous studies have shown that Risperidone may have side effects such as drowsiness and instability (McNeil *et al*., 2023).

In this study, the histological examination of the ovaries of rats treated with Risperidone revealed varying dose dependent effects. Rats treated with 2.5 mg/kg showed ovarian tissue with a normal histological appearance. The ovarian cortex contained follicles at various stages of maturity, including Graafian follicles with mature oocytes, granulosa cells, and other defined structures. The medulla contained blood vessels and lymphatics, and the corpus luteum appeared unremarkable. However, rats treated with a higher dosage of 5 mg/kg of

Risperidone showed the presence of follicles at different stages of maturity, there was evidence of cell injury with vascular congestion and white blood cell infiltration. This suggests that Risperidone, particularly at higher doses, may have a toxic effect on ovarian tissue, potentially leading to cell damage and even cell death. Oxidative stress, characterized by the excessive production of reactive oxygen species (ROS), can result in damage to proteins, fats, and nucleic acids, including DNA, ultimately leading to cell death. Antioxidant enzymes and molecules play a crucial role in neutralizing these harmful ROS. The study found a significant decrease in the mean concentration of the antioxidant enzyme Superoxide dismutase (SOD) in animals treated with 5mg/kg of Risperidone compared to the control group. This decrease suggests that there was insufficient antioxidant protection against oxidative stress in the ovarian tissue, leading to cell damage. However, the antioxidant enzymes Catalase (CAT) and Glutathione peroxidase (GPX) showed no significant change across treatment groups when compared to the control group. This suggests that CAT and GPX were effective in preventing oxidative stress and subsequent tissue damage. The study also observed a significant increase in the mean concentration of Malondialdehyde (MDA), a product of lipid peroxidation, in animals treated with 5mg/kg of Risperidone. This increase indicates oxidative damage to the tissue, likely caused by the presence of free radicals and reactive oxygen species.

## Conclusion

The study reveals that Risperidone administration in rats has a dose-dependent effect on ovarian tissue, with the higher dose of 5mg/kg indicating potential harm. While the lower dose of 2.5mg/kg did not show significant alterations, the higher dose led to cellular responses indicative of tissue injury and a heightened risk of cell death. The biochemical assays underscored the importance of antioxidant enzymes, with CAT and GPX exhibiting protective effects against oxidative stress. However, the diminished levels of SOD in rats treated with the higher dose of Risperidone suggest inadequate antioxidant defense, leading to oxidative damage as reflected by elevated MDA levels. Overall, these findings highlight the potential toxicity of Risperidone on ovarian health, particularly at higher doses, emphasizing the importance of further research to elucidate its mechanisms and implications for reproductive health.

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Nil.

**Conflicts of Interest**
Nil.

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