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Research Paper

Anxiolytic Effect of *Anethum Graveolens* Seed Extract Combined With the Antagonist of Estrogen Receptor in Female Rats With Anxiety

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ABSTRACT

Background: Anxiety as an important psychological disorder that has affected many people and has made them to spend a lot of money for its treatment.

Objectives: This study aims to assess the anxiolytic effect of the hydro-alcoholic extract of *Anethum Graveolens* L. seed (AGS) combined with the antagonist of estrogen receptor in female rats with anxiety.

Materials & Methods: In this study, 66 female Wistar rats were divided into eight groups as follow as: Negative control (10 ml/kg saline), positive control (0.6 mg/kg diazepam), five groups of AGS extract (0.1, 1, 10, 100 and 1000 mg/kg) and tamoxifen+AGS extract (10 mg/kg). Anxiety indicators were measured by the elevated plus maze (EPM) test.

Results: The percentage of time spent open arms (%OAT) and percentage open arms entry (%OAE) significantly increased after using 10 mg/Kg dose of AGS extract compared to control group. Tamoxifen could significantly reduce this increase caused by AGS extract on anxiety indices.

Conclusion: Considering the anxiolytic effect of the hydro-alcoholic extract of AGS and the reduction of this effect by the antagonist of estrogen receptor (tamoxifen), it can be concluded that the extract's anxiolytic effect may be due to interaction with estrogen receptor.

Keywords: Anxiety, *Anethum graveolens*, Estrogen receptor antagonists

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Caspian Journal of Neurological Sciences

Highlights

- The hydro-alcoholic extract of *Anethum Graveolens* L. seed had anxiolytic effects.
- The hydro-alcoholic extract of *A. Graveolens* L. seed can exert its anxiolytic effect through estrogen receptor.
- The hydro-alcoholic extract of *A. Graveolens* L. seed can be used as complementary treatment for anxiety.

Introduction

Anxiety is one of the most prevalent psychological disorders. Physiologically, anxiety is defined as complex behavioral changes in response to psychological or environmental stressors [1, 2]. The prevalence of anxiety is about 10-30% worldwide [3]. Sex hormones are also involved in the etiology of anxiety [1, 2, 4]. Changes in the level of endogenous steroid hormones, such as estrogen, can be the main cause of anxiety in females [5]. Since estrogen receptors are expressed in neurons and are involved in excitatory induction of these cells, their activation can result in reduced anxiety [6]. Moreover, steroid metabolites, including progesterone and corticosterone, have sedative, antidepressant, and anxiolytic effects [7]. Phytoestrogens are non-steroidal polyphenolic compounds that can be found in some plants such as soya, clover, fruits and vegetables [8]. Structure of hormones in these plants is similar to estrogen hormones expressed in human. Some studies have revealed the effects of the compounds of these plants on anxiety, learning, and oxidative activity [9]. The plant *Anethum graveolens* L. (dill) grows widely in West Asia and Southeast Europe. Various species of drill comprise different types of steroids and phytoestrogen with tranquilizing and anti-inflammatory properties [10]. Although, flavonoids, phenolic compounds and essential oil are the major components of dill, this plant also contain kaempferol, trans-anethole and limonene with phytoestrogenic properties [11]. In this study, we aim to assess the possible anxiolytic effect of the hydro-alcoholic extract of *A. graveolens* L. seed (AGSE) and its interaction with estrogen receptors in female rats.

Materials and Methods

Preparation of chemicals and hydro-alcoholic extract

Tamoxifen was granted by Iran Hormone Company (Tehran, Iran) and diazepam was purchased from Caspian Company (Rasht, Iran). The solvent of both materials was saline. The plant seeds were bought from herb stores in Rasht, and then cleaned, dried and powdered at room temperature. Ethanol (80%) was added to the powdered seeds (100 g) and kept for 24 h at dark. The filtered extract was concentrated by rotary evaporator under reduced pressure at 40°C. The obtained AGSE was 6.4% (g/g).

Animals

In this experimental study, 64 female Wistar rats (weighing 20±180 g) were prepared from the Animal House of the Department of Physiology, Guilan University of Medical Sciences. They were kept at room temperature (2±22°C) and a 12:12h light-dark cycle. They had free access to food except during the test periods. All animals were in their estrous cycle and were divided into eight groups of 8 including: Negative control (10 ml/kg saline), positive control (0.6 mg/kg diazepam), five groups of AGSE (0.1, 1, 10, 100 and 1000 mg/kg), and AGSE (10 mg/kg)+tamoxifen (15 mg/kg), as the antagonist of estrogen receptor. The sexual features, secretions of vagina and its cellular content were measured and estrus cycle was determined and tested by light microscope. Experiments were performed 30 minutes after intraperitoneal injection of 10 mL/kg of saline and drugs.

Behavioral evaluation

The anxiety-like behaviour of rats was measured by the elevated plus maze (EPM) test (Borj Sanat Co., Tehran, Iran) according to a previous study [12].

Statistical analysis

The data were presented as Mean±SEM and analyzed in SPSS software, version 16 using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. $P<0.05$ was considered statistically significant.

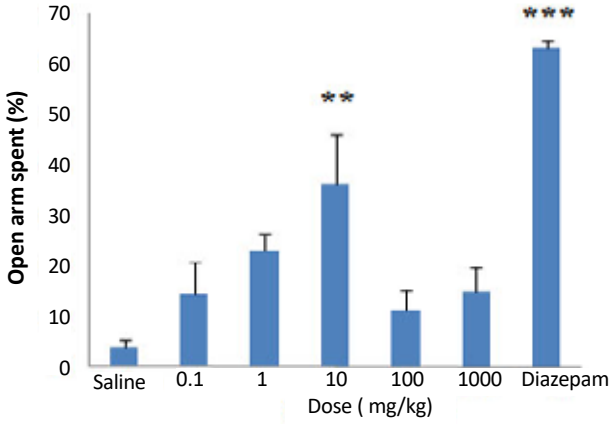

Figure 1. Effect of AGSE on the %OAT

Results

Effect of AGSE on the percentage of time spent on open arms

The different doses of AGSE increased the percentage of time spent on open arms (%OAT), but the significance effect was observed only for the dose of 10 mg/kg compared to saline group (F=13.024; P<0.01; Figure 1).

Effect of AGSE on the percentage of open arm entry

After using different doses of AGSE, significant increase in the percentage of open arm entry (%OAE) was reported for 10 mg/kg of AGSE compared to control group (F=11.118, P<0.01; Figure 2).

Effect of AGSE on the locomotor activity

The locomotor activity (LOC) is consisted of closed arms entry (CAE)+OAE. The doses of 0.1, 1, and 10 mg/kg of AGSE significantly increased the LOC compared to saline group (F=9.918, P<0.001; Figurer 3). The reason for using a dose of 10 mg/kg of the extract in the research was because of its significant effect on anxiety indicators.

Comparing the effects of AGSE and AGSE+ tamoxifen on anxiety indicators

The results of comparison showed that %OAT (F=5.081, P<0.05), %OAE (F=0.65, P<0.01), and %LOC (F=1.842, P<0.01) decreased significantly in the AGSE+tamoxifen group compared to the AGSE group as shown in Figures 4, 5 and 6.

Discussion

In recent decades, the world has witnessed an increase in occurrence of anxiety mainly due to improving technology and life style changes [3]. Anxiety affects various aspects of human life and causes various neurodegenerative dis-

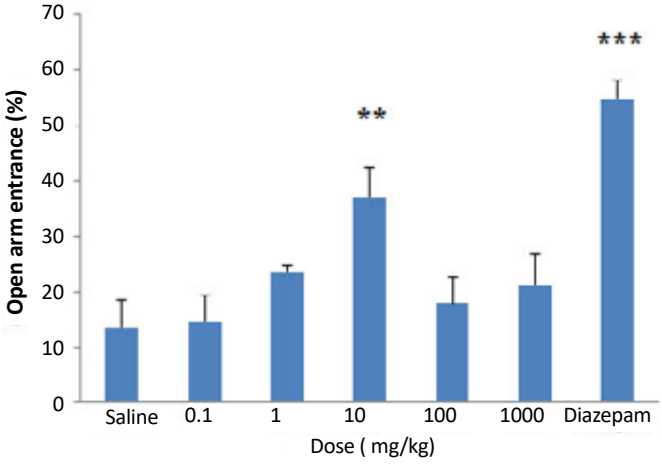

Figure 2. Effect of AGSE on the %OAE

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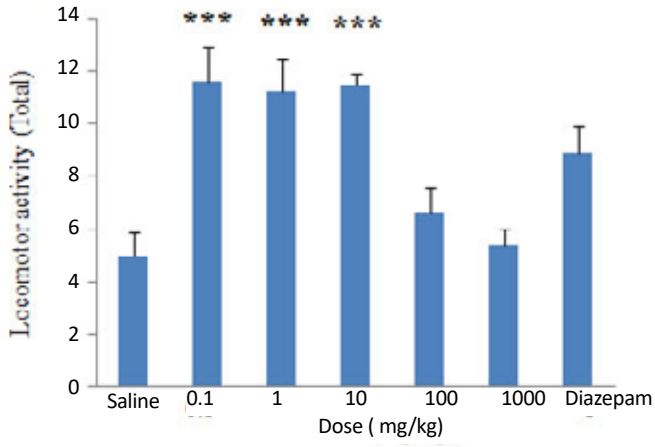

Figure 3. Effect of AGSE on the LOC

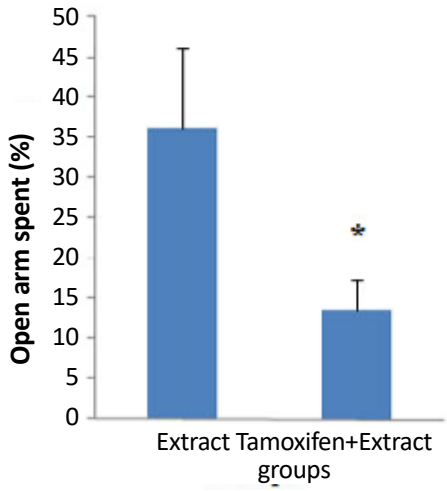

Figure 4. Comparing the effects of AGSE and AGSE+tamoxifen on the OAT

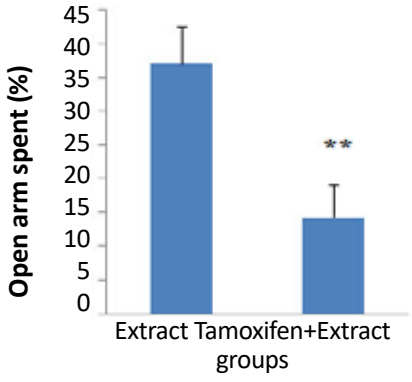

Figure 5. Comparing the effects of AGSE and AGSE+ tamoxifen on OAE

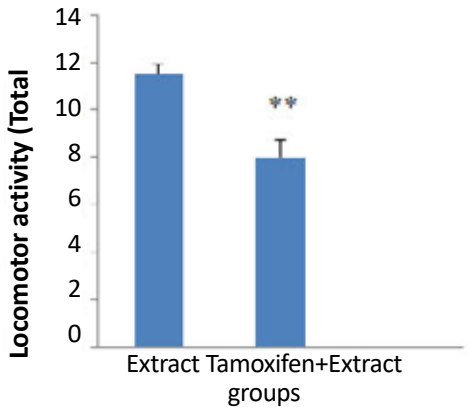

Figure 6. Comparing the effects of AGSE and AGSE+tamoxifen on the LOC

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eases [13]. Although pharmaceutical methods are preferred to treat anxiety due to their appropriate outcome, the drugs have many side effects. Therefore, due to the identification of the biological and therapeutic effects of plants and recent advances in cellular and molecular techniques, the use of traditional methods using herbal medicines has increased for treating anxiety [9]. Studies have shown that different kinds of plants especially *A. graveolens* L., contain non-steroidal estrogens named phytoestrogens which are agonist of steroid hormones [8]. A previous study reported that this plant reduced pain and improved constipation [14]; but its effect on reducing anxiety remains unclear. In this regard, we evaluated the effect of the hydroalcoholic extract of *A. graveolens* L. seeds on anxiety indicators using the EPM test. In this study, different doses of the extract did not cause any problems in the animals. Studies have shown that up to a dose of 2000 mg/kg of dill extract do not cause the death of animals [15]. In our study, the best dose of the extract to reduce anxiety was 10 mg/kg. This is consistent with results of Mansouri et al. who showed that the antidepressant and analgesic properties of lower dose of AGSE is more than the higher dose [16].

To investigate the effect of estrogen receptors, the rats were treated with the antagonist of these receptor (tamoxifen) along with AGSE. Their combined use led to significant decreases in %OAT and %OAE in comparison with the use of AGSE alone. Tamoxifen is a selective partial agonist of estrogen receptor α and a pure antagonist of estrogen receptor β [17]. In a study, the use of the selective agonist of estrogen receptor β reduced both anxiety-like behaviors and grooming time in the EPM test [4]. In addition, a study showed that animal treatment with AGSE increased serum estradiol due to the profound amount of phytoestrogens [11]. The phytoestrogens of AGSE might be applied its anxiolytic effects through activation of the estrogen receptor β.

Considering the existence of studies on the role of anxiolytic agents in increasing locomotor activity in the open arms [18], in this study, we used the EPM test to measure anxiety-like behaviors of rats. We found that the AGSE increased locomotor activity. It is possible that flavonoids present in AGSE is responsible for induction of Fos and Jun signaling pathways [19]. In addition, Monoterpenes in AGSE have been reported to have anti-anxiety effects [10].

Conclusion

In conclusion, the hydro-alcoholic AGSE can reduce anxiety-like behaviors probably via the estrogen receptor β. More study is needed to unveil the exact mechanism of anti-anxiety effect in AGSE.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee of Guilan University of Medical Sciences (Code: P.3.132.1104).

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Authors contributions

Conceptualization, supervision and funding: Mohammad Rostampour; Methodology and data collection: Rana Shahabi; Investigation and writing the original draft: All authors; Review and editing: Mohammad Rostampour and Farshid Saadat.

Conflict of interest

The authors declared no conflicts of interest.

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