

# Tamoxifen antagonizes the effects of ovarian hormones to induce anxiety and depression-like behavior in rats

Tamoxifeno antagoniza os efeitos dos hormônios ovarianos que induzem comportamento similar a ansiedade e depressão em ratos

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## ABSTRACT

The effects of tamoxifen (TAM) on anxiety and depression-like behavior in ovariectomized (OVX) and naïve female rats were investigated. The animals were divided into Sham-TAM, OVX-TAM, Sham and OVX groups. Tamoxifen (1 mg/kg) was administered for 4 weeks. In the forced swimming test, the immobility times in the OVX and Sham-TAM groups were higher than in the Sham group. In the open field, the numbers of central crossings in the OVX and Sham-TAM groups were lower than the number in the Sham group, and the number of peripheral crossings in the OVX group was lower than the number in the Sham group. In the elevated plus maze, the numbers of entries to the open arm among the animals in the Sham-TAM and OVX groups were lower than the number in the Sham group, while the number of entries to the open arm in the OVX-TAM group was higher than the number in the OVX group. It was shown that deletion of ovarian hormones induced anxiety and depression-like behavior. Administration of tamoxifen in naïve rats led to anxiety and depression-like behavior that was comparable with the effects of ovarian hormone deletion. It can be suggested that tamoxifen antagonizes the effects of ovarian hormones. It also seems that tamoxifen has anxiolytic effects on ovariectomized rats.

**Keywords:** rat, ovariectomized, tamoxifen, depression, forced swimming test, open field, elevated plus maze.

## RESUMO

Foram investigados os efeitos do tamoxifeno (TAM) no comportamento semelhante a ansiedade e depressão de ratas ooforectomizadas (OVX) e controles. Os animais foram divididos em Sham-TAM, OVX-TAM, Sham e OVX groups. Tamoxifeno (1 mg/kg) foi administrado por quatro semanas. No teste de natação forçada, os tempos de imobilidade nos grupos OVX e Sham-TAM foram maiores que aqueles do grupo Sham. No campo aberto, os números de cruzamento no centro nos grupos OVX e Sham-TAM foram menores que aquele do grupo Sham, e o número dos cruzamentos na periferia no grupo OVX foi menor que o número no grupo Sham. No labirinto elevado, os números de entradas com braços abertos entre os animais nos grupos Sham-TAM e OVX foram menores do que aqueles do grupo Sham, enquanto o número de entradas com os braços abertos no grupo OVX-TAM foi maior que aquele no grupo OVX. Foi observado que a deleção dos hormônios ovarianos induziu comportamento similar a ansiedade e depressão. A administração de tamoxifeno em ratos controle induziu a um comportamento que era comparável aos efeitos da deleção do hormônio ovariano. Pode ser sugerido que o tamoxifeno antagoniza os efeitos dos hormônios ovarianos. Parece também que o tamoxifeno tem efeito ansiolítico nas ratas ooforectomizadas.

**Palavras-chave:** rato, ooforectomia, tamoxifeno, depressão, teste de natação forçada, campo aberto, labirinto elevado.

Depression is the second most common chronic disease which has been widely expanded in the world. It is estimated that about half of the patients are unaware of their disease or their disease is diagnosed else<sup>1</sup>. Anxiety is also a complicated behavior in human and animals which may lead to a wide

range of problems in the central nervous system (CNS). It has also been reported that anxiety affects one-eighth of the population and in sever forms it has debilitating effects on the quality of life<sup>2</sup>. Besides of genetic, social, environmental and nutritional factors, it is suggested that sex hormones

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**Conflict of interest:** There is no conflict of interest to declare.

**Support:** The authors would like to thank the Vice Presidency of Research of Islamic Azad University, Isfahan (Khorasgan) Branch for financial support.

Received 19 July 2014; Received in final form 23 October 2014; Accepted 11 November 2014.

may have important roles in the pathogenesis of anxiety<sup>2</sup>. Interestingly, it is suggested that estrogen has protective roles against depressive disorders, Alzheimer's disease, Parkinson's and anxiety<sup>3,4</sup>. Similar to antidepressant drugs, estrogens are also known to increase the amounts of noradrenaline and serotonin in the CNS which may have role for regulating the mood and decreasing the susceptibility to depression<sup>5</sup>.

Selective estrogen receptor modulators (SERMs) bind to estrogen receptors and induce specific changes in their three-dimensional conformation allowing a tissue-selective recruitment of transcriptional cofactors. These compounds have been shown to exert estrogen-like neuroprotective actions in the brain. Tamoxifen, one of SERMs, is extensively used in breast cancer<sup>6</sup>. In CNS, tamoxifen has both agonistic and antagonistic effects on estrogen receptors<sup>6</sup>. There is also evidence that SERMs may affect mood, depression and anxiety<sup>7</sup>.

Regarding the modulatory effects of tamoxifen on the actions of estrogen, in the present study, the effects of tamoxifen on anxiety and depression like behaviors in ovariectomized(OVX) and sham operated rats was investigated.

## METHOD

### Animals and drugs

Sixty female Wistar rats, 12 weeks old ( $240 \pm 10$  g) were housed in 4-5 per standard cages, at room temperature ( $22 \pm 2^\circ\text{C}$ ) and a 12 h light/dark cycle. Food and water were available *ad libitum* properly. Animal handling and all related procedures were approved by the Mashhad Medical University Committee on Animal Research. The animals were divided into four groups: (1) Sham (n = 20); (2) OVX (n = 10); (3) Sham- tamoxifen (Sham-TAM; n = 20); and (4) ovariectomized- tamoxifen (OVX-TAM; n = 10). In Sham and Sham-TAM groups, 9-10 animals which had proestrous phase were selected and used for the behavioral studies. The animals in the Sham-TAM and OVX-TAM groups were treated by tamoxifen (1 mg/kg; IP, 4 w) (Iran Company, Tehran, Iran) . After the ovariectomy or sham surgeries, the rats lived in animal house for 4 weeks before conducting the experiment for diminution of the endogenous sex hormones<sup>8</sup>. The treatment in Sham-TAM and OVX-TAM groups was then started and continued for 4 weeks. The animals of Sham and OVX groups were injected by saline plus a drop of tween instead of tamoxifen.

### Surgery

The animals were ovariectomized under ketamine (Alfasan Company, Netherland) anesthesia. Abdominal incision was made through the skin of the flank and ovaries were removed. The same procedure was performed on the sham rats except that the wound was closed without removing the ovaries.

### Vaginal cytology

To ensure that the female rats were cycling, vaginal cytologies were started 1 week before each experience and continued every day. It was carried out in sham and sham-TAM groups as described previously to select 9-10 animals in each group with proestrous stage for behavioral studies. Briefly, the rats were held in a non-stress position and quickly lavaged with approximately 1ml saline. Slides were read using light microscopy, and estrous categories were classified based on cytological characteristics<sup>9</sup>.

### Behavioral procedures

Before starting the experiments, each rat was handled daily for 3 days and then the rats were accustomed to the apparatuses of the experiments.

### Forced swimming test (FST)

The animals were placed in a glass cylindrical tank with 60 cm height and 38 cm width which was filled with water ( $24 \pm 1^\circ\text{C}$ ) to the depth of 40 cm. In the first day, the rats were placed inside the water cylinder for 15 min (pre-test) and then for 5 min in the following day (test day). The time of floating (immobility) as well as the active and climbing times were recorded for 5 min<sup>9</sup>.

### Open-field

The open- field (OP) measurement was done by a pelexiglass apparatus with an area of  $100 \times 100$  cm and height of 40 cm. Inside of the apparatus was divided to 16 equal squares. In addition, within the apparatus was divided to two zones called peripheral and central zones. Each animal was placed in the central zone and its movement was recorded by a digital camera for 5 min, and the following criteria were calculated: (1) the crossing number in the central zone, (2) the crossing number in the peripheral zone, (3) the traveled distance in central zone, (4) the traveled distance in peripheral zone, (5) the time spent in central zone, (6) the time spent in in peripheral zone, (7) the total crossing number, (8) the total traveled distance<sup>10</sup>.

### Elevated Plus Maze

The elevated plus maze (EPM) was made of 4 arms (50 cm length  $\times$  10 cm width) elevated 100 cm above the floor. The two closed arms had 40 cm high dark walls and the other two open arms had 0.5 cm high edges. The between angle of arms was  $90^\circ$ . The rats were placed in the center of the apparatus facing a closed arm for 5 min. The time spent and the number of entries into the open and closed arms were measured.

### Statistical analysis

The data were expressed as mean  $\pm$  SEM. The one-way ANOVA were run followed by tukey post hoc comparisons test. The criterion for the statistical significance was  $p < 0.05$ .

## RESULTS

### Forced swimming test

The immobility time in the OVX group was higher than that of the Sham group ( $p < 0.001$ ). The immobility time in the Sham-TAM group was also higher than that of the Sham group ( $p < 0.001$ ). There was no significant difference between Sham-TAM and OVX groups. There was also no significant difference between OVX-TAM and OVX groups (Figure 1A).

The active time in OVX group was lower than that of the Sham group ( $p < 0.001$ ). In the Sham-TAM group also, the active time was lower than that of the Sham group ( $p < 0.001$ ) however, there was no significant difference between Sham-TAM and OVX groups. The active time in

OVX-TAM was also lower than that of the OVX group ( $p < 0.01$ ) (Figure 1B).

The climbing number in OVX group was lower than that Sham group ( $p < 0.001$ ). There was no significant difference between Sham-TAM and Sham groups. There was also no significant difference between OVX-TAM and OVX groups (Figure 1C).

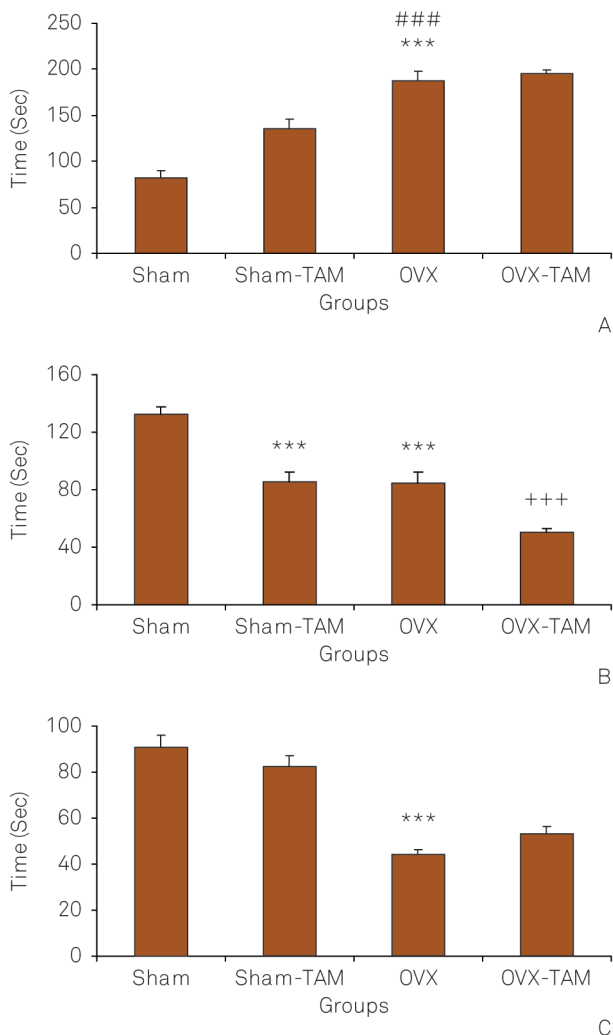
### Open-field

The number of crossing in the central zone in the OVX group was lower than that of the Sham group ( $p < 0.001$ ). As shown in Figure 2A, the crossing number of central zone in Sham-TAM group was significantly lower than that in the Sham group ( $p < 0.001$ ). The results also showed that the central crossing number in OVX group was lower than that of Sham-TAM group ( $p < 0.001$ ). The central zone crossed number by the animals of OVX-TAM group was not different from that of OVX group (Figure 2A).

The time spent and the traveled distance in the central zone by the animals of OVX group were lower than that of Sham group (both  $p < 0.001$ ). There were no significant differences neither in the time spent nor in the traveled distance in the central area between the Sham-TAM and Sham groups. The time spent and the traveled distance in the central area by the animals of OVX group was lower than that of Sham-TAM group ( $p < 0.01 - p < 0.001$ ). There was no significant difference between OVX-TAM and OVX groups when the time spent and the traveled distance in the central area were compared (Figures 2B and 2C).

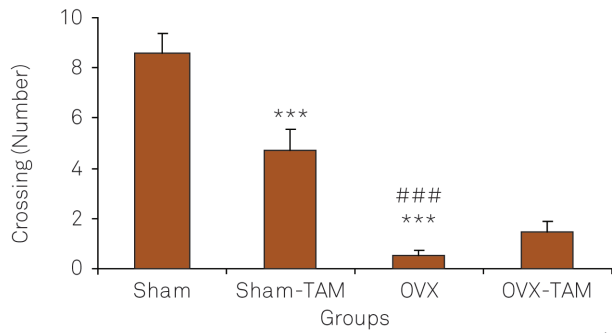
The animals of OVX group had a lower crossing number in the peripheral area compared to the Sham group (Figure 3A;  $p < 0.001$ ). The peripheral crossing number by the animals of OVX group was lower than that of Sham-TAM group however, there was no significant difference between the Sham-TAM and Sham groups. There was also no significant difference between the OVX-TAM and OVX groups (Figure 3A). The animals of OVX spent higher time in the peripheral zone compared to Sham group ( $p < 0.001$ ). The animals of OVX group spent more time in the peripheral zone compared to Sham-TAM group (Figure 3B;  $p < 0.05$ ). The traveled distance in the peripheral zone by the animals of OVX group was lower than that of both the Sham and Sham-TAM groups (Figure 3C;  $p < 0.01 - p < 0.001$ ). There were no significant differences between Sham-TAM and Sham groups and also between OVX-TAM and OVX groups (Figure 3C).

The total number of crossing in the OVX group was lower than that of the Sham group (Figure 4A;  $p < 0.001$ ). The total crossing number by the animals of OVX group was lower than that of Sham-TAM group ( $p < 0.001$ ). Treatment by tamoxifen didn't affect the total crossing number neither in Sham nor in OVX rats (Figure 4A). The total traveled distance by the animals of OVX group was lower than that of both Sham and Sham-TAM groups

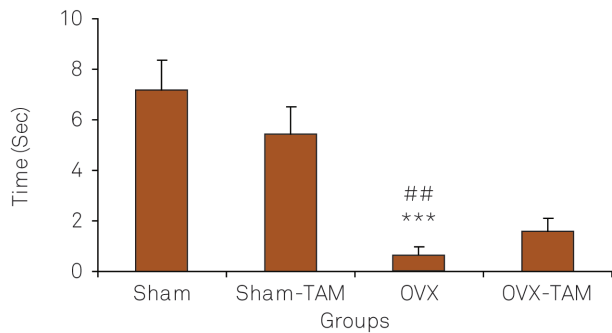


\*\*\* $p < 0.001$  compared to Sham group, ### $p < 0.001$  compared to Sham-TAM group, +++ $p < 0.001$  compared to OVX group.

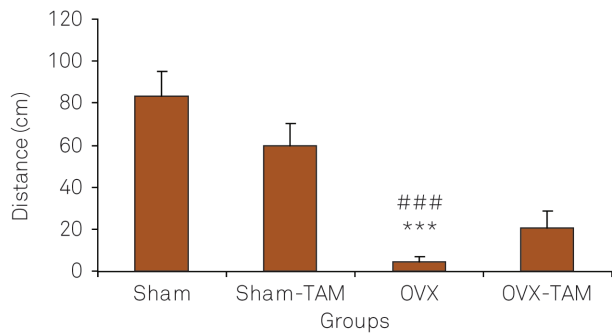
Figure 1. (A) Comparison of immobility, (B) active and (C) climbing times in the forced swimming test between four groups. Data are expressed as mean  $\pm$  SEM ( $n = 9-10$  in each group).



A



B



C

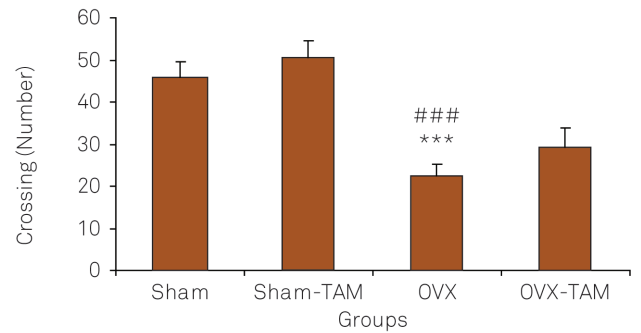
\*\*\*p < 0.001 compared to Sham group, \*\*p < 0.01, ###p < 0.001 compared to Sham-TAM group.

**Figure 2.** (A) Comparison of the number of crossing, (B) time spent and (C) traveled distance in the central zone in the open-field test. Data are expressed as mean  $\pm$  SEM (n = 9-10 in each group).

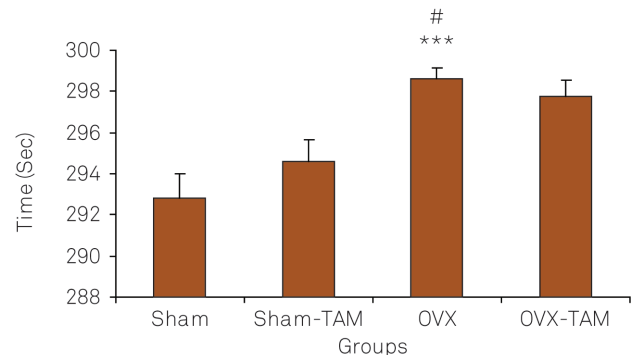
(Figure 4B; p < 0.001). There were no significant differences between the Sham-TAM compare to the Sham group and between the OVX-TAM and OVX groups (Figure 4B).

### Elevated plus maze

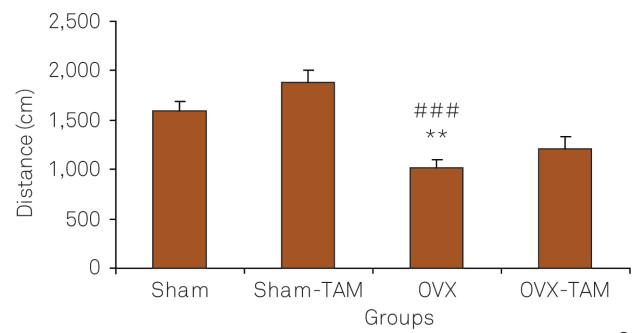
The number of entries to the open arm by the animals of OVX group was lower than that of Sham group (Figure 5A; p < 0.001). In Sham-TAM group also, the number of entries to the open arm was lower than that of Sham group (Figure 5A; p < 0.001). There were no significant differences between Sham-TAM and OVX groups. The open arm entries by the animals OVX-TAM group was higher than that of OVX group (Figure 5A; p < 0.01). The time spent in the open arms by the animals of OVX group was lower than that of Sham group



A



B



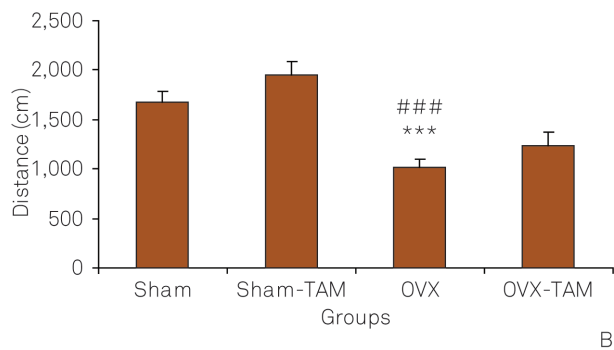
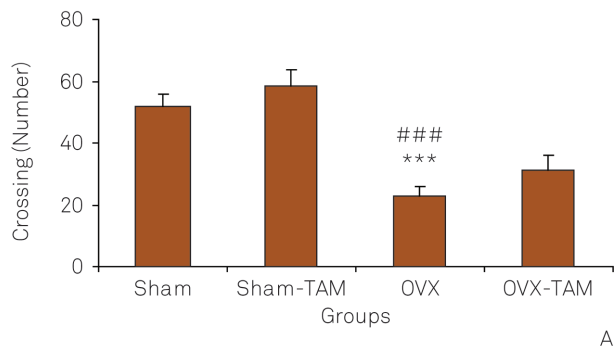
C

\*\*p < 0.01, \*\*\*p < 0.001 compared to Sham group, #p < 0.01, ###p < 0.001 compared to Sham-TAM group.

**Figure 3.** (A) Comparison of the number of crossing, (B) time spent and (C) traveled distance in the peripheral zone in the open-field test. Data are expressed as mean  $\pm$  SEM (n = 9-10 in each group).

(Figure 5B; p < 0.01). There were no significant differences between Sham-TAM and OVX groups. Treatment of sham operated rats by tamoxifen decreased while, treatment of OVX rats increased the time spent in the open arm (Figure 5B; p < 0.01).

Figure 6 shows that there were no significant differences between Sham and OVX groups in the number of entries to the closed arm. There were also no significant differences between Sham-TAM and Sham groups. The results also showed no significant differences between OVX-TAM and OVX groups. There were no significant differences between Sham and OVX groups in the time spent in the closed arm. There were also no significant differences between



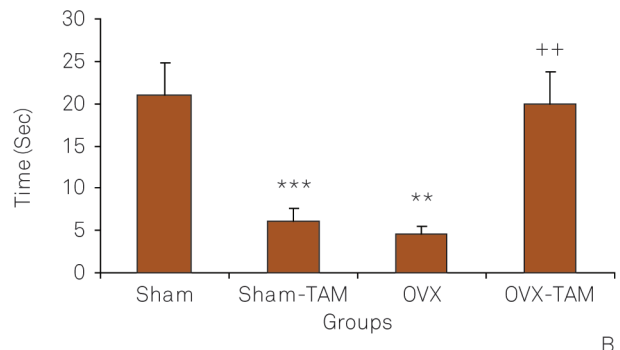
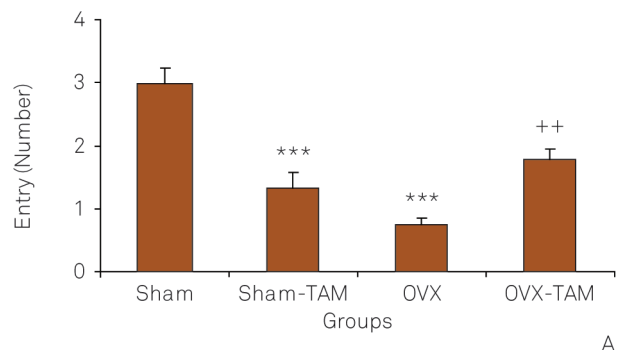
\*\*\*p < 0.001 compared to Sham group,  
### p < 0.001 compared to Sham-TAM group.

**Figure 4.** (A) Comparison of the total number of crossing and (B) total traveled distance in the open-field test. Data are expressed as mean ± SEM (n = 9-10 in each group).

Sham-TAM and Sham groups. The results showed no significant differences between OVX-TAM and OVX groups.

## DISCUSSION

Ovarian hormones not only play an important role in reproductive behavior, but also involve in emotion, memory, neuronal survival and perception of somatosensory stimuli<sup>3,11</sup>. On the other hand, mood disorders in women have been reported to be more common than men<sup>12</sup>. This fact has been attributed to hormonal changes in premenstrual and postpartum periods which have also been confirmed in hypoestrogenic conditions occurred during medical surgery or menopause<sup>13</sup>. Using FST and tail suspension tests, an increase in immobility time and a decrease in active behaviors were reported 2-4 weeks after ovariectomy in both rats and mice which were partially reduced by estradiol injection<sup>11,14</sup>. In the present study, the ovariectomized rats showed higher immobility and lower active times compared to sham operated ones when the animals were examined in FST. The results of present study also showed that exploratory behaviors in OP test were decreased in OVX rats compared to the Sham group. These data confirm the effects of deletion of ovarian hormones to induce depressive behaviors. It has



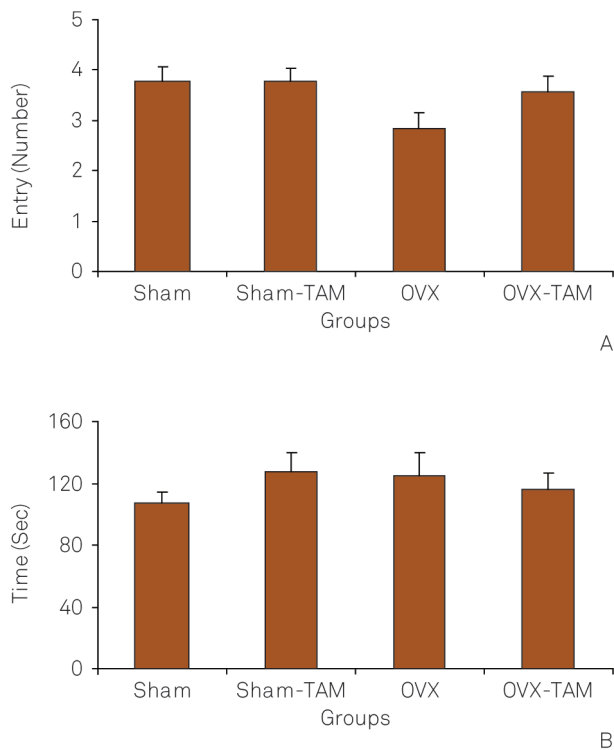
\*\*p < 0.01, \*\*\*p < 0.001 compared to Sham group,  
++ p < 0.01, +++p < 0.001 compared to OVX group.

**Figure 5.** (A) Comparison of the number of entries to and the (B) time spent in the open arms in the Elevated plus maze test. Data are expressed as mean ± SEM (n = 9-10 in each group).

been reported that estradiol increases serotonin uptake in frontal cortex and hypothalamus in ovariectomized rats<sup>4,15</sup>. Estrogen also competes with tryptophan, the precursor of serotonin, for binding sites on plasma albumin which may lead to an increased availability of this amino acid to the CNS<sup>4</sup>. In addition, estrogen reduces mono amino oxidase (MAO) activity<sup>16</sup> and decreases the expression of catechol-O-methyltransferase (COMT) thus increases the levels of catecholamines and serotonin in the brain<sup>4</sup>. Estrogen has also been shown to have a neuroprotective effect on dopaminergic neurons and prevent degeneration of neurons<sup>17</sup>. All of these findings might be considered as the mechanism(s) by which ovarian hormones elimination induces a depression like behavior in rats.

The presence of an anxiety-like behavior has also been reported in OVX rats when the animals were examined in the EPM test<sup>13</sup>. Clinical studies also show that anxiety disorders occur in 50% of menopausal women<sup>18</sup>. The results of present study also showed that the number of entries into as well as the time spent in open arm of EPM decreased after ovariectomy. The animals of OVX group also had lower crossing number in the central zone when they were examined in OP test.

The results of present study showed that the female rats with preposterior phase had a better performance in FST,



**Figure 6.** (A) Comparison of the number of entries to and the (B) time spent in the closed arms in the Elevated plus maze test. Data are expressed as mean  $\pm$  SEM ( $n = 9-10$  in each group).

OP and EPM compared to ovariectomized rats. The rat's estrous cycle is including proestrous, estrous, metaestrous and diestrous<sup>9</sup>. In proestrous phase, the estrogen levels are in high level. Therefore, it seems that a high level of endogenous estrogen attenuates anxiety and depression like behaviors. The results of present study confirm the mood improvement effects of endogenous ovarian hormones<sup>19</sup>. It has also been previously shown that female mice in proestrous have better moods than those in other phases<sup>20</sup>.

The results of present study also showed that sham operated rats treated by tamoxifen had higher immobility times in FST compared to non-treated sham ones. Treatment of the animals of sham group also decreased the active times. It seems that tamoxifen induces a depressive like behavior in the presence of ovarian hormones. In contrast, there were no significant differences between the OVX-TAM and OVX groups in immobility times. Therefore, it is suggested that tamoxifen has a dual effect in the presence and absence of ovarian hormones. The results of OP test also confirmed a depression like behavior induced by tamoxifen in the presence of ovarian hormones. In OP test the crossing number in the central area is considered as criteria for depression<sup>10,21</sup>. In the present study, the animals of Sham-TAM group had lower crossed number in the central area of OP apparatus compared to Sham group while, there were no significant differences between OVX and OVX-TAM groups. Calmarza-Font et al. (2012) showed that tamoxifen as well as the other

SERMs compound, raloxifene reduced depressive-like behavior induced by stress in ovariectomized mice<sup>22</sup> however, they didn't examine the effects of the drugs in the conditions without stress to compare with the results of present study. Raloxifene has also been reported to decrease depression-like behavior of ovariectomized rats in the FST<sup>23</sup> however, this finding has not been confirmed in other studies<sup>24</sup> and by the results of present study in which tamoxifen was examined. There are also other controversial effects about tamoxifen. Increased incidence of depressive symptoms have been reported after using of tamoxifen by the humans<sup>25</sup> and other studies have found no relationship between tamoxifen use and depression<sup>7,26</sup>.

Interestingly, the effects of both SERMs and estradiol on depressive and anxiety-like behaviors have been attributed to their actions on serotonergic system. Estradiol affects the availability and actions of serotonin by regulating the degradation of monoamine oxidase enzyme<sup>16</sup> and the expression of serotonin transporters, 5HT<sub>2A</sub> receptors and tryptophan hydroxylase enzyme<sup>5,27</sup>. Raloxifene and estradiol enhance the central serotonergic tone<sup>5,27</sup>, tamoxifen does not<sup>28</sup> and even may block some of the mechanisms involved in the regulation of serotonin uptake by estradiol<sup>29</sup>.

Recent studies suggesting a positive effect of raloxifene in the reduction of anxiety and in postmenopausal women<sup>30,31</sup> which have also been confirmed in ovariectomized rats using commercial behavioral tests<sup>24</sup>. It is suggested that raloxifene regulates adrenal function, decreasing cortisol levels in postmenopausal women and increasing the levels of the anxiolytic neuroactive steroid allopregnanolone in both ovariectomized rats and postmenopausal women<sup>32</sup>. This may also contribute to the anxiolytic effects of tamoxifen in our model which was seen in OVX rats however, the plasma cortisol level was not measured and it needs to be investigated in futures. In the present study administration of tamoxifen increased the number of entries into and the time spent in open arm in OVX rats in EPM test and had an anxiolytic effect while, it reduced the number of entries to the open arm in sham operated rats showing that it antagonizes the effects of estradiol and has an anxiogenic effects. It seems the effects of tamoxifen are different in the presence and absence of ovarian hormones.

In conclusion, the results of the present study showed that deletion of ovarian hormones induced depression and anxiety like behaviors in rats. Administration of tamoxifen in sham operated rats lead to induced depression and anxiety like behaviors which were comparable to the effects of ovarian deletion. It is suggested that tamoxifen antagonizes the effects of ovarian hormones. Tamoxifen administration reduced anxiety like behavior in OVX rats. It seems that the effects of tamoxifen are different in the presence and absence of ovarian hormones.

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