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Effect of different doses of cyclobenzaprine-HCl on the reproductive health of female Wistar rats

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Background
Women often experience significant burdens both at home and in their professional lives, which can lead to physical and mental strain. To manage these challenges, medications such as cyclobenzaprine-HCl, originally developed as a tricyclic antidepressant but reintroduced as a muscle relaxant, are sometimes used. Its effectiveness in treating muscle spasms, localized pain, and limited range of motion in acute musculoskeletal conditions has been well documented.

Objective
The objective of this study was to explore the potential consequences of cyclobenzaprine-HCl on reproductive health by assessing its influence on female Wistar rats.

Materials and methods
The study was conducted on 18 adult female Wistar rats, divided into three groups: a control group receiving distilled water and two treated groups with two doses – a low-dose group receiving 1.5 mg/kg of cyclobenzaprine-HCl and a high-dose group receiving 3 mg/kg, administered orally for 30 days. We assessed the impact of cyclobenzaprine-HCl on serum hormonal levels (progesterone, estradiol, luteinizing hormone, follicle-stimulating hormone), oxidative stress markers (glutathione, superoxide dismutase, catalase, malondialdehyde), histopathological changes in ovaries and uteri, and DNA stability using the comet assay.

Results and conclusion
The study revealed that cyclobenzaprine-HCl caused hormonal imbalances distinguished by significant increase in estradiol and follicle-stimulating hormone levels, and significant decreases in progesterone and luteinizing hormone levels. Dissruptions in oxidative stress markers were characterized by elevated malondialdehyde and reduced glutathione levels. Histopathological abnormalities included degeneration, deformed follicles, congested blood vessels, and necrosis in the ovarian tissue. In addition, diffused eosinophil infiltration, pyknotic nuclei, glandular hyperplasia, necrosis, and hypercellularity in the uterine tissue. DNA instability was observed in both dosage groups as evidenced by fragmented DNA in the shape of comets. These findings underscore the potential reproductive toxicity of cyclobenzaprine-HCl in female rats, suggesting a need for caution in its use considering its possible adverse effects on reproductive health.

Keywords:
cyclobenzaprine-HCl, histopathology, hormones, muscle relaxants, oxidative stress, reproductive health

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Introduction

After continuous sexual activity without contraception for 1 year in women under 35 years and for 6 months in women aged 35 years and older infertility arises [1]. Infertility affects everyone. Although the cause and severity vary by geography and socioeconomic position, 60–80 million couples worldwide experience infertility each year [2]. According to epidemiological studies, 10–15% of couples develop primary infertility [3]. Inhorn and Patrizio [3] describe a "belt of infertility" throughout West, Central, and Southern Africa with significant rates of infertility. Many countries in this region experience primary and secondary infertility. Endometriosis, pelvic adhesions, tubal obstruction, hyperprolactinemia, and ovulatory disorders usually cause female infertility. Other evidence suggests that ovulatory disorders cause 50% of female infertility [1]. In communities where motherhood is valued, infertility can cause long-term psychological suffering. Women

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are blamed for fertility troubles and may endure psychological and social stigma [4].

The female body undergoes countless hormonal and anatomic alterations that affect the musculoskeletal system, and these shifts can result in musculoskeletal problems, increase the risk of injury, or alter the course of preexisting conditions [4]. In addition, women are more prone to fibromyalgia [5]. Fibromyalgia is a prevalent long-term pain condition affecting the musculoskeletal system, marked by widespread pain, heightened sensitivity to pain at various tender areas, fatigue, disrupted sleep, and feelings of depression [6]. Women often bear the responsibility of childcare, household chores, and caring for family members, while also being the primary earners. According to the central agency for mobilization and statistics (CAPMAS), women lead 18.1% of households in Egypt. In the end, women are overworked inside and outside of their homes [7]. All these responsibilities and stress cause physical and psychological harm to women.

Cyclobenzaprine-HCl was first synthesized in 1960 to be used as an antidepressant [8]. However, in 1977, it was reintroduced as a muscle relaxant that is useful in enhancing muscle spasm relief, alleviating localized pain and tenderness, and expanding the range of motion in acute, painful musculoskeletal issues [9]. It mainly functions at the brainstem level within the central nervous system [10]. Cyclobenzaprine-HCl is well absorbed, widely distributed among body tissues, subject to enterohepatic circulation, metabolized extensively through oxidative and conjugative routes, well tolerated, and has only a few adverse effects [11]. It is entirely but slowly absorbed from the gut; the peak of serum levels is not reached until 3–8 h after ingestion and is slowly eliminated with a half-life of 1–3 days [12].

Unlike other muscle relaxants, cyclobenzaprine-HCl has been used to treat schizophrenia, depression, anxiety, chronic tension headache, and Parkinson's disease rigidity with limited success [11]. The impact of cyclobenzaprine-HCl on female reproductive health has yet to be extensively studied. Inevitably, there is a dying need to explore the effects of varying doses of cyclobenzaprine-HCl on the reproductive well-being of female Wistar rats. Our investigation comprehensively evaluates sex hormone levels, oxidative stress markers, histopathological changes, and DNA damage to elucidate the potential impact of cyclobenzaprine-HCl intake.

Materials and methods

Drug

Cyclobenzaprine-HCl tablets are produced by Apex Pharma (S.A.E, Badr City, Egypt). The drug is released in 5 and 10 mg tablets. The human recommended prescription is 5–10 mg three times a day, ranging from 20 to 40 mg/daily [10]. The dose should not exceed 60 mg/daily. It is soluble in distilled water. The rats' human equivalent dose was calculated according to Janhavi *et al.* [13]; it resulted in 1.5 mg/kg as a low dosage and 3 mg/kg as a high dosage.

Animals

The Institutional Animal Care and Use Committee of Cairo University Faculty of Veterinary Medicine (vet. cu011020202014) approved our study

Eighteen adult female Wistar rats weighing an average of 205±20 g were housed in the animal facility of the Faculty of Science, Cairo University, They were kept in clean cages with sawdust-covered flooring, in an environment maintained at a temperature of 25 ±2°C, relative humidity at 50±20%, and followed a 12-h light/dark cycle. Before the start of the treatment, the animals were acclimated for a week. The animals were provided unrestricted access to tap water and standard rat chow.

Methodology setup

The rats were divided into three groups (n=6). The control group received 1 ml of distilled water, the low-dose group was administered 1.5 mg/kg of cyclobenzaprine-HCl, and the high-dose group was orally administered 3 mg/kg of cyclobenzaprine-HCl for 30 days. After 24 h from the last administration, all the rats were killed with an intraperitoneal injection with 60 mg/kg of sodium pentobarbital. After dissection, the ovaries and uteri were cleaned with 0.9% saline, dried on filter paper, and weighed.

Hormonal analysis

Rats in all groups underwent heart punctures to obtain samples of blood, which were spun for 10 min at 3000 rpm to isolate the serum for hormone analysis. Using enzyme-linked immunosorbent assay (ELISA) kits from Wuhan fine Biotech Co. Ltd kits (Wuhan, 430074, Hubei, China), the levels of follicle-stimulating hormone (FSH), estrogen, progesterone, and luteinizing hormone (LH) were measured in serum. The kit applies a competitive ELISA detection technique. Standard, sample, and control (blank) were added to the plates after two rounds of washing. A volume of 50 µl of the standard, blank, or sample must be added to each well before adding the

50 µl biotin-labeled antibody working solution. To ensure complete mixing, cover the plate and lightly tap it. Incubate at 37°C for 45 min and then perform three washes of the plate. Add 100 µl HRP-Streptavidin conjugate (SABC) working solution, which is prepared by diluting the SABC with SABC Dilution Buffer at a ratio of 1 : 100. Mix them thoroughly, keep for 30 min at 37°C, and wash the plate five times. A measure of 90 µl tetramethylbenzidine substrate should be added to each well, place in a dark environment and let it sit for 10–20 min at 37°C, then add 50 µl of stop solution, and promptly measure the absorbance at 450 nm.

Oxidative stress markers

Following the removal of the ovarian and uterine tissues a polytron homogenizer prepared 10% of the homogenate in 0.05 M phosphate buffer (pH 7) at 4°C. Centrifuge the homogenate at 10 000 rpm for 20 min to eliminate the mitochondria, erythrocytes, unbroken cells, cell debris, and nuclei according to Bradford's method [14].

The supernatant was used to estimate glutathione (GSH), malondialdehyde (MDA), catalase activity (CAT), and superoxide dismutase activity (SOD), using Genei, Bangalore, protein estimation kit and ELISA plate reader (stat fax 2200, awareness technologies, Florida, USA), with a range of 490–630 nm OD.

Histopathological examination

The uteri and ovaries were fixed in 10% formalin for 24 h duration in preparation for histological examination using light microscopy. Specimens were washed with saline before being dehydrated with serially diluted ethanolic solutions. After that, they were immersed in xylene, embedded paraffin, and placed in a stifling air oven for 24 h at 56°C. Slices of 4 µm thickness were cut from paraffin beeswax tissue blocks using a sliding microtome and then stained with hematoxylin and eosin for analysis.

Comet assay

Single-cell gel electrophoresis, the comet assay, was conducted according to Speit and colleagues [15,16]. The samples were homogenized to acquire tissue solution. The comet assay was conducted under a fluorescence microscope at a magnification of ×400 using a closed-circuit digital camera as described by Tice *et al.* [17] with epifluorescence using filters 15 BP546/1 2, FT580, and LP590. They were using the comet five image analysis software made by Kinetic Imaging Ltd (Liverpool, UK) developed to measure olive moment, tail moment, DNA in the tail, tail length, and % of DNA damage to investigate the DNA damage level.

Statistical analysis

The collected data were expressed as mean±standard error of the mean (SEM). To identify differences between groups, analysis of variance and Tukey's multiple comparison post-hoc analysis were conducted with statistical significance set at *P* value less than 0.05. IBM SPSS, version 25, software was used for the analysis.

Result

The impact of cyclobenzaprine-HCl on the weights of reproductive organs

The findings in Table 1 show that neither the weights of the left and right ovaries nor the body weights have changed significantly. The uteri of the low-dose (1.5 mg/kg) group demonstrated a significant rise in both absolute and relative weights in comparison to those of the control group. Moreover, the uteri in the low-dose (1.5 mg/kg) group are significantly heavier

Table 1 Difference in body weights and relative and absolute weights of reproductive organs

	Groups			
Parameters	Control	Low dose (1.5 mg/kg)	High dose (3 mg/kg)	*P* value
Body weight change (g)	14.5±3.65	5.6±3.23	13.167±4.40	0.271
Absolute weights (g)				
Left ovary	0.043±0.00	0.04±0.00	0.07±0.02	0.238
Right ovary	0.04±0.00	0.04±0.00	0.05±0.00	0.449
Uterus	0.42±0.02	0.63±0.05[a]	0.475± 0.05	0.018
Relative weights %				
Left ovary	0.022±0.00	0.018±0.00	0.03±0.01	0.229
Right ovary	0.02±0.00	0.020±0.003	0.02±0.00	0.919
Uterus	0.20±0.01	0.296±0.028[a]	0.20±0.02[b]	0.018

All values are presented as the mean±standard error of the mean. [a]Significant difference compared with the control group, while [b]significant difference compared with the low-dose (1.5 mg/kg) group. Statistical significance was determined using analysis of variance followed by Tukey's test, with a significance level set at less than 0.05.

than in the high-dose (3 mg/kg) group in relative weight.

The impact of cyclobenzaprine-HCl on female reproductive hormones

Based on the data in Table 2, it can be observed that both low and high dosages resulted in a significant rise in estradiol levels when compared with the control group. The low dose led to a more pronounced increase (103.30±10.81 pg/ml) compared with the high dose (44.60±5.26 pg/ml). The administration of low dose resulted in a considerable decrease in progesterone levels (7.306±0.83 ng/ml) compared with the control group (20.36±1.19 ng/ml). However, the high dose (3 mg/kg) only caused a minor drop (15.97±2.47 ng/ml). The administration of a low dose resulted in a considerable elevation of LH levels (4.08±0.79 mIU/ml) compared with the control group. Conversely, the high dose increased less (1.28±0.26 mIU/ml). Both dosages of the substance caused a substantial rise in FSH levels compared with the control group. The high dose (3 mg/kg) resulted in a more pronounced increase (72.10±8.77 mIU/ml) compared with the low dose (1.5 mg/kg) (31.96±3.69 mIU/ml).

The impact of cyclobenzaprine-HCl on oxidative stress markers

The uteri and ovaries of the low-dose group, as well as the uteri of the high-dose group, when measured to those of the control group, MDA levels significantly rise while GSH levels significantly decline. The SOD and CAT levels in the uteri and ovaries of the high-dose (3 mg/kg) group are significantly lower than the control group shown in Table 3.

The impact of cyclobenzaprine-HCl on histopathology

The ovarian tissue histopathology

In the control group the developing follicles in the cortex consist of oocytes with spherical-shaped nuclei and are boarded by flattened follicular cells. The developing follicles exhibit characteristics such as granulosa cells bound to them. The mature follicles are surrounded by multilayered granulosa cells and have an antral cavity. Atretic follicles and clusters of stromal cells are visible. The secondary follicles consist of an oocyte circled by multilayers of granulosa cells with narrow fluid spaces. The moderately eosinophilic cells having giant vesicular nuclei and foamy cytoplasm comprises the large corpora lutea (Fig. 1a–c).

Table 2 Levels of reproductive hormones

	Groups			
Parameters	Control	Low dose (1.5 mg/kg)	High dose (3 mg/kg)	*P* value
Estradiol (pg/ml)	38.12±2.74	103.30±10.81[a]	44.60±5.26[b]	0.000
Progesterone (ng/ml)	20.36±1.19	7.306±0.83[a]	15.97±2.47[b]	0.000
LH (mIU/ml)	1.01±0.17	4.08±0.79[a]	1.28±0.26[b]	0.001
FSH (mIU/ml)	13.38±1.57	31.96±3.69	72.10±8.77[a,b]	0.000

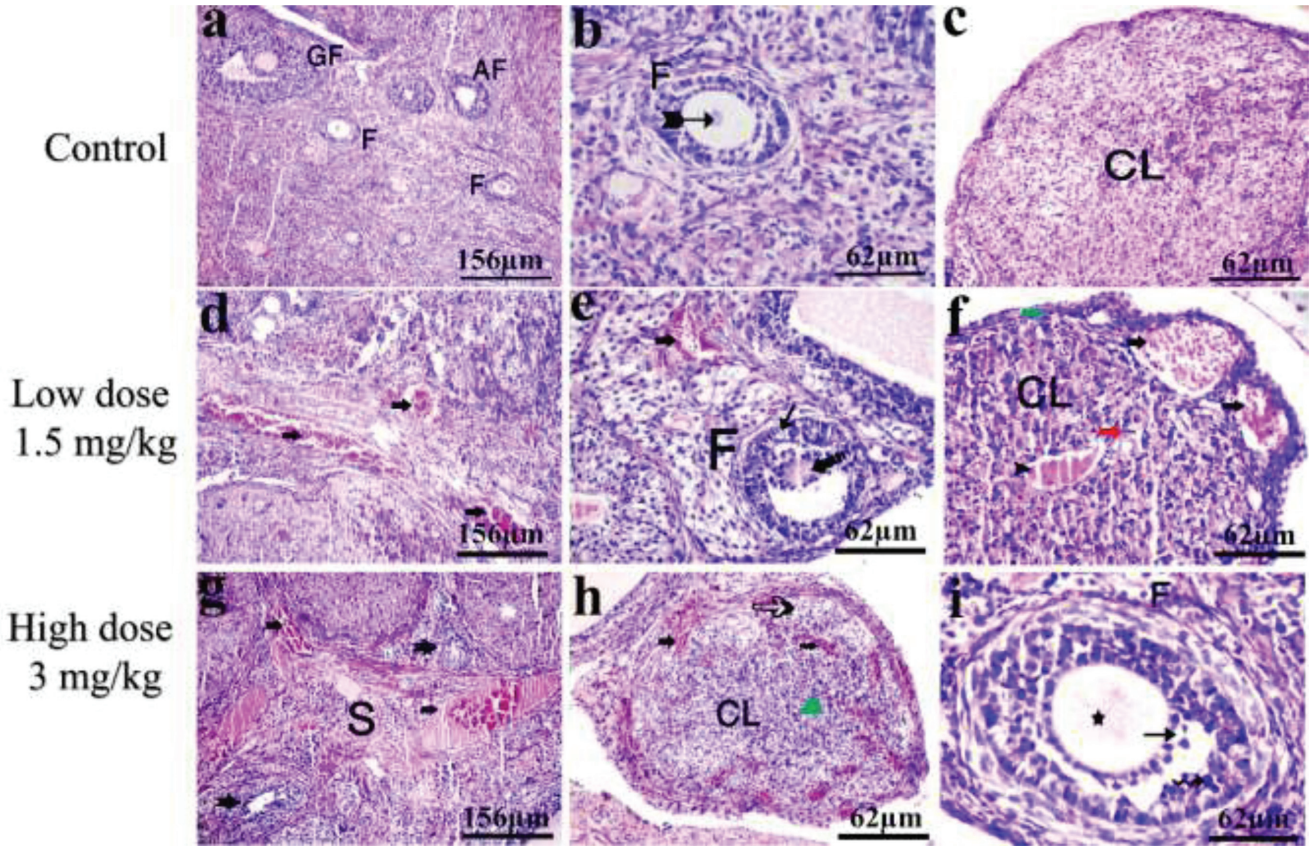
All values are presented as the mean±standard error of the mean. FSH, follicle-stimulating hormone; LH, luteinizing hormone. [a]Significant difference compared with the control group, while [b]significant difference compared with the low-dose (1.5 mg/kg) group. Statistical significance was determined using analysis of variance followed by Tukey's test with a significance level set at *P* value less than 0.05.

Table 3 Levels of oxidative stress markers

	Groups			
Parameters	Control	Low dose (1.5 mg/kg)	High dose (3 mg/kg)	*P* value
Ovary				
MDA (nmol/mg protein)	0.32±0.04	0.55±0.04	1.02±0.12 [a,b]	0.007
GSH (nmol/mg protein)	1.73± 0.05	1.45±0.05	0.85±0.06 [a,b]	0.001
SOD (U/mg protein)	2.19±0.04	1.82±0.08	1.30±0.04 [a,b]	0.004
CAT (nmol/mg protein)	3.55±0.15	3.10±0.17	1.60±0.10 [a,b]	0.003
Uterus				
MDA (nmol/mg protein)	0.36±0.01	0.55±0.04[a]	1.19±0.05[a,b]	0.000
GSH (nmol/mg protein)	1.72±0.05	1.42±0.02[a]	0.80±0.04[a,b]	0.000
SOD (U/mg protein)	2.19±0.12	1.89±0.10	1.23±0.06[a,b]	0.006
CAT (nmol/mg protein)	3.56±0.14	3.33±0. 37	2.00±0.20[a]	0.029

All values are presented as the mean±standard error of the mean. CAT, catalase; GSH, glutathione; MDA, malondialdehyde; SOD, superoxide dismutase. [a]Significant difference compared with the control group, while [b]significant difference compared with the low-dose (1.5 mg/kg) group. Statistical significance was determined using analysis of variance followed by Tukey's test, with a significance level set at *P* value less than 0.05.

Figure 1



Photomicrographs of the control group's (a–c) ovarian tissue (H&E stain) showing normal Graafian follicle (GF), the atretic follicle (AF), the follicle (F) with oocyte (bifid arrow), and corpora lutea (CL) with a foamy cytoplasm. The low-dose (1.5 mg/kg) group's ovarian tissue (d–f) showing the degenerated corpora lutea with vacuoles (red arrow), dark stained nuclei (green arrow), acidophilic hyaline (arrowhead), congested blood vessels (thick arrow), degenerated secondary follicles (F), with disintegrated oocyte (dotted arrow), and a degenerated follicular layer (thin arrow). The high-dose (3 mg/kg) group's ovarian tissue (g–i) showing a degenerated follicle (notch arrow), congested blood vessels (bold arrow) within the stroma (S), corpus luteum (CL) with blood capillaries (bold arrow), follicle (F) with vacuolation (thin arrow), ovum degeneration (star), and pyknotic granulosa cells (wavy arrow).

In the low-dose group the normal ovarian histoarchitecture has been lost. Multiple deformed and deteriorated follicles can be detected; none have an intact ovum or its normal nucleus. There are degenerated follicles, which are distinguished by degraded oocytes and zona pellucida, disintegration of granulosa cell layers as well as an increase in pyknosis. Oocyte nuclei in abnormal secondary follicles have undergone karyolysis. There is congestion in the stroma. The corpora luteal cells feature numerous vacuoles and acidophilic hyaline degeneration (Fig. 1d–f).

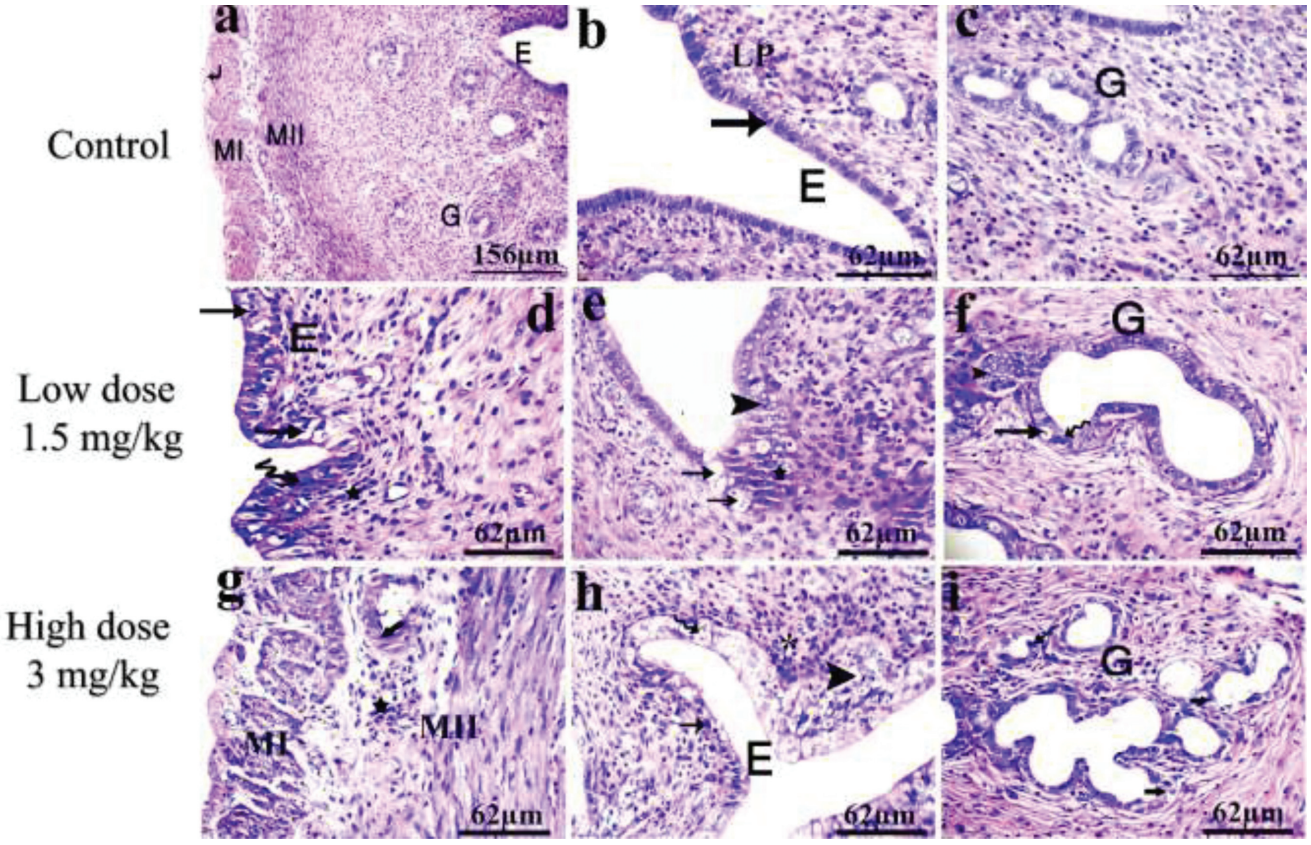
In the high-dose group there are several dilated, congested blood vessels visible in the ovarian medulla. The lumina of the blood vessels were filled with hyaline eosinophilic material. A rich network of blood capillaries exists in the corpus luteum. The stromal cells displayed vacuolation, severe degeneration, and clustering. They were degenerating follicles with desquamation of pyknotic granulosa cells, indicating increased apoptosis. Degeneration of the oocyte, corona radiate, and zona pellucida (Fig. 1g–i).

The uterine tissue histopathology

The control group was displaying layers of the uterus wall, including the endometrium, inner circular muscle, and longitudinal muscle layer. Also, the perimetrium and glandular epithelium are visible. The endometrium consists of a surface layer of simple columnar epithelial cells and a sublayer of lamina propria containing endometrial glands lined with simple columnar epithelial cells that have vesicular nuclei (Fig. 2a–c).

In the low-dose group, the perimetrium and myometrium layers had diffused eosinophil infiltration with pyknotic nuclei. Also, polymorphonuclear cell infiltrations between stromal cells and muscle fibers invade the perimetrium and myometrium layers. The glandular epithelium had

Figure 2



Photomicrographs of the control group's (a–b) uterine tissue (H&E stain) showing the layers of the uterus. Endometrium (E) lined with intact columnar epithelial cells (arrow), with lamina propria (LP), endometrium glands (G), inner circular muscle (MII), outer longitudinal muscle (MI), and the perimetrium (curved arrow). The low-dose (1.5 mg/kg) group's (d–f) uterine tissue, endometrium (E) and uterine glands (G) showing vacuolation (thin arrow), extensive epithelial proliferation and hyperplasia (arrowhead), polymorph nuclear cells infiltration (star), and pyknotic nuclei (wavy arrow). The high-dose (3 mg/kg) group's (g–i) uterine tissue showing thick-walled blood vessels (bold arrow), lamina propria with lymphocyte invasion (star), endometrial epithelium with cellular apoptosis (thin arrow), vacuolation (wavy arrow), endometrial hyperplasia (arrowhead), highly branched glands (G) with dark stained nuclei (arrow), and necrosis of the outer longitudinal muscle (MI).

vacuolation, hyperplasia, darkly stained nuclei, and extensive epithelial proliferation. Endometrial folds showed a patch of hypertrophied surface epithelium (endometrial hyperplasia), and some necrotic cells were observed, exhibiting vacuolated cytoplasm with darkly stained nuclei (Fig. 2d–f).

In the high-dose group, the connective tissue stroma showed hypercellularity, thick wall blood vessels, and outer circular muscle myometrium with severe necrosis. The endometrial glands were larger and branched with a wider lumen than the control group. Also, there is infiltration of the endometrium with abundant lymphocytes and polymorph nuclear cells. In addition, apoptosis was noted in the surface and glandular epithelial cells, characterized by cells exhibiting deep pyknotic nuclei. The luminal epithelium had proliferation with pseudostratification of its nuclei. Necrosis of epithelial cells is observed at the luminal angles, characterized by vacuolated cytoplasm and pyknotic nuclei (Fig. 2g–i).

The impact of cyclobenzaprine-HCl on the DNA integrity

The high-dose group's ovaries display a significant rise in tail DNA. At the same time, the low-dose group's ovaries show a significant increase in tail length, as shown in Table 4 and Fig. 3. According to the comet assay results in Table 4, the high-dose group's uterine tissue had DNA damage, as shown by a significantly high olive moment and tail moment. The low-dose group's uteri display a significant rise in tail DNA as illustrated in Fig. 4.

Discussion

This investigation studies the impact of cyclobenzaprine-HCl on the female Wistar rats' reproductive health by assessing levels of gonadal hormones, cellular oxidative stress, histopathological examinations, and DNA integrity.

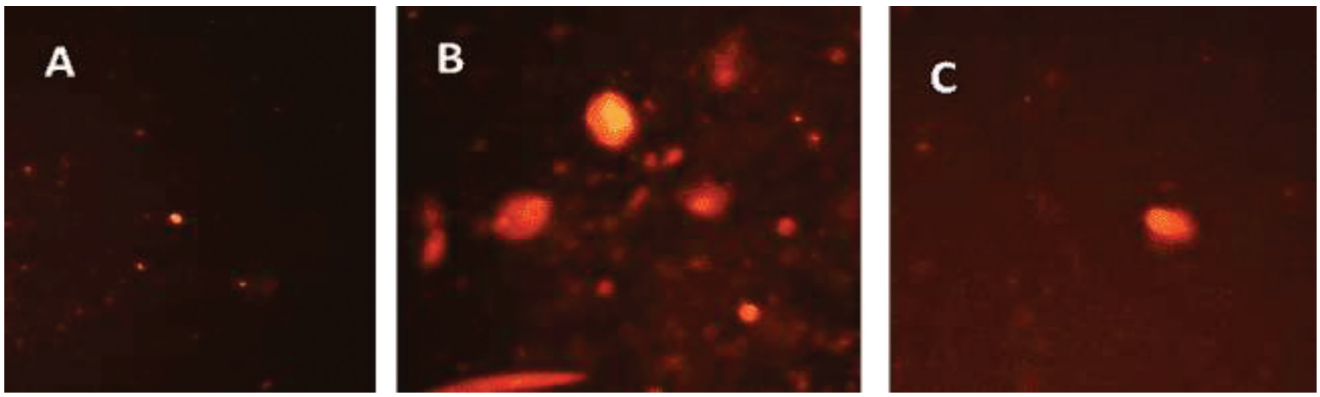
Table 4 Comet assay results

Parameters	Groups			*P* value
	Control	Low dose (1.5 mg/kg)	High dose (3 mg/kg)	
Ovary				
Olive moment	1.193±0.063	1.203±0.053	1.503±0.062[a,b]	0.017
Tail moment	0.580±0.006	0.663±0.003[a]	0.733±0.009[a,b]	0.000
DNA in tail	8.847±0.064	9.373±0.168[a]	11.423±0.090[a,b]	0.000
Tail length	6.630±0.060	8.720±0.110a	7.370±0.064[a,b]	0.000
% f DNA damage	10.700±0.058	12.467±0.067[a]	14.100±0.057[a,b]	0.000
Uterus				
Olive moment	0.453±0.007	0.623±0.009[a]	0.953±0.009[a,b]	0.000
Tail moment	9.603±0.134	11.450±0.096[a]	12.520±0.042[a,b]	0.000
DNA in tail	0.990±0.021	1.190±0.035[a]	1.447±0.062[a,b]	0.001
Tail length	5.350±0.122	6.237±0.153[a]	7.600±0.076[a,b]	0.000
% f DNA damage	8.900±0.058	12.833±0.067[a]	10.903±0.055[a,b]	0.000

All values are presented as the mean±standard error of the mean. [a]Significant difference compared with the control group, while [b]significant difference compared with the low-dose (1.5 mg/kg) group. Statistical significance was determined using analysis of variance followed by Tukey's test, with a significance level set at *P* value less than 0.05.

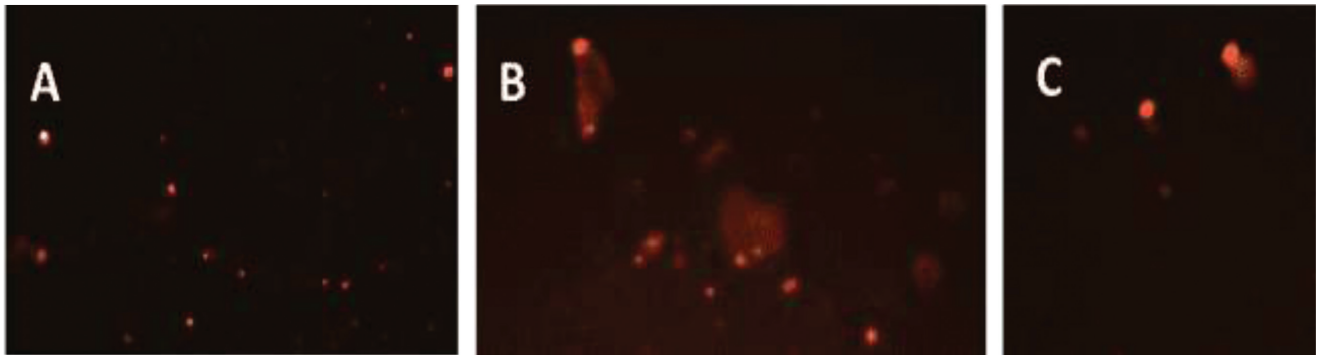
Cyclobenzaprine-HCl is a tricyclic amine that treats skeletal muscular spasms [18,19]. The cyclobenzaprine-HCl's chemical structure closely resembles amitriptyline, a well-known tricyclic antidepressant, except for an additional double bond in the central cycloheptyl ring. Cyclobenzaprine-HCl has anticholinergic and cardiac overdose side effects, the same as tricyclic antidepressants, though less severe [20].

Figure 3



Photos illustrate the DNA damage in the ovarian tissue in all groups: (a) the control group showing cells with minimal comet tails, (b) the low-dose (1.5 mg/kg) group showing cells with pronounced comet tails, and (c) high-dose (3 mg/kg) group DNA fragments forming comet tails are present.

Figure 4



Photos illustrate the DNA damage in the uterine tissue in all groups: (a) control group having no comet-like tail, (b) low-dose (1.5 mg/kg) group having long DNA tails forming a 'comet' shape, (c) high-dose (3 mg/kg) group DNA fragments forming comet tails are present.

Our study found no significant variance in the body weights of rats between the two groups who received both doses of cyclobenzaprine-HCl (1.5–3 mg/kg) compared with the control group. This finding aligns with the results reported by Nobrega and Coscina [21], who proved that tricyclic antidepressant treatment with amitriptyline (2.5 mg) resulted either in no deviation from control levels or a minor, typically nonsignificant inclination toward decreased values in the body weight of the animals.

The findings indicated that the absolute and relative weight of the uterus of the low-dose group was higher than the control group. The high dose group's left and right ovaries and the uterus exhibited no significant alteration compared with the control group.

The hormones LH and FSH considerably influence follicles' development and ovulation. Before an egg is released, FSH promotes the growth of ovarian follicles in the ovary also raising the ovaries' production of estradiol which indorses ovulation and endometrium growth [22]. Consequently, any imbalance in these hormones, especially those produced by the ovaries, can cause harmful effects on conception and fertility [23].

In this context, we measured the rats' sex hormones (FSH, LH, estrogen, and progesterone). We found that low doses demonstrated significantly higher levels of LH and estrogen and lower progesterone levels. Alternatively, the high dose had significantly elevated levels of FSH in comparison to both the control group and the low-dose group, while LH and estrogen showed lower levels than the low-dose group. Progesterone levels increased significantly in the high-dose group than in the low-dose group. This illustrates that the low dose of cyclobenzaprine-HCl may have a negative feedback on gonad hormonal levels.

Cyclobenzaprine can affect the hypothalamus, causing increased secretion of gonadotropin-releasing hormone (GnRH). GnRH causes the pituitary gland to release both LH and FSH. Increased levels of LH and FSH cause the ovaries to release more estradiol. Cyclobenzaprine may affect the feedback loops that control the HPG axis. Elevated estradiol levels typically suppress the hypothalamus and pituitary gland, resulting in decreased GnRH, LH, and FSH release. Cyclobenzaprine may interfere with this feedback mechanism, resulting in a prolonged increase of these hormones. Cyclobenzaprine can affect liver enzyme activity, altering the metabolism and clearance of estradiol, LH, and FSH. This, in turn, can cause high amounts of these compounds in the bloodstream. A change in LH and FSH levels may disrupt the normal luteal phase, resulting in lower progesterone synthesis. Elevated levels of estradiol, LH, and FSH may have a negative feedback effect on progesterone production. Estradiol and LH generally promote the development of the follicular phase, but progesterone is dominant during the luteal phase. An imbalance in this equilibrium could lead to low progesterone levels.

The Tousson *et al.* [24] experiments on amitriptyline (70 mg/kg body weight/daily) on male albino rats' reproductive health drastically reduced the levels of prolactin, testosterone, FSH, and LH hormones. The results of Tousson and colleagues are consistent with other studies, suggesting that amitriptyline (0.4–0.8 mg/dl) might be harmful to reproduction in Wistar male rats by upsetting the balance of essential sex hormones [25,26].

We evaluated MDA, GSH, SOD, and CAT oxidative damage markers in the tissues of the ovaries and uteri of female Wistar rats. In the low-dose group, the uterine tissue had a significantly high concentration of MDA [27]. Moreover, the concentration of GSH was significantly low relative to the control. Alternatively, no significant alterations were observed in any of the oxidative stress markers in the ovarian tissue of the low-dose group, implying the absence of oxidative stress. Furthermore, the high-dose group showed oxidative damage through significantly elevated levels of MDA [28]. Moreover, there were significantly low levels of GSH, SOD, and CAT in both ovarian and uterine tissues as compared with the control group. The findings indicate that the reproductive organs exhibited increased oxidative damage and inadequate antioxidant defense mechanisms, indicating a dose-dependent effect of cyclobenzaprine-HCl on both the ovary and the uterus.

Oxidative stress negatively impacts multiple aspects of reproductive functions, which significantly affects female fertility [29]. It reduces the development and quality of oocytes and increases the risk of miscarriage [30]. Furthermore, it alters granulosa cell function, which influences follicular growth and ovulation and exacerbates illnesses such as endometriosis and polycystic ovarian syndrome. Also, damage to the endometrium resulting from oxidative stress elevates the risk of implantation failure and recurrent pregnancy loss, and impedes successful implantation [31]. Beyond the reproductive system, oxidative stress-related fertility impairments have an impact on overall

health and can result in complications such as intrauterine growth restriction, hypertension, and premature birth [32].

Amitriptyline disturbs the equilibrium between oxidants and antioxidants in testicular tissue, as indicated by decreased activity of the antioxidant enzyme SOD and significant depletion of the nonenzymatic GSH in groups receiving amitriptyline. Furthermore, in the testicular tissue of male albino rats treated only with amitriptyline (70 mg/kg), crucial antioxidant enzymes like glutathione reductase (GR), glutathione peroxidase, and glutathione S-transferase show decreased activity [24]. These findings are consistent with prior research, showing that amitriptyline (4 mg/kg in adult male BALB/c mice) lowers GSH levels and increases MDA formation [33]. Also, oxidative stress affects hormone levels, including LH and testosterone [34].

Histological studies have shown that the low-dose group had defective ovarian structure and decaying follicles. Atretic follicles, with damaged oocytes and disintegrating granulosa cell layers, increase. Unusual secondary follicles had karyolysis, the disintegration of the cell nucleus. The uterine tissue revealed polymorphonuclear cells and glandular epithelium characterized by vacuolation, hyperplasia, and proliferation. The high-dose group had swollen and congested blood vessels with follicular cell degeneration and necrosis. Endometrial glands are larger and branch more. Lymphocytes, neutrophils, and polymorphonuclear cells invaded the endometrium, whereas the glandular epithelial cells showed signs of apoptosis. These findings are consistent with the histological findings of amitriptyline (70 mg/kg) on the testes of male albino rats, which indicated substantial abnormalities and irregular spermatogenesis cycles in the seminiferous tubules, and the high doses of the drug caused lowered levels of reproductive hormones, lower sperm counts, and testicular damage [35].

However, there was a lack of existing literature on the impact of cyclobenzaprine-HCl on the reproductive organs of male and female laboratory animals. Nevertheless, based on our data and findings, we can propose that cyclobenzaprine-HCl causes histopathological changes in the ovaries and uterus.

Cyclobenzaprine-HCl can induce ovarian follicle deformity, follicle degradation, oocyte destruction, and disintegration of granulosa cell layers in rat ovary histology sections. Various mechanisms bring about these effects.

Cyclobenzaprine-HCl has the potential to cause oxidative stress, which can result in the production of reactive oxygen species (ROS). Elevated amounts of ROS can potentially harm cellular constituents, including DNA, proteins, and lipids, hence leading to the degradation of follicles and damage to oocytes. ROS can induce cellular damage and result in vacuolation (the creation of vacuoles within cells) and apoptosis (programmed cell death) in glandular epithelial cells.

Cyclobenzaprine-HCl may disrupt the hormonal balance required for proper folliculogenesis. Imbalances in the levels of FSH, LH, estrogen, or progesterone can negatively impact the growth and functioning of follicles. Disruptions in the levels of estrogen and progesterone can cause hyperplasia, which is an increase in cell growth, and result in morphological alterations in endometrial glands. An overabundance of estrogen, for example, might trigger the growth and branching of endometrial glands.

The medication and its metabolites may directly cause cytotoxic effects on ovarian cells and uterine epithelial cells. The toxicity could lead to the breakdown of granulosa cells, which play a vital role in sustaining and nourishing the oocyte and also trigger apoptosis in glandular epithelial cells.

Cyclobenzaprine-HCl has the potential to induce an inflammatory reaction in the uterus. Inflammatory cytokines and other mediators can contribute to the restructuring of tissues, excessive cell growth, and programmed cell death.

We were assessing the influence of cyclobenzaprine on the DNA integrity of the ovaries and uteri of female Wistar rats. In the low-dose group DNA damage was evident in both the ovary and uterus, indicated by a notable rise in DNA tail length in the ovarian tissue and an increase in tail DNA in the uterine tissue [36,37]. Also, in the high-dose group, there was a notable elevation in tail DNA observed in the ovarian tissue and significantly high values of the olive moment and tail moment in the uterine tissue, indicating significant DNA damage. This implies DNA damage in the uterine tissue due to the administration of the cyclobenzaprine-HCl [25].

Amitriptyline induces DNA damage, specifically in the testes. The comet assay results revealed a significant

increase in DNA damage markers, incorporating tail DNA, tail moment, and tail length, in male albino rats exposed solely to amitriptyline (70 mg/kg) [38,39]. Tricyclic antidepressants have a planar molecular structure that eases their insertion between DNA bases, thereby causing DNA damage [39]. According to Korobkova and colleagues., research on tricyclic antidepressants has been conducted across various platforms, including studies in cells, animals, and humans. Exposures to imipramine and amitriptyline have revealed findings such as strand breaks, chromosome aberrations, and sister chromatid exchanges in exposed cells. Metabolism of these antidepressants can exacerbate DNA damage, contributing to the formation of breaks in DNA strands [25,40]. In murine models, these antidepressants have been shown to impede cell division in the bone marrow. Mechanistically, the generation of free radicals through oxidation processes, mainly catalyzed by peroxidases, is implicated in the DNA damage caused by these antidepressants [40]. Free radicals have the potential to interact with DNA, proteins, and other molecules, resulting in DNA cleavage, cross-linking, and the formation of stable complexes that disrupt normal DNA processes [41]. Amitriptyline disrupts the equilibrium between oxidants and antioxidants in testicular tissue, leading to negative changes such as DNA damage. This imbalance, coupled with disturbances in reproductive hormones and histopathological abnormalities, contributes to the adverse effects on male reproductive health noted in the study. The oxidative stress triggered by amitriptyline causes peroxidative harm to sperm DNA, a factor known to produce low-quality sperm, thereby increasing the likelihood of fertility issues and heightened rates of germ cell apoptosis [42].

Oxidative stress significantly influences female fertility by affecting various physiological processes in the ovary and uterus. ROS are vital in ovarian processes like steroid production, ovulation, implantation, blastocyst formation, oocyte maturation, and luteal maintenance throughout gestation. Oxidative stress also modulates the physiology of ovarian germ cells and stromal cells. The concentration of ROS is pivotal in processes like egg implantation and fertilization [43,44]. Studies have localized SOD enzymes in the ovary, with copper–zinc SOD found in granulosa cells and mature follicles. At the same time, manganese superoxide dismutase (Mn-SOD) is found within the luteal cells of the corpus luteum in rats. Reduced estrogen and progesterone levels decrease SOD expression, which leads to oxidative stress within the uterus, which in turn causes endometrial shedding and impairs implantation [45]. Oxidative stress-induced dysfunction of endothelial cells in the uterus is associated with conditions such as preeclampsia and endometriosis [46,47]. Oxidative stress profoundly impacts female fertility by inducing DNA damage in oocytes, follicles, and endometrial cells, thus compromising embryonic development, and contributing to implantation failure. The DNA damage caused by oxidative stress also disrupts gene expression and can lead to chromosomal abnormalities [48].

Conclusions

The investigation highlights the effects of cyclobenzaprine-HCl on oxidative stress, histopathological changes, hormonal levels, and DNA integrity in female Wistar rats. Cyclobenzaprine-HCl, akin to amitriptyline, raises concerns for reproductive toxicity due to its tricyclic amine structure, disrupting oxidant–antioxidant equilibrium in the ovaries and uterus, leading to oxidative stress and compromised defense mechanisms. This disruption is evident in histopathological findings, including disrupted follicular development and uterine structural abnormalities. In addition, cyclobenzaprine-HCl induces dose-dependent DNA damage in ovarian and uterine tissues, emphasizing oxidative stress's pivotal role in mediating its adverse effects on female fertility. Further research is warranted to elucidate underlying mechanisms and interventions.

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Conflicts of interest

There are no conflicts of interest.

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