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Curcumin Attenuates Development of Depressive-Like Behavior in Male Rats after Spinal Cord Injury: Involvement of NLRP3 Inflammasome

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Abstract

Objectives: The purpose of this study is investigating curcumin role in mood alterations in male rats after spinal cord injury through focusing on the involvement of NLRP3 inflammasome.

Methods: Fourteen adualt male Wistar rats (220–250 g) were divided to five animal groups (n = 8 per group): Control: healthy animals which received normal saline for 14 days; spinal cord injury: spinal cord injury-induced animals which received normal saline for 14 days; spinal cord injury + curcumin (20, 40, and 80 mg/kg/ i.p): spinal cord injury-induced animals treated with 3 doses of curcumin for 14 days. To assess the mood of animals, the elevated plus maze test, forced swimming test, tail suspension test, and open field test were performed. Graph-pad prism software was used for data analysis. Statistical analysis was done by one-way ANOVA with Tukey's post hoc test. $P < 0.05$ was statistically significant.

Results: Treatment with curcumin with two doses of 40 and 80 mg/kg increased open arm time and decreased close arm time as compared to the spinal cord injury group. The administration of curcumin (40 mg/kg and 80 mg/kg) enhanced the altered behaviors. Spinal cord injury caused an increase in the protein levels NLRP3, ASC and Casp1 in the hippocampus of animals as compared to the sham group. Curcumin regulated the protein levels of NLRP3, ASC and Casp1 in the hippocampus of animals.

Conclusion: Depression is prevalent in person with spinal cord injury and our findings indicated that curcumin appears to constitute a suitable agent to reduce neuroinflammation and through it, relieve a depressive-like state.

Keywords: Spinal cord injuries, depression, curcumin, hippocampus, inflammasome.

Introduction

Spinal cord injury, a damage to spinal cord, causes changes in its function.[1,2] This condition may occur due to traumatic accidents like sport related injuries, falls, car accident (acute or traumatic spinal cord injury) or due to illnesses like tumors, infections, degenerative disease, and myelitis (chronic or non-traumatic spinal cord injury).[3-5] Traumatic spinal cord injury includes two phase, primary and secondary phases. In the primary phase, the cells are damaged and this damage causes the initiation of secondary phase events like inflammation and stress oxidative.[6-8] Interlukine18, Interlukine1β, and Tumor Necrosis Factor α are important pro inflammatory factors and these factors play important role in inflammation of central nervous system.[9,10] The release of these factors is due to activation of inflammasome complex, specially NLRP3 inflammasome a protein complex with three subdivisions, including NLRP3, ASC, and Caspase1.[11-13] In addition to physiological injuries after injury, patients may experience mental disorders.[14] Depression, a psychiatric disorder with a highly complicated pathophysiology, is one of the important results of SCI.[15,16] According to previous studies, 18.7% to 26.3% of patients with SCI suffer from clinical symptoms of depression.[16,17] These clinical symptoms reduce their participation in activities, resulting in lower life satisfaction.[18,19] The increase in the incidence of depression after SCI may not come as a surprise given the grueling changes in life caused by the injury, but these changes have been seen in animal models of SCI, in the absence of social, financial problems or changing in their functional independence.[20,21] Symptomes of depression in animal models after injury suggests that intrinsic physiological or molecular changes in SCI may play an important role in the development of depression.[22,23] It has been demonstrated that patients with chronic inflammatory disease are more prone to depression.[24,25] Inflammatory cytokines have not only been existed in depressed patients, but have also been shown to cause depression if prescribed for other conditions, such as cancer and hepatitis.[26] Chronic inflammatory diseases are associated with symptoms of depression.[27] In fact, the endocrine and nervous systems are affected by chronic inflammation, and this causes an imbalance in neurological compounds and hormones.[28,29] Changes in these compounds may eventually affect behavior and contribute to depressive symptoms.[30] Due to the contribution of inflammation in the pathogensis of SCI, which is accompanied by increases in the systemic and brain (especially hippocampus) levels of pro-inflammatory cytokines.[31-35]

According to previous studies targeting inflammation may be effective in the treatment of depression.[36,37] Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is a polyphenol, nonsteroidal extracted of *Curcuma longa* species. It has been used as dietary spice and as a traditional Indian medicine.[38,39] Curcumin (CuC) is a multifunctional drug and it has a lot of pharmacologic effects, like

anti-inflammatory, anti-oxidant, and anti-infectious activities.[40] Studies have reported that CuC improves neurological defects by inhibiting apoptosis and neuronal cell death and reducing inflammation after SCI.[41,42] Considering the role of CuC in reducing inflammation after SCI and the role of NLRP3 inflammasome in starting of inflammation and also the role of the inflammatory process in depression after SCI, the purpose of this study is investigating curcumin role in mood alterations in male rats after SCI through focusing on the involvement of NLRP3 inflammasome.

Materials and Methods

Animals

Fourteen adualt male Wistar rats were (220–250 g) purchased from the animal laboratory of Tehran University of Medical Sciences (TUMS), Tehran, Iran. All rats were kept in standard condition (12 h light/dark cycles and controlled temperature of 22–24°C) with free access to water and food. There were five animal groups (n = 8 per group): Control: healthy animals which received normal saline for 14 days; SCI (Model): SCI-induced animals which received normal saline for 14 days; SCI + CuC (20 mg/kg/ i.p.): SCI-induced animals treated with CuC (20 mg/kg/ i.p.) for 14 days. SCI + CuC (40 mg/kg/ i.p.): SCI-induced animals treated with CuC (40 mg/kg/ i.p.). SCI + CuC (80 mg/kg/ i.p.): SCI-induced animals treated with CuC (80 mg/kg/ i.p.). This test was approved by the TUMS Animal Ethics Association (IR.TUMS.SPH.REC.1400.160). This method is summarized in Figure 1.

Spinal Cord Injury

For all groups, Adualt male rats were anesthetized with i.p. administration of ketamine/xylazine (80/15 mg/kg). After shaving, cleaning and disinfection of the surgery site, Laminectomy of T8-T9 level was done with a micro rongeur (Fine Science Tools, USA). SCI Extradural clip compression was performed by closing the aneurysm clip (Aesculap, Tuttlingen, Germany) with a 90 g calibrated closing force on right side of the spinal cord for 1 minute. After surgery, the surgical site was sutured, and rats were recovered on a 30°C heating pad, for rehydrating and preventing urinary tract infection, the rats received saline (2 ml, twice a day, subcutaneous) and Cefazoline (40 mg/kg twice on the day of surgery, i.p.). Manual urination was performed twice a day until the bladder emptying reflex improved.

Behavior Tests

To assess the mood of animals, The elevated plus maze (EPM) test, forced swimming test (FST), tail suspension test (TST), and open field test (OFT) were performed. All tests were performed according to the programs shown in Figure 1 by trained researchers who were blind to the study.

Elevated plus maze test

For measearing anxiety the EPM test was done using a plus-shaped (+) apparatus with two open (50 × 10 cm) and two enclosed arms (50 × 10 × 30 cm). Each rat was placed alone in the center of the maze, it was faced to one of the open arms, and its behavior was screened for 5 minutes. Parameters were recorded, including closed/open arm time.

Forced swimming test

For assessing depressive-like behaviors FST was done in the first day for training the test to the rats, the rats were forced to swim in cylindrical shape glass (80 cm height & 30 cm diameter) which filled with 40 cm of water (temperature 23 ± 2°C). the next day, the experiment was performed again for 4 minutes as final test and each animal's behavior was screened and recorded. The duration time of immobility, swimming, and climbing were calculated for the last 3 min of 4 min.

Open field test

Usually OFT is used For examining depression in animals.[37] The open field apparatus (100 × 100 × 50 cm) consisted of one floor with 16 equal squares. Each rat was placed in the center of the device and allowed to stand freely for 5 minutes. Behaviors were recorded and delays in central and peripheral areas were calculated.

Tail suspension test

TST is one of the other tests for assesing depressive-like behaviors. Accordingly, each rat was individually suspended by the tail from the height of 50 cm bove the ground for 6 minutes and the duration of immobility was calculated for 4 min of 6 min.

Tissue Preparation

After extraction of each rat brain. The HC was isolated and it has been kept at −80°C. The resulting tissue samples were used for total RNA extraction (n = 3 in each group) and ELISA technique (n = 4 in each group).

Quantitative Real time-PCR

The total RNA was extracted by using TriPure Isolation reagent (Takara Bio Inc., Otsu, Japan). Based on the manufacturer's instructions, 1 µg RNA was carried out for determination of reverse transcription using cDNA PrimeScript RT Reagent

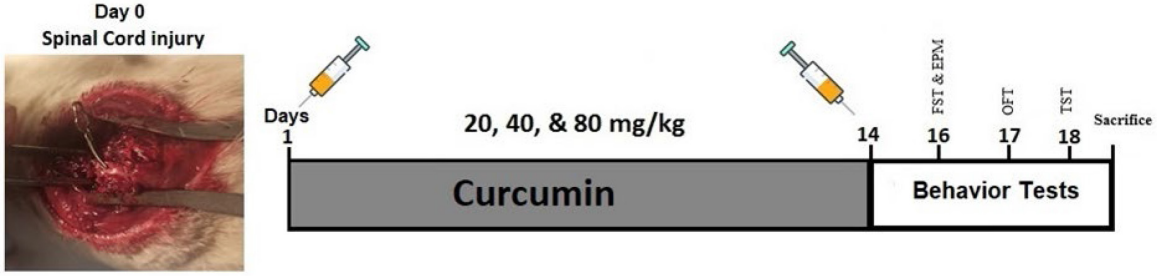

Fig. 1 **Study design.**

Kit (Takara Bio Inc.). qRT-PCR was done using SYBR-Green kit (Takara Bio Inc.) and a Cycler (Light Cycler 2.0, Roche) based on manufacturer's instructions. The levels of relative expression were measured by 2−ΔΔCt formula. Normalization was performed using endogenous. β2-microglobulin (B2M) was used as housekeeping gene. The primers used in experiments are given in Table 1.

Histopathological Investigation

After anesthetization the rats were perfused with 4% paraformaldehyde in phosphate buffer (pH 7.6). Then the brains extracted and fixed with 10% formalin. Histological processings were done and the samples were embedded in paraffin.[43,44] Five micron-thick sections were preapered and mounted on slides for nissl staining. After that the dead neurons which were characterized by karyolitic, karyorectic nucleus were evaluated under light microscop for understanding. The percentage of dark neurons in CA1, CA3, and Dentate gyrus.

Statistics

Mean ± standard deviation was used to express data. Graph-pad prism software (ver. 6) was used for data analysis. Statistical analysis was done by one-way ANOVA with Tukey's post hoc test. $P < 0.05$ was statistically significant.

Results

Anxiety and Depressive-Like Behaviors

SCI significantly increased anxiety and depression in CCI model.

Elevated plus maze test

According to the EPMT, SCI significantly decreased delay in the open arm ($P < 0.05$, Fig. 2A) and increased delay in the close arm ($P < 0.05$, Fig. 2B) in the SCI, SCI + CuC20, SCI + CuC40, and SCI + CuC80 groups compared to Sham group. Improved EPMT results were reported in SCI + CuC40, and SCI + CuC80 groups compared to SCI group ($P < 0.05$, Fig. 2A and 2B).

Forced swimming test

Results from the FST showed that SCI decreased swimming time ($P < 0.05$, Fig. 3A) and increased immobility time

Table 1. **Primers**

		Primers	Product size (bp)	Melting temperature (°C)
B2m (Reference gene)	Forward	CTTTCTACATCCTGGCTCACAC	151	82.8
	Reverse	GTCCAGATGATTCAGAGCTCC		
CASP1	Forward	CCACTCGTACACGTCTTGC	209	86.1
	Reverse	GTCAGAAGTCTTGTGCTCTGG		
NLRP3	Forward	CTGACCCATAACCAGAGCCTCC	196	83.2
	Reverse	CAGTCAGCTCAGGCTTTTCCTC		
ASC	Forward	CTCGTCAGCTACTATCTGGAGG	141	84.7
	Reverse	AGGGACACTGGTTGCAGTAG		

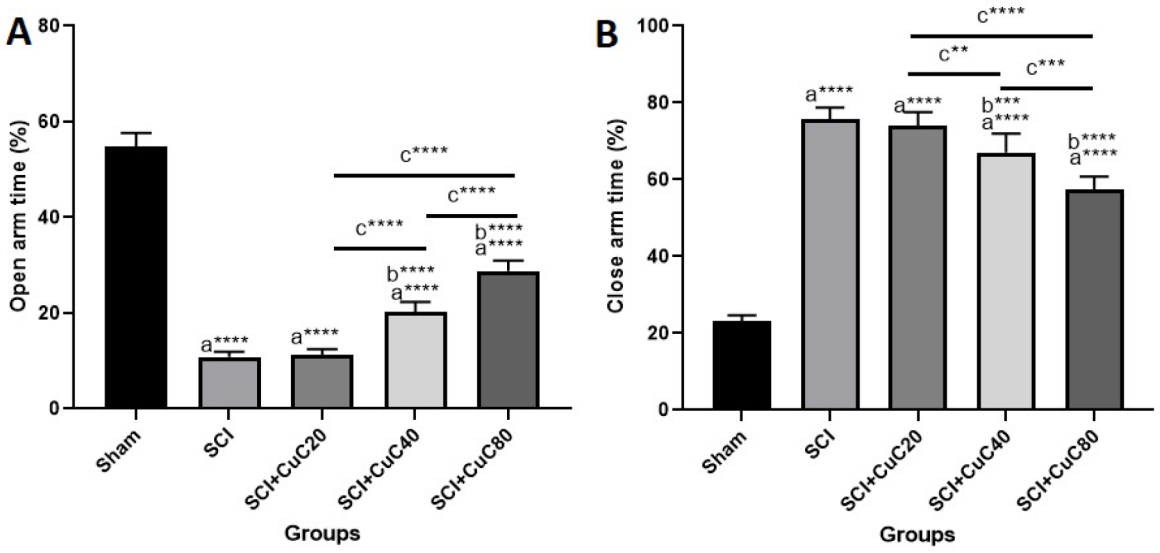

Fig. 2 **Effects of CuC on anxiety-like behaviors (elevated plus maze test) in the SCI-induced rats. Mean percentage of A) Time spent in open arm and B) Time spent in close arm (%). Values: mean ± SEM ($n = 3$). *$P < 0.05$, **$P < 0.001$, ***$P < 0.001$, & ****$P < 0.0001$; *a* vs. the Sham group; *b* 0.001 vs. the SIS group, and *c* between different doses of CuC. Sham: sham operated animals received normal saline for 14 days; SCI: SCI-induced animals received normal saline for 14 days; SCI + CuC20, 40, or 80: SCI-induced animals received CuC with doses of 20, 40, or 80 mg/kg, respectively.**

($P < 0.05$, Fig. 3B) in SCI, SCI + CuC20, SCI + CuC40, and SCI + CuC80 groups compared to the Sham group. The administration of CuC (40 mg/kg and 80 mg/kg) significantly improved the altered behaviors ($P < 0.05$, Fig. 3A and 3B).

Open field test

Based on OFT findings, SCI significantly reduced delay in the central zone ($P < 0.05$, Fig. 4A) and enhanced delay in the peripheral zone ($P < 0.05$, Fig. 4B) in SCI, SCI + CuC20, SCI + CuC40, and SCI + CuC80 groups compared to the Sham group. Improved OFT values were seen in SCI + CuC40, and SCI + CuC80 groups compared to the CCI group ($P < 0.05$, Fig. 4A and 4B).

Tail suspension test

Results obtained from TST showed that SCI enhanced immobility time ($P < 0.05$, Fig. 5) in the SCI, SCI + CuC20, SCI + CuC40, and SCI + CuC80 groups compared to the Sh group. LUT 25 and 50 mg/kg significantly improved the altered behaviors ($P < 0.05$, Fig. 5).

Gene Expression of NLRP3 Inflammasome Components in the HC

An enhanced gene expression of NLRP3 ($P < 0.05$, Fig. 6A), ASC ($P < 0.05$, Fig. 6B) and Casp1 ($P < 0.05$, Fig. 6C) was reported in the HC of animals in SCI, SCI + CuC20, SCI + CuC40, and SCI + CuC80 groups compared to the Sham

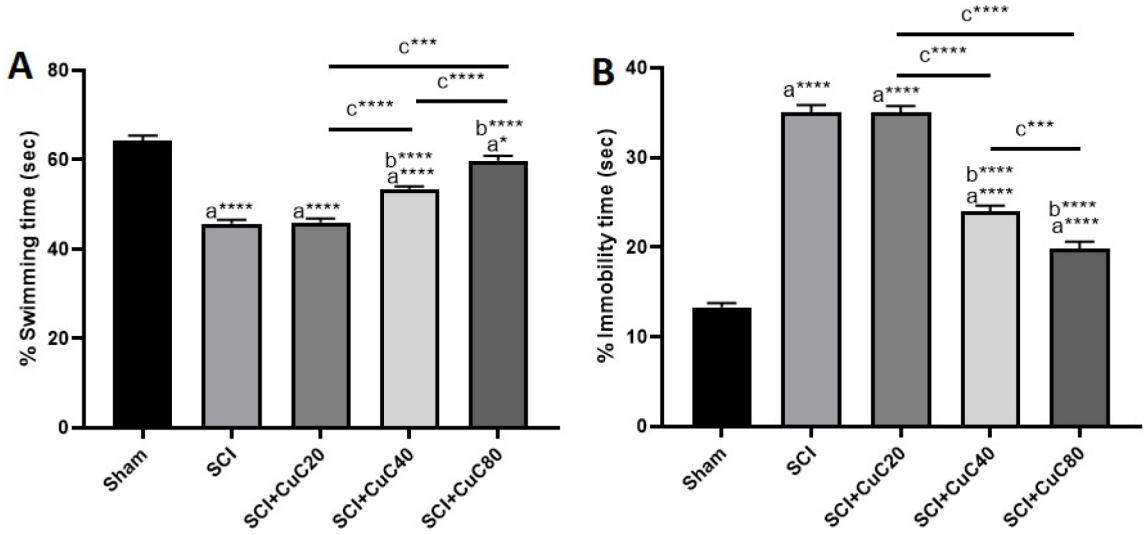

Fig. 3 **Effects of CuC on depressive-like behaviors (forced swimming test) in the SCI-induced rats. Mean percentage of A) Swimming time and B) Immobility time. Values: mean ± SEM ($n = 3$). *$P < 0.05$, **$P < 0.001$, ***$P < 0.001$, & ****$P < 0.0001$; *a* vs. the Sham group; *b* 0.001 vs. the SIS group, and *c* between different doses of CuC. Sham: sham operated animals received normal saline for 14 days; SCI: SCI-induced animals received normal saline for 14 days; SCI + CuC20, 40, or 80: SCI-induced animals received CuC with doses of 20, 40, or 80 mg/kg, respectively.**

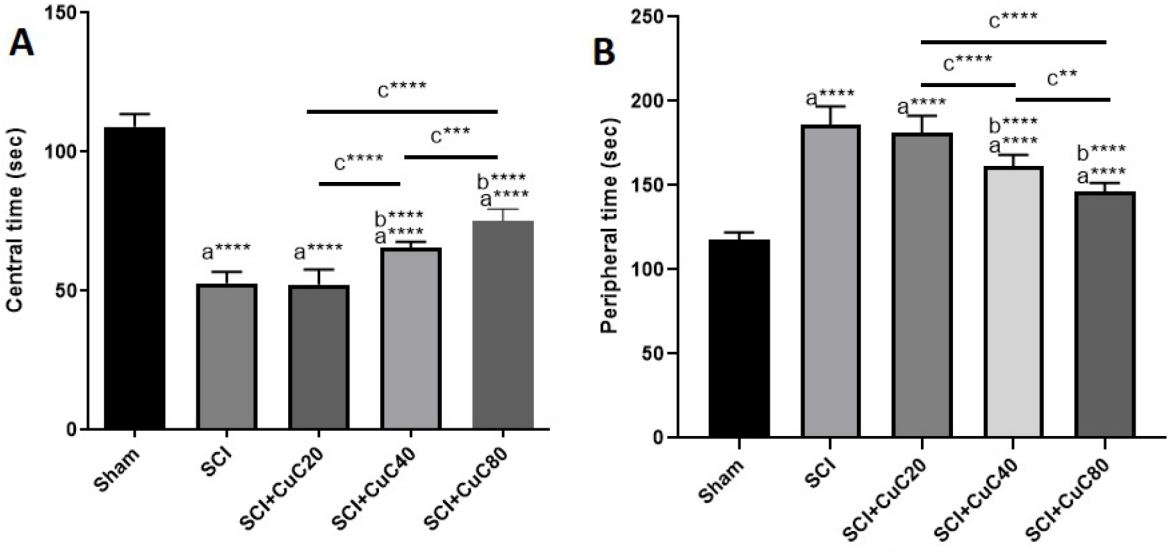

Fig. 4 **Effects of CuC on depressive-like behaviors (open field test) in the SCI-induced rats. A) Delay in the central zone and B) Delay in the peripheral zone. Values: mean ± SEM ($n = 3$). *$P < 0.05$, **$P < 0.001$, ***$P < 0.001$, & ****$P < 0.0001$; *a* vs. the Sham group; *b* 0.001 vs. the SIS group, and *c* between different doses of CuC. Sham: sham operated animals received normal saline for 14 days; SCI: SCI-induced animals received normal saline for 14 days; SCI + CuC20, 40, or 80: SCI-induced animals received CuC with doses of 20, 40, or 80 mg/kg, respectively.**

group. The treatment with CUC (40 mg/kg & 80 mg/kg) regulated the gene expression of NLRP3 ($P < 0.05$, Fig. 6A), ASC ($P < 0.05$, Fig. 6B) and Casp1 ($P < 0.05$, Fig. 6C) in the HC of animals.

Histopathological Alterations in the HC

Exposure of animals to SCI enhanced the percentage of dark neurons in the hippocampal CA1 ($P < 0.05$, Fig. 7A and 7B), CA3 ($P < 0.05$, Fig. 7A and 7C), and DG ($P < 0.05$, Fig. 7A and 7D) of SCI, SCI + CuC20, SCI + CuC40, and SCI + CuC80 groups compared to the Sham group. Treatment of animals with CuC (40 mg/kg & 80 mg/kg) significanltly increased cell survival in the CA1 ($P < 0.05$, Fig. 7A and 7B), CA3 ($P < 0.05$, Fig. 7A and 7C), and DG ($P < 0.05$, Fig. 7A and 7D) of SCI + CuC40 and SCI + CuC80 groups compared to the SCI group.

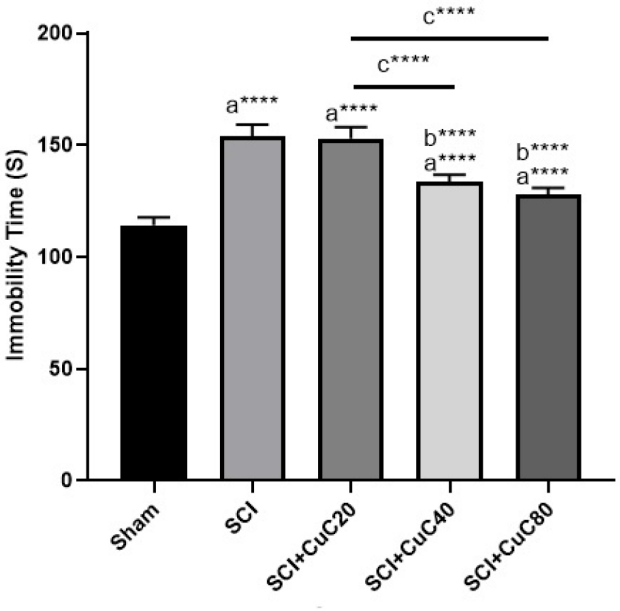

Fig. 5 **Effects of CuC on depressive-like behaviors (immobility time in tail suspension test) in the SCI-induced rats. Values: mean ± SEM ($n = 3$). *$P < 0.05$, **$P < 0.001$, ***$P < 0.001$, & ****$P < 0.0001$; *a* vs. the Sham group; *b* 0.001 vs. the SIS group, and *c* between different doses of CuC. Sham: sham operated animals received normal saline for 14 days; SCI: SCI-induced animals received normal saline for 14 days; SCI + CuC20, 40, or 80: SCI-induced animals received CuC with doses of 20, 40, or 80 mg/kg, respectively.**

Discussion

This study examined the expression of NLRP3, ASC, and Caspase-1 genes in the HC of animals in sham, SCI, SCI + CuC20, SCI + CuC40 and SCI + CuC80 groups. This study has investigated the effects of different doses of curcumin on behavioral alterations in animals with exposure to SCI.

Based on the results of EPM, FST, TST and OPT, it can be found that SCI increased anxiety and depression. According to the results of the present study, SCI raised the activity of NLRP3 inflammasome in the HC. Moreover, we found that curcumin can attenuate the depression, anxiety, and neuroinflammation via suppressing the NF-κB pathway in SCI rat model. Taken together, these results suggest that: (1) NLRP3 inflammasome may contribute to anxiety and depression and (2) the neuroprotectant, curcumin, demonstrate antidepressant-like effects via suppressing the NF-κB pathway involved with inflammation.

One of the most usual psychological issues in person with spinal cord injury is depression. In our study, according to the EPMT, we found that SCI decreased delay in the open arm and increased delay in the close arm in comparison to the sham. The results from the FST revealed that SCI decreased swimming time and increased immobility time as compared to the sham group. In line with the results of the present study, Xie et al. (2021), investigated the correlation between depression-like behavior and SCI and their findings demonstrated SCI led to decreased immobility time in the forced swim test.[45] According to the results of OFT, SCI decreased delay in the central zone and increased delay in the peripheral zone in comparison to the sham group. Similar to the results of the present study, in the study by Brakel et al. (2021), the lower levels of open field activity were observed in the SCI group.[33]

There are several common antidepressants drugs but they usually induce several side effects.[46]

Curcumin which is used as dietary spice has anti-inflammatory and anti-oxidant effects and also in previuos studies it is proved that by the activation of the ERK/BDNF neurotrophic pathway it can reduce the depressive behavior.[47] In this study, treatment with CuC with two doses of 40 and 80 mg/kg

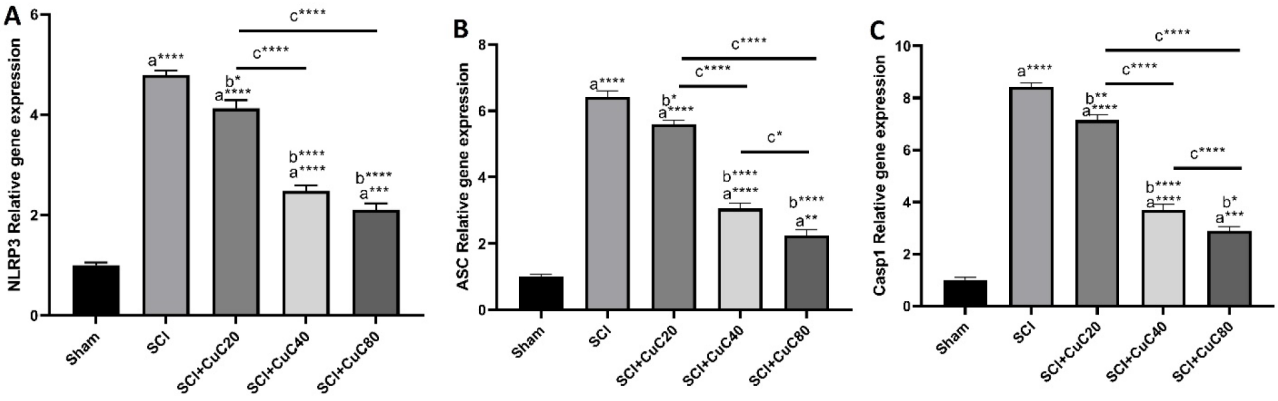

Fig. 6 **Effects of CuC on the gene expression of NLRP3 inflammasome components in the HC of SCI-induced rats. A) NLRP3, B) ASC, and C) Casp1. Values: mean ± SEM ($n = 3$). *$P < 0.05$, **$P < 0.001$, ***$P < 0.001$, & ****$P < 0.0001$; *a* vs. the Sham group; *b* 0.001 vs. the SIS group, and *c* between different doses of CuC. Sham: sham operated animals received normal saline for 14 days; SCI: SCI-induced animals received normal saline for 14 days; SCI + CuC20, 40, or 80: SCI-induced animals received CuC with doses of 20, 40, or 80 mg/kg, respectively.**

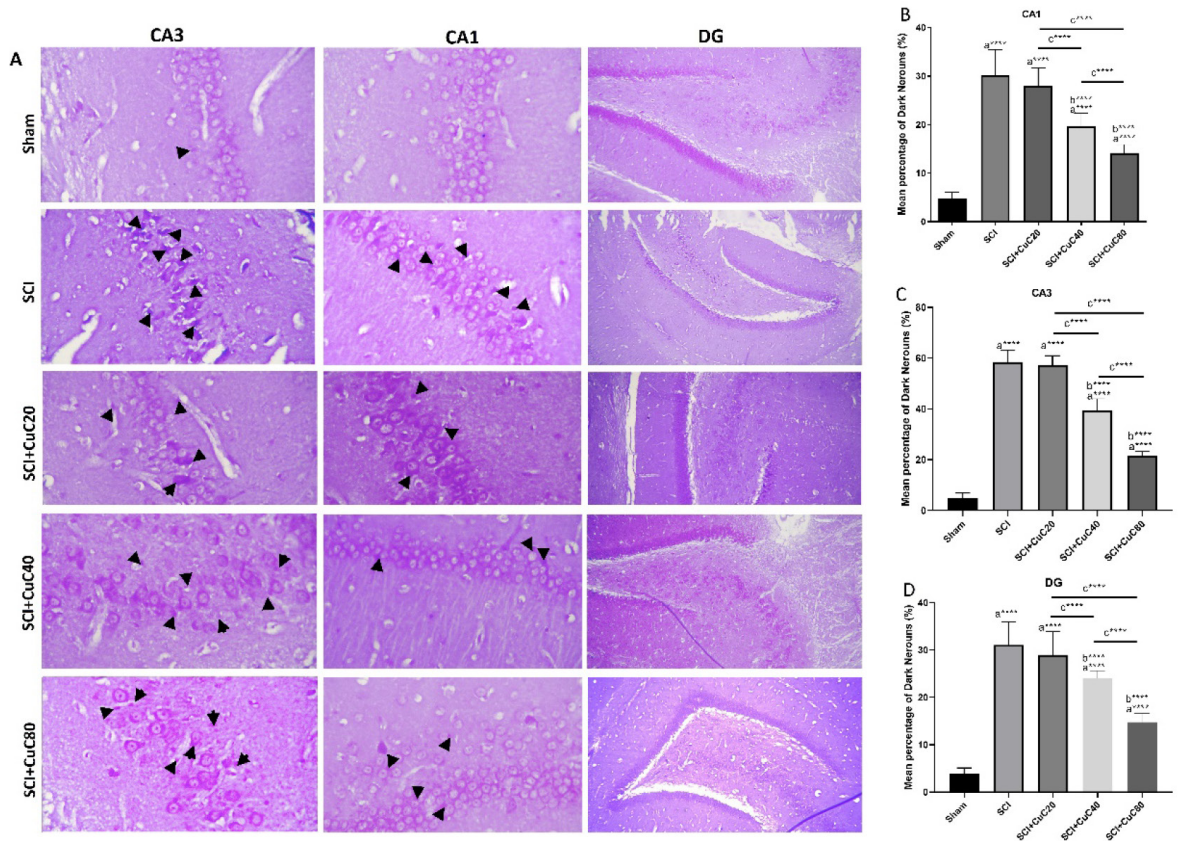

Fig. 7 **Effects of CuC on the percentage of dark neurons in the HC of SCI-induced rats. A) Nissl staining (×100 & ×400 magnification) black arrows shows dark neurons; The percentage of dark neurons in the B) CA1, C) CA3, and D) Dentate gyrus. Values: mean ± SEM (*n* = 4). **P* < 0.05, ***P* < 0.001, ****P* < 0.001, & *****P* < 0.0001; *a* vs. the Sham group; *b* vs. the SIS group, and *c* between different doses of CuC. Sham: sham operated animals received normal saline for 14 days; SCI: SCI-induced animals received normal saline for 14 days; SCI + CuC20, 40, or 80: SCI-induced animals received CuC with doses of 20, 40, or 80 mg/kg, respectively.**

increased open arm time and decreased close arm time as compared to the SCI group. Zhang et al. (2019) showed that curcumin successfully increased the percentage of time spent on the open arms in comparison to the injury group.[48] The administration of CuC (40 mg/kg and 80 mg/kg) enhanced the altered behaviors. Same as the results of this study, in the study by Zhang et al. (2019), the treatment of curcumin decreased immobility time as compared to the injury group.[48] Fan et al. (2018) reported that curcumin (40 mg/kg) demonstrated antidepressant-like actions and reduced responses related to inflammation in a rat model of depression.[49] In another study, Xu et al. (2005) investigated the curcumin treatment at 5 and 10 mg/kg on depressive-like behaviors in mice and they explained that these doses didn't impact locomotor activity and this result may be due to the use of low doses of curcumin.[50] Evidences based on the tail suspension test and the forced swimming test suggest that chronic administration of curcumin reverses levels of 3,4-dihydroxyphenylacetic acid, noradrenaline, serotonin, and 5-hydroxyindoleacetic acid in the HP region of animals, produces antidepressant effects which is related to serotonergic system and also it can improve neurogenesis in this region by involvement of BDNF and pCREB/CREB ratio.[51-53] Same as the results of EMPT and FST, improved OFT values were observed in CuC40 and CuC80 groups.

The hippocampus plays a main role in the progress of depression and also neuroinflammation in this region has been approved in depression. In the present study, SCI caused an increase in NLRP3, ASC and Casp1 gene expression in the HC of animals in comparison to the sham group. CuC20, CuC40 and CuC80 regulated the gene expression of NLRP3, ASC and Casp1 in the HC of animals. SCI caused an increase in the protein levels NLRP3, ASC and Casp1 in the HC of animals as compared to the sham group. CuC20, CuC40 and CuC80 regulated the protein levels of NLRP3, ASC and Casp1 in the HC of animals. Similar to the findings of the present study, Limcharoen et al. (2021),[54] Zhang et al. (2019)[48] and Yu et al. (2015)[55] presented that the curcumin could reduce mRNA expression of proinflammatory cytokines like IL-1β, IL-6, and TNF-α. These similarities in different studies may be due to suppress in NF-κB activation. Curcumin produces beneficial effects in SCI by inhibiting neuroinflammation by reducing the expression of proinflammatory cytokines.[56,57]

Conclusion

Our results suggested that administration of CuC80 decreased neuroinflammation. Based on the findings of the behavioral tests in the present study, because of safety and high ability to enhance the parameters related to depression, CuC80 can be considered as a treatment for depression.

Conflicts of Interest

None.

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