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Research article

Possible involvement of NMDA receptor in the anxiolytic-like effect of caffeic acid in mice model of maternal separation stress

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ABSTRACT

Background and aim: Anxiety disorders are one of the most common psychiatric disorders worldwide. Common anti-anxiety medications are associated with several side effects. Caffeic acid (CA) is a phenolic compound with several pharmacological effects. The aim of this study was to investigate the anxiolytic-like effect of CA in maternally separated (MS) mice focusing on the possible involvement of the NMDA receptor.

Materials and methods: In this study, we used the MS paradigm (as a valid animal model of anxiety) in male mice and examined their anxiety-like behavior in postnatal day (PND) 45. The animals were divided into 12 experimental groups. Mice treated with CA alone and in combination with the NMDA receptor agonist/antagonist and then using open field (OFT) and elevated plus maze (EPM) anxiety-like behavior was assessed. Finally, the expression of NMDA receptor subtypes was assessed in the hippocampus using RT- PCR.

Results: Finding showed that CA exerted anxiolytic –like effects in the OFT and EPM tests. We showed that administration of effective dose of NMDA significantly reversed the anxiolytic-like effect of effective dose of CA and co-administration of ketamine (a NMDA receptor antagonist) significantly potentiated the effect of sub-effective dose of CA. Furthermore, ketamine enhanced the CA-reducing effect on NMDA receptors in the MS mice.

Conclusion: Our finding demonstrated that, probably at least, NMDA receptors are involved in the anxiety-like properties of CA in MS mice.

1. Introduction

Anxiety is an unpleasant and elusive feeling with inquietude behavior [1]. It is estimated that about one-eighth of the world's population suffers from anxiety [2]. Repeated stress exposure during the early life is could lead to adverse behavioral effects in the adulthood [3]. Maternal separation (MS) is defined as the lack of care or short-term care during the early stages of childhood development. This paradigm can negatively affect brain development, cognition, and social behavior in adulthood [4]. In this regard, the literature shows that children who are exposed to the MS paradigm are prone to anxiety, depression and memory loss [5, 6, 7]. A substantial body of evidence suggests that early life stress, such as MS, causes long-term behavioral disorders such as anxiety [5, 8].

N-methyl D-aspartate (NMDA) receptor is one of the sites of action for glutamate [9]. The NMDA receptor plays an important role in the development of mood and cognition [10, 11]. Activation of NMDA receptors has been shown to be associated with anxiety-like behavior [12]. In this concept, previous studies have shown that administration of NMDA receptor antagonists reduced the anxiety behavior in both clinical and experimental studies [12, 13].

The hippocampus is an important structure in the brain that plays an important role in shaping behavior [14]. Previous studies have shown that activation of NMDA receptors in the hippocampus leads to anxiety-like behavior in rodents [12, 13]. Therefore, the hippocampus and its NMDA receptors can be considered a major target for the development of anti-anxiety drugs.

Although commonly used chemical drugs are highly effective however, they also have side effects [15]. Therefore, researches are needed to find more effective drugs with fewer side effects. Traditionally, medicinal plants and their active ingredients have attracted the attention of researchers in the treatment of various disorders [16]. Caffeic Acid (CA), 3, 4 hydroxycinnamic acid, is a polyphenolic compound that is widely found in fruits and plants [17]. Previous studies have introduced various pharmacological properties for CA including antitumor [18], analgesic

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[19], anti-Alzheimer's [20], and anti-inflammatory effects [21]. It has been shown that CA has neuroprotective effect in experimental neurologic disorders [22]. It has been determined that CA exerted antidepressant- and anxiolytic-like effects in rodents [23, 24]. However, the exact mechanisms are involved in the anxiolytic-like effect of CA has not been established.

Considering that 1) MS causes anxiety-like behavior, 2) NMDA receptors are involved in the pathophysiology of anxiety, 3) CA have exerted various neuroprotective effect, in this study we aimed to evaluated the anxiolytic-like effect of CA in mouse model of MS stress focusing possible contribution of NMDA receptors.

2. Material and methods

2.1. Ethics

The present study was conducted in accordance with the guide for the care and use of laboratory animals as adopted by the committee on the care and use of laboratory animals of Shahrekord University of medical science with ethical code: IR.SKUMS.REC.1396. 101.

2.2. Animals

In this study (supported by a research grant (NO: 1396-08-75-3439) from Shahrekord University of Medical Sciences, Shahrekord, Iran), 96 male NMRI mice were used. To induce anxiety-like behavior, maternal separation (MS) stress paradigm was used [25, 26]. To perform this model, mice were separated from their mothers for 3 h daily on their third day of birth and then returned to their mother's cage. This paradigm continues until the 14th day after birth. The mice were then kept in cages in groups of 6 from day 14 to day 45 after birth. Tests are performed between 45 and 47 days after birth. The tested groups are as follows:

1. Control group (non-stressed animals received normal saline), 2. Stress group (MS mice received normal saline.), Groups 3–5 non-stress animals received CA at doses of 15, 30 and 45 mg/kg, groups 6–8 MS mice received CA at doses of 15, 30 and 45 mg/kg, 9. MS mice received NMDA agonist (7.5 mg/kg, i.p.), 10. MS mice received NMDA antagonist (ketamine) (1 mg/kg, i.p.), 11. MS mice received sub-effective dose of CA plus ketamine (1 mg/kg), 12. MS mice received an effective dose of CA plus effective dose of NMDA agonist (7.5 mg/kg).

Caffeic acid was injected as single dose via intraperitoneal route 30 min before behavioral tests. NMDA receptor antagonist or agonist is administered as single dose via intraperitoneal route 60 min before behavioral assessments. Doses and time of drugs administration was chosen based on previous studies as well as our pilot study [27, 28, 29]. All experimental groups involved 8 mice for behavioral assessments and 6 mice for RT-PCR evaluations and we tried to minimize the use of animals and to improve their well-being.

2.3. Open field test (OFT)

The OFT was used as a trial to evaluate the anxiety-like behavior and motor function in rodents. OFT apparatus is a Plexiglas box (40 × 50 × 60 cm). Mice were placed individually on the center of the box, and each animal's movements monitored and recorded during 5 min. Time spent in the central zone as a scale for assessment of anxiety-like behavior was recorded [30]. Increase in time spent in central zone of the OFT indicating the anxiolytic-like behavior. Furthermore, the distance moved (horizontal activity, number of crossing by 4 foots from each square) was recorded. After each experiment, the apparatus was cleaned using ethanol 70%.

2.4. Elevated plus maze (EPM)

The EPM is an appropriate tool to assess the anxiety-like behavior in rodents. The EPM apparatus is a black opaque Plexiglas consists of two open arms and two closed arms located at a height of 50 cm above the ground. Animals were individually placed in the center of the EPM facing to closed arms. The total time spent in the open and close arms, and number of entries into the open and close arms as well as total entries was recorded over a period of 5 min [31].

2.5. Real-time PCR

At the end of the experiment, mice were sacrificed under anesthesia using diethyl ether, and the hippocampus was isolated and the gene expression of NMDA receptor subunits (NR2a and NR2b) was assessed by Real-time PCR [32]. At first total RNA using TRIzol reagent (Invitrogen) was extracted. Alterations in mRNA levels of genes were determined using qRT-PCR after reverse transcription of 1 μg of RNA from each sample using PrimeScript RT reagent kit (Takara Bio, Inc., Otsu, Japan). qRT-PCR was done on a light cycler device (Roche Diagnostics, Mannheim, Germany) using SYBR Premix Ex Taq technology (Takara Bio). Thermal cycling conditions were including an initial activation step for 30 s at 95 °C afterwards 45 cycles as well as a denaturation step for 5 s at 95 °C and a combined annealing/extension step for 20 s at 60 °C. Melting curve analysis was performed to certify whether all primers yielded a single PCR product. The reaction for each gene is in triplicate and is repeated twice. The favorite primers were designed using Primer 3 software version 0.4.0 (Table 1). The *H2afz* gene was considered as normalizer [33]. The rate of change in the expression of the target genes was compared to the control group. Finally, the data obtained from Real-time PCR are presents as fold change expression.

2.6. Statistical analysis

Statistical analysis was performed using SPSS software and the results were presented as Mean ± SEM. One-way ANOVA and Tukey's post hoc tests were used for analysis. P values < 0.05 were considered to be statistically significant.

3. Results

3.1. Caffeic acid increases the time spent in central zone as well as decreases distance moved in the OFT

One way ANOVA analysis showed that there is significant difference among the experimental groups ($F(11, 85) = 18.22$ ($P < 0.001$)). Results showed that the time spent in the center of OFT was significantly less in the MS group in compared with the control group (Figure 1, $P < 0.05$). Time spent in center of the OFT in the MS mice received CA at doses of 30 and 45 mg/kg was significantly longer than saline-received MS mice ($P > 0.01$). Furthermore, administration of NMDA (150 mg/kg) to MS mice significantly decreased the time spent in central zone in compared with saline-received counterpart ($P < 0.05$). Our findings demonstrated that co-administration of sub-effective dose of CA (15 mg/kg) plus Ket (0.25 mg/kg) significantly increased the time spent in central zone in comparison with group received sub-effective dose of CA (15 mg/kg) ($P < 0.05$). In addition, co-injection of NMDA (150 mg/kg) plus effective dose of CA (45 mg/kg) to the MS mice decreased the time spent in the central

Table 1. Sequences of primers.

name	sequence
Nr2a-F	CTCAGCATTGTCACCTTGGA
Nr2a-R	GCAGCACTTCTTCACATTCAT
Nr2b-F	CTACTGCTGGCTGCTGGTGA
Nr2b-R	GACTGGAGAATGGAGACGGCTA
H2afz-F	TCATCGACACCTGAAATCTAGGA
H2afz-R	AGGGGTGATACGCTTTACCTTTA

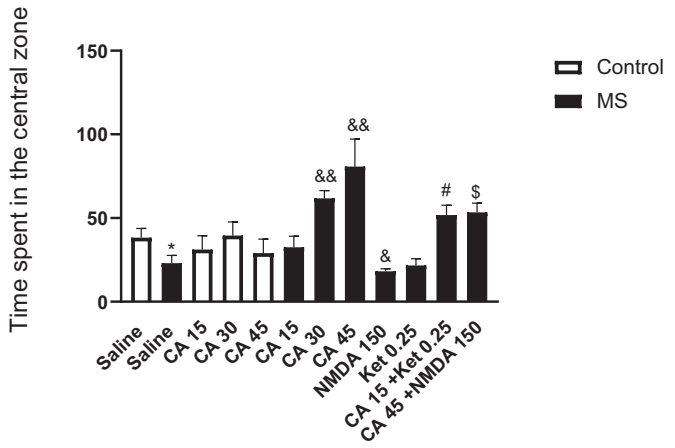

Figure 1. Comparison of time spent in the central zone of OFT in experimental groups. Data are expressed as mean ± SEM and was analysis using one-way ANOVA following Tukey post-hoc test. *P < 0.05 in compared to the saline-treated control group, & P < 0.05 and && P < 0.01compared with the saline –treated MS mice, #P < 0.05 compared with CA 15 mg/kg-received MS mice and $ P < 0.05 compared with CA 45 mg/kg-received MS mice. CA: caffeic acid, Ket: ketamine and MS: maternal separation.

zone of OFT in a significant manner in compared to the group received effective dose of CA (45 mg/kg) alone (P < 0.05).

One way ANOVA analysis showed that there is significant difference among the experimental groups (F (11, 85) = 10.13 (P < 0.01)). Results showed that the horizontal activity was significantly increased in the MS group in compared with the control group (Figure 2, P < 0.05). The horizontal activity in the MS mice received CA at doses of 30 and 45 mg/kg was significantly decreased in compared with the saline-received MS mice (P > 0.05).

3.2. Caffeic acid increases the time entries to the open arms as well as decreased the time entries to the open arms of the EPM

One way ANOVA analysis showed that there is significant difference among the experimental groups in case of open arms entries in the EPM (F (11, 85) = 12.38 (P < 0.01)). Results showed that, the time entry in EPM open arms was significantly lower in the MS group than the control group (Figure 3, P < 0.001). The time spent in EPM open arms in the MS mice received CA at doses of 30 and 45 mg/kg was significantly longer

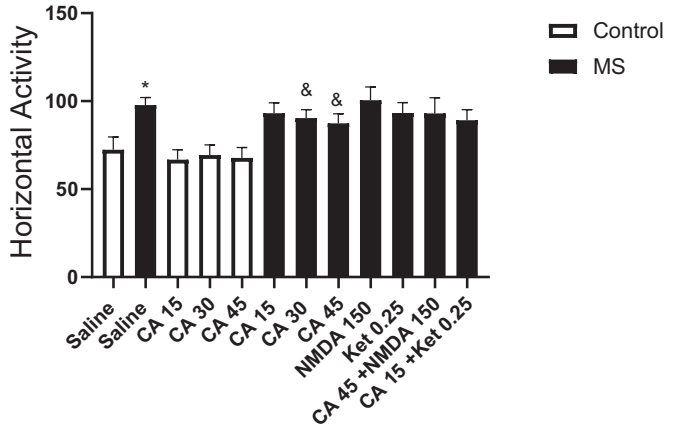

Figure 2. Comparison of the horizontal activities in the OFT in experimental groups. Data are expressed as mean ± SEM and was analysis using one-way ANOVA following Tukey post-hoc test. *P < 0.05 in compared to the saline-treated control group and & P < 0.05 compared with the saline –treated MS mice. CA: caffeic acid, Ket: ketamine and MS: maternal separation.

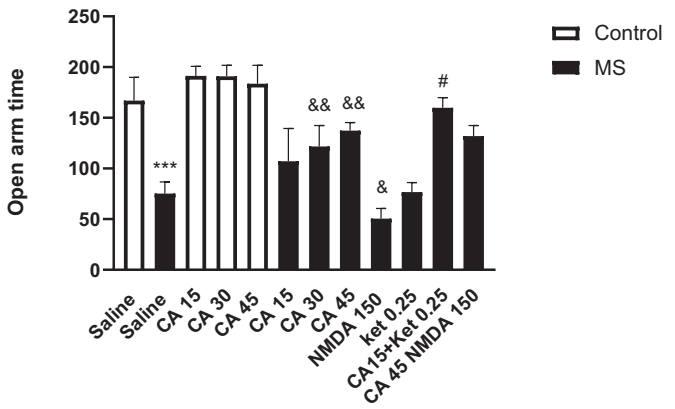

Figure 3. Comparison of time spent in the EPM open arms in experimental groups. Data are expressed as mean ± SEM and was analysis using one-way ANOVA following Tukey's post-hoc test. ***P < 0.001 in compared to the saline-treated control group, & P < 0.05 and && P < 0.01compared with the saline –treated MS mice, #P < 0.05 compared with CA 15 mg/kg-received MS mice. CA: caffeic acid, Ket: ketamine and MS: maternal separation.

than saline-received MS mice (P > 0.01). Furthermore, administration of NMDA (150 mg/kg) to MS mice significantly decreased the time spent in EPM open arms in compared to the saline-received counterpart (P < 0.05). Our findings demonstrated that co-administration of sub-effective dose of CA (15 mg/kg) plus Ket (0.25 mg/kg) significantly increased the time spent in the open arms of EPM in comparison with the group received sub-effective dose of CA (15 mg/kg) alone (P < 0.05).

One way ANOVA analysis showed that there is significant difference among the experimental groups in case of close arms entries in the EPM (F (11, 85) = 19.25 (P < 0.001)). Results showed that, the time entry in EPM close arms was significantly longer in the MS group than the control group (Figure 4, P < 0.001). The time spent in EPM close arms in the MS mice received CA at doses of 30 and 45 mg/kg was significantly decreased in compared with the saline-received MS mice (P > 0.01). Furthermore, administration of NMDA (150 mg/kg) to MS mice significantly increased the time spent in EPM close arms in compared to the saline-received counterpart (P < 0.05). Our findings demonstrated that co-administration of sub-effective dose of CA (15 mg/kg) plus Ket (0.25 mg/kg) significantly decreased the time spent in the close arms of EPM in comparison with the group received sub-effective dose of CA (15 mg/kg) alone (P < 0.05).

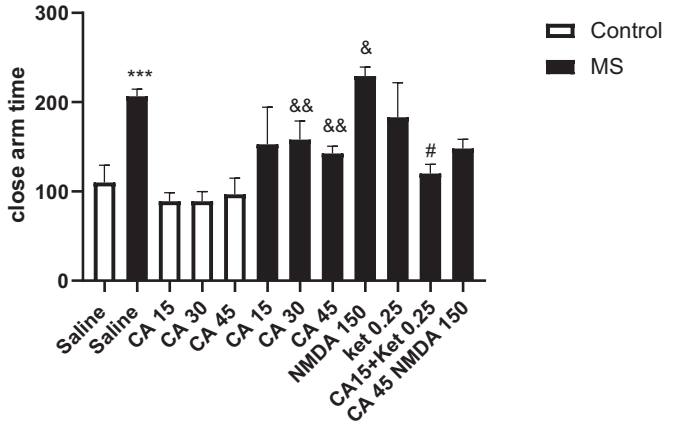

Figure 4. Comparison of time spent in the EPM close arms in experimental groups. Data are expressed as mean ± SEM and was analysis using one-way ANOVA following Tukey's post-hoc test. ***P < 0.001 in compared to the saline-treated control group, && P < 0.01compared with the saline –treated MS mice, #P < 0.05 compared with CA 15 mg/kg-received MS mice. CA: caffeic acid, Ket: ketamine and MS: maternal separation.

3.3. Caffeic acid increases the number of entries to the EPM open arms

One way ANOVA analysis showed that there is significant difference among the experimental groups in case of number of entries to the EPM open arms ($F(11, 85) = 11.41$ ($P < 0.01$)). As Figure 5 shows, the entries frequency of EPM open arms in the MS group was significantly lower than the control group ($P < 0.05$). The entries frequency of EPM open arms in the MS mice received CA at dose of 45 mg/kg was significantly higher than saline-received MS mice ($P > 0.01$). Furthermore, administration of NMDA (150 mg/kg) to MS mice significantly decreased the entries frequency of EPM open arms in compared with saline-received counterpart ($P < 0.05$). Our findings demonstrated that co-administration of sub-effective dose of CA (15 mg/kg) plus Ket (0.25 mg/kg) significantly increased the frequency of EPM open arm entry in comparison with group received sub-effective dose of CA (15 mg/kg) alone ($P < 0.05$). In addition, co-injection of NMDA (150 mg/kg) plus effective dose of CA (45 mg/kg) to the MS mice decreased the entries frequency of EPM open arms in a significant manner in compared to the MS group received effective dose of CA (45 mg/kg) alone ($P < 0.05$).

One way ANOVA analysis showed that there is significant difference among the experimental groups in case of number of entries to the EPM close arms ($F(11, 85) = 25.36$ ($P < 0.001$)). As Figure 6 shows, the entries to the EPM close arms was significantly increased in the MS group in comparison with the control group ($P < 0.001$). The entries frequency of EPM close arms in the MS mice received CA at doses of 30 and 45 mg/kg was significantly lower than saline-received MS mice ($P > 0.05$ and $P > 0.001$, respectively). Furthermore, administration of ketamine (0.25 mg/kg) to MS mice significantly decreased the entries frequency of EPM close arms in compared with saline-received counterpart ($P < 0.05$). Our findings demonstrated that co-administration of sub-effective dose of CA (15 mg/kg) plus Ket (0.25 mg/kg) significantly decreased the frequency of EPM close arm entry in comparison with group received sub-effective dose of CA (15 mg/kg) alone ($P < 0.01$). In addition, co-injection of NMDA (150 mg/kg) plus effective dose of CA (45 mg/kg) to the MS mice increased the entries frequency of EPM close arms in a significant manner in compared to the MS group received effective dose of CA (45 mg/kg) alone ($P < 0.05$).

One way ANOVA analysis showed that there is no significant difference among the experimental groups in case of total entries in the EPM ($F(11, 85) = 6.13$ ($P > 0.05$)). As Figure 7 shows, neither condition (maternal separation) nor treatments did not change the total entries to the EPM amongst experimental groups.

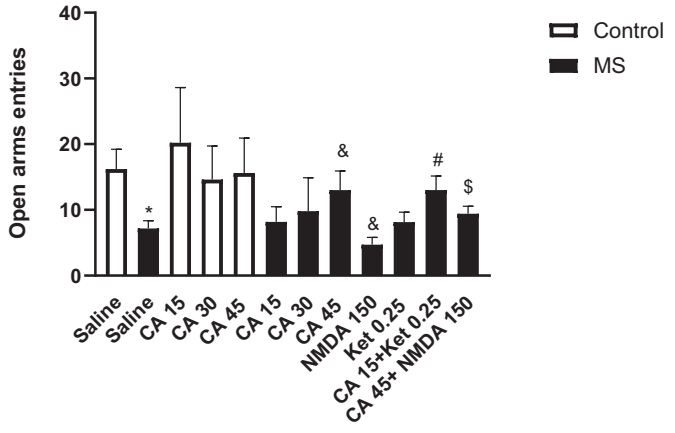

Figure 5. Comparison of the entries frequency in the EPM open arms in experimental groups. Data are expressed as mean ± SEM and was analysis using one-way ANOVA following Tukey's post-hoc test. *$P < 0.05$ in compared to the saline-treated control group, & $P < 0.05$ compared with the saline –treated MS mice, #$P < 0.05$ compared with CA 15 mg/kg-received MS mice and \$ $P < 0.05$ compared with CA 45 mg/kg-received MS mice. CA: caffeic acid, Ket: ketamine and MS: maternal separation.

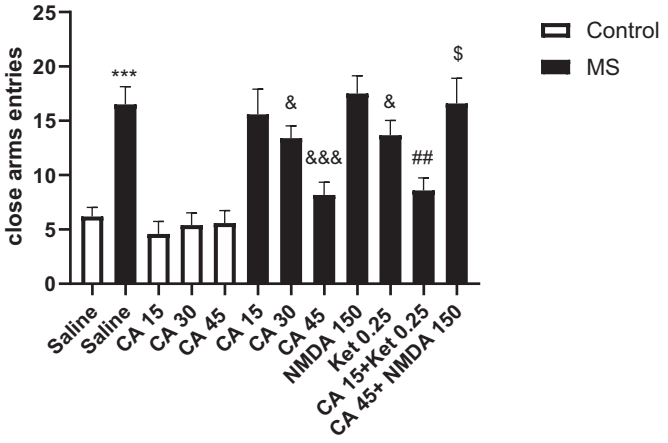

Figure 6. Comparison of the entries frequency in the EPM close arms in experimental groups. Data are expressed as mean ± SEM and was analysis using one-way ANOVA following Tukey's post-hoc test. ***$P < 0.001$ in compared to the saline-treated control group, & $P < 0.05$ and &&& $P < 0.001$ compared with the saline –treated MS mice, ##$P < 0.01$ compared with CA 15 mg/kg-received MS mice and \$ $P < 0.05$ compared with CA 45 mg/kg-received MS mice. CA: caffeic acid, Ket: ketamine and MS: maternal separation.

3.4. Caffeic acid modulates the expression of NR2a and NR2b subunits of NMDA receptors in the hippocampus

One way ANOVA analysis showed that there is significant difference among the experimental groups in case of expression of the NR2a ($F(5, 30) = 9.58$ ($P < 0.05$)) and NR2b ($F(5, 30) = 14.11$ ($P < 0.01$)). Findings showed that expression of NR2a (Figure 8) and NR2b (Figure 9) subunits of NMDA receptors was significantly increased in the MS group in comparison with the control mice ($P < 0.05$). Expression of NR2b subunit of NMDA receptors in the MS mice received CA at dose of 45 mg/kg was significantly lower than saline-received MS mice ($P < 0.05$). We demonstrated that co-administration of sub-effective dose of CA (15 mg/kg) plus Ket (0.25 mg/kg) significantly decreased the expression of NR2b subunit of NMDA receptors in comparison with group received sub-effective dose of CA (15 mg/kg) alone ($P < 0.05$).

4. Discussion

In this study, we found that the separation of infants from mothers causes anxiety-like behaviors in adulthood. MS mice had a significant

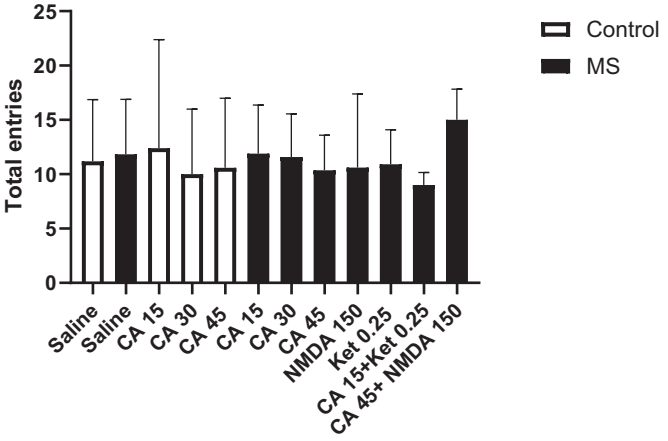

Figure 7. Comparison of the total entries to the EPM arms in experimental groups. Data are expressed as mean ± SEM and was analysis using one-way ANOVA following Tukey's post-hoc test. CA: caffeic acid, Ket: ketamine and MS: maternal separation.

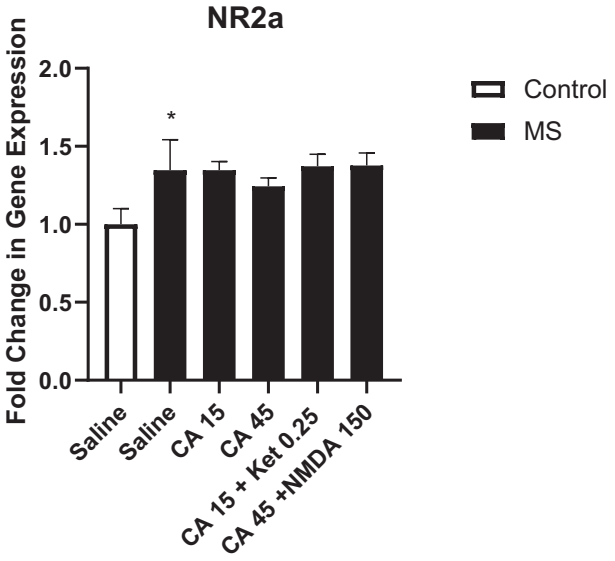

Figure 8. Comparison of the expression of NR2a subunit of NMDA receptors in experimental groups. Data are expressed as mean ± SEM and was analysis using one-way ANOVA following Tukey's post-hoc test. *P < 0.05 in compared to the saline-treated control group. CA: caffeic acid, MS: maternal separation.

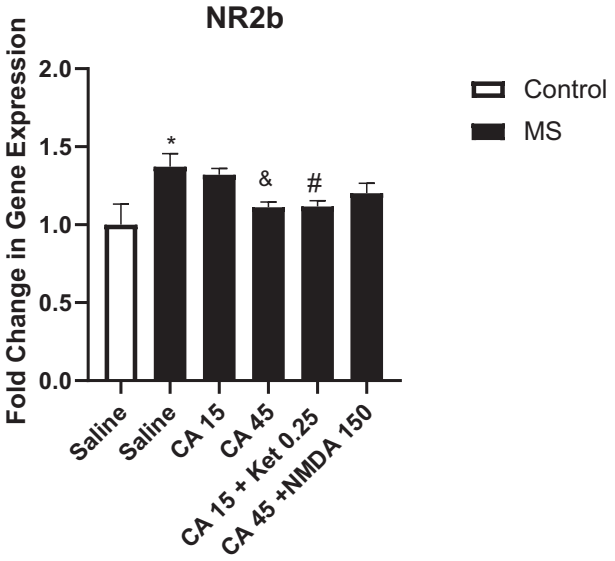

Figure 9. Comparison of the expression of NR2b subunit of NMDA receptors in experimental groups. Data are expressed as mean ± SEM and was analysis using one-way ANOVA following Tukey's post-hoc test. *P < 0.05 in compared to the saline-treated control group, & P < 0.05 compared with the saline –treated MS mice, #P < 0.05 compared with CA 15 mg/kg-received MS mice. CA: caffeic acid, MS: maternal separation.

reduction in the frequency of entries and time spent on open arms in the EPM, and also spent less time in the central zone of the OFT in comparison with the control mice. The gene expression of NMDA receptors significantly increased in the hippocampus of MS mice. We showed that administration of CA significantly reversed the negative effect of MS on behavior as well as reduced the expression of NMDA receptor subunits (NR2b) in the hippocampus. Surprisingly, we found that the beneficial effect of CA as an anxiolytic agent (at least in part) is mediated by NMDA receptors. In this concept, co-administration of NMDA receptor antagonist (ketamine) potentiated the effect of sub-effective dose of CA. In addition, administration of NMDA plus effective dose of CA significantly mitigated the anxiolytic effect of CA.

Anxiety is one of the most common psychiatric diseases [34]. Experiencing adverse events in early periods of life harmfully affects development of the brain and behavior and also, is regarded as a risk factor for susceptibility to psychiatric disorders. It has been well established that early life stress as maternal separation (MS) impaired development of the brain and behavior and led to anxiety behavior in the adulthood [35, 36, 37]. MS led to neurochemical, neurohormonal and neurostructural changes in the brain [38, 39]. Given the above changes in the brains of MS mice, it is somewhat predictable that MS mice will have a better response to factors affecting neurological and psychological disorders than normal (intact) mice.

In this study, exposure of mice to maternal separation stress was associated with a significant decrease in the time spent in the center of open field in comparison with the control group. The open field is an experiment used to measure the amount of locomotor activity and anxiety in animals (usually rodents) [40]. Indicator of anxiety-like effects is decrease in time spent in the central zone of the open field [41]. A 2018 study by Jane et al. Showed that exposure to maternal separation stress was associated with anxiety-like behavior in the open field test, which was associated with reduced time spent in the central zone of open field [42], which is consistent with the results of the present study.

Elevated plus maze (EPM) is a standardized test for evaluating anxiety-like behavior in laboratory animals. This test is commonly used to evaluate the anti-anxiety effects of pharmaceutical compounds [43]. This test is based on the animal's propensity for open arms. In the EPM, anxiety-like behavior is manifested by decreasing in the entries frequency and duration of time spent in the open arms [44]. Studies have shown that experiencing stressful conditions is associated with anxiety-related behaviors in the EPM test [42, 45], which is in line with our results.

Previous studies have introduced various pharmacological properties for CA including antitumor [18], analgesic [19], anti-Alzheimer's [20], and anti-inflammatory effects [21]. It has been shown that CA has neuroprotective effect in experimental neurological disorders [22]. In the present study, administration of CA to MS mice increased the time spent in the central zone of OFT as well as increased the time spent and the number of entries to the open arms of the EPM. It has been determined that CA exerted antidepressant- and anxiolytic-like effects in rodents [23]. In this regard, Takeda et al. demonstrated the antidepressant-like effect of CA in the forced swimming test in mice. They concluded that the anti-depressant-like effect of CA was partially mediated by neurogenesis and its neuroprotective properties [46]. In another study, Takeda et al. (2002) showed that CA dose dependently exerted antidepressant-like activity in stress-exposed mice [47].

Glutamate is an amino acid and an excitatory neurotransmitter in the brain. Studies have shown that glutamate plays an important role in the pathophysiology of anxiety [48]. In this regards, previous studies have showed that activation of NMDA receptors led to anxiety-like behaviors in mice [12]. Recent studies with genetically modified mice have shown that deletion of NMDA receptor NR1 subunits, especially from the hippocampal reduced the anxiety-like behavior. These findings indicate that NMDA receptors in the hippocampus have key role in the development of anxiety-related behaviors [12]. In line with previous studies, the expression of NR2a and NR2b subunits of NMDA receptors in the hippocampus of MS mice significantly increased. Our results showed that treatment of MS mice with CA significantly reduced the expression of the NR2b subunit of NMDA receptor. This finding indicates that the anxiolytic-like effect of CA mediated via reduction in the expression of NMDA receptor in the hippocampus.

In the present study, administration of NMDA significantly reduced the time spent in the central zone of the OFT and the entries frequency and spent time in the open arms of EPM indicating activation of NMDA receptors causes anxiety-like behavior. Salunke et al., showed that NMDA agonist aggravated anxiety-like behaviors and NMDA antagonist possessed anxiolytic-like behaviors in mice [49]. Solati et al. showed that intracerebroventricular injection of different doses of NMDA agonist

decreased the percentage of entries and time spent in open arms of EMP [50]. In this regards, Engin et al. showed that ketamine has anxiolytic- and antidepressant-like properties in animal models [51].

In the present study, we observed that MS mice received CA plus NMDA showed decrease in spent time and entries frequency to the open arms of EPM and time spent in the central zone of OFT indicating that the anxiolytic-like effects of CA partially mediated via NMDA receptors. Furthermore, we co-administrated the sub-effective dose of CA plus ketamine (a NMDA receptor antagonist), we found that entries frequency and time spent in the open arms of EPM as well as time spent in the central zone of OFT. These findings confirmed that anxiolytic-like effect of CA in MS mice partially mediated NMDA receptors. However, future studies warranted to explore more possible mechanisms are involved in the anxiolytic-like effect of CA.

5. Conclusion

In conclusion we found that CA exerted anxiolytic-like effect in EPM and OFT in maternally separated mice. Results showed that co-administration of NMDA mitigated the beneficial effect of CA and co-injection of ketamine potentiated the anxiolytic-like effect of CA. Our findings indicated that NMDA receptors, partially at least, mediated the anxiolytic-like activity of CA in mouse model of MS induced anxiety-like behavior.

Declarations

Author contribution statement

Zahra Lorigooini: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Shakiba Nasiri boroujeni: Performed the experiments; Wrote the paper.

Shima Balali-Dehkordi, Leila Ebrahimi, Mohammad Rahimi-Madiseh: Analyzed and interpreted the data.

Elham Bijad: Performed the experiments.

Hossein Amini-Khoei: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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