Introduction

Curcumin (CUR) is a non-toxic natural flavor as well as a polyphenol with widespread neuroprotective and cognition-enhancing...
Although the importance of the CUR in the treatment of memory disorders is well established due to its potential neuroprotective effects, its mechanisms of action are still being investigated. CUR supplements were shown to be beneficial in the treatment of Alzheimer’s disease and other central nervous system-related neurodegenerative disorders, such as Parkinson’s diseases, and brain malignancies.

The transcription factor cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB) exerts its main role in long-term memory and plasticity by phosphorylation at Ser 133. The phosphorylated form of CREB (p-CREB) is known as the synaptic plasticity molecular interface and the final phase of long-term potentiation. A review of the literature reveals the role of CREB phosphorylation in the neuroprotective effects of phytochemicals such as CUR. CREB signaling is involved in the CUR’s neuroprotective effects against nicotine. According to a substantial body of research, CUR protects against the impairment induced by scopolamine, nicotine, acrylamide, and alcohol-induced hippocampal neurotoxicity and neurodegeneration.

Morphine (Mor) were shown to impair memory in laboratory animals. Morphine application at different times of training and testing affects learning and memory. Since the cellular and molecular correlates of drug dependence and memory for inducing neuronal plasticity were similar, Mor-conditioned response was also associated with an alteration in the p-CREB in the hippocampus. Moreover, the importance of nitric oxide (NO) in regulating CREB phosphorylation in rats has been well documented. Furthermore, there is a functional association between NO and CREB in nervous system functions, and NO facilitates the regulation of CREB phosphorylation and expression, and there is evidence that NO affects the CUR mediation.

Emotional memory is an aversive type of memory in animals that has primarily been used in research on the mechanisms of the different phases of learning and memory. Among the existing aversive memory methods, the inhibitory avoidance (IA) method stands out.

Taken together, the interaction between opioids and NO in memory, as well as opioid involvement in the modulation of NO function, is well established. Considering that there is a functional association between NO and CREB in nervous system functions, as well as the large body of evidence on the neuroprotective effects of CUR as a probable mechanism against numerous memory impairing agents (see above), the present study was designed to evaluate the novel mechanism of CUR against Mor-induced memory impairment (MMI) and its possible mediation through the CREB-NO pathway.

Materials and Methods

This experimental study was conducted at Kashan University of Medical Sciences (Kashan, Iran), in 2018. The study was approved by the Ethics Committee for Animal Studies (code: IR.KAUMS.MEDNT.REC.1396.26), and all the animal care and behavioral tests were carried out in accordance with the Guide for the Care and Use of Laboratory Animals.

Animals

Forty male Wistar rats (weighing 180–200 g) were housed in polycarbonate cages (four rats in each cage) in the standard living conditions including controlled room temperature (22±2 ºC) with a 12-hour light/dark cycle and relative humidity (40%-60%). During the study, the animals had free access to standard food and water. All the experiments were carried out between 08:00 a.m to 03:00 p.m.

Experimental Protocol

The sample size for the study was calculated using data from a previous study. The rats were randomly assigned into four groups (n=10) and treated as follows: control (CTL), curcumin-pretreatment (CUR), morphine (Mor), and CUR-pretreatment+Mor (CUR+Mor) groups. The CTL group received saline. The rats in the CUR and CUR+Mor groups were pretreated with oral CUR (10 mg/Kg) once a day for 35 days. The Mor and CUR+Mor groups received a post-training intraperitoneal (i.p.) injection of Mor (7.5 mg/Kg/i.p.) immediately after training. A single post-training injection of Mor was administered to the CUR+Mor group one day after the CUR-pretreatment was terminated. Based on the findings of the previous study, the dosage and route of CUR administration were determined. When Mor was injected, the groups of rats that had not received Mor were injected with saline. Similarly, when rats were gavaged with CUR, those rats that had not received Mor were gavaged with saline. Following the termination of behavioral tests (IA memory and open field), the brain tissues of some rats and the hippocampi of the remaining animals in each group were removed for NO metabolites (NOx) assay and western blotting, in respective order (figure 1).

Drugs

The CUR was acquired from Sigma Aldrich.
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All of the drugs were dissolved in sterile saline 0.9% and were freshly prepared in the required concentration. Antibodies directed against phospho-CREB (9198), β-actin (4970), and secondary horse radish peroxidase (HRP)-conjugated (7074) were purchased from Cell Signaling Technology (The Netherlands). The total CREB antibody (sc-186) was obtained from Santa Cruz Biotechnology (USA). Amersham enhanced chemiluminescence (ECL)select™ (RPN2235) reagent kit and polyvinylidene fluoride (PVDF) membrane were purchased from GE Healthcare (USA).

**Inhibitory Avoidance Setup**

Given the findings of the researchers' previous study, step-through IA was utilized for the memory assessment. This apparatus was a two-chambered black/white Plexiglass apparatus (30×30×40 cm) with a grid floor consisting of parallel stainless steel rods (0.3 cm diameter, spaced 1 cm apart). The black and white chambers were separated by a guillotine door. The test consisted of a training session followed by a memory retention session carried out 24 hours after the training. During the training session, each animal was gently placed in a white chamber, and its latency to step through the guillotine door and into the dark chamber with all four paws was measured.

After entering the dark chamber and placing their four paws on the grid floor, an isolated stimulator (Technique Azma, Tabriz, Iran) was used to deliver an electric shock (50 Hz, 3 s, 1 mA). The memory retention session was conducted in the same way as the training session, with the exception that no shock was administered. The latency time (s) to enter the dark chamber was taken as a criterion for measuring memory. For latency time, a cut-off time of 300 seconds was taken. Considering the effect of environmental conditions on plasticity, all of the animals were subjected to the same experimental settings.

**Open Field**

For the locomotor activity, the animals were placed in an open-field apparatus after the completion of their training (on the first day) only for habituation purposes, and the data were collected five minutes after the termination of testing, on the second day, in an open-field chamber using a charge-coupled device video monitoring system (Technique Azma, Tabriz, Iran), as previously described. The total horizontal distance traveled (cm) in the chamber was used to obtain the data on open-field locomotor activity.

**Western Blotting**

Some of the animals were deeply anesthetized by CO₂ inhalation and then decapitated immediately after the termination of the behavioral tests. Their hippocampi (n=3) were isolated on ice over a short period of time and then were stored at -80 °C, until they were ready for molecular experiments. The hippocampi of the rats were quickly weighed and homogenized on the ice at three times the volume/weight of cold radioimmunoprecipitation assay lysis buffer containing protease and phosphatase inhibitor cocktail (Sigma, USA). The lysates were centrifuged at 13,000 rpm for 35 min at 4 °C, and the protein containing the supernatants was collected. The protein concentration was determined using Bradford's method, and bovine serum albumin (BSA) as a reference standard. The samples with equal protein concentrations (20 μg/well) were separated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. After that, the gel electrophoresis was transferred to PVDF membranes. The blots were subsequently blocked in 5% BSA-Tris Buffered Saline with Tween (TBST) and then were probed with primary antibodies (1/4000) overnight at 4 °C. The next day, after washing them with TBST, the blots were incubated at room temperature for 110 minutes with rabbit IgG conjugated to HRP (1/10,000) as the secondary antibody. The membranes were developed using ECL select™.
followed by autoradiography. A densitometric scan of the films was used to quantify the results, and the density of the bands was calculated using Image J software (National Institute of Health, USA).

Nitric Oxide Assay
Following the termination of the behavioral trials, some animals in each group (other than those remaining from the western blot analysis) were sacrificed, and their brains (five right and five left hemispheres) were extracted for NOx evaluation. The NOx assay was performed using the Griess reaction.21 For this purpose, after preparing nitrite standard curves, the brain samples were homogenized using phosphate buffer. Then, 100 μL of the tissue suspensions were added to the Griess reagent, including 100 μL of vanadium (III) chloride (VCl₃), 50 μL of sulfanilamide, and 50 μL of N-(1-Naphthyl) ethylenediamine dihydrochloride. The nitrate was reduced to nitrite using VCl₃. The proteins were subsequently precipitated with the addition of 50 μL of trichloroacetic acid 10%. After a 45-minute incubation period, the contents were centrifuged. The supernatants were then transferred to a 96-well flat-bottom microplate. Absorbance was read at 540 nm using a spectrophotometer (NanoDrop Technologies, USA), and the final values were calculated using nitrite standard curves. NOx concentrations were measured in all the groups.

Statistical Analysis
After ensuring that the data distribution was normal, the collected data were analyzed using Kruskal–Wallis and analysis of variance (ANOVA). Paired group comparisons were performed using Holm-Sidak and Dunn’s post hoc tests. The level of significance was 0.05. All analyses were conducted using SigmaPlot software (version 14.0, Systat Software, Inc. UK).

Results

The Effect of Curcumin and Curcumin+Morphine on Inhibitory Avoidance Memory
The effect of oral CUR pretreatment (10 mg/Kg for 35 days) alone and the effect of CUR+Mor coadministration on IA memory are illustrated in figure 2. The Kruskal–Wallis test showed that, while the post-training administration of Mor (7.5 mg/Kg, i.p.) significantly impaired IA memory (P=0.001), CUR+Mor coadministration prevented the MMI (P=0.075).

Locomotor Activity of the Curcumin and Curcumin+Morphine Groups in the Open Field
Figure 3 shows the effect of oral CUR pretreatment (10 mg/Kg for 35 days) alone on locomotion in the open field, as well as the effect of CUR+Mor coadministration. In this regard, the Kruskal–Wallis test revealed no differences between the groups (P=0.203).
analysis revealed that the p-CREB/CREB ratio was higher in the hippocampus (2.06 fold, \( P=0.012 \)) of the CUR+Mor coadministration group than the Mor group (figure 4a). As shown in figure 4b, the one-way ANOVA revealed that the post-training Mor group (7.5 mg/Kg, i.p.) had a lower level of p-CREB (70.55 \%, \( P=0.001 \)) than the CTL group (\( P=0.001 \)). However, the one-way ANOVA revealed a significant difference in total CREB concentration between the groups (\( P=0.011 \)). Moreover, the Holm-Sidak post hoc test revealed that the concentration of total CREB in the Mor group was higher than in the CTL group (1.54 fold, \( P=0.012 \)).

Discussion

The behavioral investigation results demonstrated that step-through latency decreased in the Mor-treated rats compared to the control group, which is indicative of MMI. The impairing effect of post-training Mor has been extensively studied in different memory paradigms, most notably in the researchers’ previous study.\(^2\)\(^2\)

Moreover, in the present study, while post-training Mor administration alone impaired IA memory, CUR+Mor coadministration prevented MMI. For the first time, the current findings demonstrated that CUR prevented IA memory in rats subjected to MMI. Liu and others administered the same dose of oral CUR over the same treatment period and reported a similar effect, which was consistent with the current findings.\(^9\) Likewise, CUR were shown
to have a preventative effect in a large number of studies, both in the short-term and long-term doses. Sarlak and colleagues, on the other hand, used low to moderate doses of CUR (5 and 15 mg/Kg, i.p.) in an IA model and reported no significant effects on rats’ memory. Since the latter study used a single i.p. administration, and the current study used an oral pretreatment of CUR for 35 days, it seems that, besides the route of administration, the duration of CUR administration is also a factor in this discrepancy. Furthermore, in the current study, the non-significant result of open field locomotor activity ruled out the possibility that our observed effects of CUR and Mor or CUR+Mor could not be secondary to the effects of Mor on general motor behavior.

Considering the ceiling effect on memory performance as a constraint to show the memory-reversing effect of some drugs, in the present study, memory impairment was induced by Mor to observe the preventive effect of CUR. Given the fact that no significant preventive effect of CUR has been reported in intact animals with the absence of memory impairing agents, evidence suggests that CUR-induced prevention mostly occurs when the intact memory were previously impaired by a memory impairing agent.

Furthermore, when comparing the Mor group to the control group, the western blot analyses in this study revealed a significant reduction in p-CREB/CREB ratios in the Mor group. CUR is found to play a regulatory role in the alteration of p-CREB/CREB ratios in the Mor group. Since the latter study used a single i.p. administration, and the current study used an oral pretreatment of CUR for 35 days, it seems that, besides the route of administration, the duration of CUR administration is also a factor in this discrepancy. Furthermore, in the current study, the non-significant result of open field locomotor activity ruled out the possibility that our observed effects of CUR and Mor or CUR+Mor could not be secondary to the effects of Mor on general motor behavior.

In agreement with the current findings, Akbarabadi and others also found that Mor administration decreased the hippocampal p-CREB expression. Following acute Mor administration, Gago and others reported a reduction in p-CREB in the medial part of the caudate. Given that evidence signifies alterations in p-CREB in different brain regions following Mor administration, the findings of this study suggest that the unequal distribution of the proteins involved in the effective phosphorylation of CREB may have a role in this difference. There is some evidence that supports this suggestion. For instance, as the main upstream kinase of CREB that also plays a role in CREB phosphorylation, learning, and memory, calcium-calmodulin kinase II (CaMKII) is highly distributed in the hippocampus and comprises nearly 2% of the total proteins.

To the best of the researchers’ knowledge, few studies on the subject of learning reported the regulation of CREB phosphorylation associated with Mor. Few studies have been done on the effect of Mor on CREB in conditioning paradigms, e.g., conditioned place preference. 

Meanwhile, the present study suggests a potential link between MMI and CREB phosphorylation regulation as a possible preventive mechanism for CUR. Nevertheless, further studies are needed to clarify the role of CUR in the CREB signaling pathway. The present study found that CUR inhibits MMI-induced memory loss in rats in the IA test. These findings indicated that the CREB signaling pathway was involved in memory impairment prevention by CUR and that Mor down-regulation of CREB phosphorylation led to memory deficits. As a result of a CUR-induced increase in CREB, Nam and colleagues reported cognitive improvement in aged mice.

Furthermore, the NOx assay results revealed decreased NOx concentrations only in the Mor group rather than the control group, and a significant increase was also observed in the CUR+Mor coadministration group compared to the Mor group. Mor-induced NOx and memory responses were also in the same direction. Accordingly, changes in NO production by different NO inducers and inhibitors affected the Mor response to memory, and the interaction between Mor and NO modulated learning and memory in the brain. In agreement with this finding, Farahmandfar and others found that the coadministration of L-arginine, as a NO precursor and pre-training Mor prevented MMI demonstrating the prevention of MMI through increased NO production. Given the fact that Mor increases and decreases the GTPase- and cGMP-related protein kinases (e.g., CAMKII) upon binding to inhibitory G-proteins, one may hypothesize that Mor-induced inhibition of adenylate cyclase decreases the cAMP; and the reduced Ca+ entry into cells thus dissociates the Ca+-calmodulin complex. By this hypothetical pathway, Mor may inhibit the neuronal NO synthase activity, resulting in reduced NO production. In support of these findings, some research demonstrated that NO mediates CUR effects. According to Yu and others, CUR prevented the memory impairment induced by aging in mice by increasing NO concentrations. Similarly, Zhu and others reported that increased NO production in the hippocampus by CUR resulted in impairment prevention via the cGMP/PKG pathway.

The failure to work on the molecules involved
in the p-CREB upstream or downstream signaling pathway is one of the limitations of this study. Working on such molecules may help researchers better understand the mechanism of CUR in Mor-induced p-CREB alteration. In addition, while this study did not use some NO inducers and inhibitors to firmly confirm the role of NO signaling, it is a preliminary work that shows the initial contribution of signaling in the process. Obviously in the next phase, understanding the details of this mechanism requires more pharmacological and/or molecular confirmations.

**Conclusion**

The present findings suggested the existence of a CUR, CREB, and NO interaction that inhibited MMI in IA memory models. In addition, targeting the NO-CREB signaling pathway may represent an interesting approach for the development of new CUR-derived drugs to prevent memory impairments caused by Mor administration.

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**Authors’ Contribution**

K.K: Performing behavioral, biochemical, and molecular experiments, artwork and data analysis; B.A: Study design, performing molecular experiments, artwork and data analysis, drafting the manuscript; A.H: Biochemical experiments; A.A: Study design, artwork and data analysis. All authors were involved in critically revising the present version and made a notable contribution to the final revision of the manuscript. All authors approved the present version of the manuscript. All authors agreed on being accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

**Conflict of Interest:** None declared.

**References**

1. Yan D, Yao J, Liu Y, Zhang X, Wang Y, Chen X, et al. Tau hyperphosphorylation and P-CREB reduction are involved in acrylamide-induced spatial memory impairment: Suppression by curcumin. Brain Behav Immun. 2018;71:66-80. doi: 10.1016/j.bbi.2018.04.014. PubMed PMID: 29704550.

2. Issuriya A, Kumarnsit E, Wattanapiromsakul C, Vongvatcharan U. Histological studies of neuroprotective effects of Curcuma longa Linn. on neuronal loss induced by dexamethasone treatment in the rat hippocampus. Acta Histochem. 2014;116:1443-53. doi: 10.1016/j.acthis.2014.09.009. PubMed PMID: 25440530.

3. Shabaninejad Z, Pouranifeh MH, Movahedpour A, Mottaghi R, Nickdasti A, Mortezapour E, et al. Therapeutic potentials of curcumin in the treatment of glioblastoma. Eur J Med Chem. 2020;188:112040. doi: 10.1016/j.ejmech.2020.112040. PubMed PMID: 31927312.

4. Lobos P, Cordova A, Vega-Vasquez I, Ramirez OA, Adasme T, Toledo J, et al. RyR-mediated Ca(2+) release elicited by neuronal activity induces nuclear Ca(2+) signals, CREB phosphorylation, and Npas4/RyR2 expression. Proc Natl Acad Sci U S A. 2021;118:110. doi: 10.1073/pnas.2102265118. PubMed PMID: 34389673; PubMed Central PMCID: PMCPMC8379958.

5. Motaghinejad M, Motevalian M, Fatima S, Faraji F, Mozaffari S. The Neuroprotective Effect of Curcumin Against Nicotine-Induced Neurotoxicity is Mediated by CREB-BDNF Signaling Pathway. Neurochem Res. 2017;42:2921-32. doi: 10.1007/s11064-017-2323-8. PubMed PMID: 28608236.

6. Eun CS, Lim JS, Lee J, Lee SP, Yang SA. The protective effect of fermented Curcuma longa L. on memory dysfunction in oxidative stress-induced C6 gliomal cells, proinflammatory-activated BV2 microglial cells, and scopolamine-induced amnesia model in mice. BMC Complement Altern Med. 2017;17:367. doi: 10.1186/s12906-017-1880-3. PubMed PMID: 28716085; PubMed Central PMCID: PMCPMC5514491.

7. Malboosi N, Nasehi M, Hashemi M, Vaseghi S, Zarrindast MR. The neuroprotective effect of NeuroAid on morphine-induced amnesia with respect to the expression of TFAM, PGC-1alpha, DeltfamosB and CART genes in the hippocampus of male Wistar rats. Gene. 2020;742:144601. doi: 10.1016/j.gene.2020.144601. PubMed PMID: 32198124.

8. Zarrindast MR, Ardjmand A, Rezayof A, Ahmadi S. The time profile of morphine effect on different phases of inhibitory avoidance memory in rat. Arch Iran Med. 2013;16:34-7. PubMed PMID: 23273234.

9. Baudonnat M, Guilou JL, Husson M, Bohbot VD, Schwabe L, David V. Morphine Reward Promotes Cue-Sensitive Learning: Implication of Dorsal Striatal CREB Activity.
Inhibitory memory prevention by curcumin via NO/CREB

Front Psychiatry. 2017;8:87. doi: 10.3389/fpsyt.2017.00087. PubMed PMID: 28611691; PubMed Central PMCID: PMC5447690.

Mohamed RMP, Kumar J, Yap E, Mohamed IN, Sidi H, Adam RL, et al. Try to Remember: Interplay between Memory and Substance Use Disorder. Curr Drug Targets. 2019;20:158-65. doi: 10.2174/138945011866170622092824. PubMed PMID: 28641520.

Muntaz F, Rashki A, Imran Khan M, Shadboorestan A, Abdollahi A, Ghazi-Khansari M, et al. Neuroprotective effect of sumatriptan in pentylenetetrazole-induced seizure is mediated through N-methyl-D-aspartate/nitric oxide and cAMP response element-binding protein signaling pathway. Fundam Clin Pharmacol. 2022;36:250-61. doi: 10.1111/fcp.12728. PubMed PMID: 34545607.

Longobardi C, Damiano S, Andretta E, Prisco F, Russo V, Pagnini F, et al. Curcumin Modulates Nitrosative Stress, Inflammation, and DNA Damage and Protects against Ochratoxin A-Induced Hepatotoxicity and Nephrotoxicity in Rats. Antioxidants (Basel). 2021;10. doi: 10.3390/antiox10081239. PubMed PMID: 34439487; PubMed Central PMCID: PMCPMC8389288.

Banafshe HR, Mohsenpour M, Ardjmand A. Effects Following Intracerebroventricular Injection of Immunosuppressant Cyclosporine A On Inhibitory Avoidance Learning and Memory in Mice. Galen Med J. 2018;7:e1044. doi: 10.22086/gmj.v0i0.1044. PubMed PMID: 34439487; PubMed Central PMCID: PMCPMC8389288.

Schrader M, Jarrett BJM, Kilner RM. Larval environmental conditions influence plasticity in resource use by adults in the burying beetle, Nicrophorus vespilloides. Evolution. 2022;76:667-74. doi: 10.1111/evo.14339. PubMed PMID: 34463348; PubMed Central PMCID: PMC59293066.

Ainalighipour A, Mazoochi T, Ardjmand A. Low-dose ethanol ameliorates amnesia induced by a brief seizure model: the role of NMDA signaling. Neurol Res. 2019;41:624-32. doi: 10.1080/01616412.2019.1602323. PubMed PMID: 30967097.

Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248-54. doi: 10.1006/abio.1976.9999. PubMed PMID: 942051.

Esmaili Z, Heydari A. Effect of acute caffeine administration on PTZ-induced seizure threshold in mice: Involvement of adenosine receptors and NO-cGMP signaling pathway. Epilepsy Res. 2019;149:1-8. doi: 10.1016/j.eplepsyres.2018.10.013. PubMed PMID: 30391360.

Tavassoli M, Ardjmand A. Pentylenetetrazol and Morphine Interaction in a State-dependent Memory Model: Role of CREB Signaling. Basic Clin Neurosci. 2020;11:557-72. doi: 10.32598/bcn.11.4.1482.1. PubMed PMID: 33613894; PubMed Central PMCID: PMCPMC7878041.

Gupta SC, Patchva S, Aggarwal BB. Therapeutic roles of curcumin: lessons learned from clinical trials. AAPS J. 2013;15:195-218. doi: 10.1208/s12248-012-9432-8. PubMed PMID: 23143785; PubMed Central PMCID: PMCPMC3535097.

Moore TL, Bowley B, Shultz P, Calderazzo S, Shobin E, Killiyan RJ, et al. Chronic curcumin treatment improves spatial working memory but not recognition memory in middle-aged rhesus monkeys. Geroscience. 2017;39:571-84. doi: 10.1007/s11357-017-9998-2. PubMed PMID: 29047012; PubMed Central PMCID: PMCPMC5745216.

Sarla Z, Oryan S, Moghaddasi M. Interaction between the antioxidant activity of curcumin and cholinergic system on memory retention in adult male Wistar rats. Iran J Basic Med Sci. 2015;18:398-403. PubMed PMID: 26019804; PubMed Central PMCID: PMCPMC4439456.

Venkatesan UM, Rabinowitz AR, Riccitello RM. Breaking the Percent Memory Retention Ceiling using Bayesian Statistics. J Int
Neuropsychol Soc. 2021;27:396-400. doi: 10.1017/S1355617720000892. PubMed PMID: 33012298.

27 Akinyemi AJ, Oboh G, Oyeleye SI, Ogun-suyi O. Anti-amnestic Effect of Curcumin in Combination with Donepezil, an Anticholinesterase Drug: Involvement of Cholinergic System. Neurotox Res. 2017;31:560-9. doi: 10.1007/s12640-017-9701-5. PubMed PMID: 28102474.

28 Sevastre-Berghian AC, Fagarasan V, Toma VA, Baldea I, Olteanu D, Moldovan R, et al. Curcumin Reverses the Diazepam-Induced Cognitive Impairment by Modulation of Oxidative Stress and ERK 1/2/NF-kappaB Pathway in Brain. Oxid Med Cell Longev. 2017;2017:3037876. doi: 10.1155/2017/3037876. PubMed PMID: 29098059; PubMed Central PMCID: PMC5643119.

29 Srivastava P, Dhuriya YK, Kumar V, Srivastava A, Gupta R, Shukla RK, et al. PI3K/Akt/GSK3beta induced CREB activation ameliorates arsenic mediated alterations in NMDA receptors and associated signaling in rat hippocampus: Neuroprotective role of curcumin. Neurotoxicology. 2018;67:190-205. doi: 10.1016/j.neuro.2018.04.018. PubMed PMID: 29723552.

30 Guitart X, Thompson MA, Mirante CK, Greenberg ME, Nestler EJ. Regulation of cyclic AMP response element-binding protein (CREB) phosphorylation by acute and chronic morphine in the rat locus coeruleus. J Neurochem. 1992;58:1168-71. doi: 10.1111/j.1471-4159.1992.tb09377.x. PubMed PMID: 1531356.

31 Akbarabadi A, Sadat-Shirazi MS, Kabbaj M, Nouri Zadeh-Tehrani S, Khalifeh S, Pirri F, et al. Effects of Morphine and Maternal Care on Behaviors and Protein Expression of Male Offspring. Neuroscience. 2018;466:58-76. doi: 10.1016/j.neuroscience.2020.04.011. PubMed PMID: 33915201.

32 Gago B, Suarez-Boomgaard D, Fuxe K, Brene S, Reina-Sanchez MD, Rodriguez-Perez LM, et al. Effect of acute and continuous morphine treatment on transcription factor expression in subregions of the rat caudate putamen. Marked modulation by D4 receptor activation. Brain Res. 2011;1407:47-61. doi: 10.1016/j.brainres.2011.06.046. PubMed PMID: 21782156.

33 Wang DM, Yang YJ, Zhang L, Zhang X, Guan FF, Zhang LF. Naringin Enhances CaMKII Activity and Improves Long-Term Memory in a Mouse Model of Alzheimer's Disease. Int J Mol Sci. 2013;14:5576-86. doi: 10.3390/ijms14035576. PubMed PMID: 23478434; PubMed Central PMCID: PMC3634479.

34 Alghamdi BS, Alshehri FS. Melatonin Blocks Morphone-Induced Place Preference: Involvement of GLT-1, NF-kappaB, BDNF, and CREB in the Nucleus Accumbens. Front Behav Neurosci. 2021;15:762297. doi: 10.3389/fnbeh.2021.762297. PubMed PMID: 34720901; PubMed Central PMCID: PMCPMC8551802.

35 Nam SM, Choi JH, Yoo DY, Kim W, Jung HY, Kim JW, et al. Effects of curcumin (Curcuma longa) on learning and spatial memory as well as cell proliferation and neuroblast differentiation in adult and aged mice by upregulating brain-derived neurotrophic factor and CREB signaling. J Med Food. 2014;17:641-9. doi: 10.1089/jmfd.2013.2965. PubMed PMID: 24712702; PubMed Central PMCID: PMCPMC4060834.

36 Farahmandfar M, Kadivar M, Naghdi N. Possible interaction of hippocampal nitric oxide and calcium/calmodulin-dependent protein kinase II on reversal of spatial memory impairment induced by morphine. Eur J Pharmacol. 2015;751:99-111. doi: 10.1016/j.ejphar.2015.01.042. PubMed PMID: 25661847.

37 Al-Hasani R, Bruchas MR. Molecular mechanisms of opioid receptor-dependent signaling and behavior. Anesthesiology. 2011;115:1363-81. doi: 10.1097/ALN.0b013e318238bba6. PubMed PMID: 22020140; PubMed Central PMCID: PMCPMC3698859.

38 Pigott BM, Garthwaite J. Nitric Oxide Is Required for L-Type Ca(2+) Channel-Dependent Long-Term Potentiation in the Hippocampus. Front Synaptic Neurosci. 2016;8:17. doi: 10.3389/fnsyn.2016.00017. PubMed PMID: 27445786; PubMed Central PMCID: PMCPMC4925670.

39 Yu SY, Gao R, Zhang L, Luo J, Jiang H, Wang S. Curcumin ameliorates ethanol-induced memory deficits and enhanced brain nitric oxide synthase activity in mice. Prog Neuropsychopharmacol Biol Psychiatry. 2013;44:210-6. doi: 10.1016/j.pnpbp.2013.03.001. PubMed PMID: 23500667.

40 Zhu W, Su J, Liu J, Jiang C. The involvement of neuronal nitric oxide synthase in the anti-epileptic action of curcumin on pentylentetrazol-kindled rats. Biomed Mater Eng. 2015;26:S841-50. doi: 10.3233/BME-151376. PubMed PMID: 26406082.