Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

The Biochemical, Genotoxic, and Oxidative effects of Duloxetine Hydrochloride Drug on the Reproductive Organs Health of Female Wistar Rats

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Abstract

Duloxetine hydrochloride is one of the most abundant antidepressants among Serotonin-Norepinephrine reuptake inhibitors. It is endorsed for treating the major depressive disorder, generalized anxiety disorder, diabetic peripheral neuropathic pain, and fibromyalgia. Our study aims to evaluate the implications of duloxetine hydrochloride on the reproductive health of female Wistar rats by measuring oxidative stress markers, histopathological alterations in reproductive tissues, and serum reproductive hormones, besides assessing the genotoxicity of duloxetine on female rats' reproductive organs using the comet assay. We conducted this study on 16 female Wistar rats divided into 2 groups; the control group; was administered distilled water, while the treated group; was administered 6.1 mg/kg of Duloxetine for a month. The results indicated a significant elevation in lipid peroxide Malondialdehyde and a significant decrease in reduced glutathione levels. A hormonal imbalance was evidenced by a reduction in Estrogen levels, while Follicle-Stimulating Hormone (FSH) levels dramatically raised. Pathogenic morphological changes appeared in the reproductive tissues of the treated group compared to the control group. Moreover, significant DNA damage in the uterus and ovaries tissues of the treated group was revealed by the comet assay. Our results suggest cumulative damage may be produced by duloxetine due to its oxidative stress induction potency.

Keywords: Antidepressant; Duloxetine; Reproductive Health; Oxidative stress; Female rats

1. Introduction

Infertility is the threat that worries every couple, it is the incompetence to obtain pregnancy after a year of regular and unprotected sexual intercourse; it could be a struggle confronting 8 to 12% of couples amid their regenerative age worldwide wide [1][2]. Due to their unsuccessful attempts at conception, people who are trying to conceive may have feelings of shame, and low self-esteem [3]. These terrible sentiments can lead to depression, and misery [4]. Women experience depression almost twice as prevalent as men [5], moreover, they endure protracted or recurring depression more frequently than depressed men do, with an early-onset age and lower quality of life. They even account for the majority of antidepressant consumers [6].

Over the last few decades, antidepressants have been increasingly used in the treatments for major depressive disorder, as 17% of individuals globally experience severe depression at some stage in their lives [7]. Selective Serotonin Reuptake Inhibitors (SSRIs) are the primary line of antidepressants, but lately, Serotonin and Norepinephrine Reuptake Inhibitors (SNRIs) have been favoured for their unique characteristics and the dual reuptake nature of both Serotonin and Noradrenaline, making SNRIs useful therapeutic alternatives, especially in severe cases experiencing depression, they're also known for their high tolerance and safety margins [8]. In many observational trials, antidepressants showed evidence of toxicity in the reproductive system and sexual functions for both males and females [9]. Long-lasting treatment with SSRIs produces oxidative

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Receive Date: 03 January 2023, **Revise Date:** 09 March 2023, **Accept Date:** 22 March 2023,
First Publish Date: 22 March 2023
DOI: 10.21608/EJCHEM.2023.185199.7418

damage to sperm they also caused an increase in the ejaculation latency that reduced sperm quality seen with all SSRIs evaluated thus far, including higher sperm DNA fragmentation [10]. It has been demonstrated that SNRIs increase male prolactin levels, which is commonly linked to their inhibitory effects on male reproduction [11].

There is an ongoing debate over whether antidepressants directly affect gonadal hormones. Bataineh et al. studied long-term exposure to the SSRI fluoxetine orally for 60 days, it resulted in lowering the testicular, epididymal, and prostate volumes as well as lowering sperm counts and motility with a significant reduction in FSH levels [12][13].

Duloxetine hydrochloride belongs to the SNRIs class, that is available in the form of [(+/)-N-methyl-3-(1-napthalenyloxy)- 2 thiophenepropanamine], it has low affinities for monoamine receptors in the central nervous system and has little effect on histaminic H1, muscarinic, α1-adrenergic, and opioid receptors, it is also one of the most potent antidepressants due to its validity, and potency [14]. Duloxetine was approved for the treatment of major depressive disorders (MDD) firstly in 2004. It's also approved to treat generalized anxiety disorder, stress urinary incontinence, variant degrees of diabetic peripheral neuropathic pain, and chronic musculoskeletal pain, as well as the general pain of fibromyalgia [15].

Regarding its long duration of administration, the safety of duloxetine on reproductive health should be considered. A prior study revealed a link between duloxetine uptake and oxidative stress induction [16]. Álvarez-González et al. demonstrated duloxetine's DNA-damaging capacity in mice's brain and liver tissues [17].

Due to the scarcity of evidence for the impact of duloxetine on female reproductive health, this study aims to estimate the safety of duloxetine hydrochloride on the reproductive system of female Wistar rats and evaluate the potential oxidative effect, histopathological and sex hormone variations, as well as clarify the genotoxic potential of duloxetine on the female uterus and ovaries cells via comet assay after a month of daily treatment.

2. Material and Methods

2.1. Ethical Approval

All the experimental approaches and chosen protocols used in this study were approved by the Cairo University, Faculty of Veterinary Medicine Institutional Animal Care and Use Committee (IACUC) (Egypt), Vet CU011020202014.

2.2. Drug Used

Cymbalta tablets produced by (El Lilly Company, Cairo, A. R. E.), each tablet contains 60 mg of duloxetine hydrochloride. The tablets were soluble in distilled water. The recommended dose for a human is 60 mg/daily [15]. The human dosage was adjusted for rats' weight according to [18] so 6.1 mg /kg was the appropriate dose.

2.3. Experimental Design

The sample size was calculated by the method of Arifin and Zahiruddin [19], 16 healthy adult female Wistar rats (*Rattus norvegicus*), approximately 2 to 2.5 months (170-180 grams) were acquired from the animal house of the Faculty of Veterinary Medicine, Cairo university- Egypt, and used for experimentation under the optimal and standard conditions (21 ± 1°C, 60 ± 20% humidity, and 12 hrs. light/12 hrs. dark cycle) with the standard rat food and free access of water. The rats were separated into two groups at random, each group had eight rats. The first one is the control group that had gotten orally distilled purified water for 30 days. The second group is the treated group which received orally 0.4 ml of duloxetine hydrochloride drug solution dissolved in distilled water for 30 days. The rats were sacrificed at the end of the experiment with anesthesia using sodium pentobarbital (40 mg/kg) via the IP injection. The ovaries and uteri were dissected out, cleaned in physiological saline solution, examined, and weighted to calculate the absolute and relative weights.

2.4. Biochemical Studies

Autopsy samples taken from ovaries and uterus were washed properly and then rinsed with ice. Tissue pieces were weighed in an analytical balance. We prepared 10% homogenate in 0.05 molar from the phosphate buffer saline (pH 7) at 4^0C using a polytron homogenizer. Then this homogenate solution was centrifuged for 20 minutes at 10 x 10^3 rpm to eliminate cellular debris, nuclei, erythrocytes, and mitochondria. The supernatant that contains cytoplasmic extract was used to calculate; Lipid Peroxidation (MDA) using the method of Kei [20], Glutathione Reduced (GSH) based on Beutler et al. study [21], Superoxide Dismutase (SOD) according to Greenwald [22], and Catalase (CAT) was measured by the method of Aebi [23].

2.5. Histopathological Studies

The dissected ovaries and uteri were settled in 10% formalin for 24 hrs. before being washed with tap water and dried out with sequential dilutions of methyl alcohol followed by ethyl alcohol, and lastly absolute ethyl alcohol. Specimens were passed on xylene and fixed in paraffin for 24 hrs. at 56ºC in a hot air oven. A Sledge microtome was utilized to obtain paraffin blocks for sectioning the thickness at 4 microns. Tissue sections were collected on glass slides, deparaffinized, and stained with the tissue stain hematoxylin and eosin (H&E) for routine examination before being examined with a light electric microscope [24]

2.6. Hormones Estimation

The whole blood was collected after heart puncturing and kept in clean tubes at room temperature for 2 hrs. and then the tubes were centrifuged for 20 minutes at approximately 1000×g, the serum was collected and stored at −20 ºC for further analysis of sex hormones. Concentrations of luteinizing hormone (LH), follicle-stimulating hormone (FSH), estrogen (E2), and progesterone (P4) were assessed using the Enzyme-Linked Immunosorbent Assay (ELISA), immunodiagnostic reagents, and ELISA kits specific for rats supplied by Wuhan Fine Biotech Co., Ltd. Kits.

2.7. Comet Assay

Comet assay was done to detect the DNA breaks in a single cell of ovaries and uterus tissues, the procedure was based on previously published guidelines by Tice et al.[25]. We used about 100 cells of well-minced tissues for each tested sample with viability of more than 80%, moreover, the magnitude of qualitative as well as quantitative DNA breaks in cells were assessed by staining DNA with ethidium bromide and determining the following parameters; the tail DNA (%), the length of tail, DNA in the tail, the tail olive moment, and the tail moment, using a 40x objective on a fluorescent microscope adapted to image analyzer software (Komet 5) developed by (Kinetic Imaging, Ltd. Liverpool, UK) and connected to a closed-circuit digital camera (Carl Zeiss Axioplan with epifluorescence using (filter 15 BP546/12, FT580, and LP590).

2.8. Statistical Analysis

All data are presented as Mean ± SEM, and the statistically significant change across the examined groups was evaluated using independent samples t-test and calculated with the SPSS version 18 for windows (SPSS, IBM, the USA). The *P*-value < 0.05 indicate statistical significance [26].

3. Results

3.1. Effect of Duloxetine on weights

The weight of rats in the two groups was measured (final weight - initial weight) and no statistically significant change was observed between the two groups. The relative weights of the right ovary and uterus of the treated group increased significantly over the control group, while there was no statistically significant difference in the relative weight of the left ovary (Table 1).

3.2. Oxidative stress results

The oxidative impact of duloxetine administration on the ovaries and uterus tissues of our treated group relative to the uterus and ovaries tissues of the control group was measured. The results illustrate a significant rise in lipid peroxidation in both organs expressed by the high levels of malondialdehyde MDA, in contrast, GSH had decreased significantly in both ovary and uterus tissues ($P < 0.05$). On the other hand, neither SOD nor CAT levels changed in the ovary or uterus tissues between groups (Table 2&3).

Table 1: The effect of 4 weeks of Duloxetine hydrochloride exposure on the body weight change and relative reproductive organs weight.

Group	Weight change (gm)	The relative weight of the right ovary (%)	The relative weight of the left ovary (%)	The relative weight of the uterus (%)
Control	14.5 ±3.7	0.023 ± 0.003	0.021 ± 0.0024	0.2 ± 0.012
Treated (6.1 mg /kg)	16.16 ±3.2	0.033 ± 0.004	0.024 ± 0.004	0.27 ± 0.02
P-value	0.74	0.033	0.46	0.017

The data were expressed as mean ± SEM. All data were calculated by the independent samples t-test. ($P < 0.05$) indicates statistically significant differences.

Table 2: The effect of 4 weeks of duloxetine exposure on ovarian oxidative stress markers (MDA, GSH, SOD, and CAT).

Group	MDA (nmol/mg)	GSH (nmol/mg)	SOD (U/mg)	CAT (nmol/mg)
Control	0.324 ± 0.05	1.74 ± 0.06	2.2 ± 0.04	3.6 ± 0.15
Treated (6.1 mg/kg)	0.62 ± 0.05	1.4 ± 0.07	2.1 ± 0.14	3.2 ± 0.18
P-value	0.029	0.028	0.535	0.199

The data were expressed as mean ± SEM. All data were calculated by the independent samples t-test. ($P < 0.05$) indicates statistically significant differences.

Table 3 : The effect of 4 weeks of Duloxetine exposure on uterine oxidative stress markers (MDA, GSH, SOD, and CAT).

Group	MDA (nmol/mg)	GSH (nmol/mg)	SOD (U/mg)	CAT (nmol/mg)
Control	0.37 ± 0.02	1.73 ± 0.06	2.2 ± 0.12	3.6 ± 0.15
Treated (6.1 mg/kg)	0.58 ± 0.05	1.42 ± 0.06	1.8 ± 0.05	3.1 ±0.18
P-value	0.04	0.019	0.078	0.13

The data were expressed as mean ± SEM. All data were calculated by the independent samples t-test. ($P < 0.05$) indicates statistically significant differences.

3.3. Histopathological results

The histopathological changes in uterine and ovarian tissues were evaluated using light microscopy; uterus tissue sections from the control group had normal morphology. Figures 1a-c represent the normal layers of the uterus wall of the control group including the endometrium which is composed of surface simple columnar epithelial cells and underlying lamina propria containing endometrial glands lined with simple columnar epithelial cells with basal oval nuclei, the longitudinal muscle layer, and the perimetrium can be seen. Various pathological features appeared in the uterus tissues of rats after duloxetine administration elucidated in the degenerated and disorganized cells in the muscle layer with congested blood vessels that have thickened hyalinized walls and numerous vacuolations were also observed (Fig. 1d&e). Endometrial infiltration with a significant proportion of lymphocytes, neutrophils, and polymorph nuclear cells. The luminal and gland epithelium had necrosis, with a large number of apoptotic and degenerated cells represented in (Fig. 1d-g). The ovaries of the control group showed the common morphologies of developing follicles (Fig. 2a-c). Granulosa cells surround the growing follicles. Multilayered granulosa cells with antral cavities surround mature follicles and the atretic follicle besides clusters of stromal cells was also evident. Moreover, ovarian tissues of the 6.1 mg/kg duloxetine-treated group exhibited pathological changes, including a degenerated oocyte and loss of cellular architecture in follicular granulosa cells whereas most of these cells had pyknotic nuclei (Fig. 2d-h). Extreme ovarian stromal hemorrhage appeared clearly in the ovarian stroma (Fig. 2e), follicular degeneration, vascular dilatation, and congestion. dramatically increased the apoptotic cells in some parts of the granulosa layer (Fig. 2d-h).

3.4. The effect of duloxetine hydrochloride on serum hormonal levels

Serum estrogen levels were significantly decreased after duloxetine administration when compared to the measured estrogen of the control group, while serum FSH levels increased significantly in the treated group than in the control group ($P < 0.05$). The serum progesterone and LH levels did not change across different groups (Table 4).

3.5. Comet assay result

The comet assay results showed elevated DNA damage after 6.1 mg/kg duloxetine exposure in the uterus and ovaries tissues of the treated group in comparison to the control group. Tail DNA (%), tail length, DNA in the tail, tail moment, and olive moment were all measured. Significant damage was observed in the treated group's comet tail length, tail DNA (%), and tail moment in the uterus (Table 5 and Fig. 3b), while a significant increase in comet tail length and DNA in the tail of the ovary's tissues in the treated group more than the control group highlighted in Table 6 and Figure 3d.

Table. 4: The effect of 4 weeks of duloxetine on serum hormonal levels.

Group	E2 (pg/mL)	P4 (ng/mL)	FSH (mIU/ml)	LH (mIU/ml)
Control	38.12 ± 2.7	20.36 ± 1.2	13.38 ± 1.6	1.01 ± 0.18
Treated (6.1mg/k)	26.54 ± 1.3	17.5 ± 2.7	58.84 ± 4.2	0.72 ± 0.13
P-value	0.01	0.37	0.00	0.22

The data were expressed as mean ± SEM. All data were calculated by the independent samples t-test. ($P < 0.05$) indicates statistically significant differences.

Table 5: The effect of 4 weeks of Duloxetine exposure on DNA damage in the uterus tissues.

Group	Tail DNA (%)	Tail length	DNA in the tail	Tail moment	Olive moment
Control	8.96 ± 0.7	4.7 ± 0.02	9.5 ± 0.03	0.45 ± 0.06	0.97 ± 0.014
Treated (6.1 mg/kg)	14.2 ± 0.7	10.6 ± 0.36	10.6 ± 0.6	1.1 ± 0.08	1.3 ± 0.14
p-value	0.006	0.003	0.22	0.006	0.16

The data were expressed as mean ± SEM. All data were calculated by the independent samples t-test. ($P < 0.05$) indicates statistically significant differences.

Table 6: The effect of 4 weeks of Duloxetine exposure on DNA damage in the ovaries tissues.

Group	Tail DNA (%)	Tail length	DNA in the tail	Tail moment	Olive moment
Control	10.05 ± 0.03	6.6 ± 0.23	8.5 ± 0.3	0.6 ± 0.05	1.12 ± 0.09
Treated (6.1 mg/kg)	11.4 ± 0.6	7.92 ± 0.28	9.8 ± 0.16	0.75 ± 0.01	1.4 ± 0.09
P-value	0.16	0.026	0.036	0.09	0.097

The data were expressed as mean ± SEM. All data were calculated by the independent samples t-test. ($P < 0.05$) indicates statistically significant differences.

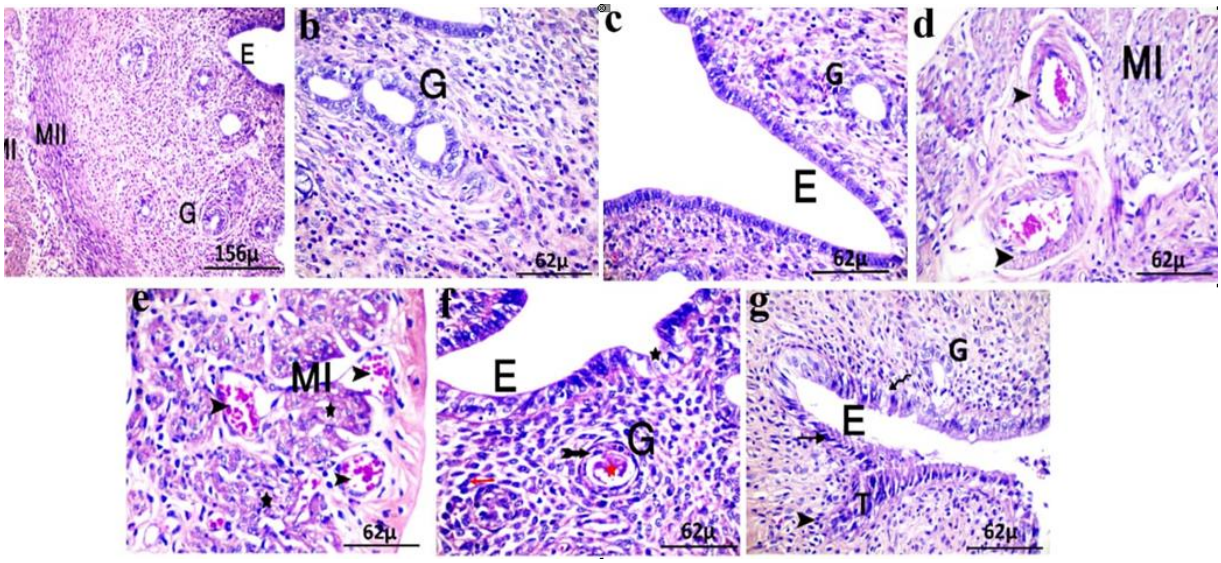

Fig. 1. A photomicrograph of H&E-stained sections of the female rat's uterus**, (**a-c) represent the uterus of the control group. (a) showing layers of the uterus' wall include endometrium (E), inner circular muscle (MII), and longitudinal muscle layer (MI) perimetrium (curved arrow). (b) the glandular epithelium can be observed (G) lined with multiple regular columnar cells with basal oval nuclei. (c) The endometrium layer is composed of surface (E) simple columnar epithelial cells and underlying lamina propria (LP) containing endometrial glands (G) lined with simple columnar epithelial cells with vesicular nuclei. (d-f) Photomicrographs of uterus sections from the treated group with 6.1 mg/kg duloxetine, (d) Showing longitudinal (MI) muscle layer, congested blood vessels were clear with hyalinized thick wall (arrowhead). (e) Longitudinal (MI) muscle layer with deteriorated cells (star) and multiple vacuolations (arrow), in addition to congested blood vessels (arrowhead). (f) Sever lymphocytes, and polymorph nuclear cells infiltration in the endometrium (red arrow). the uterine endometrium epithelium (E) with degenerated cells (black star), glandular epithelium (G) lined with syncytium epithelial wall provided pyknotic nuclei (black arrow) and its lumen filled with cellular debris and casts (red star). (g) Demonstrates necrosis and degeneration of luminal and gland epithelium with a greater incidence of apoptotic cells (arrow) and vacuoles (wavy arrow) in the endometrium, as well as severe neutrophil infiltration (arrowhead).

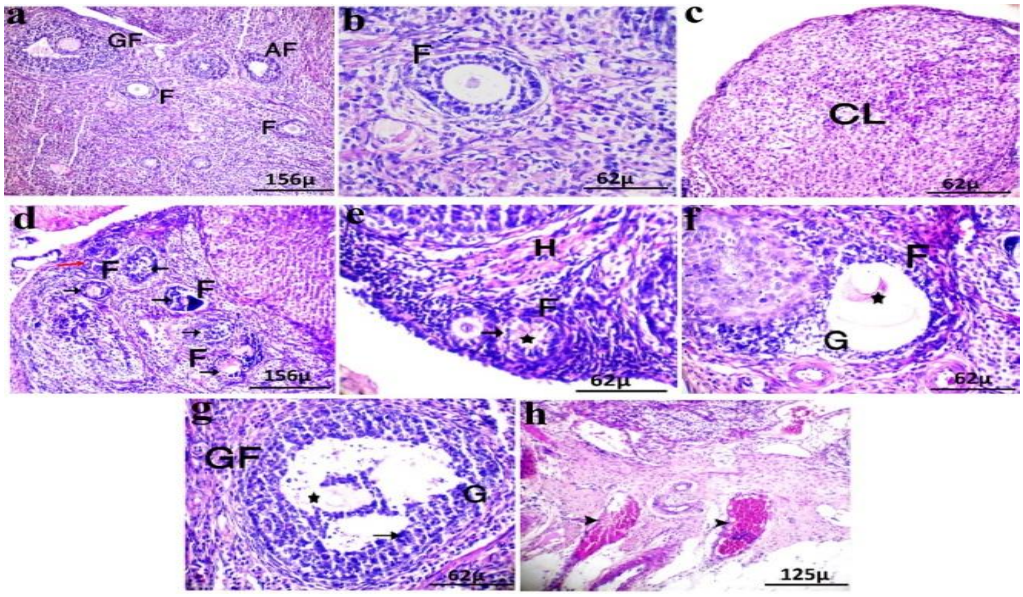

Fig. 2. Photomicrograph rats' ovarian sections stained with H&E, (a-c) represent the ovarian sections of the control group. (a) The developing follicles appear clearly in the cortex of the ovary and the granulosa cells that surround the developing follicles distinguish them. The mature follicles are unsheathed with multi-layered granulosa cells and an antral cavity. Atretic follicles (AF) and stromal cell clusters were observed. (b) Follicles are consisting of oocytes with round nuclei that are surrounded by flattened follicular cells (F). (c) Large corpora lutea (CL) were composed of moderately eosinophilic cells with large vesicular nuclei and foamy cytoplasm. Photomicrographs of ovarian sections from the treated group with 6.1 mg/kg duloxetine (d-h), (d) Representing the varying degrees of degenerative alterations, including a degenerated oocyte and disorganized follicular granulosa cells (arrow) with darkly stained nuclei, reduction in the ovarian follicles were seen, and pre-ovulatory follicle cells were degenerated (red arrow). (e) The follicle has markedly degenerated granulosa cells and multiple vacuoles (arrow) with a massive ovarian stromal haemorrhage (H). (f) Arteric follicles (F) with degenerated oocytes and zona pellucida (star), disintegrating granulosa cells (G), and destruction. (g) There was also a degenerated ovum with no visibility of the nucleus or nucleolus, as well as the absence of zona pellucida (star). Within the granulosa layer (G) of the Graafian follicle (GF), the cells appeared apoptotic, with deep acidophilic cytoplasm and pyknotic nuclei (arrow). (h) The ovary has dilatation and blood vessel congestion (arrowhead).

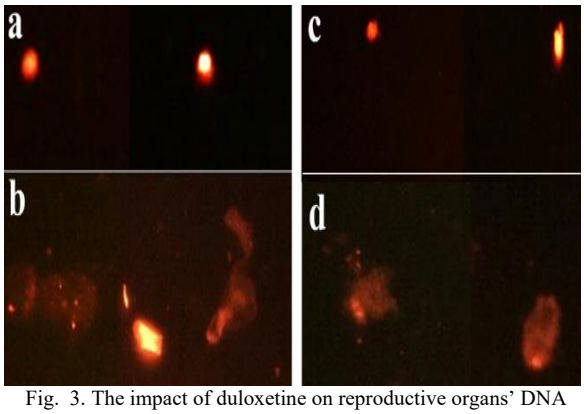

Fig. 3. The impact of duloxetine on reproductive organs' DNA damage of female rats among the studied groups. (a) The control group of the uterus cells shows intact and rounded nuclei. (b) The cells from the treated group of the uterus showed a high damage degree of the DNA. (c) The cells of the control group's ovaries show a rounded nucleus with no tail. (d) The treated group ovaries' cells show high degrees of DNA damage.

4. Discussion

It is believed that there is a strong correlation between infertility and psychological problems [27]. Duloxetine is an SNRI antidepressant that showed potent regulation of serotonin-norepinephrine uptake, and it is also effective against MDD and related conditions [15]. In general, women are more susceptible to MDD than men and are more prone to consume antidepressants [28]. Therefore, it is mandatory to confirm duloxetine's safety on female reproductive health. In this context, we evaluated the safety of duloxetine hydrochloride in the female Wistar rats' reproductive system in a comprehensive vision through histopathological examination of the uteri and ovaries tissues, observing the oxidative stress markers, measuring sex hormone changes, and the variations in the DNA molecules after a month of daily administration.

Body weight and the relative weights of uteri and ovaries were calculated to assess the impact of daily administration of duloxetine hydrochloride. Our findings revealed no change in rats' body weight (Table 1). Wise et al. observed instant weight loss in patients treated with duloxetine, followed by a slight gain during a more extended treatment period. The magnitude of the observed weight changes implies that duloxetine has minimal or no effect on weight for the majority of people [30][29]. Unlike fluoxetine, its long-term administration increased the body weight of healthy mice through increasing food intake [31].

Moreover, the relative weights of the treated group's uteri and right ovaries had risen significantly, but the relative weights of the left ovaries did not show any significant change. Müller et al. noticed that dams exposed to fluoxetine had a weight reduction in the uterus, which reveals a possible toxic effect of antidepressants on female reproductive organs' health [32].

Reactive oxygen species are by-products of aerobic respiration and metabolic processes [33]. Although a lot of studies confirm that antidepressants can decrease oxidative stress, numerous others proved the capability of several antidepressants to enhance oxidative stress levels [34][35]. Normal levels of reactive oxygen species are not harmful; they are essential for several signaling transduction pathways, including ovarian follicle maturation, embryogenesis, and embryonic implantation [36]. Agarwal et al. previously reported that an excess of free oxygen radicals is directly associated with the emergence of endometriosis, developing polycystic in the ovaries, and idiopathic causes related to infertility [37]. Another study linked oxidative stress to pregnancy-related illnesses like the resorption of the embryos, Preeclampsia, multiple miscarriages, intrauterine growth restriction, and fetal death [38].

At physiological pH, SOD enhances the superoxide anion (O_2^{-}•) dismutation to produce H_2O_2 and O_2 at a rate 10^4 times faster than the normal dismutation. CAT is the enzyme that eliminates hydrogen peroxide H_2O_2 from the cell when its concentrations exceed normal levels [39].

The GSH is a vital antioxidant that plays numerous roles in cells, including the formation of conjugates with a variety of toxic intrinsic and mutagenic chemicals, as well as cell protection against reactive oxygen and nitrogen species. GSH deficiency can cause DNA damage and increase H2O2 concentrations. When polyunsaturated fatty acid side chains combine with O2, they produce the peroxyl radical, which can cause lipid peroxidation, forming the peroxyl radical, which can then get H+ from the other fatty acid, resulting in a continual process. High rates of lipid peroxidation are linked to loss of membrane fluidity and function, as well as, stimulation of the apoptotic cascade [40].

We measured the MDA, GSH, SOD, and CAT in the ovarian and uterine tissues. We observed a significant increase in lipid peroxidation while the GSH levels decreased significantly in both uterus and ovaries in the group that administered (6.1 mg/kg) of duloxetine (Table 2&3). A prior study by Aynaoglu et al. on female rats stated that animals with high MDA and low GSH experienced infertility [41]. Reactive oxygen species can cause more severe and irreparable cell deterioration, eventually leading to cell death via necrosis or apoptosis [42].

Our histopathological findings revealed uterine and ovarian tissue damage in the treated group in comparison with the untreated control group (Fig. 1&2). High frequencies of apoptotic cells were seen in both organs' tissues after duloxetine treatment as a potential consequence of oxidative stress elevation [36]. Whereas follicular atresia and oocyte damage in the ovaries usually result from free radical oxidation [43]. Extreme ovarian stromal hemorrhage, follicle deterioration, dilatation, and congestion were

observed in the treated group's ovarian sections. In the uterine tissues of the treated group, there was cellular degeneration and necrosis, along with a substantial increase in lymphocyte infiltration. Together, this confirms the pathological potency of duloxetine on the reproductive organs of female rats. Duloxetine treatment is expected to elevate serotonin, which is assumed to be responsible for the histopathological change in the reproductive organs.

Duloxetine's toxicity was reported in the gastrointestinal and neurological systems, with hepatic cell destruction being the most prevalent [44]. Numerous studies have shown that tricyclic antidepressants, such as amitriptyline, may increase lipid peroxidation, which lowers testosterone and prolactin levels significantly. It also reduced the number of sperms present and caused pathological changes in the testicular tissues, including focal pyknosis and even damage to the lining cells of spermatogonia with deficient spermatids [45].

Our results show a significant decrease in estrogen levels, while FSH levels increased significantly, with no statistically significant variations in progesterone or LH levels observed (Table 4). Another study examined changes in male rats' testes that were given duloxetine, concluding that the drug significantly decreased testosterone, LH, and FSH levels. In addition to initiating oxidative stress that appeared in MDA elevations, moreover, it dropped GSH, SOD, and CAT levels, with testicular degeneration by reducing germ cell apoptosis [16]. Domingues et al. investigated the effects of fluoxetine on female mice at doses of 2 mg/kg and 20 mg/kg by intraperitoneal injection and discovered that animals given high doses of fluoxetine had estrous cycle disruption shortly after starting treatment. [46]. Hormonal imbalance is linked directly to infertility [27]. Ulker et al. examined the impact of two SSRIs (bupropion and paroxetine) on female rats' reproductive system and revealed that oral administration of bupropion (3.6 mg/kg) significantly reduced serum levels of the anti-Müllerian hormone and (LH). Paroxetine (3.6 mg/kg) increased the serum estradiol levels due to paroxetine's indirect effects on the pituitary gland or its direct toxic effect on gonads. The ovarian and uterine tissues from animals treated with both antidepressants showed histopathologic alterations [47].

The weight change of the reproductive organs in our treated group can be attributed to the hormonal imbalance since gonadotropic hormones can, directly or indirectly, affect the growth and development of the ovary and uterine [48]. Antidepressants can disrupt the Hypothalamic-Pituitary-Gonadal axis by inhibiting dopamine receptors, including dopamine reuptake and/or increasing prolactin secretions. The latter suppresses the gonadotropin release [49].

Antidepressants and sex hormones are somehow related [50]. An *in vitro* study found that duloxetine can cause endocrine imbalance by inhibiting the cytochrome CYP17 enzyme activity, as cytochrome CYP17 (P450 17) is a vital enzyme located upstream in the steroidogenic pathway, and the vast bulk of steroid synthesizing tissue is dependent on CYP17 function [51]. Pavlidi et al. studied several antidepressants' potential effects on testosterone and estrogen levels. Albeit some SSRIs have a magnified affinity for CYP19 (an estrogen biosynthetic enzyme) such as sertraline, others have a lower affinity for CYP19. Sertraline stimulates aromatase activity, whereas fluoxetine has the opposite effect. On the other hand, paroxetine (SSRI) administration suppresses CYP19, resulting in lower circulating estradiol levels in healthy mice. Eventually, interfering with aromatase CYP19 may be one mechanism by which SSRIs affect estrogen and androgen balance as serotonin regulates the aromatase (CYP19) [12].

Erdemir et al. investigated four SSRI drugs on male testicular parameters in rats and discovered that SSRIs impair testicular tissues, especially after paroxetine treatment, serum FSH levels were also measured and were found to be dropped significantly in rat groups given fluoxetine, escitalopram, and paroxetine groups, and peaked with sertraline. The same study explained the potential ability of SSRIs to worsen semen parameters that serotonin receptors are frequent in the vas deferens, epididymis, testis, Sertoli, and sperm cells [52].

In terms of the genotoxic consequences of antidepressants, this area added new impute for excessive studies to show the relatedness between antidepressants and genome and molecular alterations. The outcomes revealed disruptions in mitotic and meiotic processes, as well as chromosomal aberrations, following antidepressant treatment [53]. On the cellular and mitochondrial levels, Silva et al. noticed gene expression alterations that are directly related to DNA damage, DNA repair, energy metabolism, antioxidant protection mechanism, cell progression as well as, and cell apoptosis after SNRI consumption [54]. Many studies have shown duloxetine's ability to cause genotoxicity explicated in congenital malformations in infants during the first trimester of pregnancy in females given duloxetine [55]. Gao et al. investigated the neural cytotoxicity of duloxetine in N2a cells, finding that it caused alterations in the cellular markers such as viability, biochemistry, and morphological features, and duloxetine also decreased the CYP levels [56].

The single-cell gel electrophoresis, comet assay, is one of the most reliable, low-cost, easy-to-handle, as well as integrable, and accurate methods not only to detect DNA strand breaks but also to discover incomplete excision repair events, alkaline labile spots, and cross-linking events [57]. As long as there have been few studies of duloxetine's genotoxicity in

female reproductive organs, we applied the alkaline comet assay on rat uterus and ovaries' cells. The results showed elevated DNA breaks appeared clearly in the tail length of the treated group $P < 0.05$ in both organs besides the tail DNA percent and tail moment in the uterus, while DNA in the tail was significantly increased in ovaries tissues, revealing a potential genotoxic effect of duloxetine (Table 5&6) (Fig. 3).

Álvarez-González et al. showed Duloxetine's DNA-damaging capacity in brain and liver cells and explained these findings to its oxidative potential, since duloxetine is exposed to oxidation, methylation, and conjugation pathways due to the action of enzymes such as CYP2D6 and CYP1A1, and the formation of metabolites that include the glucuronide conjugate of 4-hydroxy duloxetine on it, in addition to, the sulfate conjugate of 5-hydroxy, 6-methoxy duloxetine, which explains the destructive effect of duloxetine in hepatic cells whereas the biotransformation of the drug occurs [17]. In acute and sub-chronic assays in mice, Madrigal-Bujaidar et al. discovered that the number of polychromatic micro-nucleated red blood cells or rather micro-nucleated normochromic erythrocytes increased significantly after duloxetine treatment [58]. The same authors confirmed their findings by showing that this medication can stimulate sister chromatid reciprocal exchanges, and cellular division and proliferation kinetics in mouse bone marrow at three different doses of duloxetine (2, 20, and 200 mg/kg) [35][59].

5. Conclusion

Our study suggests possible adverse implications of duloxetine on the reproductive health of female rats. The significant change in oxidative stress negatively impacted the sex hormones balancing and caused cellular damage to ovarian and uterine tissues. In addition, our data correlated the potential direct link between duloxetine administration and genotoxicity. These findings recommend caution regarding duloxetine's adverse effect on female reproductive health.

6. Conflict Of Interest

The authors declare that they have no conflicts of interest.

7. Author Contributions

All authors have shared the study design, methodology assessment, data analysis, and drafting. All authors have read and agreed to the submitted version of the manuscript.

8. Data Availability Statement

The data relating to this study are available on request from the corresponding author.

9. Acknowledgment

The authors would like to thank Sara Ibrahim Galal, a graduate of the Biotechnology/Biomolecular Chemistry program, Faculty of Science, Cairo University for her treasured contribution to the laboratory work.

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