Introduction
Chronic stress is known to produce significant behavioral, endocrinological, and neurobiological changes. N-acetyl-cysteine (NAC) is commonly used as a safe mucolytic drug. Sulpiride is a selective dopamine antagonist with antipsychotic and antidepressant activity.

Aim
As the mechanism of stress is not known the aim of this work was to evaluate the histological and immunohistochemical changes that might occur in rat ovaries after stress exposure and the possible protective effect of sulpiride and NAC.

Materials and methods
Thirty-six rats were divided into four groups: group I was the control group (n=6); group II (n=6) was injected with sulpiride at 0.28 mg/kg body weight/day for 1 month; group III (n=6) was injected with NAC at 150 mg/kg body weight intraperitoneally for 5 days; group IV (the stress group) (n=18) was divided into three equal subgroups: subgroup IVa, in which rats were exposed to crowding only for 1 month, subgroup IVb, in which rats were exposed to crowding along with sulpiride treatment for 1 month, and subgroup IVc, in which rats were exposed to crowding for 1 month along with NAC treatment for 5 days.

Results
Stress resulted in a significant increase in luteinizing hormone, follicle stimulating hormone, and prolactin levels and a significant decrease in progesterone and estrogen levels. Sulpiride treatment resulted in a significant decrease in progesterone and estrogen levels and increase in prolactin, with decreased reaction for estrogen receptor. NAC led to a significant increase in the level of estrogen and progesterone, with no effects on other parameters, and increased reaction for estrogen receptor. The ovary of both the stress and the sulpiride group revealed cystically dilated follicles lined with granulose cells separated by a proliferation of ovarian stroma (luteinized stromal cells). NAC-treated group showed decreased number of atretic follicles and thickness of theca layer.

Conclusion
NAC had a protective role against the induced damage but sulpiride did not counteract the impairing effects of stress. It is beneficial to use NAC in people exposed to stress.

Keywords:
crowding, N-acetyl-cysteine, sulpiride
living in the same area and/or by reducing their living space. Crowding stress induces complex changes at the behavioral, physiological, and molecular levels [7,8]. Sulpiride is the most common drug used to manage stress [9]. Containment of the damage from stress exposure may be achieved through administration of synthetic antioxidant compounds such as N-acetyl-cysteine (NAC). NAC comes from the amino acid l-cysteine. Amino acids are the building blocks of proteins and have many uses such as in medicine and it is the acetylated precursor of both amino acid l-cysteine and reduced glutathione [10,11]. Moreover, NAC is a clinically proven safe dietary supplement that may be used in radiation therapy to prevent radiation-mediated genotoxicity [10]. Thus, the present study dealt with the possible protective effect of N-acetyl-cysteine and sulpiride against stress in female albino rats from the histological, immunohistochemical, and biochemical point of view.

Materials and methods

Experimental animals and grouping

Thirty-six female albino rats weighing 200±30 g were obtained from the animal house, Moshtohor Faculty of Veterinary Medicine, Benha University. They were given a balanced diet with free access to water. All animal procedures were performed according to approved protocols and in accordance with the recommendations for the proper care and use of laboratory animals. The animals were kept under observation for 1 week before the beginning of the experiment to acclimatize. They were divided into four groups:

(1) Group I (n=6): this group consisted of normal rats that served as negative controls injected with saline for 1 month and kept in a cage measuring (30x30x30 cm).

(2) Group II (n=6): this group consisted of rats injected with sulpiride at 0.28 mg/kg body weight/day for 1 month.

(3) Group III (n=6): this group consisted of rats injected with NAC at 150 mg/kg body weight intraperitoneally for 5 days.

(4) Group IV (the stress group): this group was divided into three equal subgroups (n=18) – subgroup IVa, in which rats were exposed to crowding without treatment for 1 month; subgroup IVb, in which rats were exposed to crowding along with sulpiride treatment for 1 month; and subgroup IVc, in which rats were exposed to crowding for 1 month along with NAC treatment for 5 days.

Application of stresses

Application of crowding

Crowding stress was induced by placing six rats from group IV in a cage measuring 20x20x20 cm [12].

Drugs

N-acetyl-cysteine administration

NAC was injected at a dose of 150 mg/kg body weight intraperitoneally. The NAC solutions were present at 0.5% (100 mg/kg) (Sigma Company, Cairo, Egypt) [13].

Sulpiride administration

Sulpiride, dissolved in normal saline, was administered orally by means of a gastric tube at a dose of 0.28 mg/kg body weight/day for 1 month. The dose for the rat was calculated according to Paget’s formula on the basis of the human dose (Sanofi Company, Cairo, Egypt) [14].

Experimental parameters

Biochemical examination

At the end of experiment, the animals were killed by an overdose of ether and blood samples were collected from their tail veins. The samples were collected in clean, dry graduated centrifuge tubes and left for 10 min to clot, and then centrifuged at 5000 rpm for 15 min. Using a pasteur pipette about half of the supernatant serum was transferred into clean dry tubes for subsequent tests. The serum was separated and kept at −20°C until analysis. Serum follicle stimulating hormone (FSH), luteinizing hormone (LH), serum prolactin, serum progesterone, and serum estradiol (E2) were measured according to the method described by Tietz [15].

Histological studies

Rats from the control, stressed, and treated groups were sacrificed around 4 weeks in either proestrus or diestrous stage, and small specimens of their ovary were taken for histological examination. These specimens were fixed in 10% neutral buffered formal solution for histological and immunohistochemical studies. Paraffin sections were prepared at 5 μm thickness and stained with H&E [16].

Immunohistochemical examination

Sections were preincubated for 15 min in a 1% solution of H2O2 before being incubated overnight at 25°C in 0.3% Triton X-100 and 0.5 mg/ml bovine serum albumin with the primary Hsp90 polyclonal antibody at 1 : 100 dilution (Assay Designs and Stressgen; Enzo Life Sciences, Exeter, UK). The sections were incubated for 120 min with anti-rabbit secondary antibodies (1 : 200 each; Vector Laboratories Inc., Burlingame, California, USA) and then treated with an avidin–biotin–peroxidase complex for 1 h at room temperature. After several rinses, the sections were mounted onto slides and counterstained with hematoxylin, dehydrated, and then coverslipped.

To examine the cellular expression of Estrogen receptor ER in the ovaries after treatment, the sections were processed for immunohistochemical analysis. The primary antibody used was anti-ER [17,18].

Statistical analysis

Experimental data were analyzed by one-way analysis of variance using SPSS (version 16.0; SPSS Inc., Chicago, Illinois, USA) software. Significant differences among the treatments were compared by means of the T test. P values less than 0.05 were considered significant, whereas P values less than 0.01 were considered highly significant.
Comparative study on the effect of N-acetyl-cysteine versus sulpiride
Faruk and Abd Elsalam Morsy

**Results**

**Biochemical results**

The crowding exposure group revealed a very highly significant ($P<0.01$) decrease in serum estrogen and progesterone and a highly significant ($P<0.05$) increase in serum LH, FSH, and prolactin. The animals treated with sulpiride recorded a highly significant ($P<0.05$) decrease in progesterone and estrogen levels with no effect on serum LH or FSH and a very highly significant ($P<0.01$) increase in prolactin. Treatment with NAC resulted in highly significant ($P<0.05$) increase in the level of estrogen and progesterone, with significant effects on other parameters (Table 2).

**Macroscopic results**

No animal death occurred in any group during the period of the experiment. The body weight of the group exposed to crowding, the group exposed to crowding along with NAC treatment, and the group exposed to crowding along with sulpiride treatment showed a highly significant decrease ($P<0.01$, <0.05 and <0.01 respectively) in comparison with group I. Rats treated with NAC alone and rats treated with sulpiride alone showed high body weight gain ($P<0.01$) (Table 1).

**Table 1. Percentage of body weight change in female albino rats in group I and group II**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Sulpiride</th>
<th>N-acetyl-cysteine</th>
<th>Crowding</th>
<th>Crowding and sulpiride</th>
<th>Crowding and N-acetyl-cysteine</th>
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<td>Body weight change</td>
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<tr>
<td>Mean</td>
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<td>123</td>
<td>15.8</td>
<td>26.5</td>
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<tr>
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<td>1.6</td>
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<td>0.8</td>
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<td>$P$</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
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<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
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</table>

Rats treated with sulpiride alone and those treated with NAC alone showed high body weight gain ($P<0.01$), but the crowding exposure group and the crowding exposure treated with sulpiride group recorded highly significant body weight loss ($P<0.01$).

**Table 2. Serum levels of estrogen, progesterone, prolactin, follicle stimulating hormone and luteinizing hormone in all groups and group IIC compared with group IIB**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Sulpiride</th>
<th>N-acetyl-cysteine</th>
<th>Crowding</th>
<th>Crowding and sulpiride</th>
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<tr>
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<td>11</td>
<td>12</td>
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<tr>
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<tr>
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<td>Mean</td>
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<td>63.70</td>
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<td>Highly significant</td>
<td>Highly significantly</td>
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<td>21.9</td>
<td>39.1</td>
<td>21.93</td>
<td>29.21*</td>
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<tr>
<td>FSH level (miu/ml)</td>
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<tr>
<td>Mean</td>
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<td>21.9</td>
<td>22.4</td>
<td>49.3</td>
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<td>1.3</td>
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<tr>
<td>$P$-value</td>
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<td>&lt;0.01</td>
<td>&lt;0.05</td>
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<td>Significant</td>
<td>Highly significant</td>
<td>Significant</td>
<td>Significant</td>
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</tbody>
</table>

FSH, follicle stimulating hormone; LH, luteinizing hormone, miu/ml, mili international unit.

*The crowding exposure group demonstrated a very highly significant ($P<0.01$) decrease in serum estrogen and progesterone and a highly significant ($P<0.05$) increase in serum LH, FSH, and prolactin. Sulpiride treatment resulted in a highly significant ($P<0.05$) decrease in progesterone and estrogen levels with no effect on serum LH or FSH and a very highly significant ($P<0.01$) increase in prolactin. The group subjected to crowding with NAC treatment recorded a highly significant ($P<0.05$) increase in the level of estrogen, progesterone with significant effects on other parameters.*
Histological results
The ovary of rats in the control group showed a normal architecture. It was covered with germinal epithelium with underlining tunica albuginea; the cortex was filled with spindle-shaped normal stromal cells and many ovarian follicles such as primordial follicles, a primary follicle, a secondary follicle, and mature Graafian follicles with many corpus albicans (Fig. 1).

The group treated with sulpiride alone showed spindle-shaped normal stromal cells, atretic follicles, luteinized stromal cells, and many cysts containing clotted blood (Fig. 2).

The group treated with NAC alone showed a normal ovary covered with regular epithelium with many mature follicles and corpus luteum (Fig. 3).

The ovaries of the stress group showed that the cortex was occupied by many spindle-shaped normal stromal cells and luteinized hyperplastic theca cells with thick blood vessels, atretic follicles, and many cysts containing clotted blood (Figs 4 and 5). The ovary also contained corpus luteum (Fig. 5). The ovaries in the group treated with sulpiride, had multiple cysts and atretic follicles (Fig. 6).

NAC-treated stressed ovaries showed irregular germinal epithelium, many growing follicles, and corpus luteum (Fig. 7), in addition to many primary follicles, corpus luteum, many dilated congested blood vessels, atretic follicles, and hyperplasia of theca interna. The ovaries were also occupied by many spindle-shaped normal stromal cells with foci of luteinizing stromal cells (Fig. 8).

Immunohistochemical results
Strong positive reaction for ER protein was detected in the nuclei of epithelial cells (granulose and theca cells) of the control group (Fig. 9). Compared with the control group, ER immunostaining was markedly decreased in the stress group and in sulpiride-treated rats (Figs 10 and 12), whereas in the NAC-treated rats the ER immunostaining was mildly to moderately decreased (Fig. 11). There was severe to moderate decrease in the ER immunostaining in sulpiride-treated group (Fig. 13) but the NAC-treated group mild decrease in ER immunostaining (Fig. 14). The mean area% of ER immunostaining is shown in Table 3.

<table>
<thead>
<tr>
<th>Estrogen receptors</th>
<th>Control</th>
<th>Sulpiride</th>
<th>N-acetyl-cysteine</th>
<th>Crowding</th>
<th>Crowding and sulpiride</th>
<th>Crowding and N-acetyl-cysteine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean area%</td>
<td>8.184</td>
<td>2.482</td>
<td>7.784</td>
<td>0.988</td>
<td>2.312</td>
<td>6.543*</td>
</tr>
<tr>
<td>SD</td>
<td>1.779</td>
<td>0.801</td>
<td>0.518</td>
<td>0.669</td>
<td>1.581</td>
<td>1.563</td>
</tr>
<tr>
<td>P value</td>
<td>0.026</td>
<td>0.001</td>
<td>0.008</td>
<td>0.001</td>
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<td>0.001</td>
</tr>
<tr>
<td>Significance</td>
<td>HS</td>
<td>HS</td>
<td>HS</td>
<td>HS</td>
<td>S and HS*</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. The mean area% of estrogen immunostaining, SD and P values in group II compared with the control group (group I) and group IIc compared with group IIb

HS, highly significant; S, significant

**Figure 1.** A photomicrograph of a section of a rat ovary from the control group (group I) showing germinal epithelium (g), tunica albuginea (t), primordial follicle (pr), primary follicles (p), secondary follicles (s), mature Graafian follicle (m) and corpus albican (c). H&E, ×200.

**Figure 2.** A photomicrograph of a section of a rat ovary from group II showing multiple follicular cysts containing clotted blood (c), hyperplasia of stromal cells (s) with foci of luteinization (i), and atretic follicles (f). Note the presences of thick-walled arterioles (v) and irregular tubules lined with epithelium (r). H&E, ×200.
Figure 3. A photomicrograph of a section of a rat ovary from group III showing a normal structure covered with regular germinal epithelium (g), primordial follicles (pr), mature Graafian follicles (m), and corpus luteum (l).

H&E, ×200.

Figure 4. A photomicrograph of a section of a rat ovary from the group IVa showing spindle-shaped stromal cells with flat nuclei (s), hyperplastic stromal cells (h) with focal luteinization (l), atretic follicles (arrows), and thick blood vessels (v). Note the presence of multiple follicular cysts containing clotted blood (c) and irregular tubules lined by flattened epithelium (r).

H&E, ×200.

Figure 5. A photomicrograph of a section of a rat ovary from the group IVa showing many atretic follicles at different stages: atretic follicles lined with one-layered epithelium (c) and others with multiply layered epithelium (f). Note that corpus albican (a).

H&E, ×200.

Figure 6. A photomicrograph of a section of a rat ovary from group IVb showing numerous follicular cysts (c) and atretic follicles (f) with hyperplastic stromal cells (i). Note that primary (p) and secondary (s) follicles.

H&E, ×200.

Figure 7. A photomicrograph of a section of a rat ovary from the group IVc showing irregular germinal epithelium (g), primary (p), and secondary follicles (s), corpus luteum (c).

H&E, ×200.

Figure 8. A photomicrograph of a section of a rat ovary from the group IVc showing many growing follicles (f), corpus luteum (CL), luteinized stromal cells (l), and many dilated congested blood vessels (v).

H&E, ×200.
Figure 9. A photomicrograph of a section of a rat ovary from the group I showing strong positive nuclear estrogen receptor immunoreactivity in the granulose cells of growing follicles (arrow) and in the stromal cells (L).

Figure 10. A photomicrograph of a section of a rat ovary from group II showing negative nuclear estrogen receptor immunoreactivity in the luteinized stromal cells (arrows).

Figure 11. A photomicrograph of a section of a rat ovary from the group III showing moderate to strong nuclear estrogen receptor immunoreactivity in the granulose cells of growing follicles (arrow), the corpus luteum (L) and germinal epithelium (g).

Figure 12. A photomicrograph of a section of a rat ovary from the group IVa showing weak nuclear estrogen receptor immunoreactivity in the stromal cells (arrows).

Figure 13. A photomicrograph of a section of a rat ovary from the group IVb showing negative nuclear estrogen receptor immunoreactivity in the stromal cells (arrows). Note that presence of multiple cysts (c).

Figure 14. A photomicrograph of a section of a rat ovary from the group IVc showing moderate to strong nuclear estrogen receptor immunoreactivity in the stromal cells (arrow) and in the germinal epithelium (g).
Discussion

Of all types of environmental pollutants, crowding is the most prevalent and insidious natural pollutants that causes deleterious physiological and structural effects. Hence, the present study was conducted to examine the possible protective effect of NAC and sulpiride against crowding stress in female albino rats.

Moreover, cystically dilated follicles lined by granulose cells separated by normal ovarian stroma, similar to those seen in polycystic ovarian syndrome, were seen in the present study in both stress and sulpiride-treated groups.

There were also numerous irregular tubules lined with epithelium. These tubules were called rete ovaries. This may be due to increase in androgen level by the cystic ovary.

Park et al. [19] recorded that this syndrome was associated with various conditions of ovarian dysfunction characterized by an increase in androgen level, which could be attributed to elevated LH levels. This alteration in ovarian steroidogenesis with increased inhibin production led to decreased FSH levels. Moreover, Dissen et al. [20] recorded that with increased stress there was excessive production of nerve growth factor of the sympathetic nervous system. This might have a role in the development of polycystic ovaries in rats through regulation of follicle formation by induction of FSH receptors. Immunohistochemical and biochemical studies of the stress and sulpiride-treated groups revealed significant decrease in reactivity of granulose cells to estrogen receptors. Also Ruder et al. [21] recorded that increase in androgen receptor expression with decreased estrogen receptor expression in stress was a result of increased release of heat shock protein 90 by stressed cells. This protein played an important role in regulation of gonadotropin activity through its binding to steroid receptors. This protein might be involved in the induction of polycystic ovarian syndrome [9,10]. In the above-mentioned group, there was absence of corpus luteum indicating absence of ovulation. This was confirmed by decrease in estrogen and prolactin levels in the present study. Increase in prolactin level in the present study was due to loss of estrogen and progesterone and the inhibitory effect on prolactin.

Hyperplasia of stromal cells with foci of luteinizing stromal cells was detected in the stress and sulpiride groups of the present study. This might be attributed to the follicular atresia resulting from nonavailability of steroid hormones [22]. Moreover, Aguirre-Samudio et al. [23] stated that stress induced a marked expression of corticotropic-releasing hormone in stromal cells. Many investigators stated that disturbance in hormone levels in the stressed group might be due to generation of free radicals that caused cell damage by lipid peroxidation and protein degradation of granulosa cells [24–26]. Thus, the present study suggested that treatment of chronic stress with sulpiride could enhance the toxic effect of stress instead of eliminating it. The histological result in NAC treatment showed ovaries regaining their normal ovarian structure. The antiapoptotic effect of NAC was due to its sulfhydryl component, which acted as chelating agent. Also NAC enhanced antioxidant enzymes like glutathione [27].

The crowding treated with NAC group revealed an irregular surface for the ovary, presence of growing follicles, and corpus albicans indicating ovulation, whereas the group treated with sulpiride revealed smooth-surfaced ovaries, no growing follicles, no corpus luteum indicating anovulation, and decreased immunoreactivity of estrogen receptors.

This disruption in the growth and differentiation of preovulatory follicles may be due to the nonavailability of steroidal hormones [28], which are essential for their maturation and differentiation because of secretion of androgens by cystic follicles [29], imbalanced endogenous steroid and protein hormones, or because of the generation of free radicals like reactive oxygen species (ROS) [30]. Oxidative stress (OS) can initiate apoptosis in mammalian oocytes [31]. Intracellular accumulation of ROS can damage cells by causing nucleic acid strand breaks, lipid peroxidation, protein degradation, and ultimately cell death [3]. It has been suggested that steroidogenically active cells such as granulosa cells of the antral follicles require high levels of energy production and thus generate large amounts of ROS [32]. Therefore it is possible that OS is involved in the mechanisms that trigger apoptosis in healthy steroidogenic antral follicles [33]. Vascular congestion and atretic follicles have been observed and characterized in the ovary. This was in agreement with Aguirre Samudio et al. [34], who found that the same changes occurred after exposure to cold and immobilization stress. Other studies showed that sulpiride induces an increase in follicular atresia in ovaries when administered during gestation [33]. Rats treated with the antioxidant NAC exhibit higher numbers and quality of oocytes. Appropriate NAC treatment may have reduced apoptosis and losses of healthy follicles during ovarian aging. Although we speculate that the beneficial effects of NAC are mediated by its activity as an antioxidant, it is possible that NAC may act by increasing glutathione levels. However, the protective effects by antioxidant activity also could include function on the hypothalamus–pituitary–gonad axis, in addition to the ovary also in addition, the antiapoptotic effects of NAC [35]. FSH release is increased when estrogens are decreased and elevation in FSH can result in abnormal estrous cycles and ovarian follicular maturation [36]. The study by Lappas et al. [36] supports our suggestion that decreased estrogens was observed in stress-induced groups compared with NAC-treated groups, probably resulting in the elevation of FSH release. The crowding stress results in stimulation of adrenocorticotropic hormone, adrenal hyperactivity, and increased corticosterone secretion with LH elevation, and this may be one of the pathophysiological mechanisms involved in follicular cyst pathogenesis [37]. Chronic exposure to stressors increases hypothalamus–pituitary–adrenal axis activity and concurrently reduces hypothalamus–pituitary–gonad
axis activity [25]. The decrease in the serum levels of E2 during sulpiride administration is probably secondary to hyperprolactinemia, as sulpiride is a dopamine antagonist and thus increases prolactin levels, which leads to decreased androgen and progesterone levels [26]. The present work showed a significant increase in the percentage of body weight gain in the drug-treated groups compared with the control group. This may be because of the action of sulpiride, which mainly interacts with dopamine D2–D3 receptors in the brain [38]. This was in agreement with previous studies [9]. A significant decrease in the percentage of body weight gain of rats after exposure to crowding stress may be because chronic exposure to crowding stress decreases body weight and food intake, and also due to nonavailability of either gonadotropic or steroid hormones, or both, or due to OS [26]. From the foregoing, it could be concluded that stress caused alterations in ovarian tissues, which were hormonal in nature and elevated FSH, LH, and progesterone receptor PL but lowered estrogen concentration. These results may have implications on the interruption of the estrous cycle associated with the diagnosis of polycystic ovary syndrome PCOS. Finally, our results suggest that stress may have affected the ovary and alterations occurred in steroid hormone receptors and reproductive hormones, and that these changes can together lead to abnormal follicle development. Also our studies clearly show that estrogen is necessary for reproductive protection against stress toxicity.

Conclusion and recommended ovarian changes
Stress and sulpiride induced ovarian changes in the form of development of polycystic ovaries. NAC has a protective role against the induced damage but sulpiride did not counteract the impairing effects of stress. It is beneficial to use NAC in people who exposed to stress. Stress exposure should be avoided as much as possible for good health.

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Conflicts of interest
There are no conflicts of interest.

References
المختصر العربي

مقارنة بين عقار السليبريد والان استيل سستين على الضغط العصبي في مبايض الفئران البيضاء

دراسة نسيجية ونسيجية كيميائية مناعية وكيماوية حيوية

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المقدمة:
يعتبر الضغط العصبي من المواثرات الشديدة التأثير على الإنسان لذا تهدف هذه الدراسة لمقارنة دواء السليبريد والان استيل سستين على الفئران المعرضة للضغط العصبي وتوضيح أثارهم على المعايير النسيجية والنسيجية الكيميائية المناعية والكيميائية الحيوية.

الهدف:
تهدف هذه الدراسة لدراسة تأثير الضغط العصبي ومقارنة تأثير دواء السليبريد والان استيل سستين والمقارنة بينهم في مبايض الفئران البيضاء البالغة.

وصفت ستة وثلاثون فارسا وقسمت الى أربع مجموعات كالاتي:
المجموعة الأولى مجموعة ضابطة.
المجموعة الثانية حققت بعقار السليبريد.
المجموعة الثالثة حققت بعقار الان استيل سستين.
المجموعة الرابعة عرضت للضغط العصبي وقسمت الى ثلاث مجموعات فرعية.
- اولا مجموعة معرضة للضغط العصبي فقط.
- ثانيا مجموعة معرضة للضغط العصبي وحققت بعقار السليبريد.
- ثالثا مجموعة معرضة للضغط العصبي وحققت بعقار الان استيل سستين.

النتائج:
أظهرت النتائج ان عقار السليبريد والضغط العصبي كان له تأثير متلازمة تعدد الأكياس المبيضية حيث وجد نقص في مستوى هرمون الاستروجين والبروجسترون وارتفاع نسبة مستوي هرمون البرولاكتين بينما الفئران التي حققت بعقار الان استيل سستين كان لها التأثير الإيجابي على المبايض. حيث أظهر نسيج المبيض مع الضغط العصبي وعقار السليبريد وجود حويصات متعددة وازيد في خلايا السيلا ووجود ارتفاع في أنسجة المبيضية وذلك لزيادة نسبة انتردروجين.

الخلاصة:
أن الضغط العصبي وعقار السليبريد يحدث تأثيرات ضارة على التركيب النسجي والكيميائي الحيوي للمبايض بينما الناس استيل لأثر إيجابي لذا ينصح باستخدامه وتجنب الضغوط العصبية.