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Ovarian Gene Transcriptional Responses To Antidepressant Drugs (Imipramine And Fluoxetine) In Female Wistar Rats

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ABSTRACT

This study was designed to investigate ovarian gene transcriptional responses to selected antidepressant drugs (imipramine and fluoxetine) in female rats. Fifteen female rats (120 – 140 g) were used for this study. Imipramine (0.71 mg/kg) and fluoxetine (0.57 mg/kg) were given orally for 50 days. The method of RT-PCR was employed to investigate the expressions of FSH-R, p53 and Bcl-2 genes. Graphics were generated as mean +/- SEM using GraphPad Prism version 8.0. Results of the study revealed that the FSH-R, p53 and Bcl-2 expressions were significantly ($p<0.05$) up-regulated in the imipramine-treated rats relative to their controls. Conclusively, it can be suggested that imipramine induced follicular growth and apoptosis in female Wistar rats.

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1 Introduction

Antidepressants are a group of drugs used to cure depression, anxiety, pain, and to treat addictions (Jennings, 2018). Their side effects are xerostomia, obesity, sexual impairment (Healy et al. 2018) and emotional disorder (Sansone and Sansone 2010). Taking these drugs can lead to a higher chance of suicide thought by people of different age categories. Stoppage of antidepressant medication may lead to discontinuation syndrome (Gabriel and Sharma 2017).

Some research that has been carried out previously revealed the efficacy of antidepressant agents in adults (Barth et al. 2016), while some other researchers have given contrary results (Jakobsen et al. 2020); however, the evidence of their usefulness in children and adolescents has not been proven (Cipriani et al. 2016). In Nigeria, the most often prescribed antidepressant agents are twenty-one in number, and they give better results than a placebo for short-term treatment of mature patients suffering from depressive ailment (Cipriani et al. 2018). Investigation concerning the efficacy of antidepressant agents is carried out on people suffering from severe symptoms; this group of people show lower responses to placebo, which indicates that the outcome cannot be generalized to the whole people suffering from this ailment (Cipriani et al. 2018).

The effects of antidepressant drugs on rats' neurogenic regions (Nasrallah et al. 2010), trained rats to discriminate centrally given isoproterenol (Alicia and James 2002), sexually induced side effects (Dimitry et al. 2017), rats prenatally stressed (Jordan et al. 2014), mice hippocampus (Filiou et al. 2014), genetics of mice (Kazuko et al. 2013), pregnant mice (Rahn et al. 2019), DNA damage (Eduardo et al. 2022) as well as on cognition and cardiovascular system (Ali, 2022) have been well studied. But, as a result of limited information obtained from the literature concerning the effects of antidepressant drugs (imipramine and fluoxetine) on ovarian gene expression in female rats, this research intends to bridge this gap.

2 Materials and Methods

2.1 Experimental Animals

Fifteen female rodents of weight range 120 – 140 g raised in the Animal Holding of ABUAD were used in the current study. These rodents were accommodated in a conducive laboratory atmosphere with an unlimited feed and water supply for two weeks before starting the experiments. The Helsinki Declaration on animal experimentation was used for animal experiments.

2.2 Drugs

Antidepressant drugs Imipramine (Dellwich Healthcare Ltd) and fluoxetine (MedreichPlc, UK) were purchased from Danax Pharmacy, Ibadan, Nigeria. Among these, imipramine (25 mg) and fluoxetine (20 mg) were liquefied in l0 ml of distilled water to produce concentrations of 2.5 mg/ml and 2.0 mg/ml, respectively. The dosages of the antidepressant drugs considered in this research were as per the suggestions of the manufacturing industries.

2.3 Experimental Design

Fifteen matured female rats (five per group) used in this study received the oral doses of the antidepressant drugs and distilled water (control) for 50 days as per the predefined group as follows (i) Group I rodents (control group) were given 5 mL/100 g of water (distilled), (ii) Group II rodents were given 0.71 mg/kg of imipramine, and (iii) Group III rodents were given 0.57 mg/kg of fluoxetine.

On the next day after the last treatment (day 51), the rodents were euthanized by overdosing with diethyl ether; ovaries were harvested with the fatty tissue removed and quickly transferred into TRIzol reagent (ThermoFisher Scientific) for total isolation of RNA.

2.4 Isolation of RNA

RNA was isolated from whole tissues as described by Omotuyi et al. (2018). In summary, the ovaries were homogenized in TRI reagent at cold 4 ℃. Partitioning Total RNA in chloroform was done by centrifuging at 15,000 rpm for 15 minutes. The supernatant containing RNA was removed from the solution with isopropanol of the same volume. Ethanol (70%) was used to wash the extracted RNA twice, which was then dehydrated for 5 minutes before being re-suspended in the buffer.

2.5 Conversion of cDNA

Spectrophotometer was used to determine the purity and quantity of total RNA at an absorbance of $A_{260}/A_{280,}$ as described by Omotuyi et al. (2018).

2.6 Polymerase chain reaction (PCR)/Electrophoresis

FSHR, p53 and Bcl-2 genes were amplified by PCR targeting primers highlighted in the table below. A software called Primer3 was used to design the primers. The PCR amplification process was carried out as described by Omotuyi et al. (2018).

Amplification products were Electrophoresis in agarose gel (1.5%) using 0.5X TBE (Tris-borate EDTA, JHD chemicals, China) containing ethidium bromide at 100V for 60 minutes. The gel was visualized with UV light with a photo documentation system fitted with a camera. Gel images were analyzed using the keynote platform described by Omotuyi et al. (2020), and Image J software was used to quantify them. Graph-pad prism version 8.0 was used to plot the graphs as average +/- SEM.

Table 1 List of used primers

Primers	Sequence	Product length	Annealing temperature
FSHR	F:ATTCTTGGGCACGGGATCTG R:TGGTGAGCACAAACCTCAGTT	140	55.09 °C
P53	F: TCTCCAGATTCGGCAGCAAG R: GGCCCGTCAGAGCTTTCAT	126	55.10 °C
BCL-2	F:GCGTCAACAGGGAGATGTCA R:TTCCACAAAGGCATCCCAGC	119	55.47 °C

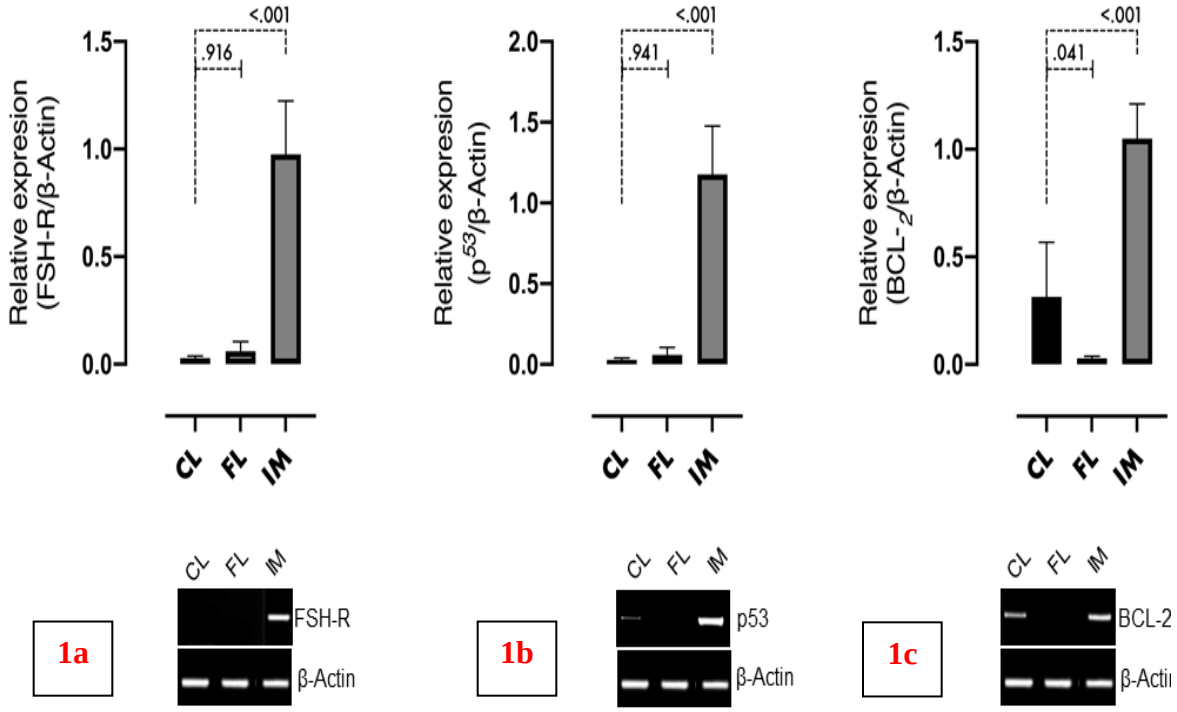

Figure 1 Comparative expression of (1a) FSH-R, (1b) p53, (1c) Bcl-2 in the ovary of rats treated with fluoxetine (FL) and imipramine (IM) 1a as well as gel image expression patterns of FSH-R and β-actin for fluoxetine (FL) and imipramine (IM) treated rats (β-actin served as the internal control). The band density (image J) was plotted as a bar graph (n=5 p<0.05).

3 Results

Results presented in Figure 1a revealed that FSH-R expression was significantly up-regulated ($p<0.05$) in the imipramine-treated rats relative to the control. Furthermore, the results presented in Figure 1b suggested that the expression of p53 was significantly up-regulated ($p<0.05$) in the imipramine-treated rats relative to the control. Similarly, figure 1c also suggested that Bcl-2 expression was up-regulated significantly ($p<0.05$) in the imipramine-treated rats relative to the control.

In addition, results presented in Figures 1a and 1b revealed that FSH-R and p53 expressions were nonsignificantly ($p>0.05$) up-regulated in the fluoxetine-treated rats compared to the control. In contrast, Bcl-2 expression was significantly ($p<0.05$) down-regulated in the fluoxetine-treated rats as compared to the control (Figure 1c).

4 Discussion

The FSH-R expression was significantly up-regulated in the imipramine-treated rats, which probably indicates that the imipramine induced follicular growth and these results were corroborated with the findings of Dewailly et al. (2016) and Jamnongji and Hammes (2006) while the findings of Jue et al. (2014) in Pacific Oyster treated rats are contrary to the findings of the present study. Similarly, the p53 expression was significantly up-regulated in the imipramine-treated rats, which suggests that imipramine induced apoptosis and these results are validated by the assertion of Fridman and Lowe (2003). Ukwade et al. (2020) reported a similar effect in the *Byrsocarpus coccineus* treated ovarian cancer cell line. In addition, Bcl-2 expression was also significantly up-regulated in the imipramine-treated rats, which probably indicates that imipramine stimulated or induced apoptosis in ovarian tissue, and these results were corroborated by the

assertions of Boise et al. (1993), Oltvai et al. (1993), and Choudhuri et al. (2002) while the findings of Majid et al. (2019) in *Olea europaea* are contrary to the present study.

Unlike imipramine, the expression of FSH-R was insignificantly down-regulated in the fluoxetine-treated rats, which probably indicates that fluoxetine inhibits follicle growth, and these results corroborated with the findings of Dewailly et al. (2016), Jamnongji and Hammes (2006), and Seyedeh-Roza et al. (2021). Similarly, the Bcl-2 expression was significantly down-regulated in the fluoxetine-treated rats, which probably indicates that fluoxetine prevented apoptosis in ovarian tissue, and these findings are supported by the previous study of Boise et al. (1993), Oltvai et al. (1993), Ebrahim et al. (2016), and Choudhuri et al. (2002) while contrary to the result reported by Mohammad et al. (2022) in minocycline treated rats. Further, the expression of p53 was nonsignificantly up-regulated in the fluoxetine-treated rats, suggesting that fluoxetine induced apoptosis. Similar results were reported by Fridman and Lowe (2003), while the findings of Nori-Garavand et al. (2020) in selenium-treated mice contradict the results of this study.

Conclusively, it can be suggested that imipramine induced follicular growth and apoptosis in female rats, while fluoxetine probably inhibited follicular growth and apoptosis in female rats.

Conflict of Interest

There is an absence of conflicting interests in this research work.

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