Journal of Pharmaceutical Research International

33(47B): 661-676, 2021; Article no.JPRI.75412
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Putative Role of Monoaminergic Systems in Antidepressant and Anxiolytic Effects of Naringin in Mice: An Interaction Study with Receptor Antagonists

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i47B33168
Editor(s):
(1) Dr. Rafik Karaman, Al-Quds University, Palestine.
Reviewers:
(1) Nagham Mahmood Aljamali, Iraq.
(2) Enrico Pessina, Dipartimento di Saute Mentale, Itlay.
Complete Peer review History: https://www.sdiarticle4.com/review-history/75412

Original Research Article

Received 02 August 2021
Accepted 06 October 2021
Published 04 November 2021

ABSTRACT

Aim: Stress-related disorders like depression and anxiety represent one of the greatest therapeutic challenges globally. Although previous studies have revealed the antidepressant-like potentials of naringin, the neurotransmitter receptor interaction mechanisms of action have not been studied, hence, this study was carried out to evaluate the role of neurotransmitter-receptor antagonists in the antidepressant-like effects of naringin in mice.
Method: Male Swiss mice were subjected to chronic unpredictable mild stress (CUMS) apart from mice in the control group. The mice were then pretreated with different neurotransmitter antagonists; metergoline (4 mg /kg i.p.), a 5-HT_1 - and 5-HT_2 -receptor antagonist; propranolol; (0.2

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mg/kg i.p.), $\beta_{1,2}$-noradrenoceptor antagonist or haloperidol (0.2 mg/kg i.p.), D 2 -dopaminergic receptor antagonists prior to the administration of naringin or vehicle (10 mL/kg). The antidepressant-like and anxiolytic effects of naringin were evaluated 30 min later using the tail suspension test (TST), open-field test (OFT), sucrose preference test (SPT) and elevated plus maze (EPM) tests paradigms.

Results: Administration of naringin following CUMS significantly decrease immobility time and locomotion activity in TST and OFT respectively, relative to control while increasing preference to sucrose in SPT, open arm entries as well as time spent in open arm in EPM, relative to control suggesting antidepressant-like property. Pretreatment with metergoline, propranolol, and haloperidol following CUMS increased immobility time in TST, locomotor activity in OFT and IOAA in the EPM. Reduced preference for sucrose in SPT, open arm entry and duration in EPM relative to control ($p < 0.05$), however, these effects were attenuated by naringin.

Conclusion: These findings suggest that the antidepressant-like activity exhibited by naringin might be mediated via interactions with 5-HTergic, noradrenergic, and dopaminergic receptors, while the anxiolytic effect might involve interaction with both 5-HTergic and noradrenergic receptors.

Keywords: Depression; anxiety; Naringin; monoaminergic system; antidepressant; Neurotransmitter-receptor antagonists.

1. INTRODUCTION

Stress-related disorders such as depression and anxiety represent one of the greatest therapeutic challenges in the twenty-first century. It is burdensome as it has a high lifetime prevalence rate and a high level of comorbidity with other psychiatric diseases [1]. There is a growing research interest on the treatment of these stress-related disorders, albeit findings in drug discovery research have implicated the involvement of neuroactive peptides in the pathophysiology of these disorders [2-4]. Depression is associated with anhedonia, low self-esteem, guilty feelings, worthlessness, social impairment, sadness and suicidal tendencies [5-6], while anxiety disorder is associated with excessive fear and panic attacks [5]. Neuropsychiatric disorders are seen as a complex disorder, the mechanisms underlying their pathogenesis remain unclear although some peptide neurotransmitters in the brain has been implicated in previous researches [7-9]. Incidentally, common findings suggest that monoamine oxidase inhibitors (MAOI) in monoamine hypotheses play a major role in depression [10], as monoamine oxidase inhibitors has been used to relieve some of the depressive symptoms and anxiety [11].

The symptoms of depression and anxiety steams from disturbance in the transmission of neuro-peptide neurotransmitters like serotonin (5-HT), noradrenaline (NA) and dopamine (DA) all of which play important roles in transmission of chemical information (impulses) across the synapse [10,12]. Recent works on the treatment of depression and anxiety demonstrate that the administration of selective serotonin reuptake inhibitors, dopamine inhibitors and/or serotonin–noradrenaline reuptake inhibitors, augments the level of the neurotransmitters (serotonin, dopamine and/or noradrenaline) in the central nervous system as an important step and strategy for the treatment of this disorder [9,13-14]. Furthermore, non-synaptic approach of a selective and moderate induction of the carrier-mediated release of noradrenaline and serotonin is also proposed for the treatment of depression as active antidepressants [12].

The hypothalamus–pituitary–adrenal (HPA) axis and corticosteroid receptor signaling dysregulation has been reported to be the hallmark of major depressive and anxiety disorders [15]. This dysregulation is as a result of the increased production and secretion of corticotropin-releasing hormone (CRF) [16], triggered by chronic exposure to unpredictable stress. The corticosteroid receptor hypothesis of depression has led to another line of thought and research, focusing on the brain neuropeptide receptors, and especially the CRF receptors as drug target [17]. A variety of psychopharmacological agents are used to treat patients with major depressive disorder and anxiety disorders. Foremost among these medications are the anxiolytics and antidepressants such as imipramine, iproniazid, fluoxetine, sertraline, duloxetine etc (Saltiel et al., 2015; Montoya et al., 2016) [18-19]. The most widely used antidepressant medications have traditionally been the selective serotonin

reuptake inhibitors (SSRI), which act primarily by enhancing serotonin neurotransmission [20]. However, currently used antidepressants are the serotonin-norepinephrine-dopamine reuptake inhibitor (SSNDRI), also referred to as triple reuptake inhibitor (TRI). This medication act as a combined reuptake inhibitor of the monoamine neurotransmitters, serotonin, noradrenalin and dopamine [20]. Various studies have shown the role of monoamines such as 5-HTergic, noradrenergic, and dopaminergic systems in the pathophysiology of depression [21-23,4]. The principal therapeutic benefit of TRI lies in their ability to increase the availability and effect of the three neurotransmitters to relieve depressive symptoms and enhance cognitive balance, which act via mechanisms related to modulation of 5-$HT_{1,2,6}$, $\alpha_{1,2}$ and $\beta_{1,2}$-noradernergic and D1,2-dopaminergic receptor systems in particular [24]. The monoamine neurotransmitter (5-HT, α, β and D) receptors are exclusively distributed in specific areas in the central nervous system with high distribution in limbic and cortical areas and are connected post-synaptically to GABAergic, glutamatergic, dopaminergic, and noradrenergic interneurons [24]. Therefore, ligands of these monoamine receptors exert their pharmacological activity via interactions with each other and other neurotransmitter receptors [25]. Pool of evidence abound on the interaction of biomolecules like phenolic compounds with monoaminergic and nonmonoaminergic receptors to induce their psychopharmacological activities [25]. Considering that some bio-substances have antidepressant-like effect and anxiolytic potential, this potential could probably be due a modulation of the monoaminergic pathways via neurotransmitter-receptor interactions.

Naringin, is a flavonoid (flavanone-7-O-glycoside) compound formed from the flavanone naringenin and the disaccharide neohesperidose, is one of the main active ingredients found in grape fruit and other citrus fruits [26]. It is a bioactive phenolic compound with several neuro-therapeutic potentials. Several studies have shown that naringin possessed strong antioxidant, anti-inflammatory, antidepressant, anxiolytic, antihypertensive, anticancer, antidiabetic and memory enhancing [27-35]. Similarly, it has shown efficacy in several neurological disorders such as Alzheimer's [36], Parkinson's disease [37], polyglutamine disease [38], diabetic neuropathy [32], antiepileptic [39], anxiety and depression [40]. Remarkably, majority of the beneficial effects of naringin have been partly attributed to its free radical scavenging and antioxidant activities [41,28].

Although studies have shown that naringin enhances calcium/calmodulin-dependent protein kinase II activity in a mouse model of Alzheimer's disease [42], attenuates mitochondrial oxidative damage against aluminum-induced neurotoxicity in rats [43], modulates cholinergic and nitric oxide pathways in stressed mice [44], improved long-term memory deficits [42,43,44], and exhibit antidepressant-like effect via monoaminergic system [45], however, the neurotransmitter receptor interaction mechanism of naringin remain unexplored. Furthermore, literature search revealed that no information exists on the monoaminergic and nonmonoaminergic psychopharmacologic receptor interactions. Hence, we investigated the role of monoaminergic and nonmonoaminergic neurotransmitter receptors interaction in the antidepressant-like and anxiolytic effects of naringin in swiss mice.

2. MATERIALS AND METHODS

2.1 Laboratory Animals

Adult male Swiss mice (20–25 g; 7 weeks old) were housed, acclimatized and maintained in plastic cages (42 × 30 × 27 cm), in controlled temperature (25 ± 1°C) with a 12-h light/ dark cycle. They were allowed to have access to standard rodent pellet food and water ad libitum throughout the experimental period.

2.2 Drug and treatments

Metergoline – MET, propranolol – PRO, haloperidol – HLP, and naringin – NAR (Cas No. 3H1/X0,6/S/16/3/3AM 12358-M3) used in this study were all purchased from Sigma-Aldrich, St. Louis MO, USA. All drugs were dissolved in normal saline immediately before use and administered intraperitoneally (i.p.). The dose of naringin (10 mg/kg, most effective dose) used in this study was based on results obtained from previous study (Ben-Azu et al., 2018b) [46]. The doses of receptor blocker(s): metergoline (4 mg/kg i.p), propranolol (1 mg/kg i.p), haloperidol (0.2 mg/kg i.p), and the routes of administration used were chosen based on documented data in [10,47,48,23]. Normal saline solution was also administered intraperitoneally in a volume of 10 mL/kg per body weight, as normal control. All receptor blocker(s) were administered 15 min

prior to treatment with naringin, for the purpose of blocking different neurotransmitter receptors [23] in order to elucidate the neurochemical receptor activity behind the antidepressant-like properties, locomotor activity as well as anxiolytic effect of mice in the tail suspension test (TST), sucrose preference test (SPT), Open field test (OFT) and elevated plus maze. All behavioral tests were carried out between 8:00 – 14: 00; 30 min after naringin administration [49].

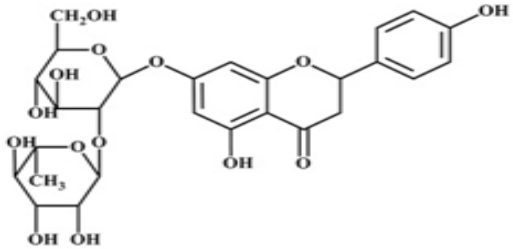


Fig. 1. Schematic diagram of Naringin [4]

2.3 Experimental Design

2.3.1 Role of serotonergic, noradrenagic and dopaminergic systems in antidepressant and anxiolytic activity of naringin

In a bid to show the possible contribution of the serotonergic noradrenagic and dopaminergic systems in the antidepressant-like and anxiolytic activity of NAR in the TST, OFT, SPT and EPM, the animals were separated into 3 sets (sets A-C) of 5 treatment groups with 5 mice in each group. All the groups except group 1 in each of the 3 sets, were subjected to chronic unpredictable mild stress (CUMS), for 21 days. Twenty-four (24) hours after the last day of CUMS, group 1 was treated with 10 mL/kg of vehicle; Group 2 was left untreated to serve as reference group. Group 3 was administered naringin (10 mg/kg, i.p.) in each of the 3 sets. Group 4 was pretreated with metergoline (4 mg/kg, i.p.; a nonselective 5-HT_1 and 5-HT_2 receptor antagonist), propranolol (1 mg/kg, i.p.; a β-adrenoceptor antagonist), and haloperidol (0.2 mg/kg, i.p.; dopaminergic D2 receptor antagonist) for sets A-C respectively. Group 5 was pretreated with metergoline (4 mg/kg, i.p.), propranolol (1 mg/kg, i. p) and haloperidol (0.2 mg/kg, i.p) respectively, 15 min before the administration of naringin (10 mg/kg, i.p.). Thirty minutes later [2], all animals were evaluated in the tail suspension, open field, sucrose preference and elevated plus maze models as previously described in literature [50,51,52,53,54,4].

2.4 Tail Suspension test

This test was carried out according to the method of Steru et al.and Sherman et al., [50,51]. A new set of animals were treated with the various drugs as described earlier. Thirty minutes after treatment, mice were suspended individually on a retort stand, placed 50 cm above the floor with the help of an adhesive tape placed approximately 1 cm from the tip of the tail. The total duration of immobility was recorded during the last 4 min of the 6 min test. An animal was considered to be immobile when it did not show any movement of the body and hangs passively.

2.5 Open-field test

This was engaged as previously described by [53]. The OFT consists of a wooden box (28 × 28 × 25 cm) with visible lines drawn to divide the floor into 16 (7 × 7 cm) equal squares with a frontal glass wall, and placed in a sound free room. The animals were placed in the rear left square and left to explore it based on number of line crossings for 5 min. Thereafter, the observation chamber was cleaned with 70% ethanol to remove residual odor

2.6 Sucrose Preference Test

For the sucrose intake test, mice were trained to consume 2% sucrose solution prior to the start of the experiment. They were exposed to 2% sucrose solution for 24 h period in their home cages without any food or water available. Prior to the test, mice were food and water deprived for 12 h. after a 12-h period of food and water deprivation, mice were given two bottles containing water or 2% (wt-vol) sucrose solution. Six hours later, the volumn of water and sucrose consumed will be measured. The mice' sensitivity to reward will be calculated as the percentage of total liquid intake attributed to the 2% sucrose solution, according to the following, equation:

Sucrose solution (g)/ (Sucrose solution [g] + water [g]) x 100% [54].

2.7 Elevated-plus Maze

The elevated plus maze test was used to assess for possible anxiety component of the

depression. It is a widely used behavioral test to assess anxiogenic or anxiolytic effects of pharmacological agents. Animals conduct anxiety-like behaviors usually show the reductions both in the number of entries and in the time spent in the open arms, along with an increase in the amount of time spent in the closed arms in the EPM. The apparatus consists of a central square platform (5 x 5 cm) from which emanated two open arms (30 x 5 x 0.25 cm) and two closed arms (30 x 5 x 15 cm) directly opposite each other, respectively. The entire apparatus was elevated to a height of 50 cm above floor level. Mice were placed at the junction of the four arms of the maze, facing an open arm and allowed to explore the maze for 5 min. During the test period, the following measurements were recorded: 1) number of open arm entries, b) number of closed arm entries, c) time spend in open arms, and d) time spent in closed arms. Entry by an animal into an arm was defined as the condition in which the animal has placed its four paws in that arm. Ethanol (70%) was used to clean the maze after each test session to prevent residual odor bias. Percentage of time spent on the open arms was calculated as percentage of the total time the rat spent on the maze [open arm time % = 100 × (OA time/total time)]. Furthermore, percentage of the total open arm entries by mice was calculated as percentage of total entries in the open arm [open arm entry % = 100 X (OA entries/total entries)]. The Index of open arms avoidance [IOAA] was determined using IOAA = 100 - (% time spent in open arms + % entries into open arms)/2 as described by [52].

2.7.1 Chronic Unpredictable Mild Stress (CUMS) Procedure

In CUMS, the mice are chronically exposed to variable unpredictable mild stressors to induce behavioral deficit (depressive- and anxiety-like behaviors) [55]. Prior to the start of the CUMS procedure, all of the mice were given 2% sucrose water for 24 hr to avoid neophobia for sucrose consumption training [54].

Mice were exposed to the following stressors daily for 21 days: 24 hr food deprivation, 24 hr water deprivation, 7 hr cage tilt (45° inclined), 1 min tail pinch with push pine, 0.45 x 12 mm (1 cm from the end of the tail), 7 hr wet bedding in cage (200 mL water in 100 g sawdust bedding), Hypoxia [15 min inside an air-tight hypoxic transparent plastic-container (height 23 cm, diameter 10 cm), overnight illumination (12 hr), Exposure to predator (12 hr). Control (unstressed) mice were undisturbed except for necessary procedures such as routine cage cleaning. Twenty-four hrs after the last application of unpredictable stressor, the different treatments to access the involvement of serotonergic, noradrenagic and/or dopaminergic pathways were given.

2.8 Statistical Analysis

Data were expressed as mean ± S.E.M. (standard error of mean). Data were analyzed using one-way analysis of variance (ANOVA) followed by the Bonferroni post-hoc test for multiple comparisons where appropriate using the Graph Pad Prism software version 5. A level of $p < 0.05$ was considered as statistically significant for all tests.

3. RESULTS

3.1 The Involvement of 5-hydroxytryptaminergic (5-TH) Neurotransmission on the Actions of Naringin in the TST, OFT, SPT and EPM

Effects of pretreatment with metergoline on the actions of naringin in the TST, OFT, SPT and EPM neuro-behavioral activities in CUMS-induced depressed mice is shown in Fig. 2. According to the results in Fig. 2 (a-e), CUMS significantly ($p < 0.05$) decreased locomotor activity, preference for sucrose intake, percentage open arm entries and duration in OFT, SPT and EPM in mice respectively while increasing immobility time and the IOAA in TST and EPM respectively as compared to the control (vehicle) group.

Pretreatment with naringin, following CUMS significantly ($p < 0.05$) reversed the decreased preference to sucrose, decreased open arm entries and duration in open arms in SPT and EPM respectively in relation to CUMS group ($P< 0.001$), and control (vehicle) group ($P< 0.05$). Furthermore, it significantly ($p < 0.05$) reduced the index of open arm avoidance in the EPM as compared with both the control (vehicle) and CUMS groups. Pretreatment with naringin also significantly ($p < 0.05$) decreased the immobility time in TST, in relation to the CUMS and control (vehicle) group.

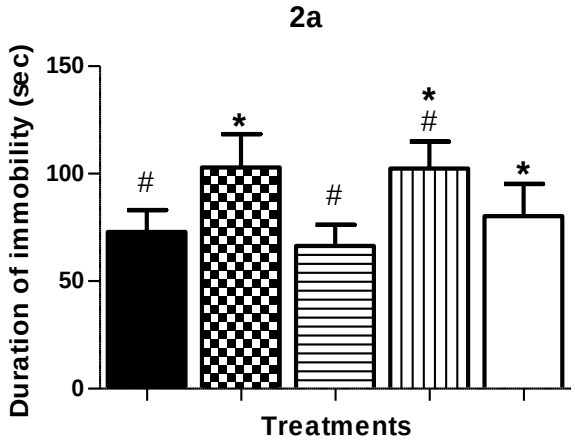
Treatment with metergoline, a 5-HT 1 - and 5-HT 2 -receptor antagonist (4 mg/kg, i.p.) had no

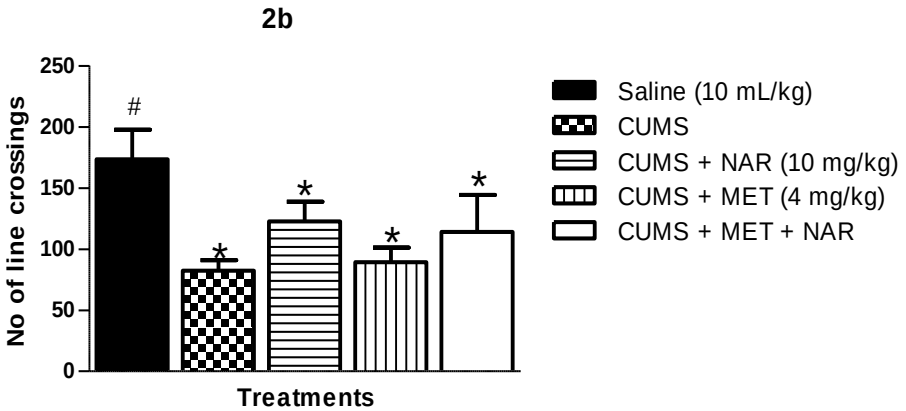
significant effect on immobility time, locomotion activity, open arm entries, duration and IOAA in TST, OFT, SPT and EPM respectively as compared with the CUMS group but significantly ($p < 0.05$) suppressed locomotion activities, sucrose preference and open arm entries and duration when compared to the control (vehicle) group.

Post-hoc analysis revealed that pretreatment with MET (4 mg/kg, i.p.) prior to naringin (10 mg/kg, i.p.) following CUMS significantly ($p<0.05$) reversed all the CUMS-induced depressive-like symptoms when compared with CUMS group.

3.2 Effect of Noradrenergic Pathway on the Actions of Naringin in the TST, OFT, SPT, and EPM Neuro-behavioral Activities

The effect of pretreatment of mice with propranolol (0.2 mg/kg, i.p.) on the actions of naringin in the TST, OFT, SPT, and EPM is shown in Fig. 2. As shown in Fig. 2a - e, two-way ANOVA and the Bonferroni post-hoc test revealed that CUMS alone and propranolol (0.2 mg/kg, p.o) pretreatment with following CUMS significantly reduced the locomotor activity ($p < 0.001$) in the OFT, percentage open arm entries ($p < 0.001$) and duration ($p < 0.001$) in EPM, while increasing immobility time ($p < 0.001$) in TST, and IOAA ($p < 0.001$) in EPM relative to the control (vehicle) group. Administration of naringin (10 mg/kg, p.o) following CUMS and pretreatment with propranolol (0.2 mg/kg, i.p) x naringin (10 mg/kg, p.o) 30 mins after following CUMS significantly abolished the locomotor suppression activity in OFT and percentage open arm entry and duration in EPM relative to CUMS. Furthermore, Administration of naringin (10 mg/kg, p.o) following CUMS and pretreatment with propranolol (0.2 mg/kg, i.p) prior to treatment with naringin (10 mg/kg, p.o) following CUMS significantly ($p < 0.05$) suppressed the enhanced immobility time in TST and IOAA in EPM. Propranolol had no significant effect on sucrose intake in SPT in relation to both the control (vehicle) group and CUMS only group.





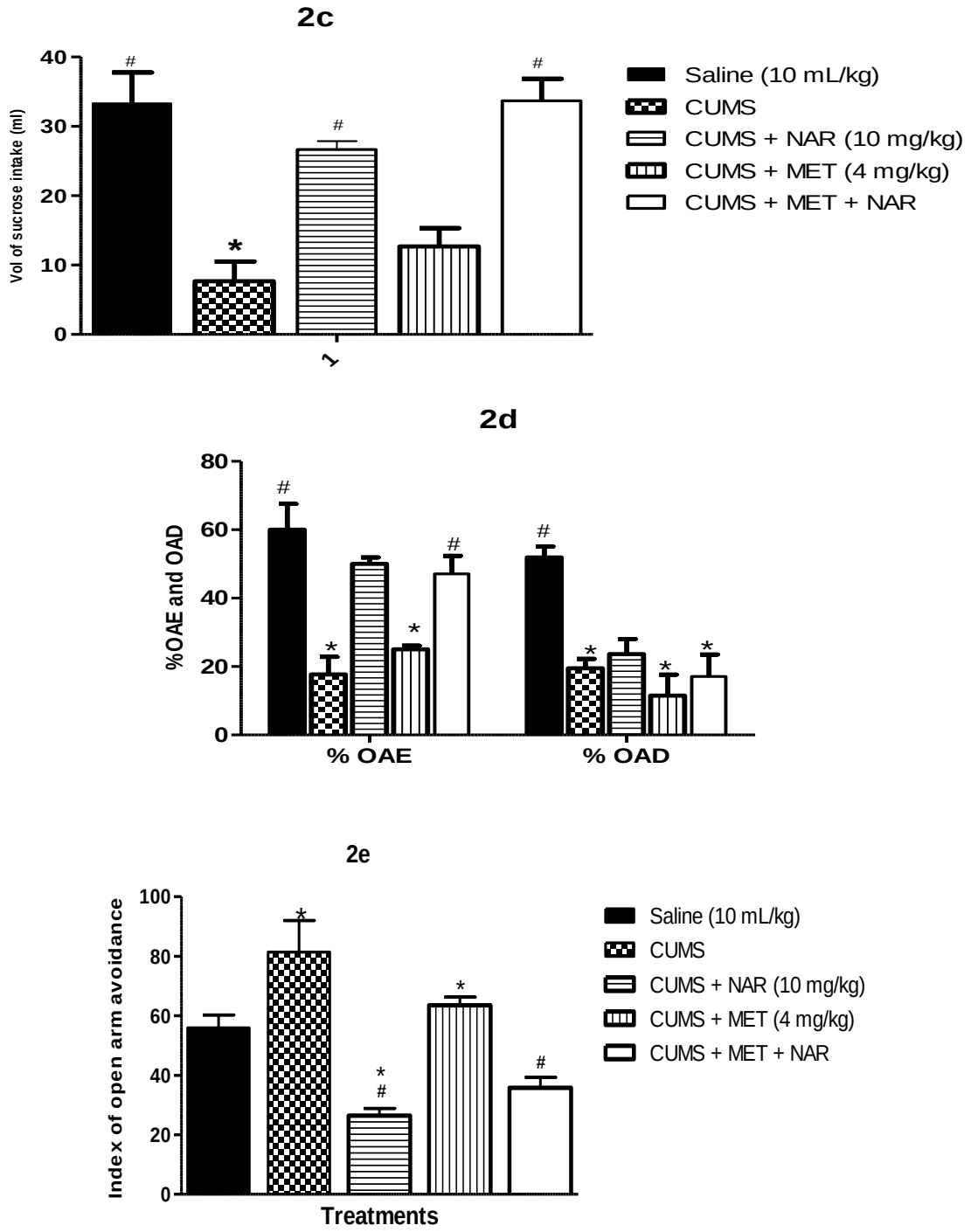


Fig. 2. Effects of pretreatment of mice with metergoline on the actions of naringin in the TST, OFT, SPT and EPM in mice (a): effect of naringin on immobility time in tail suspension test. (b): effect of naringin on locomotion in open field test (c): effect of naringin on sucrose intake in sucrose preference (d): effect of naringin on % AOE and OA duration in EPM (e): effect of naringin on IOAA in EPM

Bars represent the mean ± S.E.M of 5 animals/group. Statistical analysis was carried out by one-way ANOVA, followed by the Bonferroni post-hoc test. (#) denotes significantly different from CUMS group at P < 0.05 while (*) denotes significantly different from saline treated group at P < 0.05. VEH, vehicle; NAR, naringin; MET, metergoline; OAE, open arm entery; OAD, open arm duration; IOAA, index of open arm avoidance

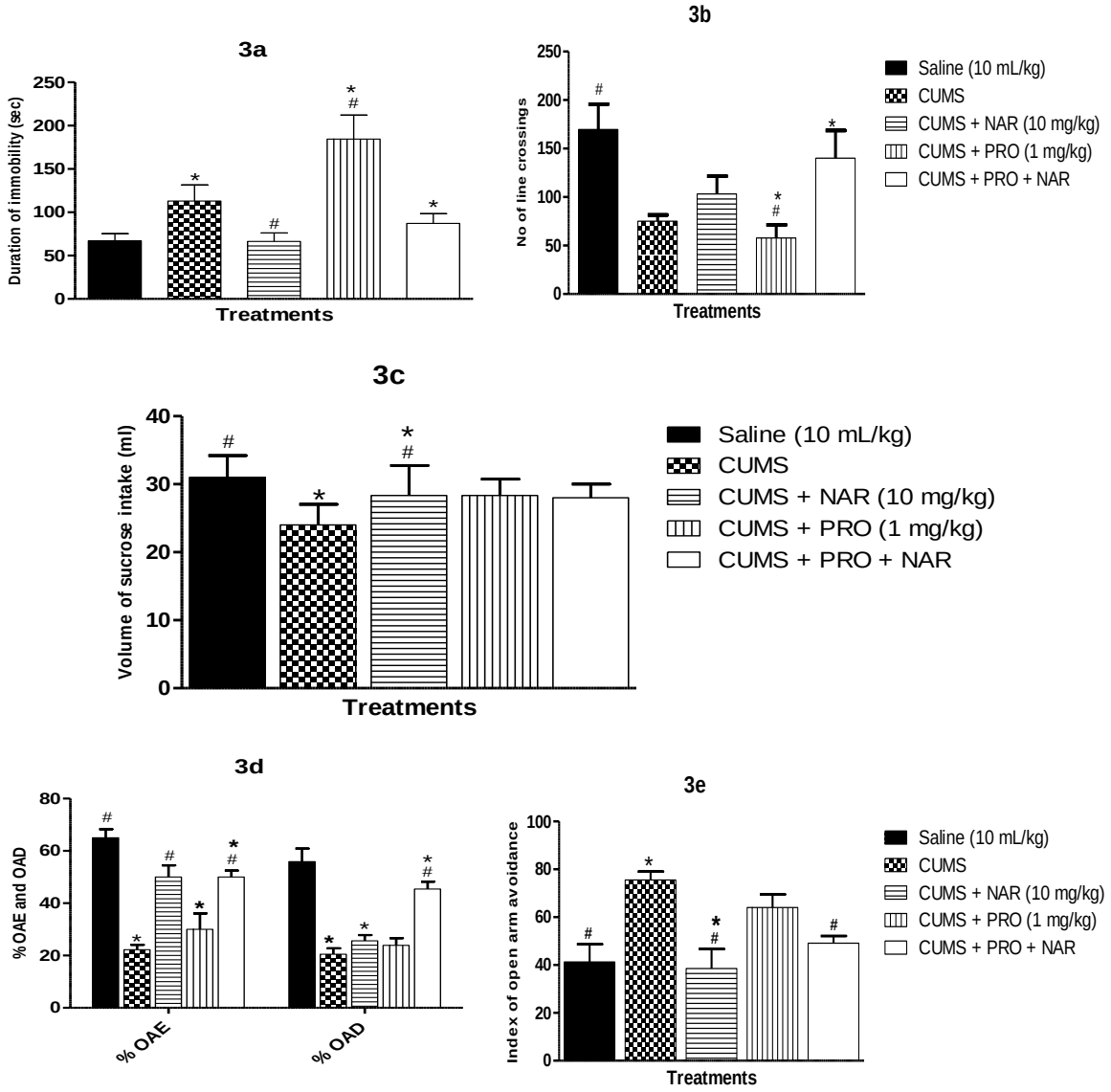


Fig. 3. Effects of pretreatment of mice with propranolol (PRO) on the actions of naringin in the TST, OFT, SPT and EPM in mice (a): effect of naringin on immobility time in tail suspension test. (b): effect of naringin on locomotion in open field test (c): effect of naringin on sucrose intake in sucrose preference (d): effect of naringin on % AOE and OA duration in EPM (e): effect of naringin on IOAA in EPM

Bars represent the mean ± S.E.M of 5 animals/group. Statistical analysis was carried out by one-way ANOVA, followed by the Bonferroni post-hoc test. (#) denotes significantly different from CUMS group at P < 0.05 while (*) denotes significantly different from saline treated group at P < 0.05. VEH, vehicle; NAR, naringin; PRO, propranolol; OAE, open arm entery; OAD, open arm duration; IOAA, index of open arm avoidance

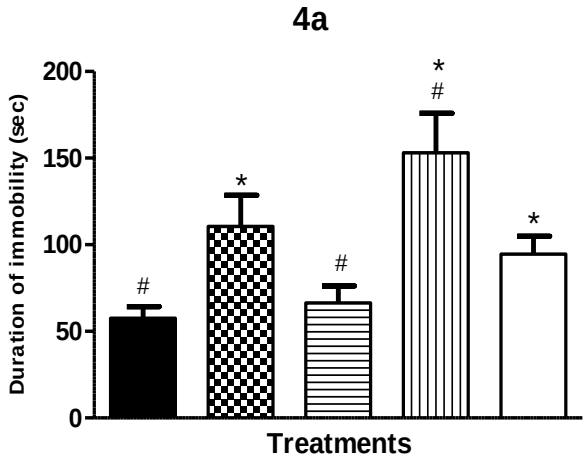
3.3 Involvement of Dopaminergic System on the Activities of Naringin in the TST, OFT, SPT and EPM

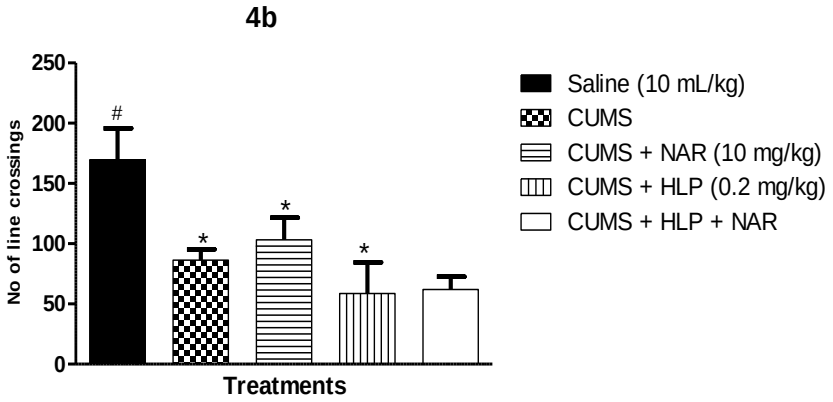
The effect of pretreatment of mice with haloperidol (0.2 mg/kg, i.p.) on the actions of naringin in the TST, OFT, SPT and EPM neuro-behavioral activities is shown in Fig. 4.

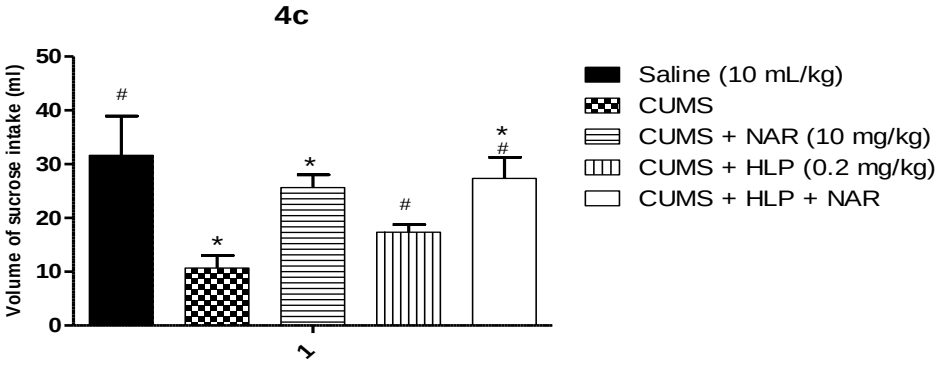
CUMS application and pretreatment with haloperidol (0.2 mg/kg, i.p.) significantly decreased locomotor activity ($p<0.001$) in the OFT and preference to sucrose ($p<0.001$) in SPT in relation to the saline treated mice. It also decreased significantly the percentage of entries in the open arm ($p<0.001$ and percentage time

spent in the open arm ($p<0.001$ of the EPM as compared with the control or saline treated mice. Interestingly, naringin treatment following CUMS was able to enhance the suppressed locomotor activity and sucrose preference in OFT and SPT respectively relative to the mice in CUMS group, albeit not significant ($p> 0.01$). The increase in percentage OAE and duration in EPM Naringin treatment following CUMS was also not significant when compared to the mice in both CUMS and saline treated groups.

Post-hoc analysis by the Bonferroni test showed that pretreatment with haloperidol (0.2 mg/kg, i.p.) prior to treatment with naringin (10 mg/kg, i.p.) significantly reversed the reduced preference for sucrose (in SPT and percentage open arm entry in EPM) which was induced by CUMS and HLP (0.2 mg/kg, i.p.) pretreatment, relative to the mice in CUMS group, but did not affect locomotion activities in OPT, immobility time in TST and IOAA in EPM relative to the mice in CUMS and saline treated groups.







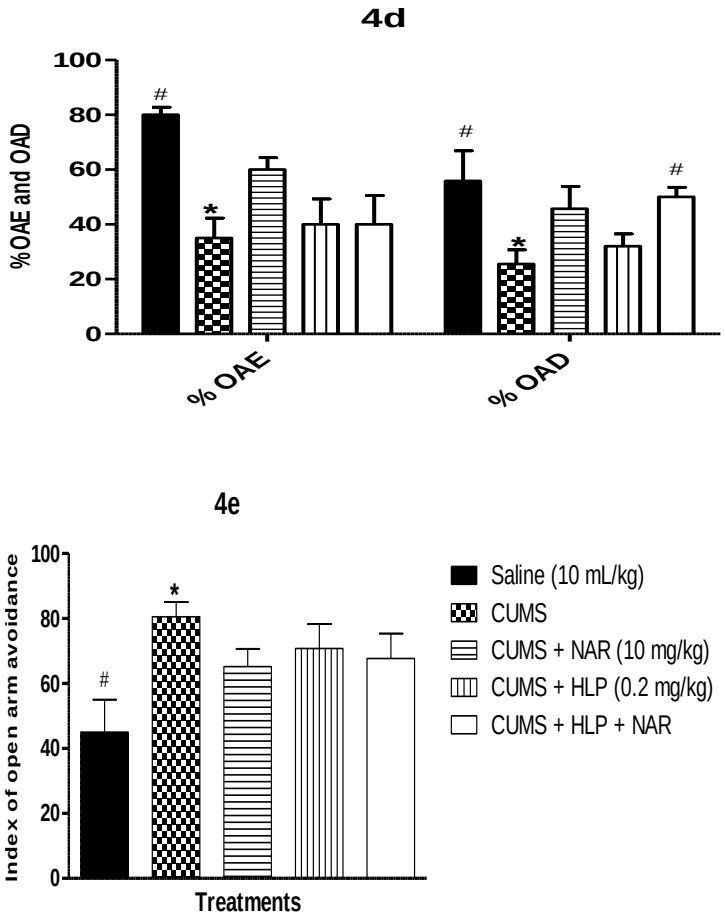


Fig. 4. Effects of pretreatment of mice with haloperidol (HLP) on the actions of naringin in the TST, OFT, SPT and EPM in mice (a): effect of naringin on immobility time in tail suspension test. (b): effect of naringin on locomotion in open field test (c): effect of naringin on sucrose intake in sucrose preference (d): effect of naringin on % AOE and OA duration in EPM (e): effect of naringin on IOAA in EPM

Bars represent the mean ± S.E.M of 5 animals/group. Statistical analysis was carried out by one-way ANOVA, followed by the Bonferroni post-hoc test. (#) denotes significantly different from CUMS group at P < 0.05 while (*) denotes significantly different from saline treated group at P < 0.05. VEH, vehicle; NAR, naringin; HLP, haloperidol; OAE, open arm entery; OAD, open arm duration; IOAA, index of open arm avoidance

4. DISCUSSION

The monoaminergic system has been implicated as the neurochemical pathway involved in the pathophysiology and treatment of depression [7,11], hence, we assessed the involvement of the serotonergic, noradrenergic and dopaminergic receptor interaction in the action of naringin on the TST, OFT, SPT and EPM behavioral activities in Swiss mice. Specific behavioral features and symptoms of anxiety and depression has been associated with deficiency or malfunction of certain monoamine neurotransmitters [56-57]. Depletion of the 5-HT, NE and/or DA is used as a model to test the involvement of monoaminergic systems in depression and anxiety. Therefore, to induce a state of depletion in the monoaminergic systems, we used enzyme-blocking agents to decrease the production of the monoamines. Metergoline was used to block 5HT 1 - and 5-HT 2 - receptors, propranolol was used to block β1,2-nor-adrenoceptor while haloperidone was used to block D2 -dopaminergic receptor) [22-23].

Interestingly, increased 5-HT activity has been associated with certain symptoms such as fatigue, while 5-HT deficiency is common in anxiety, obsessions, and compulsions [58,59]. The involvement of the serotonergic system in

the antidepressant-like and anxiolytic effects of naringin was studied using metergoline in TST, OFT, SPT and EPM paradigms. TST and SPT are validated behavioral models for assessing behavioral despair, hoplesness and anhedonic behaviors [50], for the detection of compounds with antidepressant property in rodents and elucidating their possible mechanisms of action [51,54], while the OFT and EPM are established behavioral model for assessing locomotion activities and anxiety-related behaviors respectively for the detection of compounds with anxiolytic property in rodents [52,53].

The result of this study shows that CUMS induced depression and anxiogensis in mice but pretreatment with naringin, following CUMS significantly ($p < 0.05$) alleviated the depressive and anxiogenic behaviors evidenced by the increased preference to sucrose and open arm entries and duration in SPT and EPM respectively (Fig. 2 c, d) in relation to control (vehicle) group ($P< 0.05$). It significantly ($p < 0.001$) reduced the immobility time in TST (Fig. 2a), locomotion activities in OFT (Fig. 2b) and index of open arm avoidance in the EPM (Fig. 2e) when compared to mice in the vehicle control group. Treatment with metergoline, a 5-HT 1 and 5-HT 2 -receptor antagonist (4 mg/kg, i.p.) significantly attenuated all the antidepressant-like effects of naringin as compared with the control (vehicle) group.

Studies have established that pretreatment of experimental animals with 5-HT_{2A} antagonists attenuates the antidepressant-like effects of known antidepressants like fluoxetine and imipramine [58,60]. On the contrary, 5-HT_{2A} receptor agonists has been shown to enhance the antidepressant-like effect of known antidepressant agents [61]. Therefore, the attenuated antidepressant-like effects such as increased immobility time in TST, decreased preference to sucrose and open arm entries and duration recorded in SPT and EPM were all reversed by naringin as shown in this study (Fig. 1a, c-e). These reversals of attenuated antidepressant and anxiolytic effects of naringin by pretreatment with metergoline, a 5-HT_{2A} receptor antagonist suggest the participation of 5-HT_{2A} receptors in the antidepressant-like and anxiolytic effects of naringin in TST, SPT and EPM. Similar observations were made by [4,23] in their works investigating the involvement of monoamine neurotransmitters in the antidepressant-like effects of morin and methyl jasmonate respectively [4,23].

The enhancement of NA neurotransmission is one of the mechanisms of action of some antidepressant drugs [56]. Several lines of evidence have linked reduced NA neurotransmission with decreased alertness, low energy, problems of inattention and concentration [62-63]. However, in this study, we use propranolol (a β1,2- adrenoceptor antagonist) to evaluate the involvement of noradrenergic pathway in the antidepressant-like and anxiolytic effects of naringin in TST, OFT, SPT and EPM paradigms.

Preclinical and clinical studies have shown that decreased exploratory activity and increased behavioral despair are key features associated with depressive-like behavior [63-65], and antidepressant drugs are known to increase exploratory activity and also decrease behavioral despair via increase in serotonergic and noradrenergic neurotransmissions [58,62].

In the present study, we observed that CUMS induced depression and anxiogensis in mice but pretreatment with naringin, following CUMS significantly ($p < 0.05$) alleviated the depressive and anxiogenic behaviors evidenced by the increased percentage open arm entries and duration in EPM (Fig. 3d) and immobility time in TST (Fig. 3a). According to the results, there was no recorded significant effect on sucrose intake of the mice in SPT (Fig. 3c) when compared to mice in the vehicle control group. Most importantly, we found that treatment with naringin (10 mg/kg, i.p.) and propranolol (1 mg/kg, i.p.) a β1,2- adrenoceptor antagonist, following CUMS had both significantly antidepressant-like (decreased immobility time) and anxiolytic-like (i.e., increase of open arm time) effect in TST and EPM (Fig. 3a and d) as seen in previous works [23,48]. Furthermore, Treatment with propranolol, had no significant effect on sucrose preference in SPT (3c) as compared with the control (vehicle) group. The manner of result observed in this study is similar to observations established and recorded with some antidepressant compounds in previous literatures [22,47-48]. This could be the evidence that there is possible involvement of β1,2- adrenoceptor receptors in the antidepressant-like potential of naringin.

Previous studies have shown that excitatory neurotransmitter systems play an important role in the regulation of locomotion, and increased motor activity is suggestive of enhanced excitatory neurotransmission [4,23]. Supportive

evidence has also shown that alteration in excitatory neurotransmitters (e.g., dopaminergic and glutamate) have been shown to play a prominent role in several neuropsychiatric diseases including depression, schizophrenia, Parkinson's disease among others [13,37,66]. Thus, the ability of naringin to increase locomotor activity in the OFT based on increased number of line crossing after repeated administration suggests modulation of excitatory neurotransmitter systems.

The dopaminergic pathway is implicated in mood and reward motivated behaviors and it plays a major role in the pathophysiology and treatment of depression [13]. Clinical evidences revealed that reduced dopaminergic activity has been linked to decreased incentive motivation [67-68], anhedonia (loss of pleasure) [69-70], and loss of interest [71-72], whereas increased functional dopaminergic activity has been linked to positive affect [73]. Furthermore, it has been shown that plasma levels of dopamine metabolites were significantly lower in the depressed patients suggestive of diminished dopamine turnover in depression [74].

We used haloperidol (0.2 mg/kg i.p.), a selective D2 antagonist to demonstrate the involvement of dopaminergic system in the antidepressant-like and anxiolytic potentials of naringin. Administration of D2 antagonists has been established to cause exacerbate depressive and anxiogenic behaviours (). Contrarily, D2 agonists attenuate these behaviors. Our results demonstrated significant increase in sucrose preference (Fig. 4c) and decrease in immobility time (Fig. 4a) by naringin following CUMS. This is suggestive of depressive-like behavior. Pretreatment with haloperidol prior to naringin following CUMS attenuated the sucrose preference and enhanced immobility time as observed in TST and SPT respectively. This is suggestive of the inhibition of the antidepressant-like effect of naringin in a manner similar to previous results with other antidepressant agents [4,23,49,75], this suggests its possible interaction with both D1 and 2- doperminergic receptors. The decrease in dopaminergic activity due to antagonism of central D2 and 2 receptors may perhaps explain the reduced sucrose preference and anti-immobility effect of naringin caused by haloperidol in this study.

5. CONCLUSION

This present study is of particular interest, being that, it demonstrated the involvement of monoaminergic system in the antidepressant and anxiolytic effects of naringin in mice following CUMS. Taken together, the results of our study provide evidences, which suggest that naringin produces its action in the treatment of depressive and anxiety disorders via mechanisms related to serotonergic, adrenergic and dopaminergic transmissions. Furthermore, we can affirm that there is an effect on some behaviour of mice, but to confirm the effect, a clinical study of the antidepressant study of naringin should be demonstrated.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The experimental protocols were approved by the University's College of Medicine Research Ethics Committee (COMRAC). Five animals per group were used for the study. The experiment was performed in accordance with the regulatory protocol of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985). Also, efforts were made to minimize the suffering of the animals during CUMS experimental protocols and behavioural tests.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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