The anorectic agent, lorcaserin, disturbs estrous cyclicity and produces endometrial hyperplasia without affecting ovarian population in female rats

Raghda T. Abdel-Latif, Sawsan A. Zaitone, Yousra Abdel-mottaleb, Nabila N. El-Maraghy

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Aims: The present study aims to investigate the effect of the new anorectic agent, lorcaserin, on estrous cyclicity, reproductive hormones and folliculogenesis in female mature rats.

Materials and methods: Rats were divided into four groups; Group i: control group. Group ii-iv: rats treated with lorcaserin (5, 10 or 30 mg/kg/day, p.o.), respectively. The treatment continued for 28 days.

Key findings: Lorcaserin (5 or 10 mg/kg) caused estrous cycle disturbance in 40% of treated rats while the high dose (30 mg/kg) produced disturbances in 100% of the treated rats. Lorcaserin (5–30 mg/kg) altered some of female hormones where it enhanced estradiol but reduced luteinizing hormone. Minimal edema with congested vessels was observed in the medulla of ovarian sections. Further, epithelial and uterine sections showed hyperplasia.

Significance: Taken together, the present results demonstrated that lorcaserin affected some reproductive hormones, disturbed estrous cyclicity and induced histopathological changes in the ovaries and uteri without affecting the ovarian populations. Therefore, lorcaserin should be used with caution in women of child bearing potential until adequate clinical safety data are available.

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1. Introduction

Lorcaserin (Belviq) has been approved by Food and Drug Administration (FDA) in 2012 for management of obesity. Lorcaserin acts selectively on 5-hydroxytryptamine (5-HT2C) receptors in the central nervous system especially the hypothalamus. This results in production of pro-opiomelanocortin (POMC) in arcuate nucleus which cleaves into α-melanocyte stimulating hormone (α-MSH) with subsequent reduction in food intake [1]. Lorcaserin at the human therapeutic level has higher affinity to 5-HT2C than 5-HT2A and 5-HT2B receptors thus, reducing adverse effects accompanied with stimulation of the last two receptors like mood alteration and heart valve diseases, respectively [2]. Previous anorectics like fenfluramine and sibutramine were withdrawn from the market due to valve disease induced by 5-HT2B receptors agonism [3].

Some studies have been published on the impact of lorcaserin in male rats [4]. However there are relatively little researches conducted on female rats [5,6]. Although physiological, hormonal and genetic differences between male and female impact the stage of disease, benefits of treatment and occurrence of side effects, scarce gender studies have been conducted on the subject. Additionally, FDA declared that women are more prone to common adverse effects than men.

From 1997 till 2001, ten drugs have been drawn back from the market of United States due to the incidence of adverse reactions which occurred more in women than in men [7]. Women use anorectics more than men. Therefore, more gender directed research should be conducted for anorectic drugs such as lorcaserin.

Previous studies illustrated that stimulation of 5-HT receptors by serotonin inhibits release of dopamine and norepinephrine in some brain areas [8]. It was also proven that stimulation of 5HT2C receptors results in reducing firing of ventral tegmental area (VTA) cells and consequently decreases expression of dopamine in nucleus accumbens and enhances prolactin release [9,10]. This may lead to prediction that serotonin agonists inhibit dopamine leading to hyperprolactinemia. Moreover, a case of sibutramine (serotonin and norepinephrine reuptake inhibitor) induced hyperprolactinemia was previously reported [11]. Accordingly, it is anticipated that lorcaserin may enhance the serum...
level of prolactin hormone causing the same side effects as sibutramine. Additionally, sibutramine alters ovarian and uterine histology of female rats [12], while phentermine (sympathomimetic stimulant with anorectic property) abolishes estrous cyclicity in female rats [13].

In spite of FDA approval of lorcaserin in 2012, till now a paucity of information is available in the literature with regards to potential hazards and risks to women of child bearing potential. Therefore, the present study displayed the influence of lorcaserin on estrous cyclicity and reproductive hormones in female rats and examined the harmful effects on ovarian and uterine histology.

2. Materials and methods

2.1. Experimental animals

Twenty female adult Wistar rats (145–205 g, 3–4 months of age) were provided by Modern Veterinary Office for Laboratory Animals (Cairo, Egypt). Animals were permitted to adapt 2 weeks before the use of the drug. Rats were accommodated in pairs in well-ventilated, clean, stainless steel cages at 25 ± 3 °C throughout the experiment. Rats were kept on normal light/dark cycle with standard diet pellets (Pyramid poultry Co., Egypt) contained not <23% proteins, 4% fiber, 3.7% fat, 6.5% ash and a vitamin mixture and water provided ad libitum. The wood shaving covering the bottom of the cages were changed every day. The experimental protocols were approved by the research ethics committee at Faculty of Pharmacy, Suez Canal University.

2.2. Experimental design

Twenty adult female rats were classified into four groups of five rats as follows: Group 1: rats received distilled water orally. Group 2: rats were treated daily with lorcaserin (5 mg/kg, p.o.), Group 3: rats were treated daily with lorcaserin (10 mg/kg, p.o.), Group 4: rats were treated daily with lorcaserin (30 mg/kg, p.o.) [14–17].

Lorcaserin powder (Beijing Mesochen Technology Co., Beijing, China) was dissolved in distilled water. Each rat received the dose of lorcaserin in a volume equals 1 ml/kg/day whereas; control rats received an equal volume of distilled water. The weight of each rat was determined at the beginning of the experiment and at the day of the sacrifice to calculate the percent of change in body weight.

2.3. Detection of irregularity in estrous cycle

Identification of estrous cycle phases is of great importance to estimate the percentage of rats displaying irregular estrous cycle. This is accomplished by collecting vaginal cytology sample. The vaginal smear was taken early in the morning (8:30–9:30 a.m.) daily for at least 2 consecutive cycles [18] using H2O moistened cotton bud. After the cotton bud had been inserted into the vagina about 1 to 2 in., it was gently rotated 2 or 3 times to confirm that the smear was rolled on a slide and allowed to completely dry at room temperature. As soon as the smears were performed and dried, they were immersed for 1 or 2 min in a staining jar containing 70% alcohol in order to be fixed. The smears were then stained immediately by methylene blue stain (Loba chemie, Mumbai, India) (0.5% aqueous methylene blue solution) for 3–4 min. The slides were then washed in tap water and investigated under the microscope [19].

The characteristic phases of the rat estrous cycle can be categorized as estrus (E), metestrus (M), diestrus (D) and proestrus (P). The classification of each stage was determined mainly by the proportion among three primary cell types found in the vaginal smear: nucleated epithelial cells, cornified squamous epithelial cells and leukocytes. Estrus stage consists of large numbers of cornified cells, generally found in clumps and sheets, metestrus contains entirely high numbers of leukocytes and fewer numbers of large, non-nucleated, non-granular epithelial cells, diestrus composes mainly of leucocytes but with high numbers of small cornified and epithelial cells, proestrus is distinguished by nucleated epithelial cells. The length of regular cycle is usually 4 days in the following sequence [20].

<table>
<thead>
<tr>
<th>Cycle day</th>
<th>E</th>
<th>M</th>
<th>D</th>
<th>P</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (new cycle)</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>1</td>
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</table>

A small number of female rats have regular 5-day cycles; either between other cycles of 4 days or, scarcely, frequently and systematically. The 5-day cycles occur due to the presence of a 2nd day of any of the four stages mostly estrus or diestrus in the following order:

<table>
<thead>
<tr>
<th>Cycle day</th>
<th>E</th>
<th>M</th>
<th>D</th>
<th>P</th>
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<tr>
<td>1 (new cycle)</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>1</td>
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If the length of estrus cycle is shorter than 4 days or longer than 5 days, the cycle is considered irregular. Such cycles may be detected between regular 4 or 5-day cycles or repeated frequently [20].

2.4. Blood and tissue sample collection

After 28 days, rats at estrus stage were anesthetized with ether and blood samples were assembled from the retro-orbital plexus of rats’ eye. Rats were then sacrificed by cervical dislocation, and then the ovaries and uteri were dissected. Autopsy samples were taken from the ovaries and uteri of rats were fixed in paraformaldehyde solution for 1 day. Blood samples were centrifuged at 3000 × g for a period of 10 min. Serum samples were isolated and kept at −80 °C until analysis.

2.5. Biochemical assays

A rat prolactin ELISA kit Abnova (California, USA) was used for estimating the circulating levels of prolactin. Measurement of serum estradiol (E2) was performed by commercially available rat estradiol (E2) ELISA kit Phoenix Pharmaceuticals Inc. (California, USA). Additionally, serum progesterone level was determined using rat progesterone ELISA kit Usn, Life science Inc. (Hubei, China). Serum FSH measurements were done by rat FSH ELISA kit from Elabscience Biotechnology (Hubei, China). LH level was assayed using rat LH ELISA kit from Cusabio (Hubei, China). Reagents and standard preparation followed the manufacturer’s instruction. Automated ELISA reader was used to determine the absorbance in order to assess the reactions.

2.6. Histopathological staining and examination

Autopsy samples were taken from the ovaries and uterus of rats in different groups and fixed in 10% formalin for 24 h. Sections were rinsed in tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were utilized for desiccation. Sections were cleared in xylene and embedded in paraffin at 56 °C in hot air oven for 1 day. Paraffin bees wax tissue blocks were prepared for sectioning at 4-μ thickness by a slide microtome. The provided tissue sections were assembled on glass slides, deparaffinized, and stained by hematoxylin & eosin (H&E) stain for examination through the light microscope [21].

2.7. Evaluation of the folliculogenesis process

The fifth cut was selected in all ovarian samples to determine the number of follicles and consequently, to assess the maturation of follicles using digital microscope camera (Tusen ISH1000 digital camera) using Olympus® CX21 microscope. Folliculogenesis was evaluated by identifying different types of follicles (primordial, preantral, antral and atretic follicles) and counting them in each group. Primordial follicles
are distinguished by an oocyte enfolded with even pregranulosa cells, preantral follicles are characterized by an oocyte with a clear nucleolus, from 2 to 4 layers of granulosa cells and absent an antral space. Antral follicles were characterized by an oocyte with a clear nucleolus, >5 layers of granulosa cells and the presence of an antral space which is a fluid-filled cavity adjacent to the oocyte. Follicles are identified as atretic follicles if they contained a degenerating oocyte or karyopyknosis of granulosa cells [22].

2.8. Measuring endometrial thickness

Uterine sections stained with H&E and examined under microscope for measurement of thickness of endometrium in rats treated with lorcaserin (5, 10 or 30 mg/kg) and compared with control to confirm whether the drug induce hyperplasia in endometrium or not in addition to the microscopical examination. Uterine sections were measured using calibrated software ISCapture and calibration was done using slide micrometer.

2.9. Statistical analysis

Data are presented as the mean ± S.E.M. and parametric data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer as a post-hoc test using GraphPad Prism (ISIH software, USA) version 6. Further, data of body weight were not in normal distribution and therefore, they were expressed as medians and quartiles and analyzed using Kruskal Wallis test followed by Mann-Whitney U test. However, qualitative data were analyzed using the Chi square test. The 0.05 level of probability was used as the criterion for significance.

3. Results

Daily treatment with lorcaserin (5, 10 or 30 mg/kg) for 28 days, did not produce any observed abnormality in animal gait, vitality or general activity and mortality. Rats in all the experimental groups survived until the end of the experiment (100% survival).

3.1. Alteration in estrous cycling by changes in vaginal smears

Different stages of estrous cycle (estrus, metestrus, diestrus and proestrus) were identified (Fig. 1) as this step is considered a critical step in the evaluation of estrous cyclicity regularity in each group. Estrous cycle disruptions were apparent in 40% of rats treated with lorcaserin (5, 10 mg/kg). 40% of rats treated with lorcaserin (5 mg/kg) showed 6-days estrous cycles due to both increasing length of estrus stage by 1 day and increasing length of diestrus by 1 day or increasing length of diestrus by 2 days. Meanwhile, 40% of rats treated with lorcaserin (10 mg/kg) displayed 6–7 days estrous cycles where estrus stage was increased by 1–2 days and diestrus increased by 1 day. Furthermore,

Fig. 1. Images showing the cytological assessment of vaginal smears for identification of the estrous stage. Three main cell types were detected in vaginal smear samples: cornified squamous epithelial cells (red arrow), nucleated epithelial cells (black arrow) and leukocytes (yellow arrow). The ratio of these cell types in the smear identifies rats in (A) estrus, (B) metestrus, (C) diestrus and (D) proestrus cycle. Methylene blue stain at ×10. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
all rats (100%) treated with lorcaserin (30 mg/kg) showed 8–13 days estrous cycles where (metestrus and diestrus) stages were extended to 7–11 days while proestrus stage was disappeared in 8 days estrous cycles (Table 1). However this disruption in estrous cycle was not permanent and occurred between regular cycle of 4 or 5 days.

3.2. Percent change in body weight in rats

Fig. 2 demonstrates the percentage of change in body weights in experimental groups. Data were not in normal distribution with median values 4.38, 2.6, 0.9 and –2.41 for normal and lorcaserin (5, 10 and 30 mg/kg) groups, respectively. Statistical analysis indicated that the difference between the study groups was not of statistical significance.

3.3. Serum level of reproductive hormones

It is noteworthy to mention that lorcaserin did not induce significant changes in hormone levels except for LH level and estradiol (E2), where serum LH level in lorcaserin (5, 10 or 30 mg/kg) groups was significantly reduced compared to control (1.98 ± 0.59, 2.21 ± 0.44, 2.02 ± 0.48 versus 4.76 ± 0.53, respectively). On the other hand, serum estradiol level in lorcaserin (5, 10 or 30 mg/kg) groups was significantly increased compared to control group (39.37 ± 0.89, 30.67 ± 0.45, 35.48 ± 0.85, versus 21.45 ± 0.65, respectively, P < 0.05, Table 2).

3.4. Histopathological picture for rat ovaries

Ovarian sections from the control group exhibited a normal ovarian structure which was characterized by cellular spindles stromal cells with different types of follicles starting from primordial follicles to mature antral follicles and atretic follicles with few corpora lutea. Follicles lined by granulosa cells showing uniform rounded nuclei. Stromal cells showed thick walled hiliar vessels of average caliber (Fig. 3a&b).

Ovarian sections from lorcaserin (5 mg/kg) treated group showed mild edema and vacuolation which were more evident in atretic follicles and corpora lutea. Moreover, stromal cells revealed minimal edema and few congested blood vessels (Fig. 3c&d). In ovarian sections from lorcaserin (10 mg/kg) treated group, granulosa cells showed mild edema and vacuolation which were more evident in atretic follicles and corpora lutea. Stromal cells exhibited minimal edema with moderately congested vessels. Extravasated red blood cells were seen in few ovarian samples (Fig. 3e&f).

In ovarian sections from lorcaserin (30 mg/kg) treated group, mild edema and vacuolation were detected in the follicles. Minimal edema as well as moderate congested vessels was recorded in stromal cells. Extravasated red blood cells were detected in few ovarian samples (Fig. 3g&h). In ovarian sections treated with different doses of lorcaserin, various stages of follicles maturation starting from primordial follicles to mature antral follicles and atretic follicles and corpora lutea were observed.

3.5. Testing folliculogenesis through determination of follicle population

Morphometric analysis during the estrus stage, exhibited that lorcaserin treated ovaries showed no consequential change in the count of different types of follicles: primordial, preantral, antral, atretic and corpus luteum when compared to control group (Table 3).

3.6. Histopathological picture of the uterine specimens stained with hematoxylin and eosin

Furthermore, uterine sections obtained from control group exhibited normal endometrium structure of high columnar epithelial cells with numerous cytoplasmic vacuoles indicating functioning glands while the underlying spindled stroma showed active glands. Myometrium formed of smooth muscle cells layers of average thickness and intervening vessels and intact serosal covering (Fig. 4A-a). Uterine section treated with lorcaserin (5 mg/kg) depicted mild increase in number of glands. Moreover, the columnar epithelial cells lining the glands revealed decreased cytoplasmic vacuoles indicating declining function and early hyperplasia (Fig. 4A-b). Uterine sections treated with lorcaserin (10 mg/kg) showed moderate increase in number of glands lined by hyperplastic tall columnar epithelial cells with stratification and less cellular spindled stroma (Fig. 4A-c). Animals treated with lorcaserin (30 mg/kg) recorded moderate increase in number of glands. The glands were arranged closely back to back and lined by hyperplastic tall columnar epithelial cells with stratification. Stromal cells were less cellular spindled (Fig. 4A-d).

The endometrial thickness of rats treated with lorcaserin (5, 10 or 30 mg/kg) was significantly increased 1.25, 1.32 and 1.26 fold, respectively when compared to the control group; this confirm that different doses of lorcaserin induced hyperplasia (P < 0.05, Fig. 4B).

4. Discussion

Although the impact of lorcaserin on many organs has been previously depicted [23], its influence or possible adverse effects on female reproductive system are debatable till now. The current study is the first to assess the in vivo reproductive adverse effect of lorcaserin in female rats. This was achieved through estimating estrous cycle irregularity, any change in serum level of the female reproductive hormones; prolactin, FSH, LH, estradiol and progesterone and the alteration in ovarian and uterine histology including measuring the endometrium thickness in addition to studying the effect of lorcaserin on the folliculogenesis process.
Table 2
Effect of lorcaserin (5, 10 or 30 mg/kg/day) for 28 days on serum level of female reproductive hormones in rats.

<table>
<thead>
<tr>
<th></th>
<th>Prolactin (ng/ml)</th>
<th>Estradiol (E2) (pg/ml)</th>
<th>Progesterone (ng/ml)</th>
<th>FSH (ng/ml)</th>
<th>LH (mIU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>67.76 ± 1.23</td>
<td>21.45 ± 0.65</td>
<td>64.93 ± 0.55</td>
<td>10.38 ± 0.48</td>
<td>4.76 ± 0.53</td>
</tr>
<tr>
<td>Lorcaserin (5 mg/kg)</td>
<td>72.77 ± 2.53</td>
<td>39.37 ± 0.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67.16 ± 1.13</td>
<td>7.46 ± 0.92</td>
<td>1.98 ± 0.59&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lorcaserin (10 mg/kg)</td>
<td>71.48 ± 1.5</td>
<td>30.87 ± 0.45&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>66.58 ± 0.67</td>
<td>7.51 ± 0.55</td>
<td>2.21 ± 0.44&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lorcaserin (30 mg/kg)</td>
<td>67.49 ± 2.96</td>
<td>35.48 ± 0.85&lt;sup&gt;a,b,c&lt;/sup&gt;</td>
<td>67.42 ± 1.47</td>
<td>7.51 ± 0.85</td>
<td>2.02 ± 0.48&lt;sup&gt;a&lt;/sup&gt;</td>
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</table>

FSH: follicle stimulating hormone, LH: luteinizing hormone. Data are expressed as Mean ± SEM and analyzed using one-way ANOVA followed by Tukey-Kramer as a post-hoc test at \( P < 0.05 \).

<sup>a</sup> Compared to control group.
<sup>b</sup> Compared to lorcaserin (5 mg/kg) group.
<sup>c</sup> Compared to lorcaserin (10 mg/kg) group.

Fig. 3. Representative photomicrographs of hematoxylin and eosin-stained ovarian tissue sections. (a & b) Histological sections of control group exhibit normal ovarian structure with different stages of follicles: primordial follicles (dashed arrow), growing follicles (2 arrow heads) and antral follicles (solid arrow). Spindles stromal cells with thick walled hilar vessels (grey arrow) are observed in control group. (c & d) Histological sections of lorcaserin (5 mg/kg) group showed mild edema and vacuolation in corpus luteum (blue arrow), congested blood vessels (red arrow), edema in stromal cells (black arrow). (e & f) Histological sections of lorcaserin (10 mg/kg) group showed minimal edema in stroma (black arrow) with moderate congestion in blood vessels (red arrow). Mild edema and vacuolation in granulosa cells lining follicles (yellow arrow) are also seen in lorcaserin (10 mg/kg). (g & h) Histological sections of lorcaserin (30 mg/kg) group showed edema in stroma (black arrow) with moderate congestion (red arrow) and edema and vacuolation in granulosa cells lining follicles (yellow arrow). St: stromal cells, CL: corpus luteum. Photos are captured at 100× (Figure a and c only) and 400× magnification. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
The present study displayed the effect of various doses of lorcaserin (5, 10 or 30 mg/kg) [14–17]. Doses performed for effect of lorcaserin on female rat fertility were 5, 15 and 50 mg/kg. It was found that doses ≥50 mg/kg enhanced turnover of erythrocytes which results in increased reticulocytes, mild anemia, elevated pigmented macrophages from spleen and hematopoiesis outside bone marrow [14].

The disturbance of estrous cycle can be identified as the inability to change to estrus stage and remains predominantly in diestrus which leads to prolonging of estrous cycle >4–5 days [24]. Estrous cycle abnormality is affected by female hormones. The current results indicated that estrous cycle disruptions were obvious in rats treated with lorcaserin doses (5, 10 or 30 mg/kg).

In addition to the prolongation of the estrous cycle to >4 days with the three doses of lorcaserin, dose-dependent discrepancies where observed where lorcaserin (5 and 10 mg/kg) expanded estrus and diestrous stages while lorcaserin (30 mg/kg) increased duration of metestrus and diestrous stages. Although the effects of the lorcaserin treatment on the estrous cycle depended on the doses, the hormone levels at the end of the study were affected in the same way by lorcaserin at doses of 5, 10 and 30 mg/kg. Further studies will be important to verify the hormonal level changes during each estrous cycle and correlate it with the expansion of estrus and/or diestrous stages caused by lorcaserin.

It is noteworthy to mention that this result is in accordance with former studies performed on other serotonergic drugs like fenfluramine and fluoxetine. 88% of fenfluramine treated rats showed disturbance in estrous cycle versus 62% of rats in control group where fenfluramine treated rats were put on food reduction program and applied to running wheels [24]. This disturbance was attributed to decrease in the level of neuropeptide Y (NPY) by fenfluramine where NPY enhances LH hormone [25,26]. Thus fenfluramine reduces NPY which in turns, decreases LH hormone and consequently disturbs the estrous cycle. Fluoxetine disturbed estrus cycle in 40% of mature Sprague Dawley rats [27,28]. As both fenfluramine and fluoxetine increases serotonin level and lorcaserin acts as 5HT2C agonist. Therefore, it is logic that serotonergic drugs may have a possible role in disturbing estrous cycle.

In the present study, all the utilized doses of lorcaserin decreased LH level. It was reported that activation of 5-HT2C receptors by lorcaserin leads to expression of POMC [29] known to produce many bioactive peptides including α-MSH, and β-endorphin [30], where β-endorphin conduce in the tonic suppression of LH secretion by restraining the LH releasing hormone surge [31–34].

Our result is in agreement with a study performed on immature female rats where this study proved that serotonin contributed in regulation of LH hormone secretion as morphine suppresses LH secretion through enhancing serotonergic activity [35]. However, our result is contradictory to previous study conducted on mature female rats which showed that serotonin (2.5 mg/kg, i.p) enhances LH level in estrus stage [36].

The current study highlighted that lorcaserin enhanced estradiol (E2) level. This result is in agreement with previous studies showing the effect of SSRIs on estrogen level. Fluoxetine (200 ng/l) was reported to enhance esterified estradiol level by 1.5 times in zebra mussel [37]. Fluoxetine (0.5–5 mg/kg) modulate endogenous estradiol in rats [38].

The mechanism by which serotonergic agents affect estradiol level is unclear till now. However, a previous clinical study conducted with paroxetine on 44 postmenopausal women where paroxetine (10 mg/day) was administrated for one week then the dose increased to 20 mg/daily for the rest of the duration therapy, results highlighted greater estradiol level [39]. The authors suggested that effect of paroxetine on estradiol may be due to the relation between SSRIs and cytochrome P-450 system where SSRIs inhibit a subtype of cytochrome P-450 enzymes that is responsible for metabolism of endogenous steroids such as estradiol [40,41]. In agreement, an in vitro study on human liver microsomes proved that sibutramine is potent inhibitor to cytochrome P450 2B6 [42]. Recently, a research performed on a new 5HT2C agonist, (2-Phenylcyclopropyl) methylamines showed inhibition to cytochrome P-450 enzymes [43]. Indeed, there is a lack of literatures concerning the adverse effects of lorcaserin on liver or whether lorcaserin reduces the steroid hormone binding globulin secretion which inhibit the function of estrogen.

In the current study, lorcaserin increased serum estradiol level and it is known from the literature that increment of estradiol causes negative feedback on LH and FSH. In our current study, LH level was significantly reduced while FSH showed non-significant reductions (FSH was reduced by 28.13%, 27.65%, 27.65% in lorcaserin treated rats). Therefore, we cannot confirm that decrease in LH is correlated to estradiol negative feedback and it is may be attributed to other pharmacologic or adverse effects of lorcaserin.

Ovarian sections of lorcaserin (5, 10 or 30 mg/kg) treated rats showed minimal edema in stromal cells associated with congestion in blood vessels. This change in the ovarian sections can be related to the stimulation of 5-HT receptor by lorcaserin, where it was proven that serotonin possess vasospastic properties similar to histamine. Consequently serotonin enhances permeability of vessels, which lead to edema [44].

In this study, lorcaserin did not affect the ovarian population. This could be explained as the drug did not cause damage or atresia to any type of follicles where the count of atretic follicles was not elevated. Moreover, the primordial follicles’ stock was not affected and this seems compatible with FSH results as FSH is mainly responsible for early maturation of primordial follicles and transforms it to early preantral follicles which results in rapid depletion of primordial reserve (FSH-driven follicle recruitment). The effect of lorcaserin on the count of ovarian follicles is inconsistent with a previous study performed on sibutramine (5 mg/kg/day) for 1 month where ovaries of sibutramine treated rats showed high count of atretic follicles [12].

Lorcaserin induced endometrial hyperplasia which may be attributed to the increase in estradiol (E2) hormone since estradiol causes thickening of lining mucosal epithelium of the uterus [45]. Due to the fact that endometrial hyperplasia is a critical risk factor for endometrial cancer [46,47]. Further studies should be done to investigate the possible risk of endometrial cancer by lorcaserin.

The histopathological changes occurring with lorcaserin are in agreement with the previous study conducted on sibutramine where adult Wistar female rats treated with sibutramine showed several changes in uterine histology such as: reduction in the thickness of

<table>
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<th>Table 3</th>
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<tr>
<td>Effect of lorcaserin (5, 10 or 30 mg/kg) on morphometric analysis of ovarian follicle populations in rats.</td>
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<tr>
<td>Control</td>
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<tr>
<td>Lorcaserin (5 mg/kg)</td>
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<td>Lorcaserin (10 mg/kg)</td>
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<td>Lorcaserin (30 mg/kg)</td>
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Tissue specimens from the ovaries were stained with hematoxylin and eosin and investigated for the level of maturation of the follicles at ×100. Primordial follicles comprised of an oocyte with a clear nucleolus, 5 layers of granulosa cells, and absent an antral space. Antral follicles were characterized by an oocyte with a clear nucleolus, 5 layers of granulosa cells and absent an antral space. Antral follicles were distinguished by a degenerating oocyte or granulosa cells’ pyknoty. Data are the mean number of each type of follicles ± SEM. Data and analyzed using one-way ANOVA followed by Tukey–Kramer as a post-hoc test. No significant difference was observed between the study groups at P < 0.05.

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luminal epithelium, elevation in area of the lumen, reduction in tissue layers thickness and edema [12]. Despite the different mechanisms of action of either drug, it is noteworthy to highlight the need for more data on the effect of serotonergic drugs on the female reproductive system.

Regarding the percent change in body weight throughout the experiment lorcazerin (5, 10 or 30 mg/kg) did not display significant decreases in weight gain than control. This result can be explained in light of a previous research performed by Smith et al. (2007) on male Sprague Dawley rats (250–400 g) fed with low fat diet and treated with lorcazerin (9, 18, 36 mg/kg, b.i.d.) for 28 days [16]. In the last study, the dose 9 and 18 mg/kg (b.i.d) did not produce significant reductions in weight gain when compared to control. However, the highest dose 36 mg/kg (b.i.d) produced 8.51% reduction in weight gain than the control. The highest dose utilized in our study was 30 mg/kg; it is smaller than one half of that used by Smith et al. (36 mg/kg, b.i.d). Other factors that may explain the difference in influence of lorcazerin on body weight is the gender difference and original body weight at

Fig. 4. Effect of lorcazerin on uterine histopathology in the experimental groups. A) Photomicrographs of uterine sections stained with hematoxylin and eosin (a) Sections from uteri of control rats showing normal glandular (yellow arrow) and epithelial structures (black arrow). (b) Sections from uteri of lorcazerin (5 mg/kg) group exhibit slight increase in number of glands. The columnar epithelial cells lining the glands showed decreased cytoplasmic vacuoles indicating early stage of hyperplasia. (c) Sections taken from uteri of lorcazerin (10 mg/kg) group showing moderate increase in number of glands lined by hyperplastic tall columnar epithelial cells with stratification and less cellular spindled stroma. (d) Sections from uteri of lorcazerin (30 mg/kg) group showing moderate increase in number of glands. The glands were arranged closely back to back and lined by hyperplastic tall columnar epithelial cells with stratification and less cellular spindled stroma. m: myometrium. Photos are captured at 100 x magnification. B) Mean of endometrium thickness from the experimental groups. The thickness of endometrium were determined from digital images in randomly selected and regularly spaced six fields and expressed as percentage of control. Data are expressed as mean ± SEM and analyzed using one-way ANOVA followed by Tukey–Kramer as a post-hoc test at \( P < 0.05 \). *Compared to control group, \( n = 5 \). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
the start of the therapeutic regimen; our experiment was conducted on female rats (175–200 g) while Smith et al. (2007) performed their study on male rats (250–400 g). It was confirmed that reduction in body mass is more observed in male performing physical exercise than female doing exercise [48].

5. Conclusions

Our work highlighted the effect of lorcaserin on reproductive function of female rats where it disrupts estrous cycle, alters ovarian and uterine histology and changes the level of some reproductive hormones. Therefore, its use should be closely monitored in women wishing to achieve weight loss while maintaining the reproductive potential until sufficient clinical safety data are available. Further studies are required to depict possible molecular mechanisms on effect of lorcaserin on female hormones, estrus cycle and histology of ovary and uterus in obese animals. Moreover, further studies should be warranted to assess the impact of lorcaserin on fecundity by mating males (untreated with lorcaserin) with lorcaserin treated females as well as investigating the effect of the drug on the pregnancy outcome.

Conflict of interests

None.

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