Estrogen-dependent effect of soy extract on pentylenetetrazole-induced seizures in rats

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OBJECTIVE: To study the different effects of soy extract on pentylenetetrazole (PTZ)-induced seizures in the presence and absence of ovarian hormones in rats, and the gender-dependent differences in the effects of phytoestrogens on behavior.

METHODS: Male and female Wistar rats were randomly divided into nine groups with eight in each, namely, male-saline (M-saline), male-low-dose soy (M-LDS), male-high-dose soy (M-HDS), sham-saline (Sh-saline), sham-low-dose soy (Sh-LDS), sham-high-dose soy (Sh-HDS), ovariectomized-saline (OVX-saline), ovariectomized-low-dose soy (OVX-LDS) and ovariectomized-high-dose soy (OVX-HDS). The rats of groups 7 to 9 were ovariectomized under ketamine anesthesia. The rats of groups 2, 5, and 8 were treated by 20 mg/kg of soy extract while the animals of groups 3, 6 and 9 received 60 mg/kg of soy extract for two weeks. In groups 1, 4 and 7, saline was injected instead of soy extract. The animals were then injected by a single dose of PTZ (90 mg/kg body weight, intraperitoneally) and placed in a plexiglas cage and the latency to minimal clonic seizure (MCS) and generalized tonic-clonic seizure (GTCS) was recorded.

RESULTS: Both MCS and GTCS latency in M-LDS and M-HDS groups was significantly lower than that in M-saline group (P<0.05 or P<0.01). Treatment for female sham rats by soy extract did not affect MCS and GTCS latency. The animals of OVX-LDS and OVX-HDS groups had lower MCS and GTCS latency in comparison with OVX-saline group (P<0.05 or P<0.01).

CONCLUSION: It is concluded that the phytoestrogens of soy affect seizure severity induced by PTZ, but their effects are different in the presence or absence of ovarian hormones. Further studies are necessary to be done.

KEYWORDS: soybeans; seizures; ovariectomy; rat

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Recently, hormone replacement therapy (HRT) is commonly used in order to attenuate the menopausal symptoms. But accumulating evidence indicates that the benefits of this method are accompanied with an increased risk of cardiovascular diseases, and uterine and breast cancers especially when it is used for a long-term period\(^1\). Thus, it seems that soy isoflavones which contain omega-3 and omega-6 fatty acids, glycine, daidzein and genisteinand glucoconjugates, are suitable alternatives for HRT\(^1\). These molecules are similar to estrogen and therefore, they interact with estrogen receptors, mainly the estrogen receptor \(\beta\)\(^1\). Phytoestrogens can act as estrogen agonists or antagonists\(^4,5\). Many clinical studies suggest that soy diet ameliorates menopausal problems, prevents prostate cancer, has neuroprotective effects, promotes bone health and improves cognitive function\(^6,7\). It has been reported that soy isoflavones modulate the effects of neurotransmitters such as acetylcholine, dopamine, gamma-amino butyric acid (GABA) and glycine. They have also an inhibitory effect on tyrosine kinase, topoisomerase and angiogenesis\(^8\). It has been suggested that phytoestrogens could reverse the increased Na\(^+\)-K\(^+\)-ATPase activity in rat striatum\(^9\). The results of our previous study showed that treatment of ovariectomized (OVX) rats by soy extract fastened the onset of minimal clonic seizure (MCS) and generalized tonic-clonic seizure (GTCS) in pentyleneetrazole (PTZ)-induced seizure model\(^10\). There is evidence that the effect of phytoestrogens on behavior is probably gender-dependent\(^11,12\). It has also been shown that the soy isoflavone has a gender-specific effect on food intake in rats\(^13\). Regarding this fact and the agonistic and antagonistic effects of phytoestrogens, the purpose of this study was to examine the different influences of soy extract on PTZ-induced seizures under various estrogen conditions using female (standard estrogen condition), male (naturally low estrogen condition), and OVX (artificially induced estrogen deficiency condition) rats.

1 Materials and methods

1.1 Animals and grouping

This experimental research was done in Mashhad University of Medical Sciences, Iran, according to ethics committee guidelines and all the protocols of animal experiments have been approved by the Institution’s Animal Care Committee.

In this study, 72 virgin female and male Wistar rats, \((200\pm20)\) g in weight and two months old, were used. The animals were maintained at the animal house under controlled conditions including a 12-hour light and dark cycle, 22 to 24 °C temperature and 50% relative humidity with laboratory chow and water provided \textit{ad libitum}.

Rats were randomly divided into 9 groups with 8 in each as follows: male-saline (M-saline), male-low-dose soy (M-LDS), male-high-dose soy (M-HDS), sham-saline (Sh-saline), sham-low-dose soy (Sh-LDS), sham-high-dose soy (Sh-HDS), OVX-saline ovarietomized, OVX-low-dose soy (OVX-LDS) and OVX-high-dose soy (OVX-HDS). The animals of groups 7 to 9 were OVX under ketamine (Alfasan Company Holand) \((150\ \text{mg/kg, intraperitoneally})\)\(^1\) and anesthesia while the surgery was carried out in groups 1 to 6 except removing the ovaries. At least 30 d before the experiment applied, all the rats were placed in the laboratory as adjustment and attenuation of endogenous sex hormones. Then the animals were treated respectively with saline and 20 and 60 mg/kg of soy extract for two weeks, and then administered a single dose of 90 mg/kg of PTZ (St. Louis, USA) intraperitoneally. All rats were observed for 60 min to detect behavioral seizures, examined in a Plexiglas arena\(^1\).

1.2 Plant extracts

Soy was from Gorgan City, Golestan Province, Iran and scientifically identified by the Department of Botany of Ferdowsi University of Mashhad, Iran and voucher specimen of the soybean was deposited. To prepare hydroalcoholic extract, 50 g of the crumbled, dried plant was extracted with 300 mL ethanol-water \((70/30, \text{ volume ratio})\). Then the solution was transferred to a Soxhlet apparatus, using a rotary vacuum evaporator in order to reduce extracts to dryness\(^1\).

1.3 PTZ-induced seizures

In order to observe epileptic behavior, 90 mg/kg of PTZ was injected...
intraperitoneally and the rats were placed in plexiglas arena (30 cm × 30 cm × 30 cm) on the day of the experiment. The rats were observed during the first 60 min after PTZ administration. Behavioral responses of the rats to PTZ administration were evaluated using the criteria including latency to first MCS, incidence of MCS, latency to the first GTCS, incidence of GTCS, protection percentage against GTCS and protection percentage against mortality[16,10].

1.4 Statistical analysis Instat software was used for statistical analysis. Data were expressed as mean ± standard error of mean and analyzed by using one-way analysis of variance followed by Tukey’s post-hoc comparison test. P values less than 0.05 were considered to be statistically significant.

2 Results

2.1 Comparison of MCS and GTCS latency among male, female, and OVX rats All groups showed MCS and GTCS following 90 mg/kg of PTZ. MCS latency in both Sh-saline and OVX-saline groups was shorter than that in M-saline group (P < 0.01). There was no significant difference between Sh-saline group and OVX-saline group in MCS latency. The animals of both Sh-saline and OVX-saline groups had shorter GTCS latency in comparison with that of M-saline group (P < 0.01 and P < 0.05, respectively). GTCS latency in OVX-saline group was longer than that in Sh-saline group (P < 0.05). See Table 1.

Table 1 Latency to MCS and GTCS onsets in male-saline, sham-saline and OVX-saline groups (Mean ± standard error of mean, s)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>MCS latency</th>
<th>GTCS latency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male-saline</td>
<td>8</td>
<td>62.20 ± 3.26</td>
<td>102.36 ± 11.14</td>
</tr>
<tr>
<td>Sham-saline</td>
<td>8</td>
<td>45.05 ± 2.00</td>
<td>68.73 ± 1.81</td>
</tr>
<tr>
<td>OVX-saline</td>
<td>8</td>
<td>45.30 ± 1.86</td>
<td>85.20 ± 3.21</td>
</tr>
</tbody>
</table>

* P < 0.05, ** P < 0.01, vs male-saline group; † P < 0.05, vs sham-saline group. MCS: minimal clonic seizure; GTCS: generalized clonic seizure; OVX: ovariectomized.

2.2 Effects of soy extract on MSC and GTCS latency in male rats MCS latency of male rats treated by 20 and 60 mg/kg soy extract (M-LDS and M-HDS groups) was significantly shorter than that of saline-treated male rats (P < 0.01). MCS latency in M-HDS group was shorter than that of M-LDS group (P < 0.05). GTCS latency in M-LDS and M-HDS groups was also shorter than that in M-saline group (P < 0.05 and P < 0.01, respectively). However, there was no significant difference in GTCS (latency) between the effects of two doses. See Table 2.

Table 2 Latency to MCS and GTCS onsets in male-saline, male-LDS and male-HDS groups (Mean ± standard error of mean, s)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>MCS latency</th>
<th>GTCS latency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male-saline</td>
<td>8</td>
<td>62.20 ± 3.26</td>
<td>102.36 ± 11.14</td>
</tr>
<tr>
<td>Male-LDS</td>
<td>8</td>
<td>44.60 ± 1.88</td>
<td>81.40 ± 5.22</td>
</tr>
<tr>
<td>Male-HDS</td>
<td>8</td>
<td>34.20 ± 1.81*</td>
<td>72.00 ± 2.22*</td>
</tr>
</tbody>
</table>

* P < 0.05, ** P < 0.01, vs male-saline group; † P < 0.05, vs male-LDS group. MCS: minimal clonic seizure; GTCS: generalized tonic-clonic seizure; LDS: low dose of soy; HDS: high dose of soy.

OVX rat treated by 20 and 60 mg/kg soy extract (OVX- LDS and OVX-HDS groups) had significantly shorter MCS latency in comparison with OVX rats treated by saline (both P < 0.01). MCS latency in animals of OVX-HDS group was significantly shorter than that of OVX-LDS group (P < 0.01). GTCS latency in OVX-LDS and OVX-HDS groups was shorter than that in OVX-saline group (P < 0.05 and P < 0.01, respectively). See Table 3.

Table 3 Latency to MCS and GTCS onsets in OVX-saline, OVX-LDS and OVX-HDS groups (Mean ± standard error of mean, s)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>MCS latency</th>
<th>GTCS latency</th>
</tr>
</thead>
<tbody>
<tr>
<td>OVX-saline</td>
<td>8</td>
<td>45.30 ± 1.86</td>
<td>85.20 ± 3.21</td>
</tr>
<tr>
<td>OVX-LDS</td>
<td>8</td>
<td>34.90 ± 0.70</td>
<td>69.90 ± 4.36</td>
</tr>
<tr>
<td>OVX-HDS</td>
<td>8</td>
<td>24.30 ± 1.81</td>
<td>67.80 ± 3.05</td>
</tr>
</tbody>
</table>

[16] P < 0.05, [17] P < 0.01, vs OVX-saline group; [18] P < 0.05, vs OVX-LDS group. MCS: minimal clonic seizure; GTCS: generalized tonic-clonic seizure; OVX: ovariectomized; LDS: low dose of soy; HDS: high dose of soy.

3 Discussion

Both male and female gonadal hormones affect the organization, development and function of nervous system and play an important role in some neurological disorders including epilepsy[16]. The influence of the sex hormones on seizure threshold and frequency as well as the sex dependent differences in the severity of seizures have been well documented[19]. The results of the present study showed that seizure severity in male rats was lower than that in female ones; both MCS and GTCS latency in male rats was longer than that in females. The results are in agreement with the results of Finn and Geo[20] who showed that male rats had higher threshold in chemical-induced seizures in comparison with females. In contrast, it has been shown that cocaine-treated male rats were more susceptible to PTZ-induced seizures compared to females[21]. Other studies have also shown that the males were more susceptible to the convulsive effects of kainic acid and pilocarpine[22]. Conversely, in the model of electroconvulsive shock, females exhibited a lower threshold to seizures[23]. No difference between male and female rodents in seizure thresholds in bicuculline-induced seizure model has also been reported[24].

Ovariectomy has been known as the most general animal model of postmenopausal conditions which
seems to affect the seizure patterns in female rats[21]. In this study, ovariectomy resulted in a significant decrease in PTZ-induced seizure susceptibility. The results of present study confirmed the results of our previous study[10,15]. It has been shown that estrogen increases neuronal excitability and may have pro-convulsant effects[20]. However, no effect[20] or even attenuating effect[27] of estrogen on the severity of seizures has been reported. These findings confirm that seizure is easily affected by gonadal hormones, although the exact mechanism has not been clear. In this regard, there are reports that sex hormones could influence brain through adjusting several neurotransmitters and their receptors such as acetylcholine, dopamine, GABA, N-methyl-D-aspartate, opioid receptors, and directly and/or indirectly modulation of adenosine receptors[20]. It has been shown that estradiol in physiological doses affects GABA receptors and glycine receptors[20] as well as GABA synthesis and release[10]. Modulation of analgesic effects of morphine by gonadal hormones may confirm this hypothesis[20].

Because of the structural similarity between soy isoflavones and estrogen[5,9] it seems that they could be a good substitute for the synthetic estrogens especially to treat menopausal problems without exerting the hormone therapy side effects. Thus, soy isoflavones are able to mimic the functions of estrogen. The neuroprotective effects of omega-3 and -6 fatty acids, daidzein and genistein and their glucoconjugates which are the main isoflavone compounds have been attributed to their protective effects against oxidative stress OVX female rats[64]. Memory improving effects of soy or its constituents have also been reported[9]. The other effects of phytoestrogens include their effects on neuronal survival and growth, synaptic plasticity[22] and apoptosis of cortical cells[13]. Attenuation of infarct size in middle cerebral artery occlusion model[41], prevention of prostate cancer and promotion of bone health have also been reported. It has been also reported that estrogen and soy isoflavones have the same effects on neurotrophic factors and acetylcholine transferase mRNA levels in the frontal cortex of OVX rats[54]. The interaction of estradiol and soy with the brain-derived neurotrophic factor[53] has also been reported. The results of present study confirmed the results of previous study that soy phytoestrogens can also mimic the estrogen’s pro-convulsant effects[10]. It might be suggested that they affect this proconvulsant processes mediated by estrogen receptors[9]. Several studies indicate that Na<sup>+</sup>-K<sup>+</sup>-ATPase activity changes in cerebral ischemia[1] and in epilepsy[11]. It has been reported that ovariectomy enhanced the Na<sup>+</sup>-K<sup>+</sup>-ATPase activity in rat striatum[11]. Moreover, estrogens and estrogen-like molecule activities modulate the functions of Na<sup>+</sup>-K<sup>+</sup>-ATPase[21]. Therefore our findings may at least in part be related to this mechanism.

The results of present study showed that both 20 and 60 mg/kg doses of soy extract shortened MCS and GTCS latency in male and OVX female rats, however, it was not effective in sham-operated female ones. It has been previously shown that the soy isoflavones act as estrogenic agents in estrogen-deficient conditions, whereas in estrogen sufficient situations their function is as antiestrogenic agents or even they show no effect at all[11]. The results of the present study also confirmed this hypothesis and showed that the effects of soy isoflavone are different under various estrogen conditions using female (standard estrogen condition), male (naturally low estrogen condition), and OVX (artificially induced estrogen deficiency condition) rats. It is widely recognized that the estrogenic effects of isoflavones are weaker than those of endogenous estrogens[2]. The greater sensitivity of estrogen receptors or increasing in their expression in low estrogen conditions[53] may be another explanation for different effects of soy extract which was seen in the present study. Like the results of the present study, it has been previously reported that the effect of soy on food intake is different in male, female and OVX rats[31]. However, the mechanism of the differences which was observed for the effects of soy extract cannot be concluded from the results of present study; our results maybe an explanation that the soy phytoestrogens may act on estrogen receptors in the nervous system. The gender-dependent difference in the effect of isoflavones on food intake has been attributed to the differences in the metabolism of these compounds[30]. Sex differences in the biotransformation and excretion of soy isoflavones have also been suggested[42]. It is suggested that these mechanisms may have a role in the results of present study.

In conclusion, the results of present study show that proconvulsant effect of phytoestrogens is different in male and female and also in the presence and absence of ovarian hormones, and further investigations are necessary to elucidate the mechanism involved in the interaction of gonadal hormones with soy isoflavones.

4 Acknowledgements

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5 Competing interests

The authors declare that they have no competing interests.

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黄豆提取物的雌激素依赖作用对戊四唑诱发的大鼠癫痫的影响

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3. Department of Anatomy, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

目的: 研究黄豆提取物对戊四唑(pentylentetrazole, PTZ)诱发癫痫发作的女性荷尔蒙缺乏雌性大鼠与正常雄性大鼠的不同作用，以及性别差异引起的植物雌性激素对行为的影响。

方法: 雌性 Wistar 大鼠被随机分为雌性生理盐水组，雌性低、中、高剂量黄豆提取物治疗组，每组 8 只；雄性 Wistar 大鼠随机分为假手术生理盐水组，假手术低、中、高剂量黄豆提取物治疗组，去卵巢生理盐水组，去卵巢低、中、高剂量黄豆提取物治疗组，每组 8 只。去卵巢大鼠在氯胺酮麻醉下行卵巢切开术。分别给予各组大鼠生理盐水及不同剂量黄豆提取物治疗 2 周后腹腔内注射戊四唑。将大鼠放置在树枝玻璃笼内，记录最小阵挛性癫痫发作(minial clonic seizure, MCS) 潜伏期和强直性阵挛性癫痫发作 (generalized tonic-clonic seizure, GTCS) 潜伏期。

结果: 与雌性生理盐水组大鼠比较，雌性低、中剂量黄豆提取物治疗组的 MSC 和 GTCS 潜伏期显著缩短 (P<0.05 或 P<0.01)。雌性假手术大鼠在给予黄豆提取物治疗后，其 MSC 和 GTCS 潜伏期没有显著改变。与去卵巢生理盐水组比较，去卵巢低、中剂量黄豆提取物治疗组的 MSC 和 GTCS 潜伏期明显缩短 (P<0.05 或 P<0.01)。

结论: 黄豆的植物雌激素能影响由 PTZ 诱发的癫痫发作的轻重程度，但其影响程度与卵巢激素水平有关。

关键词: 黄豆；癫痫发作；卵巢切除术；大鼠