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RESEARCH

Anxiolytic-like effects of orientin in mice: behavioral and neurochemical investigations

Orientinin fare modellerinde anksiyolitik benzeri etkileri: davranışsal ve nörokimyasal incelemeler

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Abstract

Purpose: Orientin, a water-soluble flavonoid C-glycoside found in various medicinal plants, exhibits diverse pharmacological properties. This study aimed to evaluate its anxiolytic-like effects in mice and to explore the potential roles of adrenergic, serotonergic, and GABAergic neurotransmitter systems in mediating these effects.

Materials and Methods: Orientin was administered intraperitoneally to mice at 20, 40, or 80 mg/kg. Anxiolytic-like effects were assessed using the light–dark box, elevated plus maze, hole-board, and open-field tests. For mechanism studies, separate groups received α2-adrenergic antagonist yohimbine (5 mg/kg), 5-HT1$_A$ antagonist WAY-100635 (1 mg/kg), or GABA$_A$ antagonist flumazenil (3 mg/kg) prior to 20 mg/kg orientin.

Results: Orientin at 20 mg/kg elicited significant anxiolytic-like effects in the hole-board, light–dark box, and open-field tests. The 40 mg/kg dose produced a significant effect only in the hole-board test, whereas the 80 mg/kg dose failed to elicit significant changes in any behavioral paradigm. The pronounced efficacy observed at 20 mg/kg suggests a bell-shaped dose–response profile. Pretreatment with α2-adrenergic, 5-HT$_{1A}$ serotonergic, or GABA$_A$ receptor antagonists partially or completely attenuated the effects of orientin, with the degree of reversal varying among the behavioral assays.

Conclusion: The present findings provide compelling evidence that orientin exerts anxiolytic-like effects, potentially mediated via α2-adrenergic, 5-HT$_{1A}$ serotonergic, and GABA$_A$ receptor pathways.

Keywords: Anxiolytic effects, flumazenil, orientin, WAY-100635, yohimbine.

Öz

Amaç: Orientin, çeşitli tıbbi bitkilerde bulunan suda çözünebilen bir flavonoid C-glikozididir ve geniş bir farmakolojik aktivite yelpazesine sahiptir. Bu çalışma, orientinin anksiyolitik benzeri etkilerini değerlendirmeyi ve bu etkilerde adrenerjik, serotonerjik ve GABAerjik sistemlerin potansiyel rolünü araştırmayı amaçladı.

Gereç ve Yöntem: Orientin, farelere intraperitoneal olarak 20, 40 veya 80 mg/kg dozlarında uygulandı. Anksiyolitik benzeri etkiler, aydınlık-karanlık kutu, yükseltilmiş artı labirent, delikli tahta ve open-field testleri ile değerlendirildi. Mekanizma çalışmalarında, ayrı gruplara 20 mg/kg orientin uygulanmadan önce α2-adrenerjik antagonist yohimbine (5 mg/kg), 5-HT1$_A$ antagonist WAY-100635 (1 mg/kg) veya GABA$_A$ antagonist flumazenil (3 mg/kg) verildi.

Bulgular: 20 mg/kg orientin, delikli tahta, aydınlık-karanlık kutu ve açık alan testleri testlerinde anlamlı anksiyolitik benzeri etkiler göstermiştir. 40 mg/kg doz, yalnızca delikli tahta testinde anlamlı bir etki üretirken, 80 mg/kg doz hiçbir davranışsal parametrede anlamlı değişiklik oluşturamamıştır. 20 mg/kg'de gözlenen belirgin etki, çan şeklinde bir doz-cevap profilini işaret etmektedir. α2-adrenerjik, 5-HT$_{1A}$ serotonerjik veya GABA$_A$ reseptör antagonistleri ile ön tedavi, orientinin etkilerini kısmen veya tamamen azaltmış, geri dönüşün derecesi davranış testleri arasında değişiklik göstermiştir.

Sonuç: Orientin, büyük ölçüde α2-adrenerjik, 5-HT$_{1A}$ serotonerjik ve GABA$_A$ reseptör yolları aracılığıyla anksiyolitik benzeri etkiler göstermektedir.

Anahtar kelimeler: Anksiyolitik etkiler, flumazenil, orientin, WAY-100635, yohimbin.

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INTRODUCTION

Anxiety is a prevalent psychiatric condition characterized by persistent, excessive worry and heightened physiological arousal. Its global prevalence remains substantial, with anxiety disorders ranking among the most commonly diagnosed psychiatric illnesses[1]. Clinically, these disorders often present with chronic and disproportionate fear, avoidance behaviors, and heightened vigilance, which may arise in the absence of actual threat or in response to specific stimuli [2]. Among all mental health conditions, they rank among the most frequently diagnosed psychiatric disorders [3].

Current pharmacological treatments include benzodiazepines and serotonin–noradrenaline reuptake inhibitors; however, their use is frequently associated with adverse effects such as sedation, cognitive impairment, motor coordination deficits, hypotension, and a high potential for dependence. Moreover, therapeutic efficacy is not consistently achieved [4, 5]. These limitations highlight the need for continued research into novel, safer, and more effective anxiolytic agents.

Medicinal plants have been utilized for centuries across diverse cultures as folk remedies, dietary supplements, and alternative therapeutic agents. They are frequently employed in the management of conditions such as depression, anxiety disorders, insomnia, pain, inflammation, and cerebrovascular insufficiency. Notably, species such as *Valeriana officinalis* (valerian), *Passiflora incarnata L.* (passionflower), and *Piper methysticum* (kava kava) have demonstrated anxiolytic efficacy in the realm of complementary and alternative medicine.

Passiflora species, in particular, are traditionally esteemed for their anxiolytic and sedative properties in regions where they are endemic [6]. Despite their longstanding use, the phytochemical composition of *P. incarnata*, its definitive anxiolytic potential, and its precise effects on the central nervous system remain to be fully elucidated[7]. Orientin, a major bioactive constituent of *P. incarnata*, has been reported to exhibit antioxidant, anti-aging, antiviral, anti-inflammatory, vasodilatory, cardioprotective, neuroprotective, and antinociceptive activities. Given this broad pharmacological profile, orientin—a water-soluble flavonoid C-glycoside—is regarded as a promising candidate for therapeutic development [8–10].

Although *P. incarnata* and its constituents have been investigated for their potential anxiolytic properties, the specific contribution of orientin to anxiety regulation remains insufficiently defined, and its underlying mechanisms are yet to be clarified. To date, no comprehensive behavioral investigation employing multiple validated animal models has been undertaken to address this gap in knowledge.

We hypothesized that orientin would exert significant anxiolytic effects, potentially through modulation of central neurotransmitter systems. Accordingly, the present study was designed to evaluate the anxiolytic potential of orientin and to elucidate its mechanisms of action using a range of well-established animal models of anxiety. The results provide novel experimental evidence supporting the therapeutic promise of plant-derived flavonoids as alternative anxiolytic agents.

MATERIALS AND METHODS

This study was conducted at Anadolu University under standardized laboratory conditions. Approval for the experimental protocol was obtained from the Anadolu University Local Ethics Committee (Decision No. 2017-13, Approval Date: 15/12/2017). All procedures were performed in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

This research forms part of the doctoral dissertation of Tuğçe Selcen Arslan. All research personnel received specialized training in laboratory animal care and handling, ensuring full compliance with ethical and scientific standards.

Chemicals

Orientin (TRC, Canada), diazepam (Diazem®, Deva, Turkey), WAY-100635 (Sigma, USA), flumazenil (Sigma, USA), yohimbine (Sigma, USA), and dimethyl sulfoxide (DMSO) (Sigma, USA) were used in this study.

Animals

Female Swiss albino mice, weighing 25–30 g, were used in all experiments. Animals were housed in well-

ventilated rooms maintained at 22 ± 1°C with a 12-hour light/dark cycle. Mice were acclimated to the laboratory environment for one week prior to experimentation. All experiments were conducted between 10:00 and 16:00 under constant temperature, noise, and lighting conditions. Standard feed pellets and tap water were provided ad libitum.

Drug administration and experimental procedure

Mice were randomly assigned to 12 experimental groups (n = 8 per group). All compounds were administered intraperitoneally (*i.p.*). Orientin was dissolved in 10% DMSO and administered at doses of 20, 40, and 80 mg/kg. The control group received an equivalent volume of 10% DMSO, while diazepam (1 mg/kg, *i.p.*) served as a positive control.

Behavioral assessments—including the elevated plus maze, hole-board, open field, and activity cage tests—were conducted 30 minutes after orientin administration. Based on its consistent anxiolytic-like effects, the 20 mg/kg dose was selected for subsequent mechanistic investigations. For these studies, mice were pretreated intraperitoneally with either the α2-adrenergic receptor antagonist yohimbine (5 mg/kg), the serotonin 5-HT_{1A} receptor antagonist WAY-100635 (1 mg/kg), or the $GABA_A$ receptor antagonist flumazenil (3 mg/kg). Thirty minutes following antagonist administration, orientin (20 mg/kg) was injected. Anxiety-related behaviors were subsequently evaluated 30 minutes later using the elevated plus maze, hole-board, and open field tests in sequence [11].

All experimental procedures were conducted between 10:00 and 16:00 under standard laboratory conditions by personnel trained and certified in laboratory animal handling.

Hole-Board Test

The hole-board apparatus (Ugo Basile, No. 46653; Varese, Italy), measuring 15 cm in height and containing 16 evenly spaced holes, was employed. Mice were placed individually at the center of the board to explore the holes. Both the number of holes visited and the total number of head dips were recorded using a video camera for five minutes[12]. Manual scoring was also performed, as occasional tail entry into a hole could interfere with automated detection. After each session, the surface of the apparatus was cleaned with 70% ethanol (v/v).

Light-Dark Box Test

The light-dark box (Ugo Basile, No. 47443; Varese, Italy) consisted of anthracite-colored plexiglass (44 × 44 × 35 cm) and was divided into equal dark, enclosed and brightly lit, open compartments. The duration spent in each compartment was recorded over a five-minute period. The apparatus was sanitized with 70% ethanol (v/v) after each trial.

Open-field test

The open-field apparatus (Ugo Basile, No. 47432; Varese, Italy) was constructed from anthracite-colored plexiglass (44 × 44 × 30 cm) with the floor divided into 16 equal squares. Mice were placed individually at the center of the arena. The parameters recorded included the number of squares crossed in the center (the four central squares), time spent in the center, number of squares crossed in the periphery, and time spent in the periphery[12]. The apparatus was cleaned with 70% ethanol (v/v) prior to testing each animal.

Elevated Plus-Maze Test

The elevated plus-maze (Ugo Basile, No. 40143; Varese, Italy) consisted of two open arms, two closed arms, and a central platform, positioned 50 cm above the floor. During a five-minute session, the time spent in open and closed arms, the number of entries into each arm, and the percentage of time spent in open arms were recorded[13]. The percentage of time in open arms was calculated as: ([time in open arms / total time in arms] × 100). The apparatus surface was disinfected with 70% ethanol (v/v) after each trial.

Spontaneous Locomotor Activity Test

A plexiglass activity cage (Ugo Basile, No. 47420; Varese, Italy) was used to assess locomotor activity. The apparatus emits infrared beams along opposite vertical edges, and the animal's movements interrupt these beams, which are recorded by photosensors. Data were logged at predetermined intervals[14]. Thirty minutes after administration, mice treated with 10% DMSO, orientin (20, 40, or 80 mg/kg), or diazepam (1 mg/kg) were individually placed in the activity cage and monitored for ten minutes.

Statistical analysis

All data are expressed as mean ± standard error of the mean (SEM). Behavioral test results (open-field, hole-board, light-dark box, elevated plus-maze, and activity cage) were analyzed using one-way ANOVA,

followed by Tukey's post hoc test for multiple comparisons. For mechanistic experiments, in which both orientin and receptor antagonists (flumazenil, WAY-100635, naloxone, yohimbine) were evaluated, two-way ANOVA was performed to assess interactions between treatment and antagonist administration. The Bonferroni post hoc test was applied for pairwise comparisons. Statistical significance was set at $P < 0.05$. All analyses were conducted using GraphPad Prism version 5.0.

RESULTS

Anxiolytic effects of orientin

Hole-Board Test

The anxiolytic-like effects of orientin at doses of 20, 40, and 80 mg/kg are shown in Fig. 1A–B. Administration of 40 mg/kg orientin significantly increased the number of head dips compared with the control group ($^{++}P < 0.01$). Similarly, 40 mg/kg significantly increased the number of holes explored ($^{++}P < 0.01$). Notably, 20 mg/kg orientin also produced significant increases in head dips ($^{++}P < 0.01$, Fig. 1A) and in the number of holes explored ($^{+++}P < 0.001$, Fig. 1B), suggesting a potential bell-shaped dose–response relationship.

Light-Dark Box Test

In this widely used model for assessing anxiolytic effects, the duration spent in the light compartment was evaluated (Fig. 1C). Mice treated with 20 mg/kg orientin spent significantly more time in the light compartment compared with controls ($P < 0.05$).

Open-Field Test

In the open-field test, both the duration spent in the center and the number of central square crossings were analyzed. Administration of 20 mg/kg orientin significantly increased the time spent in the center ($^{++}P < 0.01$, Fig. 1D) and the number of central crossings ($^{++}P < 0.01$, Fig. 1E) relative to controls.

Elevated Plus-Maze Test

Time spent in open and closed arms was recorded, and the percentage of time spent in open arms was calculated as: (time in open arms / total arm time) × 100. Although orientin produced a slight increase in the percentage of time spent in open arms compared with controls, this difference did not reach statistical significance (Fig. 1F).

Activity Cage Test

Horizontal and vertical locomotor activity, as well as sedation levels, were evaluated using the activity cage apparatus. Both horizontal and vertical movements were quantified (Fig. 2). Administration of diazepam or orientin at doses of 20, 40, and 80 mg/kg did not induce statistically significant changes in spontaneous locomotor activity compared with the control group.

Role of the GABAergic system

Evaluation of the behavioral tests indicated that orientin exerted its most pronounced effects at a dose of 20 mg/kg. Therefore, this dose was selected for mechanistic investigations. Flumazenil (3 mg/kg, i.p.) was employed to assess the contribution of the GABAergic system to orientin's anxiolytic effects. Flumazenil did not produce a significant change in the number of head dips measured in the hole-board test (Fig. 3A). However, pretreatment with the antagonist slightly attenuated the anxiolytic effect of orientin in terms of the number of holes explored (Fig. 3B, $^{*}P < 0.05$). In the open-field test, no significant changes were observed in either time spent in the center or the number of central square crossings (Fig. 3D). In the light-dark box test, only a partial reversal of orientin's anxiolytic effect was observed (Fig. 3C).

Role of the serotonergic system

The 5-HT1A receptor antagonist WAY-100635 (1 mg/kg) was used to evaluate the contribution of the serotonergic system to the anxiolytic effects of orientin. In the hole-board test, both the number of head dips ($^{**}P < 0.01$) and the number of holes explored ($^{**}P < 0.01$) were significantly reduced by WAY-100635, indicating a critical role of the serotonergic system in mediating orientin's anxiolytic-like effects (Fig. 4A–B). In the light-dark box test, pretreatment with WAY-100635 partially attenuated the anxiolytic effect of orientin, producing a statistically significant reversal ($^{*}P < 0.05$, Fig. 4C). Similarly, in the open-field test, the anxiolytic-like effect of orientin on the time spent in the center was significantly antagonized by WAY-100635 ($^{*}P < 0.05$, Fig. 4D), whereas the number of central square crossings remained unaffected (Fig. 4E).

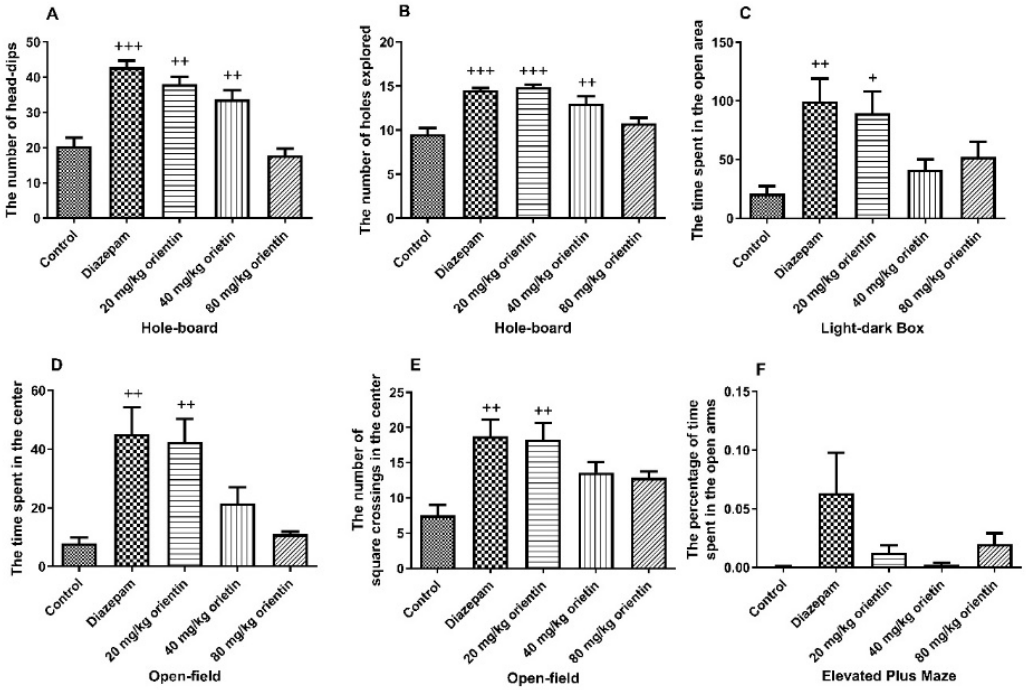

Figure 1. (A) Number of head dips and (B) number of holes explored in the hole-board test; (C) time spent in the light compartment in the light-dark box; (D) time spent in the center and (E) number of central square crossings in the open-field test; (F) percentage of time spent in the open arms in the elevated plus-maze.

Data are presented as mean ± SEM (n = 8 per group). Statistical analyses were performed using one-way ANOVA followed by Tukey's post hoc test for multiple comparisons. $^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$ indicate significant differences from the control group.

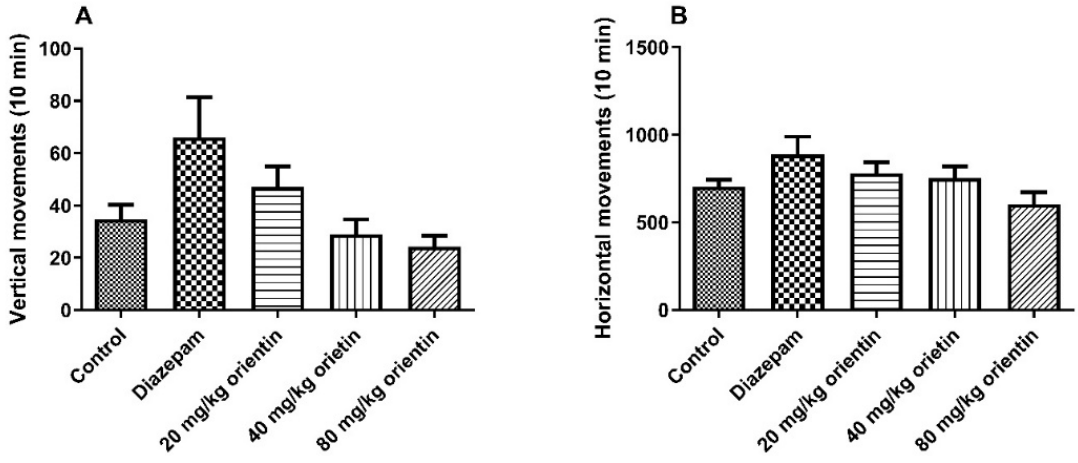

Figure 2. (A) Vertical and (B) horizontal locomotor movements in the activity cage.

Data are presented as mean ± SEM (n = 8 per group). Statistical analyses were performed using one-way ANOVA followed by Tukey's post hoc test for multiple comparisons.

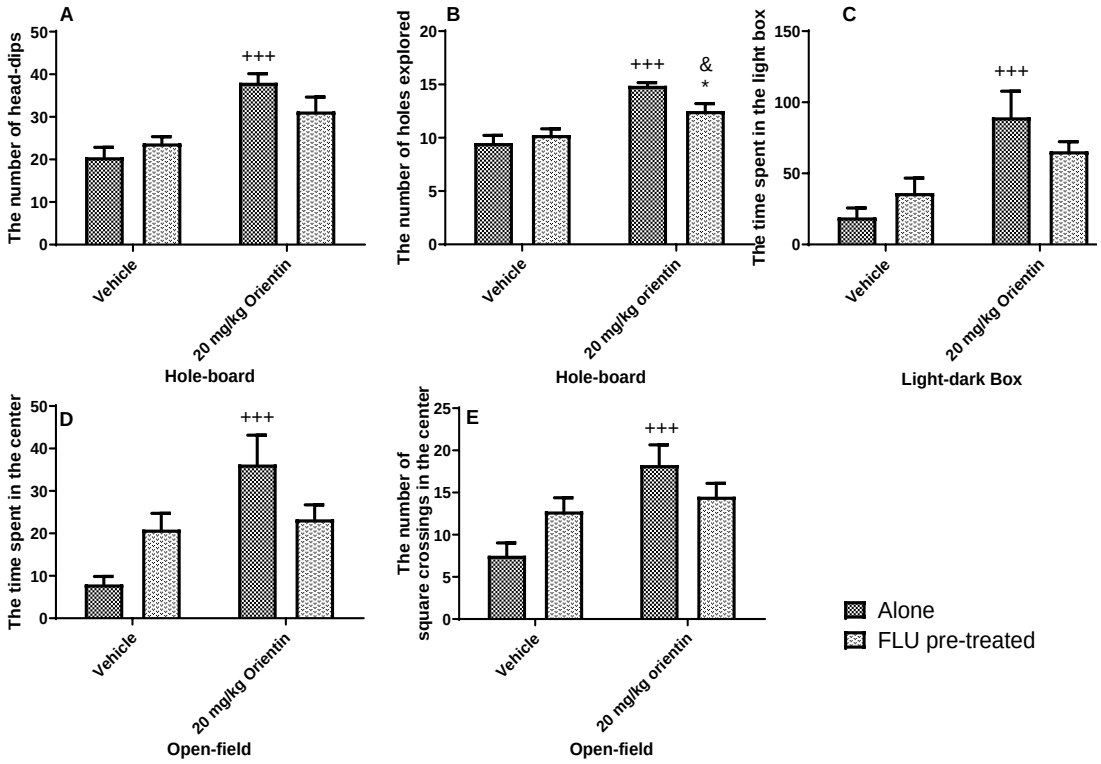

Figure 3. (A) Number of head dips and (B) number of holes discovered in the hole-board test; (C) time spent in the light compartment in the light-dark box; (D) time spent in the center and (E) number of central square crossings in the open-field test. Groups: Vehicle (10% DMSO), Vehicle + FLU (3 mg/kg flumazenil pre-treatment before 10% DMSO), Orientin (20 mg/kg), Orientin + FLU (3 mg/kg flumazenil pre-treatment before 20 mg/kg orientin).

Data are presented as mean ± SEM (n = 8 per group). Statistical analyses were performed using two-way ANOVA followed by Bonferroni's post hoc test. $^{+++}P < 0.001$, significant difference versus vehicle; $^{*}P < 0.05$, Orientin + FLU versus Orientin; $^{\&}P < 0.05$, Orientin + FLU versus FLU. FLU: flumazenil.

Role of the adrenergic system

The contribution of the adrenergic system to the anxiolytic-like effects of orientin (20 mg/kg) was evaluated using the 5 mg/kg yohimbine pretreatment in the hole-board test (Fig. 5A–B). Yohimbine significantly attenuated orientin-induced increases in the number of head dips ($^{***}P < 0.001$, Fig. 5A) and the number of holes explored ($^{***}P < 0.001$, Fig. 5B). In the light-dark box test, yohimbine significantly reversed the anxiolytic effect of orientin ($^{**}P < 0.01$, Fig. 5C). Similarly, in the open-field test, yohimbine markedly reduced the time spent in the center ($^{***}P < 0.001$, Fig. 5D) and the number of central square crossings ($^{**}P < 0.01$, Fig. 5E). A slight anxiolytic-like effect of yohimbine alone was observed compared with the vehicle control group ($^{\&}P < 0.05$, Fig. 5E), suggesting its potential to exert mild anxiolytic-like activity.

Notably, orientin produced significant anxiolytic-like effects, particularly at a dose of 20 mg/kg. In the hole-board test, anxiety-related behaviors were significantly reduced, as evidenced by increases in both head dips and the number of holes explored. In

the light-dark box test, mice administered 20 mg/kg orientin spent significantly more time in the illuminated compartment, indicating decreased anxiety. Open-field test results further demonstrated increased time spent in the center and a higher number of central square crossings, reflecting anxiolytic-like behavior. The elevated plus-maze test showed a moderate increase in the percentage of time spent in open arms, consistent with anxiolytic activity. Mechanistic investigations revealed that flumazenil, a $GABA_A$ receptor antagonist, had a modest effect, whereas the $5\text{-}HT_{1A}$ receptor antagonist WAY-100635 and the α_2-adrenergic receptor antagonist yohimbine significantly attenuated orientin's anxiolytic effects.

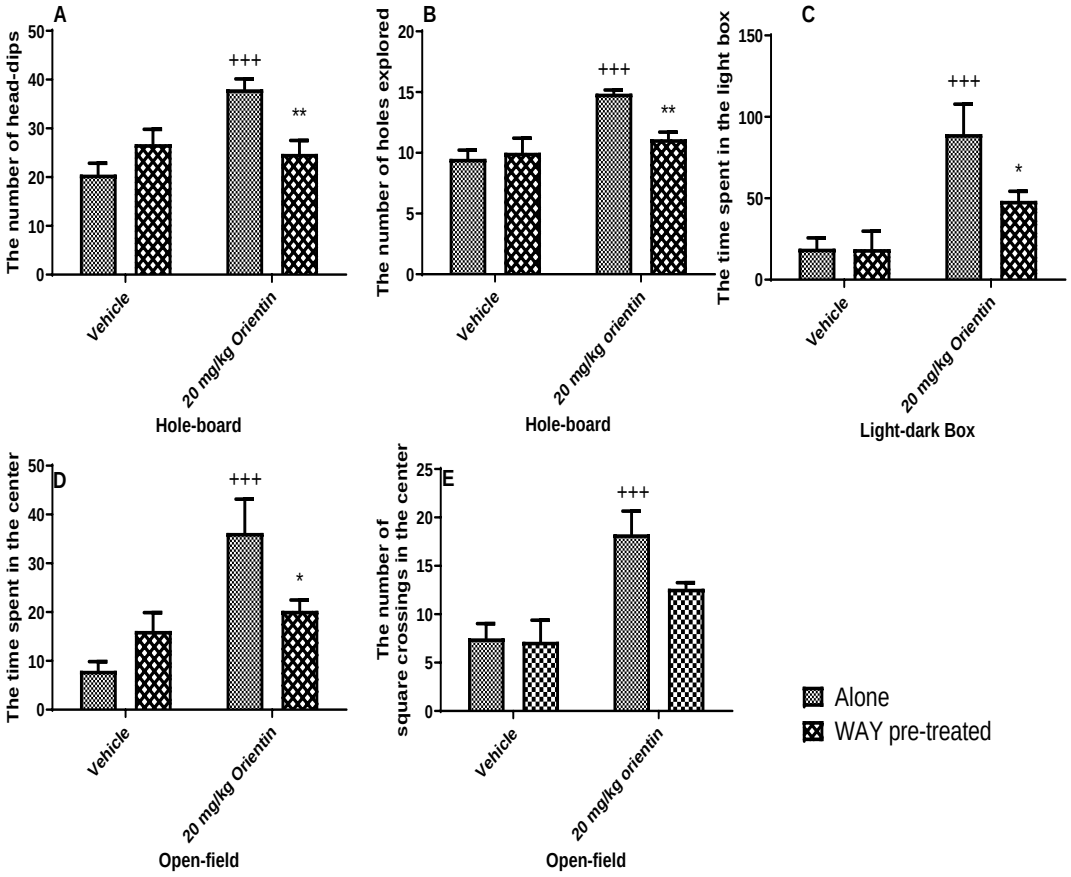

Figure 4. Involvement of the serotonergic system. (A) Number of head dips and (B) number of holes discovered in the hole-board test; (C) time spent in the light compartment in the light-dark box; (D) time spent in the center and (E) number of central square crossings in the open-field test. Groups: Vehicle (10% DMSO), Vehicle + WAY (1 mg/kg WAY-100635 pre-treatment before 10% DMSO), Orientin (20 mg/kg), Orientin + WAY (1 mg/kg WAY-100635 pre-treatment before 20 mg/kg orientin).

Data are presented as mean ± SEM (n = 8 per group). Statistical analyses were performed using two-way ANOVA followed by Bonferroni's post hoc test. $^{+++}P < 0.001$, significant difference versus vehicle; $^{*}P < 0.05$, $^{**}P < 0.01$, Orientin + WAY versus Orientin. WAY: WAY-100635.

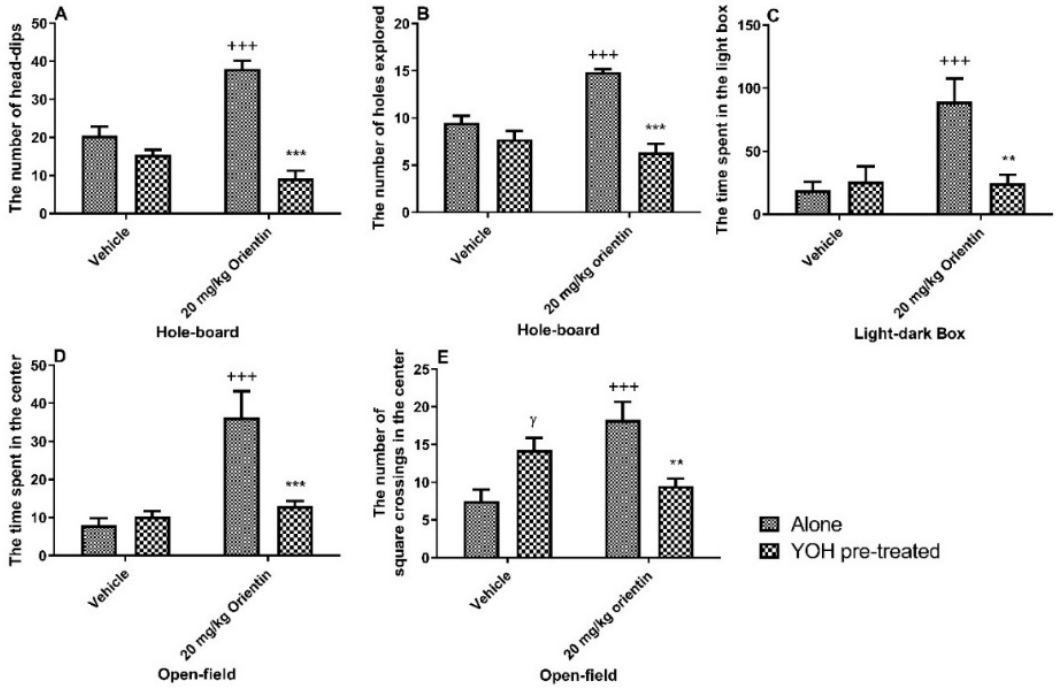

Figure 5. Involvement of the adrenergic system. (A) Number of head dips and (B) number of holes discovered in the hole-board test; (C) time spent in the light compartment in the light-dark box; (D) time spent in the center and (E) number of central square crossings in the open-field test. Groups: Vehicle (10% DMSO), Vehicle + YOH (5 mg/kg yohimbine pre-treatment before 10% DMSO), Orientin (20 mg/kg), Orientin + YOH (5 mg/kg yohimbine pre-treatment before 20 mg/kg orientin).

Data are presented as mean ± SEM (n = 8 per group). Statistical analyses were performed using two-way ANOVA followed by Bonferroni's post hoc test. $^{+++}P < 0.001$, Orientin versus vehicle; $^{**}P < 0.01$, $^{***}P < 0.001$, Orientin + YOH versus Orientin; $^{\gamma}P < 0.05$, YOH versus vehicle. YOH: yohimbine.

DISCUSSION

In this study, orientin exerted anxiolytic-like effects across most behavioral models, particularly at a dose of 20 mg/kg. These effects were not observed at 40 or 80 mg/kg when all tests were considered. Mechanistic experiments using flumazenil, yohimbine, and WAY-100635 supported these findings, indicating that orientin's anxiolytic-like effects are likely mediated primarily through serotonergic and noradrenergic modulation.

The hole-board test is widely employed to assess exploratory behavior in rodents, particularly mice. Reduced exploratory activity is generally interpreted as an indicator of increased anxiety[15]. Based on the number of holes explored, orientin at doses of 20 and 40 mg/kg produced significant anxiolytic-like effects.

The light-dark box test evaluates the natural aversion of rodents to illuminated areas, an innate anxiety-related behavior. In this model, increased time spent in the light compartment and delayed transitions to the dark compartment are indicative of anxiolytic activity [16, 17]. Administration of 20 mg/kg orientin significantly increased the time spent in the illuminated area, confirming its anxiolytic-like effect.

In the open-field test, anxious rodents typically remain in the periphery, avoiding the center. This model assesses responses to social isolation and exposure to novel, unprotected environments [18, 19]. Analysis of time spent in the center and the number

of central crossings demonstrated that orientin at 20 mg/kg produced significant anxiolytic-like effects.

Overall, the results suggest a bell-shaped dose–response relationship, with maximal anxiolytic efficacy observed at 20 mg/kg and reduced effects at higher doses. Such dose–response curves are often seen with compounds acting on multiple targets or binding sites, and with agents exhibiting both agonist and antagonist properties at different concentrations [11, 20, 21].

Results from the activity cage test indicated no significant alterations in locomotor activity, highlighting a key advantage. This finding suggests that the anxiolytic-like effects of orientin can be attributed specifically to its neurobehavioral actions rather than to confounding motor impairments.

Regarding its mechanism of action, the involvement of the GABAergic, noradrenergic, and serotonergic systems was assessed using flumazenil, yohimbine, and WAY-100635, respectively.

GABA plays a critical role in maintaining the balance between neuronal excitation and inhibition, and its dysregulation has been implicated in anxiety and depression. Flumazenil pretreatment did not significantly alter orientin's effects in the light-dark box or open-field tests. In the hole-board test, flumazenil only reversed the number of holes explored. These results suggest that orientin's effects are partially mediated via the GABAergic system, with additional pathways likely contributing [22, 23].

The 5-HT_{1A} receptor is a well-established target for the treatment of psychiatric disorders, particularly anxiety and depression [24, 25]. WAY-100635 significantly antagonized orientin's effects in the hole-board, light-dark box, and open-field tests, supporting a key role for the serotonergic system. Notably, the observed bell-shaped dose–response profile aligns with previous reports linking 5-HT_{1A} autoreceptor activity to similar pharmacological effects, further reinforcing this mechanism.

Yohimbine markedly reversed orientin's anxiolytic-like effects across multiple anxiety models, indicating a predominant role of the noradrenergic system. As a presynaptic α_2-adrenergic receptor antagonist, yohimbine increases noradrenaline release and activity in the locus coeruleus[26]. Although elevated noradrenaline generally promotes anxiety, activation of presynaptic α_2 receptors can inhibit locus coeruleus activity, thereby reducing fear-related behavior. Dysregulation of noradrenaline signaling is strongly implicated in both psychiatric and neurodegenerative disorders [27, 28].

Collectively, these findings indicate that orientin's anxiolytic-like effects are primarily mediated through serotonergic and noradrenergic systems, with a measurable contribution from the GABAergic system.

It should be noted that the elevated plus-maze may yield inconsistent results for some clinically used anxiolytics [29, 30]. As a limitation, antagonist data from this test could not be graphically presented due to statistical constraints, and experiments were not repeated.

In conclusion, orientin demonstrated promising anxiolytic-like effects. The bell-shaped dose–response profile and involvement of multiple neurotransmitter systems suggest a complex mechanism of action. Further studies are warranted to elucidate receptor-binding dynamics, evaluate long-term safety, and investigate potential synergistic effects with conventional anxiolytics. Ultimately, bridging preclinical and clinical research will determine whether orientin can be developed as a viable therapeutic option for anxiety disorders.

Author Contributions: Concept/Design : RA, NB; Data acquisition: HE, TSA; Data analysis and interpretation: RA, NB, FAA; Drafting manuscript: RA, NB, HE, FAA; Critical revision of manuscript: RA, HE; Final approval and accountability: TSS, FAA, NBT, RA; Technical or material support: TSS; Supervision: RA, NB; Securing funding (if available): n/a.
Ethical Approval: The experiments were conducted with approval from the Anadolu University Local Ethics Committee (Decision no: 2017-13, Approval Date: 15/12/2017.
Informed Consent: Informed consent was obtained from all participants.
Peer-review: Externally peer-reviewed.
Conflict of Interest: The authors have no conflicts of interest to declare.
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