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ORIGINAL RESEARCH ARTICLE

Antioxidant effects of curcumin in unilateral spinal cord injury model in adult male rats

Babak Ebrahimi[1], Atousa Yarahmadi[1], Neda Ghaffari[1], and Gholamreza Hassanzadeh[1,2*]

[1]Department of Anatomy, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

[2]Department of Neurosciences and Addiction Studies, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran

Abstract

Inflammatory responses and oxidative stress (OS) play a significant role in the development of spinal cord injury (SCI), as evidenced by both pre-clinical and clinical studies. This research aimed to assess the potential antioxidant and anti-inflammatory properties of curcumin (CuC) as a therapeutic approach in a unilateral SCI model using male rats. We used 40 adult male Wistar rats (each weighing 220 – 250 g) that were randomly assigned to one of the five experimental groups: (1) Control (Con), (2) Model (SCI animals), (3) Model+CuC20, (4) Model+CuC40, and (5) Model+CuC80. Accordingly, the SCI animals in Model+CuC20, Model+CuC40, and Model+CuC80 groups received 20, 40, and 80 mg/kg/day of CuC through the intraperitoneal route, respectively. We assessed functional recovery, measured OS indicators, including malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD), and total antioxidant capacity (TAC) in the blood, and evaluated protein levels of caspase 1, NOD-like receptor family pyrin domain-containing 3 (NLRP3), and apoptosis-associated speck-like protein containing a CARD (ASC) in the spinal cord tissue. The CuC treatment groups showed a significant enhancement in functional recovery, a marked decrease in MDA levels, and a notable elevation in SOD activity relative to the SCI animals. Model+CuC40 and Model+CuC80 animals exhibited a significant improvement in GSH activity and TAC level as compared to the SCI animals. The results also showed a dramatic decrease in the protein concentration of NLRP3, ASC, and Casp1 in the Model+CuC40 and Model+CuC80 groups relative to the Model group ($P < 0.0001$). In conclusion, the treatment with CuC significantly enhanced functional recovery in SCI rats by effectively mitigating OS and reducing inflammatory markers.

Keywords: Curcumin; Spinal cord injury; Neuroinflammation; Oxidative stress

***Corresponding author:**
Gholamreza Hassanzadeh
(hassanzadeh@tums.ac.ir)

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1. Introduction

The pathogenesis of spinal cord injury (SCI) entails two distinct phases: primary injury, which refers to the immediate impact resulting from the initial trauma, and secondary injury, characterized by a cascade of pathological processes that ensue post-trauma.[1] The ensuing complications of SCI include oxidative stress (OS), inflammation, and

neurotoxicity, with OS being a significant contributor to secondary SCI.[2-4] OS occurs when there is an excess of free radicals in the body's cells, overwhelming the antioxidant defenses that typically neutralize these harmful molecules.[5] Free radicals, primarily oxygen-based molecules such as superoxide and hydroxyl radicals, are naturally produced during cellular processes. However, after SCI, there is a significant increase in free radical production due to factors like ischemia (lack of blood flow) and hypoxia (lack of oxygen).[6,7]

In addition to OS, neuroinflammation is a fundamental factor in the onset and progression of SCI. One of the key components of this inflammatory mechanism is the activation of the NOD-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome, which recruits pathways that enhance the secretion of inflammatory cytokines.[8] This activation results in pyroptosis, a specific type of programmed cell death, occurring at the site of injury, thereby worsening tissue damage and inflammation.[9] Furthermore, previous research has shown NLRP3 activation following SCI in animal and clinical studies.[3,10]

The body's antioxidant mechanisms are unable to cope with the substantial surge in free radicals, resulting in the irremeable devastation of neuronal cells. This damage leads to neuronal death and tissue destruction.[11,12] Research indicates that preventing free radical production after SCI can preserve nerve cells from oxidative damage, potentially mitigating the pathological processes associated with SCI and promoting nerve repair and regeneration.[13,14] Pro-oxidants are often linked to the onset of various diseases, while antioxidants are typically employed to prevent or mitigate these conditions.[15,16]

Commonly found as a spice in the Indian kitchen and used in traditional Chinese medicine, curcumin (CuC) is a natural compound that constitutes 60 – 70% of the curcuminoids derived from the rhizomes of *Curcuma longa*.[17] CuC has been well-researched and is known for its diverse bioactive properties applied in traditional medicine.[18] It acts as a potent antioxidant through two primary mechanisms: first, by directly scavenging free radicals such as superoxide anions and hydroxyl radicals to prevent oxidative damage, and second, by enhancing cellular defenses against OS. This enhancement occurs through the reduction of lipid peroxidation, an increase in glutathione (GSH) levels, and the stimulation of key proteins such as superoxide dismutase (SOD) and catalase (CAT), which collectively contribute to its protective effects against OS in several pathological conditions beyond SCI.[3,19-22] Despite the aforementioned beneficial effects, the ingestion of high doses of CuC can lead to adverse gastrointestinal reactions, including stomach upset, nausea, gastroesophageal reflux disease, diarrhea, and dizziness.[23,24]

This current research aimed to investigate the antioxidant and anti-inflammatory impacts of CuC in a unilateral SCI model using male rats. Recognizing the detrimental roles of free radicals and inflammatory cytokines in SCI, we explored how different doses of CuC could modulate OS and inflammation. Specifically, we assessed the impacts of CuC on OS indicators and components of the NLRP3 inflammasome, which are critical in mediating inflammatory responses.

2. Materials and methods

2.1. Animals

A total of 40 adult male Wistar rats, each weighing 220 – 250 g, were procured from the animal room at the Tehran University of Medical Sciences (TUMS) in Tehran, Iran. These rats were housed under carefully controlled standard laboratory conditions, with a consistent ambient temperature maintained at 22 – 24°C. They were provided with unlimited access to both food and fresh water, ensuring their well-being throughout the study. The study protocol received approval from the Institutional Research Ethics Committee at TUMS (Approval No.: IR.TUMS.SPH.REC.1400.160), and the ethical treatment of animals was adhered to in the present study.

2.2. Study design

The animals were divided into separate experimental groups (n = 8 in each group) to facilitate comparative analyses of the treatment effects: (1) Control group, in which the animals underwent laminectomy without any induction of SCI, serving as a baseline for evaluating the effects of the treatments. (2) Model group, in which the animals underwent the unilateral SCI procedure and received a vehicle treatment, allowing researchers to assess the effects of SCI without any therapeutic intervention. (3) Model + CuC20, Model + CuC40, and Model + CuC80 groups, in which the animals that had been induced with unilateral SCI received CuC at 20, 40, and 80 mg/kg/day, administered through intraperitoneal injection, which is pivotal in evaluating the potential therapeutic effects of CuC on recovery following SCI. Each of these groups was carefully monitored throughout the study to assess behavioral, functional, and histological outcomes, contributing vital information to the understanding of SCI treatments and the role of CuC in neuroprotection and recovery.

2.3. Surgery procedure

The animals underwent anesthesia using a carefully calibrated mixture administered through intraperitoneal

injection consisting of 80 mg/kg of ketamine and 15 mg/kg of xylazine. This combination was selected for its efficacy in providing both sedation and analgesia, ensuring the animals remained unconscious and free from pain throughout the surgical procedure. Following anesthesia, the fur over the surgical area was shaved to ensure a sterile environment, minimizing the risk of infection. A laminectomy was then performed at the T8 – T9 vertebral levels using a precision tool known as a micro rongeur (Fine Science Tools, USA). This step involved removing a section of the vertebral bone to access the spinal cord directly. To induce SCI, an extradural clip was employed for compression, requiring the placement of an aneurysm clip on the right side of the spinal cord. This clip exerted a controlled closing force of 0.90 N for a duration of 1 min, simulating the impact of a traumatic injury. After the surgical procedure was completed, the incision site was meticulously suture-closed to promote healing and reduce the risk of complications during the recovery phase. This careful approach ensures both the integrity of the surgical site and the welfare of the animals involved in the study.

2.4. Motor behavior analysis

The progression of functional recovery in each animal was meticulously evaluated using the Basso, Beattie, and Bresnahan (BBB) test, a widely recognized and validated behavioral assessment tool for measuring locomotor function following SCI in rodent models. The BBB test scores range from 0 to 21, with higher scores indicating better locomotor function. Each rat was observed for specific behaviors such as movement, coordination, and weight-bearing ability during a series of standardized tasks, which included walking on a flat surface and navigating obstacles. Assessments began 24 h post-SCI and continued at 48 h and 72 h, with additional evaluations conducted weekly throughout the duration of the study (4 weeks).

2.5. Sample preparation

After the completion of the experimental procedures (after 4 weeks), the animals were humanely sacrificed in accordance with ethical guidelines to minimize suffering. This process typically involved administering an overdose of anesthetic, ensuring that the animals were unconscious and pain-free before euthanasia. Following sacrifice, spinal cord tissue and blood samples were collected for further analysis. The spinal cord tissues were carefully excised and stored in sterile containers, whereas blood samples were obtained through cardiac puncture, a method that allows for the collection of blood directly from the heart, ensuring high-quality samples. Once collected, the blood samples were centrifuged at 6000 rpm for 20 min to separate the plasma from the cellular components. The supernatants, which contain biochemical markers, were carefully gathered for subsequent assays. To preserve the integrity of the samples, both tissue and blood supernatants were stored at –80°C.

2.6. Measurement of OS

The present study focused on several key parameters, including malondialdehyde (MDA), GSH, SOD, and total antioxidant capacity (TAC). These parameters were measured using specific kits from ZellBio (Germany), and quantitation of OS and antioxidant levels in biological samples was conducted according to the manufacturer's protocols. The MDA assay (CAT No. ZB-MDA-96A) was used to assess lipid peroxidation, indicating oxidative damage. The GSH assay (CAT No. ZB-GSH-96A) measures the levels of this critical antioxidant, whereas the SOD assay (CAT No. ZB-SOD-96A) evaluates the activity of this enzyme that protects against OS. Finally, the TAC assay (CAT No. ZB-TAC-96A) provides a comprehensive measure of the overall antioxidant capacity of the samples. Following the manufacturer's instructions for each assay ensured accurate and reproducible results, contributing to the understanding of the biochemical changes associated with SCI and the potential therapeutic effects of the treatments administered.

2.7. Enzyme-linked immunosorbent assays (ELISA)

To measure the amounts of NLRP3, apoptosis-associated speck-like protein containing a CARD (ASC), and caspase 1 (Casp-1) proteins, ELISA assays were employed to analyze freshly extracted spinal cord tissue samples. Upon collection, the spinal cord tissues were carefully homogenized in a suitable lysis buffer to extract the proteins. Following homogenization, the samples were centrifuged to remove cellular debris, leaving a clear supernatant that contained the soluble proteins for analysis. ELISA kits for NLRP3 (MyBiosource, CA, United States), ASC (MyBiosource, CA, United States), and Casp-1 (Cusabio, Wuhan, China) that are specific for rats were used to measure the protein levels of these three proteins according to the manufacturers' protocol.

2.8. Statistical analysis

We used IBM® Statistical Package for the Social Sciences Statistics software (ver. 20) to analyze data, and the results are expressed as mean ± standard deviation. A one-way analysis of variance was conducted followed by Tukey's *post hoc* test to calculate the differences between groups. A $P < 0.05$ was considered to indicate statistical significance.

3. Results

3.1. Functional recovery

Our results showed that animals with SCI had significantly lower BBB scores relative to the Control group animals

($P < 0.05$). No meaningful difference in the BBB scores was reported between the Model+CuC20 animals and the Model group animals ($P > 0.05$). Model+CuC40 and Model+CuC80 animals demonstrated improvement in functional recovery compared to the Model group animals (Figure 1, $P < 0.0001$ for comparisons). These results indicate that higher doses of CuC may promote functional recovery in SCI, highlighting its potential therapeutic benefits.

3.2. Blood levels of OS indicators

3.2.1. MDA level

Our findings indicated a significant rise in the level of MDA in the Model group animals relative to the Control group animals ($P < 0.0001$). In contrast, a notable reduction in MDA concentrations was observed in the Model+CuC20 group animals relative to the Model group animals ($P = 0.01$). In addition, both the Model+CuC40 and Model+CuC80 groups exhibited significant reductions in MDA levels when compared to the Model animals (Figure 2, $P < 0.0001$ for both treatments). These results imply that CuC treatment may effectively reduce oxidative damage in SCI, contributing to improved outcomes in cellular health.

3.2.2. SOD activity

Our results also indicated a significant rise in SOD activity in the Model group animals compared to the Control group animals ($P < 0.0001$). No statistically significant differences in SOD activity were reported in the Model+CuC20 group relative to the Model group ($P > 0.05$). Notably, following the administration of 40 and 80 mg/kg of CuC, there was a significant modulation in SOD levels relative to the SCI animals (Figure 3, $P < 0.0001$ for both treatments). These findings suggest that the intervention not only mitigated OS but also likely enhanced overall cellular health.

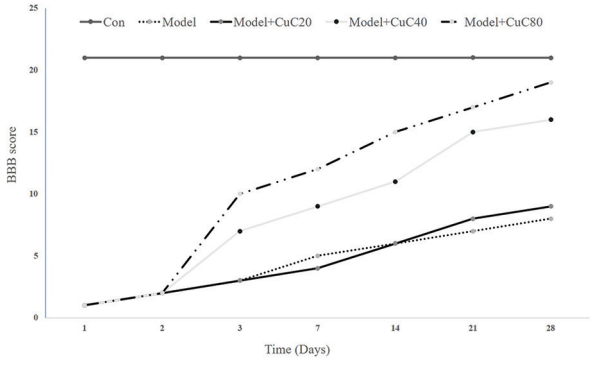


Figure 1. CuC effects on the BBB scores in experimental animals
Abbreviations: BBB score: Basso, Beattie, and Bresnahan score; Con: Control; CuC: Curcumin.

3.2.3. GSH activity

The activity of GSH was raised in the SCI-induced animals relative to the Control group animals ($P < 0.0001$). Mean GSH activity was similar between the Model+CuC20 and the Model groups ($P > 0.05$). Furthermore, both the Model+CuC40 and Model+CuC80 animals demonstrated a substantial increase in GSH activity relative to the Model group (Figure 4, $P < 0.0001$ for both treatments). These findings suggest that the intervention may have effectively alleviated OS and contributed to improved cellular health.

3.2.4. TAC level

In comparison to the Control group animals, TAC was diminished in the SCI animals ($P < 0.0001$). No significant differences in TAC levels were observed between the

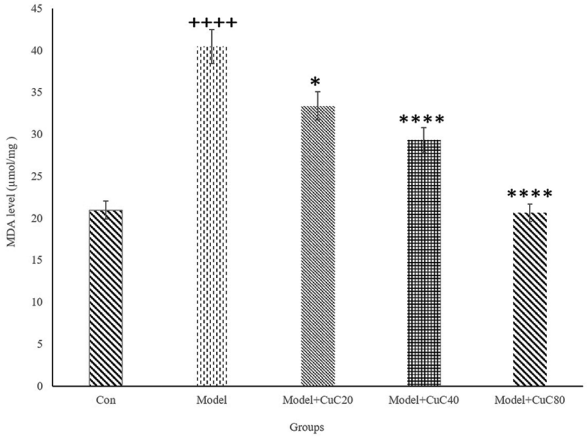


Figure 2. CuC effects on the blood concentration of MDA in the SCI-induced animals. $^{++++}P<0.0001$ compared to Control group; $^{*}P<0.05$, $^{****}P<0.0001$ compared to Model group.
Abbreviations: Con: Control; CuC: Curcumin; MDA: Malondialdehyde; SCI: Spinal cord injury.

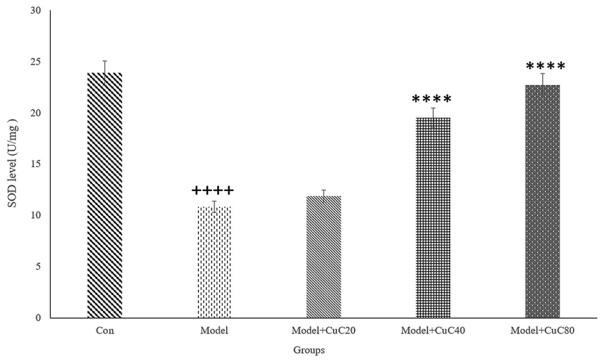


Figure 3. CuC effects on the blood concentration of SOD in the SCI-induced animals. $^{++++}P<0.0001$ compared to Control groups; $^{****}P<0.0001$ compared to Model group.
Abbreviations: Con: Control; CuC: Curcumin; SCI: Spinal cord injury; SOD: Superoxide dismutase.

Model+CuC20 and the Model groups ($P > 0.05$). However, both the Model+CuC40 and Model+CuC 80 groups demonstrated a significant elevation in TAC levels relative to the Model group (Figure 5, $P < 0.0001$ for both treatments). These results regarding TAC levels suggest that the intervention enhances the body's natural defense mechanisms, thereby improving resilience against oxidative damage.

3.3. Protein concentrations of inflammatory mediators

3.3.1. NLRP3

In comparison to the control animals, the protein concentrations of NLRP3 were significantly elevated in the tissue samples of Model group animals ($P < 0.0001$). No statistically significant difference was observed in the NLRP3 protein levels of the Model+CuC20 group animals relative to the Model group animals ($P > 0.05$). However, both the Model+CuC40 and Model+CuC80 groups demonstrated a prominent decrease in NLRP3 protein levels relative to the Model group (Figure 6, $P < 0.0001$ for both treatments).

3.3.2. ASC

Significant differences in the tissue concentration of ASC protein were observed between the Model groups and the Control group ($P < 0.0001$). In contrast, no meaningful differences in the ASC protein level were found between the Model+CuC20 and the Model groups ($P > 0.05$). Following treatment with 40 and 80 mg/kg of CuC, there was a significant reduction in the protein concentration of ASC relative to the Model group animals (Figure 7, $P < 0.0001$ for both treatments).

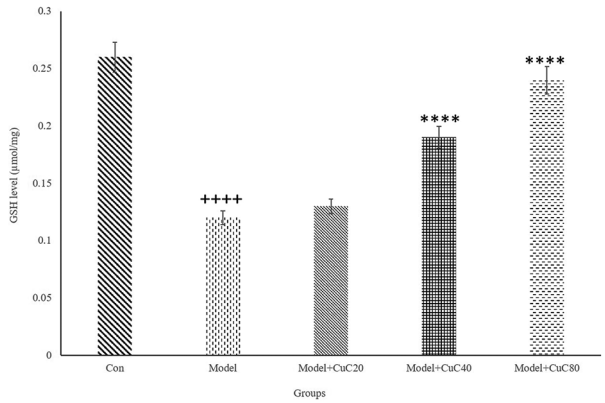


Figure 4. CuC effects on the blood concentration of GSH in the SCI-induced animals. $^{++++}P<0.0001$ compared to Control group; $^{****}P<0.0001$ compared to Model group.
Abbreviations: Con: Control; CuC: Curcumin; GSH: Glutathione; SCI: Spinal cord injury.

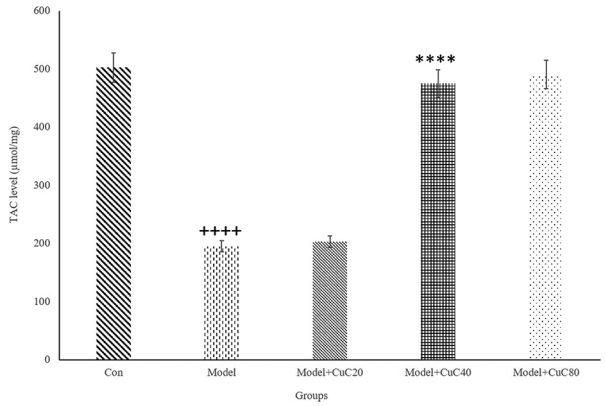


Figure 5. CuC effects on the blood concentration of TAC in the SCI-induced animals. $^{++++}P<0.0001$ compared to Control group; $^{****}P<0.0001$ compared to Model group.
Abbreviations: Con: Control; CuC: Curcumin; SCI: Spinal cord injury; TAC: Total antioxidant capacity.

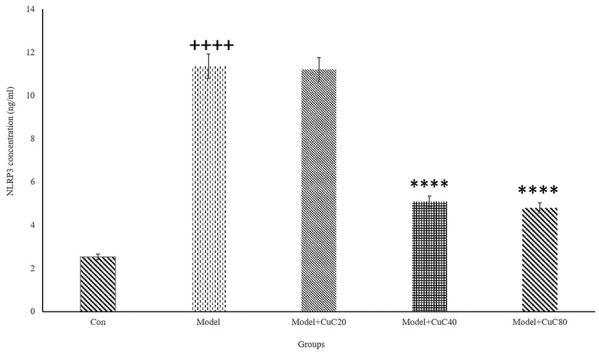


Figure 6. CuC effects on the protein concentration of NLRP3 in the SCI-induced animals. $^{++++}P<0.0001$ compared to Control group; $^{****}P<0.0001$ compared to Model group.
Abbreviations: Con: Control; CuC: Curcumin; NLRP3: NOD-like receptor family pyrin domain-containing 3; SCI: Spinal cord injury.

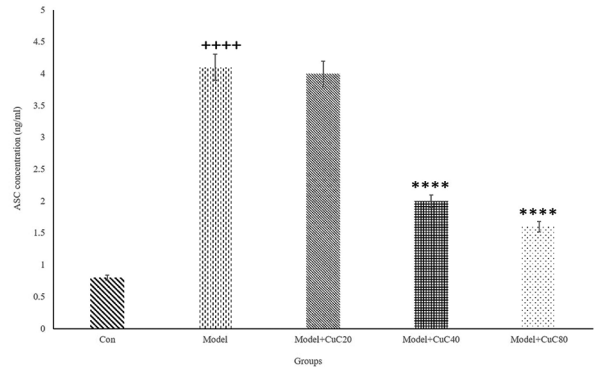


Figure 7. CuC effects on the protein concentration of ASC in the SCI-induced animals. $^{++++}P<0.0001$ compared to Control group; $^{****}P<0.0001$ compared to Model group.
Abbreviations: ASC: Apoptosis-associated speck-like protein containing a CARD; Con: Control; CuC: Curcumin; SCI: Spinal cord injury.

3.3.3. Casp-1

The outcomes demonstrated a prominent increase in the protein concentration of Casp-1 in the tissue samples of Model group animals relative to those of Control group animals ($P < 0.0001$). Meanwhile, no significant differences in the protein concentration of ASC were found between the Model+CuC20 and the Model groups ($P > 0.05$). Both Model+CuC40 and Model+CuC80 groups showed significant reductions in the protein concentration of Casp-1 when compared to the Model group (Figure 8, $P < 0.0001$ for both treatments).

4. Discussion

In this research, we investigated the antioxidant effects of varying doses of CuC in a SCI model utilizing male Wistar rats. Our findings revealed that SCI significantly elevates the levels of MDA, a well-established marker of OS, indicating increased lipid peroxidation and cellular damage. Concurrently, we observed a marked reduction in the body's antioxidant defenses, evidenced by decreased levels SOD, GSH, and TAC. These results underscore the oxidative imbalance that occurs following SCI, highlighting the detrimental impact of OS on recovery. Furthermore, our study revealed that SCI led to the upregulation of the NLRP3 inflammasome, characterized by increased protein levels of NLRP3, ASC, and Casp-1. This upregulation reflects an enhanced neuroinflammatory response following SCI, contributing to the overall pathological process.

SCI poses a significant global health challenge, with thousands of new cases diagnosed annually, frequently resulting in profound and debilitating effects on individuals' quality of life.[25] The intricate pathophysiological mechanisms that underlie SCI, especially the complex interactions between primary and secondary injury processes, are not yet fully understood. This knowledge gap presents considerable obstacles to the development of effective therapeutic strategies aimed at improving patient outcomes.[26,27] While the initial trauma and primary injury mechanisms are often unpredictable, there is an urgent need for targeted interventions that can mitigate the detrimental effects of secondary injury, particularly those related to OS and neuroinflammation.[28] Pre-clinical research has provided compelling evidence for the role of the NLRP3 inflammasome in the pathogenesis of SCI. The NLRP3 inflammasome is formed by the assembly of NLRP3, ASC, and Casp-1 in response to both external infections and internal signaling stimuli. This assembly leads to the activation of Casp-1, resulting in the release of pro-inflammatory cytokines such as interleukin (IL)-1β and IL-18.[29] Moreover, prior studies have demonstrated that inhibiting NLRP3 inflammasome activity can effectively reduce neuroinflammation and enhance functional recovery in animal models of SCI.[30] While the initial trauma and primary injury mechanisms are often unpredictable, our research emphasizes the urgent need for targeted interventions that can mitigate the detrimental effects of secondary injury, particularly those related to OS and neuroinflammation. These findings suggest that targeting the NLRP3 inflammasome may represent a promising therapeutic approach to mitigate the adverse effects of neuroinflammation and improve recovery following spinal cord injuries.

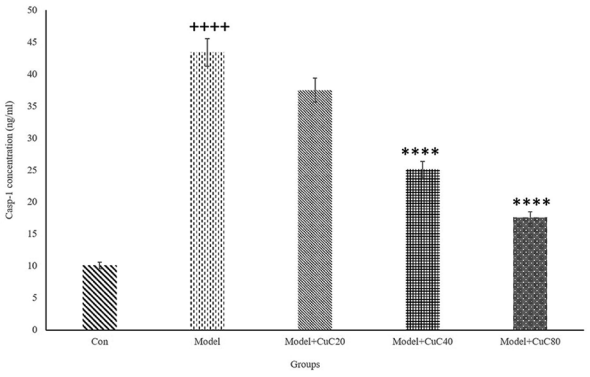


Figure 8. CuC effects on the protein concentration of Casp-1 in the SCI-induced animals. $^{++++}P<0.001$ compared to Control group; $^{****}P<0.001$ compared to Model group.
Abbreviations: Casp-1: Caspase 1; Con: Control; CuC: Curcumin; SCI: Spinal cord injury.

In this study, SCI was induced in rats by applying an aneurysm clip to the right side of the spinal cord for a duration of 1 min. In the treatment groups, CuC was administered through intraperitoneal injection at three different doses: 20, 40, and 80 mg/kg. These doses were selected to evaluate the dose-dependent effects of CuC on recovery and neuroprotection following the SCI induced. The findings of this study demonstrated that by induction of SCI, the MDA level was increased, and the SOD and GSH activities and TAC levels were decreased in the animals. Consistent with our findings, Kim *et al.*[31] demonstrated that following the SCI, the amount of oxidant parameters was increased, and the levels of antioxidant parameters were decreased.

CuC ($C_{21}H_{20}O_6$) is a polyphenol extracted from turmeric, a common spice and traditional Chinese medicine. CuC exhibits promising effects against inflammation, OS, apoptosis, and neurodegeneration.[22] Our findings revealed that CuC-treated groups exhibited a significant reduction in the level of MDA relative to the untreated SCI animals. Model+CuC40 and Model+CuC80 animals demonstrated a significant increase in the levels of SOD, GSH, and TAC in comparison to the SCI model animals. Gao *et al.*[32] showed

that CuC treatment can decrease the levels of MDA, induce the activation of GSH peroxidase, and ameliorate OS in rat models. Dong *et al.*[33] investigated the effects of CuC in a mouse wound model by administering it into the abdominal cavity. Their findings indicated that CuC exhibited a protective effect on nerve tissue. This effect was related to the upregulation of nuclear factor erythroid 2-related factor 2 (Nrf2), a key regulator of the antioxidant response. The study also noted the upregulation of downstream antioxidant enzymes, suggesting that CuC may enhance the body's ability to combat OS and promote nerve protection and recovery following injury. In 2018, Caillaud *et al.*[34] conducted research to explore the impact of continuous topical treatment with low doses of CuC on nerve function and regeneration after sciatic nerve injury in mice. These findings indicate that CuC has a protective effect against OS by reducing the secretion of ROS generated by macrophages. In addition, it lowers lipid peroxidation levels and boosts the expression of the transcription factor Nrf2, which plays a crucial role in regulating antioxidant responses. Furthermore, applying low doses of CuC locally shows potential as an effective treatment for peripheral nerve regeneration.[34] These findings are consistent with the results of our current research.

In addition to the assessment of OS parameters, functional recovery was evaluated in the present research. The BBB test outcomes showed a notable reduction in scores of BBB for animals with SCI in comparison to the Control group animals. Among the animals treated with 40 and 80 mg/kg CuC, we observed a meaningful elevation in the BBB scores relative to the untreated SCI animals. Kim *et al.*[31] assessed the effects of CuC on the development of SCI in a rat model. They found that CuC (200 mg/kg) significantly improved functional recovery in the early stages following SCI. Furthermore, they presented that CuC decreased the MDA levels, increased the activity of SOD, and reduced the inflammation. The results of this study are in agreement with the findings of Kim *et al.*'s research.[31] The findings of the mentioned studies are in line with the outcomes of the present study. Research conducted by Alvarado-Sanchez *et al.*[35] demonstrated that CuC effectively reduced concentrations of hydroxyl radicals, nitric oxide, and lipid peroxidation following SCI, although it did not significantly affect SOD activity. Our results align with these findings; however, we observed significant effects of CuC on SOD activity, which contrast with the outcomes reported by Alvarado-Sanchez *et al.*[35] This discrepancy may be attributed to the timing of the OS parameter measurements. In our study, we assessed the levels of OS parameters 4 weeks post-SCI and treatment, whereas their study evaluated these parameters just 24 h after SCI.

We also investigated the protein concentrations of inflammatory mediators, specifically ASC, NLRP3, and Casp-1, at the injury site. Our findings revealed a significant increase in these markers after SCI. The results also showed a dramatic reduction in the concentrations of NLRP3, ASC, and Casp1 proteins in the animals receiving 40 and 80 mg/kg of CuC, in comparison to the Model group animals. This suggests a noteworthy decrease in the levels of these inflammatory proteins. Prior studies have demonstrated that CuC can substantially enhance recovery following SCI by fostering the development of new nerve cells (neurogenesis) and modulating inflammatory pathways. For example, research conducted by Lee *et al.* revealed that administering CuC orally at a dosage of 0.4 mg/kg for 2 weeks led to an increase in neurogenesis by affecting brain-derived neurotrophic factor concentrations in the hippocampus, a crucial area of the brain involved in learning and memory.[36] The results of another study revealed that administering a low dose of CuC (exceeding 0.2 mg/kg) resulted in an increase in the generation of new cells within the hippocampus, thereby enhancing neurogenesis in adult mice.[37] Barati *et al.*[38] investigated the CuC effects on rats with SCI. They found that CuC might help improve movement in the early stages of the injury by promoting nerve repair and reducing the inflammation caused by astrocyte activity. The results of the mentioned studies were in accordance with the results of this study related to the inflammasome complex.

Numerous studies have consistently shown that CuC effectively mitigates OS. This protective effect is achieved through multiple mechanisms including: (1) reducing lipid peroxidation products such as MDA, 4-hydroxynonenal, and protein carbonyls, (2) enhancing the activity of GSH peroxidase and SOD, which are essential antioxidant enzymes, and (3) activating the Nrf2, a crucial regulator of the body's antioxidant response.[39-41] Despite the potential health benefits CuC can offer, taking large amounts can lead to unpleasant side effects such as stomach upset, nausea, dizziness, gastroesophageal reflux disease, and diarrhea, and our findings proved that consuming CuC at moderate dose could mitigate SCI development.

5. Conclusion

This study found that moderate and high doses of CuC effectively reduced OS and inflammation caused by SCI. Since a marked decrease was not noted between animals receiving varying CuC doses, a moderate dose of CuC is recommended.

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Conflict of interest

The authors declare that they have no competing interests.

Author contributions

Conceptualization: Gholamreza Hassanzadeh
Formal analysis: Babak Ebrahimi
Investigation: Babak Ebrahimi, Gholamreza Hassanzadeh
Methodology: Babak Ebrahimi, Atousa Yarahmadi, Neda Ghaffari
Writing–original draft: Atousa Yarahmadi, Neda Ghaffari
Writing–review & editing: Gholamreza Hassanzadeh

Ethics approval and consent to participate

This research project was approved by the Ethical Committee of Tehran University of Medical Sciences (IR.TUMS.SPH. REC.1400.160).

Consent for publication

Not applicable.

Availability of data

Data are available from the corresponding author upon reasonable request.

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