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Original Article

Therapeutic Effects of N-Acetylcysteine on Social Isolation-Induced Liver Injury: Role of Tumor Necrosis Factor-Alpha Suppression and Antioxidant Enhancement

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Abstract

Introduction: N-acetylcysteine (NAC) improves liver enzyme levels and function while reducing oxidative stress and inflammation in mouse offspring, highlighting its therapeutic potential. This study aimed to examine how NAC influences liver function and behavioral outcomes in the offspring of mice subjected to social isolation.

Methods: Forty-eight male and female pups (BALB/c mice) were divided into six groups (n=8 per group), with three groups for males and three for females. The control group received standard saline solution at 10 mL/kg to serve as the baseline comparison. A group exposed to stress induced by social isolation received saline solution at 10 mL/kg. The group exposed to social isolation stress (SIS) received NAC at a dosage of 150 mg/kg as part of the treatment protocol. Behavioral tests, including marble burying and the elevated plus maze, were conducted, and biochemical tests measured antioxidant capacity, liver malondialdehyde (MDA) levels, serum nitrite levels and tumor necrosis factor-alpha (*TNF-α*) gene expression in the liver.

Results: The NAC-treated group demonstrated increased duration spent in the open arms of the elevated plus maze ($P=0.0204$) and a reduced number of marbles being buried compared with the SIS group ($P=0030$). Moreover, this group showed enhanced hepatic antioxidant activity ($P=0.0431$) and reduced liver MDA levels ($P=0.0203$). Finally, NAC decreased serum nitrite levels in the liver ($P=0.0286$) and serum ($P=0.0291$) and downregulated hepatic *TNF-α* gene expression ($P=0.0029$).

Conclusion: NAC mitigates liver and behavioral impairments linked to SIS by counteracting cellular damage and enhancing hepatic performance.

Keywords: Elevated plus maze, Marble burying test, Malondialdehyde, Nitrite, Antioxidant capacity, Mice

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Introduction

The liver, an essential organ, is vital for numerous physiological functions, including detoxification, metabolic regulation, and the synthesis of important substances such as proteins and bile. Moreover, it processes nutrients from the digestive tract, stores energy, and regulates blood sugar levels. In addition to its other functions, this organ significantly contributes to the breakdown and removal of toxins and metabolic waste from the body. Additionally, it produces clotting factors necessary for blood coagulation and synthesizes cholesterol and triglycerides. Furthermore, the ability of the liver to regenerate and repair itself is remarkable, making it a key player in maintaining overall health and homeostasis (1). Being cut off from meaningful interpersonal connections has been linked to serious consequences for both physical and mental well-being. It increases the risk of cardiovascular diseases, cognitive decline, anxiety, and depression. Accordingly, addressing social isolation is crucial for improving overall health and well-being (2). The elevated plus maze assesses anxiety-like behavior in rodents by monitoring their preference for closed versus open arms in a cross-shaped setup. Likewise, the marble burying test examines anxiety and compulsive behaviors by recording how many marbles are hidden beneath the bedding. Overall, these behavioral assays are commonly employed to investigate the impact of anxiolytic and anti-compulsive interventions (3). Nitric oxide (NO) serves as an important signaling molecule that participates in diverse physiological functions such as vasodilation, neurotransmission, and immune regulation. By promoting relaxation of blood vessels, NO contributes to blood pressure control and facilitates cellular communication. Further, maintaining proper NO levels is vital for maintaining cardiovascular health and reducing the risk of disease (4). NO and nitrite play crucial roles in liver health by regulating blood flow, reducing oxidative stress (OS), and modulating inflammation. Additionally, elevated levels of NO and nitrite can protect against liver damage and improve liver function. Nevertheless, disruptions in balance may lead to hepatic disorders, including non-alcoholic fatty liver disease (NAFLD) and cirrhosis (5). In the liver, OS arises when reactive oxygen species (ROS)

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outweigh antioxidant defenses, causing cellular injury and contributing to the development of liver disorders. Depending on its concentration and the oxidative environment, NO plays a dual role, as it can both protect against and contribute to liver damage. Thus, maintaining a balance of NO levels is crucial for maintaining liver health and preventing disease progression (6). The liver's antioxidant capacity is crucial for neutralizing ROS and preventing OS. MDA is a marker of lipid peroxidation and oxidative damage. Elevated levels of MDA indicate increased OS, a factor associated with liver diseases such as NAFLD and cirrhosis (7). In addition, TNF-α is a key pro-inflammatory cytokine involved in the pathogenesis of liver disorders. Elevated TNF-α level are associated with liver disorders such as NAFLD and hepatitis, contributing to inflammation, hepatocyte apoptosis, and fibrosis. Hence, measuring TNF-α offers important information about disease severity and progression (8). NAC acts as a strong antioxidant, safeguarding the liver by alleviating OS and inflammation. It enhances glutathione levels, thereby helping detoxify harmful substances and improving liver function. NAC has shown therapeutic benefits in managing hepatic conditions resulting from metabolic dysfunction and adverse reactions to medications. (9). This study aims to examine how NAC affects liver function and behavioral outcomes in the offspring of mice subjected to social isolation.

Materials and Methods

Laboratory Animals

The experiment was conducted from September 1, 2024, to March 31, 2025. Following ethical approval, twenty BALB/c mice (ten males and ten females) were obtained from the Pasteur Institute in Tehran, Iran. Each male was paired with a female for breeding, and their offspring were maintained under standardized laboratory conditions, including a 12-hour light/dark cycle, a stable temperature of 22 ± 1°C, and ad libitum access to food and water. The progeny were then divided into six groups, with three male and three female groups, each consisting of eight mice.

The study design included six groups: (1) female control, (2) female isolation + saline, (3) female isolation + NAC, (4) male control, (5) male isolation + saline, and (6) male isolation + NAC. The female control group received normal saline (NS) at 10 mL/kg, and the female isolation + saline group was subjected to social isolation stress (SIS) and treated with saline (10 mL/kg). Further, the female isolation + NAC group was exposed to isolation and administered NAC at 150 mg/kg, and the male control group received saline at 10 mL/kg. Moreover, the male isolation + saline group was subjected to isolation stress and treated with saline (10 mL/kg), and the male isolation + NAC group was exposed to isolation and given NAC at 150 mg/kg. Behavioral testing was performed once the offspring reached sexual maturity, around two months of age. Blood samples were collected following anesthesia with ketamine (100 mg/kg) and xylazine (10 mg/kg). Next, the animals were euthanized by decapitation, and liver tissues were extracted for subsequent biochemical and histological analyses (Figure 1).

Social Isolation Stress Model

Beginning at the conclusion of the third week, the mice were kept in individual cages for four weeks. To reduce visual stimuli, the cages were wrapped in black paper on all sides except the top. In addition, to limit human interaction, a single handler removed each mouse from its cage only once per week for cleaning purposes (10).

Treatment With N-acetylcysteine

NAC was delivered through intraperitoneal injection at a dose of 150 mg/kg once per day for four weeks, starting in the third week alongside the onset of SIS (11).

Behavioral Tests

Marble Burying Test

Clear Plexiglas cages with dimensions of 30.5 × 35.6 × 20.3 cm were employed in this experiment. A layer of material approximately 5 cm thick was spread across the base of each cage. Initially, each mouse was placed inside the enclosure and given 10 minutes to freely explore its surroundings. After this acclimation period, the animals were moved to a holding cage while 18 marbles, each with a diameter of 1.3 cm, were arranged within the test cage. Subsequently, the mice were reintroduced into the setup, and their activity was monitored using a video recording system. Typically, healthy mice do not engage in marble burying, whereas those exhibiting signs of psychological distress (e.g., anxiety or depression) tend to conceal a greater number of marbles (12).

Raised Plus-Shaped Maze

Anxiety-like behavior was assessed using a wooden elevated plus-maze apparatus composed of four arms. Each mouse was positioned in the center of the maze, oriented toward an open arm, and permitted to explore for five minutes. The number and duration of entries into open and closed arms were documented throughout this time. Mice exhibiting lower levels of anxiety typically spent more time in the open arms, whereas those with heightened anxiety showed a preference for the closed arms (13).

Gene Expression

Liver tissues were collected and processed for analysis. Ultimately, the hepatic expression of *TNF-α* genes was quantified through real-time polymerase chain reaction, following the design and validation of specific primers suitable for the assay (14). Table 1 presents the designed primers.

Biochemical Tests

Hepatic Antioxidant Potential

The ferric reducing antioxidant power assay was utilized to evaluate antioxidant capacity through three reagent solutions. Further, reagent A was prepared as a buffer by combining 1.55 mL sodium acetate with 8 mL acetic acid and diluting to 500 mL using distilled water. Likewise,

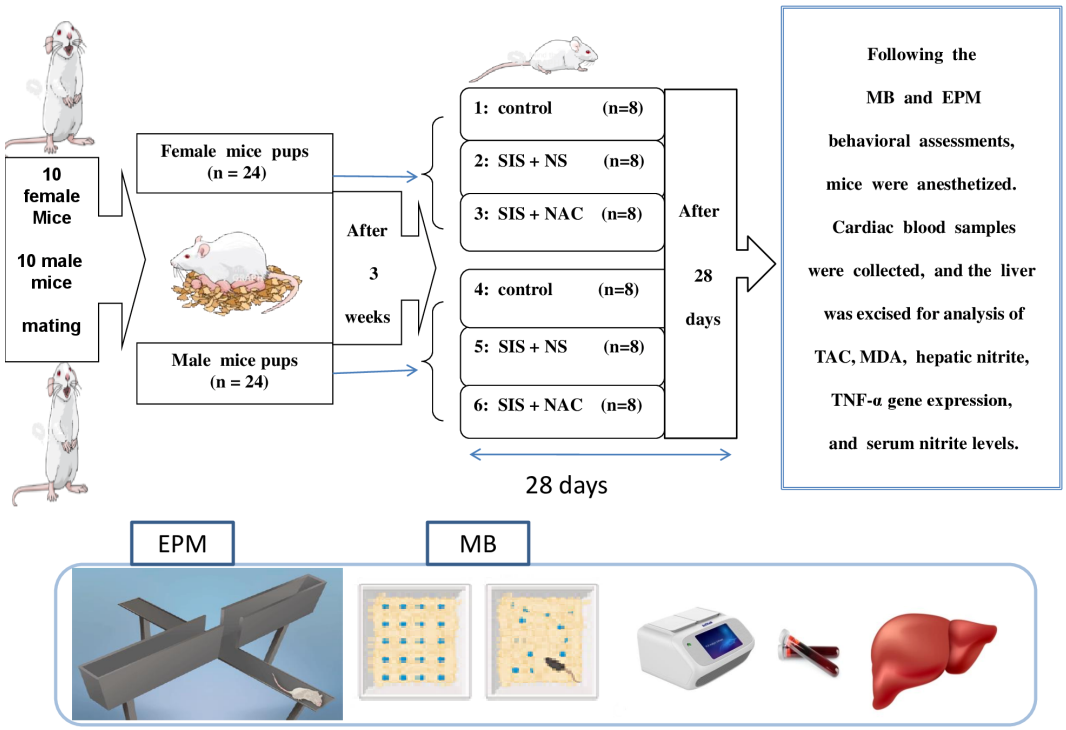

Figure 1. Visual Representation of the Research Structure
Note. NS: Normal saline; SIS: Social isolation stress; NAC: N-acetylcysteine; N: Number; MB: Marble burying; EPM: Elevated plus-maze; TAC: Total antioxidant capacity; MDA: Malondialdehyde.

Table 1. Designed Primers

Row	Gen Name	Forward Sequence	Reverse Sequence
1	*TNF-α*	TCTTCTCATTCCTGCTTGTGGC	TGATCTGAGTGTGAGGGTCTGG
2	*B2m*	CGTGATCTTTCTGGTGCTTGTC	GGAAGTTGGGCTTCCCATTCT

Note. TNF-α: Tumor necrosis factor alpha; B2M: Beta-2 microglobulin.

reagent B was obtained by dissolving 270 mg of ferric chloride hexahydrate ($FeCl_3 \bullet 6H_2O$) in 50 mL distilled water, while Reagent C was made by dissolving 47 mg triazine in 40 mL of 40 mM hydrochloric acid. The working solution consisted of 10 mL of reagent A, 1 mL of reagent B, and 1 mL of reagent C. Additionally, 25 µL of liver homogenate was introduced into this mixture and incubated at 37˚C for 10 minutes, and absorbance was recorded at 593 nm (15).

Liver Malondialdehyde Measurement

MDA levels were determined by mixing 1 mL of liver homogenate with trichloroacetic acid, thiobarbituric acid, and hydrochloric acid adjusted to pH 1.6–1.7. The mixture was incubated at 95˚C for 45 minutes, cooled for one hour, and then combined with 4 mL of n-butanol. After centrifugation at 1,000 rpm for 10 minutes, the absorbance of the supernatant was measured at 532 nm. Concentrations were calculated using the following formula:

$$\text{MDA (nmol/g)} = \text{absorbance} \div 1.56 \times 10^5 \text{ (16)}.$$

Nitric Oxide Metabolites

Considering that NO has a short half-life, direct measurement is challenging; thus, its levels are typically inferred from stable metabolites such as nitrite and nitrate. In this study, NO was quantified using a microplate-adapted Griess assay. Further, protein removal was achieved by treating 400 µL of each sample with 6 mg zinc sulfate. For total nitrite and nitrate determination, 100 µL of serum or deproteinized liver homogenate was placed into enzyme-linked immunosorbent assay (ELISA) microplate wells, followed by 100 µL of a 1:1 mixture of sulfanilamide and N-1-naphthylethylenediamine dihydrochloride. After incubation at 37˚C for 30 minutes to allow color development, absorbance was read at 540 nm using an ELISA reader, and concentrations were calculated against a sodium nitrite standard curve (100–3.125 µmol/L) (17).

Data Analysis

Statistical evaluation was performed using PRISM software version 8. Data were presented as mean values with standard deviations (mean ± SD). To determine significance, a two-way analysis of variance (ANOVA) was conducted, followed by Tukey's post-hoc multiple comparison test. Differences were considered statistically significant at $P < 0.05$.

Results

The Influence of N-Acetylcysteine on the Frequency of

Entries Into the Open Arms During the Elevated Plus-Maze Test

In the elevated plus-maze, socially isolated mice administered NS exhibited a pronounced reduction in open-arm entries compared with non-stressed controls ($P<0.01$). In contrast, treatment with NAC significantly increased open-arm entries in isolated mice relative to the saline-treated isolation group ($P<0.05$) (Figure 2a).

Impact of N-Acetylcysteine on the Duration of Open-Arm Exploration in the Elevated Plus-Maze

In the elevated plus-maze, saline-treated socially isolated mice displayed a significant decrease in open-arm duration compared with controls ($P<0.01$). Conversely, NAC treatment in isolated mice led to a notable increase in time spent in the open arms relative to the saline isolation group ($P<0.05$) (Figure 2b).

Impact of N-acetylcysteine on Obsessive-Compulsive Behaviors Assessed Through the Marble Burying Test

The one-way ANOVA revealed a significant variation in the duration of obsessive-compulsive behaviors across the treatment groups ($P<0.01$, $P<0.001$). Subsequent analysis with Tukey's post-hoc test demonstrated that mice exposed to SIS and given NS exhibited a marked elevation in compulsive activity during the marble burying test compared with the saline-treated controls ($P<0.001$). Contrarily, treatment with NAC in socially isolated mice could notably reduce the time spent engaging in these behaviors relative to the isolation group administered saline ($P<0.01$) (Figure 3).

Influence of N-Acetylcysteine on Hepatic Antioxidant Levels

ANOVA with subsequent Tukey's post-hoc testing confirmed a pronounced reduction in antioxidant concentrations in the SIS group administered NS compared to the control group receiving saline ($P<0.001$, $P<0.01$). In contrast, socially isolated mice treated with NAC showed a significant elevation in hepatic antioxidant levels when compared with the isolation group given saline ($P<0.05$) (Figure 4a).

Influence of N-Acetylcysteine on Hepatic Malondialdehyde Levels

Based on the findings, mice subjected to SIS and administered NS exhibited a substantial elevation in liver MDA content compared with the saline-treated control group ($P<0.001$). However, socially isolated mice receiving NAC displayed a significant reduction in MDA concentrations relative to the isolation group treated with saline ($P<0.05$) (Figure 4b).

Evaluation of N-Acetylcysteine on Hepatic Nitrite Concentrations

The findings revealed that liver nitrite levels were substantially elevated in mice exposed to SIS and administered NS compared with the control group receiving saline ($P<0.01$). Conversely, treatment of stressed mice with NAC resulted in a notable decrease in hepatic NO concentrations relative to the isolation group given saline ($P<0.05$) (Figure 5a).

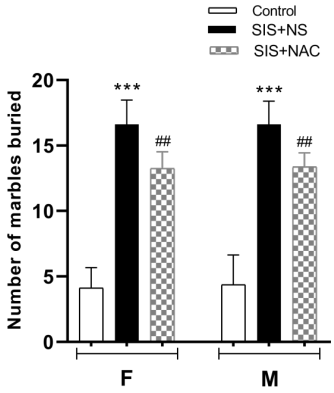

Figure 3. The Effect of NAC on Marble-Burying Behavior Evaluated by Recording the Number of Marbles Hidden During the Assay
Note. The results from groups of eight mice are presented as means ± standard deviations and analyzed employing two-way ANOVA followed by Tukey's post-hoc comparisons. A triple asterisk (***) implies a highly significant difference ($P<0.001$) compared with the control group, whereas double hash marks (##) denote a significant change ($P<0.01$) relative to the SIS group treated with NS. ANOVA: Analysis of variance; NS: Normal saline; SIS: Social isolation stress; NAC: N-acetylcysteine; F: Female; M: Male.

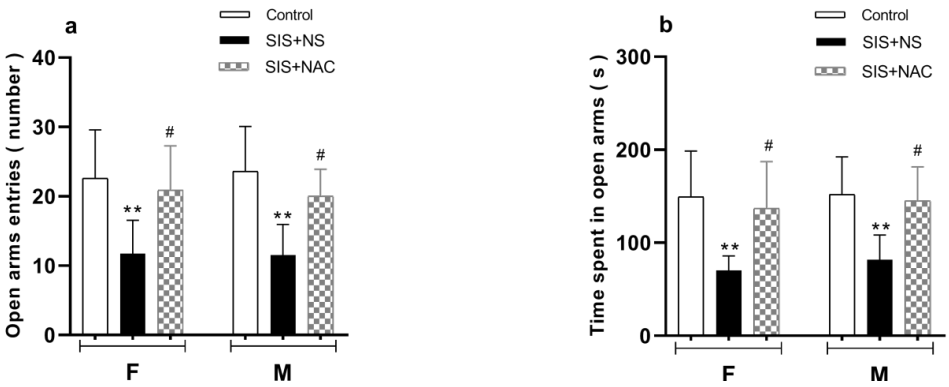

Figure 2. Effect of NAC on the Number of Entries Into the Open Arms **(a)** and the Duration Spent in the Open Arms **(b)**
Note. The results are expressed as means ± standard deviations from groups of eight mice. Statistical evaluation was performed using two-way ANOVA followed by Tukey's post-hoc comparison. A double asterisk (**) indicates a significance level of $P<0.01$ versus the control group, while a hash symbol (#) represents $P<0.05$ compared with the SIS group treated with saline. ANOVA: Analysis of variance; NS: Normal saline; SIS: social isolation stress; NAC: N-acetylcysteine; F: Female; M: Male.

Assessment of N-Acetylcysteine on Serum Nitrite Levels

The investigation indicated that serum nitrite levels were markedly higher in mice exposed to SIS and administered NS compared with the control group treated with saline ($P<0.001$, $P<0.01$). In contrast, treatment of stressed mice with NAC produced a significant decline in serum nitrite concentrations relative to the isolation group receiving saline ($P<0.05$) (Figure 5b).

Effect of N-Acetylcysteine on TNF-α Gene Expression

The evaluation demonstrated notable differences in *TNF-α* gene expression among the experimental groups ($P<0.01$, $P<0.001$). Mice subjected to SIS and treated with NS displayed a considerable elevation in TNF-α expression compared with the saline-treated controls ($P<0.01$, $P<0.001$). Contrarily, administration of NAC to the socially isolated group significantly reduced *TNF-α* gene expression relative to the isolation group receiving saline ($P<0.01$) (Figure 6).

Discussion

This study investigated the effects of NAC on liver function and behavioral outcomes in the offspring of mice exposed to SIS. Extended periods of social isolation have been shown to disrupt liver function, increase OS, and promote inflammatory responses, all of which play a role in the onset of NAFLD.

The current results demonstrated that supplementation with NAC markedly enhanced hepatic function by strengthening antioxidant defenses, decreasing MDA levels, and lowering both nitrite and TNF-α concentration. In addition, NAC mitigated anxiety-like behaviors and compulsive actions in socially stressed mice, highlighting its combined therapeutic potential for addressing both liver dysfunction and neurobehavioral impairments induced by isolation. The liver is essential for sustaining physiological homeostasis, functioning in detoxification, metabolic control, protein synthesis, and bile secretion. Furthermore, it processes nutrients absorbed from the gastrointestinal tract, stores glycogen, and helps regulate glucose levels. Additionally, it is vital for filtering and eliminating toxins and metabolic waste from the body (18). Social isolation, defined as a lack of meaningful social interaction and connection, has been linked to numerous adverse health outcomes, including heightened risks of cardiovascular disease, cognitive impairment, anxiety, depression, and immune system dysfunction. Chronic isolation can elevate stress hormones and contribute to

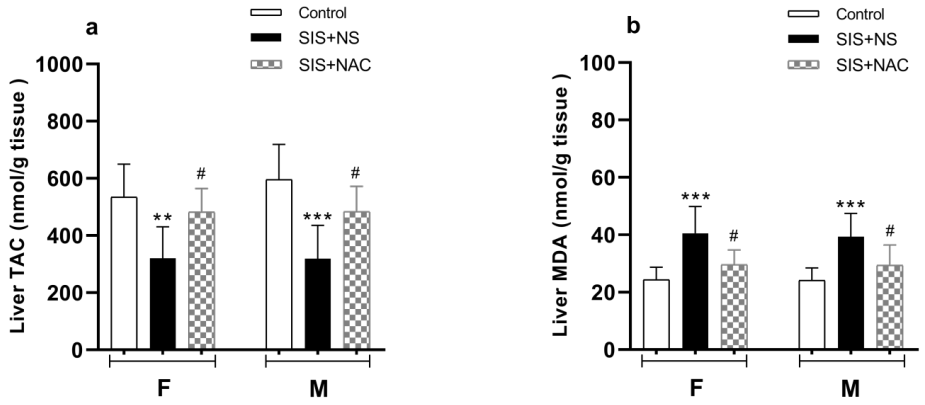

Figure 4. The Effects of NAC on Liver Antioxidant Activity **(a)** and MDA Levels **(b)**
Note. The results from groups of eight mice are shown as means ± standard deviations and analyzed utilizing two-way ANOVA followed by Tukey's post-hoc comparisons. A double asterisk (**) represents statistical significance at $P<0.01$, while a triple asterisk (***) denotes $P<0.001$ compared with the control group. Furthermore, the hash symbol (#) indicates $P<0.05$ relative to the SIS group administered NS. ANOVA: Analysis of variance; NS: Normal saline; SIS: Social isolation stress; NAC: N-acetylcysteine; F: Female; M: Male; MDA: Malondialdehyde.

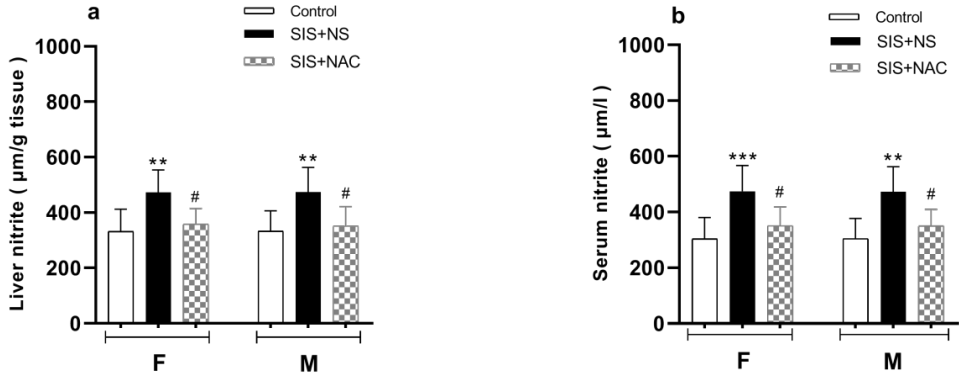

Figure 5. Effect of NAC on Nitrite Levels in Hepatic Tissue **(a)** and Serum **(b)**
Note. Data from groups of eight mice are presented as means ± standard deviations and evaluated applying two-way ANOVA followed by Tukey's post-hoc comparisons. A double asterisk (**) indicates statistical significance at $P<0.01$, while a triple asterisk (***) represents $P<0.001$ compared with the control group. Additionally, the hash symbol (#) denotes $P<0.05$, and a double hash (##) signifies $P<0.01$ relative to the SIS group administered NS. ANOVA: Analysis of variance; NS: Normal saline; SIS: Social isolation stress; NAC: N-acetylcysteine; F: Female; M: Male.

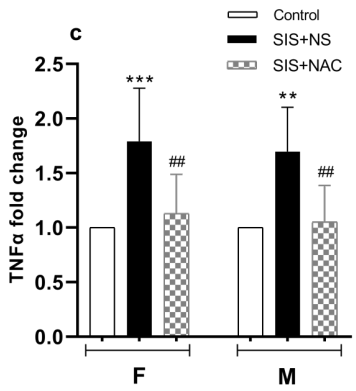

Figure 6. Effect of NAC on Liver *TNF-α* Gene Expression
Note. Data from groups of eight mice are presented as means ± standard deviations and evaluated using two-way ANOVA followed by Tukey's post-hoc analysis. A double asterisk (**) indicates statistical significance at $P<0.01$, while a triple asterisk (***) represents $P<0.001$ compared with the control group. Moreover, the double hash symbol (##) denotes $P<0.01$ relative to the SIS group administered NS. ANOVA: Analysis of variance; NS: Normal saline; SIS: Social isolation stress; NAC: N-acetylcysteine; F: Female; M: Male.

systemic inflammation, ultimately compromising overall health. Likewise, previous studies have associated social isolation with liver dysfunction, particularly through mechanisms involving OS and inflammatory signaling (19-21), which aligns with the outcomes observed in this study. Nitrite accumulation in liver tissue serves as a biomarker for oxidative and nitrosative stress, both of which are implicated in liver pathologies such as NAFLD and cirrhosis. High nitrite concentrations contribute to the generation of reactive nitrogen species, which can cause hepatocellular injury. In the present study, social isolation markedly elevated hepatic nitrite levels, whereas administration of NAC significantly lowered them (5). Hepatic NO levels are vital for maintaining liver health. Excessive NO production has been linked to the progression of liver disorders, including cirrhosis and chronic hepatitis. Moreover, NO can contribute to liver damage by promoting OS and inflammation, leading to cellular injury. However, NO also has protective effects, such as enhancing hepatic blood flow and reducing platelet aggregation. Accordingly, maintaining balanced NO levels is critical for proper liver function and prevention of liver disease progression. In this study, NAC reduced liver nitrite levels (22). NO, while essential for vascular regulation and immune responses, can become detrimental when overproduced. Similarly, excessive NO contributes to OS and inflammation, exacerbating liver injury cirrhosis and chronic hepatitis conditions. Thus, maintaining NO homeostasis is critical for liver integrity. NAC's ability to lower nitrite levels in this study suggests its role in restoring this balance. The liver's antioxidant defenses play a crucial role in counteracting ROS and protecting against oxidative injury. In addition, a compromised antioxidant capacity can lead to hepatocyte injury and progression of liver diseases. Enhancing this defense mechanism is a promising strategy for liver protection. NAC, known for replenishing intracellular glutathione, significantly improved antioxidant capacity in this study, reinforcing its hepatoprotective properties (23). MDA is a well-established marker of lipid peroxidation and OS. Elevated MDA levels are commonly observed in liver disorders such as NAFLD and cirrhosis, indicating increased membrane lipid damage and inflammation. In our study, NAC administration markedly reduced MDA levels, suggesting decreased oxidative injury to liver cells (24). TNF-α is a major pro-inflammatory cytokine that contributes to liver inflammation and fibrotic changes. Increased TNF-α levels are linked to hepatocyte death and the advancement of liver disorders. The current findings demonstrated that NAC substantially downregulated *TNF-α* gene expression in the liver, highlighting its anti-inflammatory potential (25). Collectively, NAC's multifaceted actions, including enhancement of antioxidant defenses, suppression of lipid peroxidation, reduction of nitrosative stress, and modulation of inflammatory cytokines, contribute to improved liver function and protection against stress-induced hepatic damage. These biochemical improvements were paralleled by behavioral benefits, as evidenced by increased exploratory behavior in the elevated plus maze and reduced compulsive marble-burying activity. Likewise, these behavioral changes indicate that NAC may also counteract the neurobehavioral consequences of social isolation (26-28). In summary, this study provided compelling evidence that NAC, through its antioxidant and anti-inflammatory mechanisms, can effectively mitigate liver dysfunction and behavioral disturbances resulting from SIS. These findings are supported by recent literature emphasizing NAC's therapeutic promise in managing oxidative and inflammatory liver conditions.

Conclusion

The findings of this study highlighted the promising role of NAC in mitigating both behavioral and liver dysfunctions caused by SIS. NAC demonstrated significant efficacy in reducing stress-related behavioral abnormalities, likely through its robust antioxidant and anti-inflammatory actions. These effects were reflected in elevated total antioxidant capacity and decreased levels of MDA and nitrite in both liver and serum samples. Furthermore, NAC markedly suppressed hepatic *TNF-α* gene expression, indicating its role in modulating inflammatory pathways associated with liver dysfunction. Overall, the findings confirmed that NAC holds significant promise in counteracting the harmful impact of social isolation on liver function and behavioral outcomes.

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Artificial Intelligence Use Disclosure

During the preparation of this manuscript, the authors employed ChatGPT for language and grammatical editing. All content generated with the assistance of these tools was precisely reviewed, validated, and revised by the authors to ensure accuracy, originality,

and scholarly rigor. Ultimately, the authors assume full responsibility for the integrity and final content of the published articles.

Authors' Contribution

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Writing–Review & Editing: Mehrdad Shahrani Korrani

Competing Interests

The authors declare that they have no competing interests to disclose.

Ethical Approval

All experimental procedures involving mice were conducted with the approval of the Ethics Committee at Islamic Azad University, Shushtar Branch (ethical code IR.IAU.AHVAZ.REC.1403.444). The study was also documented in compliance with ARRIVE guidelines.

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References

1. Fu Y, Jiang C, Li Z, Shi X, Lv P, Zhang J. Association between the composite dietary antioxidant index and non-alcoholic fatty liver disease: evidence from National Health and Nutrition Examination Survey 2005-2016. Front Nutr 2025;12:1473487. doi:10.3389/fnut.2025.1473487
2. Kawatake-Kuno A, Morishita H. [Childhood social isolation and social brain development]. Brain Nerve 2025;77(2):155-60. doi:10.11477/mf.1881609607700201555
3. Lee SM, Choi Y, Kim D, Jeong HJ, Do YH, Jung S, et al. Developmental deficits, synapse and dendritic abnormalities in a Clcn4 KO autism mice model: endophenotypic target for ASD. Transl Psychiatry 2025;15(1):28. doi:10.1038/s41398-024-03201-6
4. Haghighat Naeini A, Nassireslami E, Shariatpanahi M, Chamanara M, Abdolali A, Aghsami M. Necroptosis signaling in spatial memory impairment caused by aflatoxin B1 in male mice; involvement of the nitric oxide pathway. Toxicon 2025;256:108272. doi:10.1016/j.toxicon.2025.108272
5. Wahyuningsih SP, Mwendolwa AA, Winarni D, Anggreini RW, Mamuaya BK. Protective effect of red okra (*Abelmoschus esculentus* (L.) Moench) pods against sodium nitrite-induced liver injury in mice. Vet Med Int 2021;2021:6647800. doi:10.1155/2021/6647800
6. Rostami S, Arefhosseini S, Tutunchi H, Khoshbaten M, Ebrahimi-Mameghani M. Does myo-inositol supplementation influence oxidative stress biomarkers in patients with non-alcoholic fatty liver disease? Food Sci Nutr 2024;12(2):1279-89. doi:10.1002/fsn3.3842
7. Engin AB, Engin A, Engin ED, Memis L. Does lithium attenuate the liver damage due to oxidative stress and liver glycogen depletion in experimental common bile duct obstruction? Toxicol Appl Pharmacol 2023;466:116489. doi:10.1016/j.taap.2023.116489
8. Zhou X, Yang X, Liu X, Jiang L, Jiang Y. Hexavalent chromium (Cr6+) induces oxidative stress, inflammatory response, apoptosis, and DNA damage in the liver of largemouth bass by inhibiting the Nrf2-Keap1 signal pathway. Fish Physiol Biochem 2025;51(2):53. doi:10.1007/s10695-025-01469-z
9. Sun J, Chen Y, Wang T, Ali W, Ma Y, Yuan Y, et al. Corrigendum to "Baicalin and N-acetylcysteine regulate choline metabolism via TFAM to attenuate cadmium-induced liver fibrosis" [Phytomedicine. 2024 Mar;125:155337. Phytomedicine 2025;136:156285. doi:10.1016/j.phymed.2024.156285
10. Amini-Khoei H, Tahmasebi-Dehkordi H, Bijad E. Resocialization mitigates depressive behaviors induced by social isolation stress in mice: attenuation of hippocampal neuroinflammation and nitrite level. Brain Behav 2024;14(6): e3604. doi:10.1002/brb3.3604
11. Lu J, Zhao P, Ding X, Liu Y, Li H. N-acetylcysteine assists muscle development in offspring of mice subjected to maternal heat stress during pregnancy. J Sci Food Agric 2024;104(13):7895-906. doi:10.1002/jsfa.13620
12. Yao X, Chen R, Chen H, Koleske A, Xiao X. Impact of Abl2/Arg deficiency on anxiety and depressive behaviors in mice. Behav Brain Res 2024;468:115022. doi:10.1016/j.bbr.2024.115022
13. Kruk-Slomka M, Dzik A, Biala G. The effects of indirect and direct modulation of endocannabinoid system function on anxiety-related behavior in mice assessed in the elevated plus maze test. Molecules 2025;30(4):867. doi:10.3390/molecules30040867
14. Soumya K, Haridas KR, James J, Sudheesh S. Isolation of a novel quercetin derivative from *Terminalia chebula* and RT-PCR-assisted probing to investigate its DNA repair in hepatoma cells. Res Pharm Sci 2024;19(3):303-18. doi:10.4103/rps.Rps_56_23
15. Felicianna, Lo EK, Chen C, Ismaiah MJ, Zhang F, Leung HK, et al. Alpha-aminobutyric acid ameliorates diet-induced metabolic dysfunction-associated steatotic liver disease (MASLD) progression in mice via enhancing AMPK/SIRT1 pathway and modulating the gut-liver axis. J Nutr Biochem 2025;140:109885. doi:10.1016/j.jnutbio.2025.109885
16. Naseem A, Majeed Khan H, Umar A, Elshikh MS, Aljowaie RM, Gancarz M. Mechanistic insights of methylcinnamate in improving oxidative stress and inflammation in acetaminophen-induced hepatotoxic mice by upregulating Nrf2 pathway. J Pharm Pharmacol 2025;77(3):418-29. doi:10.1093/jpp/rgaf001
17. Zhang Y, Ye Y, Jia X, Wang P, Xiong Z, Zhu H. The protective effects of salusin-α against oxidative stress and inflammatory response in mice with gestational diabetes mellitus (GDM). Arch Physiol Biochem 2025;131(3):493-502. doi:10.1080/13813455.2025.2456876
18. Gao J, Lu W, Xin Y, Ma H, Sheng X, Gao G, et al. Liver-specific Bcl3 knockout alleviates acetaminophen-induced liver injury by activating Nrf2 pathway in male mice. Cell Mol Gastroenterol Hepatol 2025;19(6):101483. doi:10.1016/j.jcmgh.2025.101483
19. Zhao X, Li F, Cheng C, Bi M, Li J, Cong J, et al. Social isolation promotes tumor immune evasion via β2-adrenergic receptor. Brain Behav Immun 2025;123:607-18. doi:10.1016/j.bbi.2024.10.012
20. Miao Y, Kong X, Zhao B, Fang F, Chai J, Huang J. Loneliness and social isolation with risk of incident non-alcoholic fatty liver disease, UK biobank 2006 to 2022. Health Data Sci 2025;5:0220. doi:10.34133/hds.0220
21. Zahir M, Shariatzadeh S, Khosravi A, Alshaikh FA, Moradi P, Ghaderi M, et al. High risk of drug toxicity in social isolation stress due to liver dysfunction: role of oxidative stress and inflammation. Brain Behav 2021;11(8): e2317. doi:10.1002/brb3.2317
22. Kaur G, Kushwah AS. Sodium orthovanadate protects against ulcerative colitis and associated liver damage in mice: insights into modulations of Nrf2/Keap1 and NF-κB pathways. Naunyn Schmiedebergs Arch Pharmacol 2025;398(2):1557-74. doi:10.1007/s00210-024-03335-w
23. Ren Y, Sun Y, Javad HU, Wang R, Zhou Z, Huang Y, et

al. Growth performance of and liver function in heat-stressed Magang geese fed the antioxidant zinc ascorbate and its potential mechanism of action. Biol Trace Elem Res 2025;203(2):1035-47. doi:10.1007/s12011-024-04220-6

24. Qi S, Zhang C, Yan J, Ma X, Zhong Y, Hou W, et al. Ethyl acetate extract of *Cichorium glandulosum* activates the P21/Nrf2/HO-1 pathway to alleviate oxidative stress in a mouse model of alcoholic liver disease. Metabolites 2025;15(1):41. doi:10.3390/metabo15010041
25. Shariati S, Mohtadi S, Khodayar MJ, Salehcheh M, Azadnasab R, Mansouri E, et al. Quinic acid alleviates liver toxicity induced by acetaminophen in mice via anti-oxidative and anti-inflammatory effects. Naunyn Schmiedebergs Arch Pharmacol 2025;398(8):10367-78. doi:10.1007/s00210-025-03869-7
26. Har-Zahav A, Tobar A, Fried S, Sivan R, Wilkins BJ, Russo P, et al. Oral N-acetylcysteine ameliorates liver fibrosis and enhances regenerative responses in Mdr2 knockout mice. Sci Rep 2024;14(1):26513. doi:10.1038/s41598-024-78387-2
27. Zhao M, Han M, Guo S, Tang Z. CXCL12 as a potential hub gene for N-acetylcysteine treatment of T1DM liver disease. Biomolecules 2025;15(2):176. doi:10.3390/biom15020176
28. van Asseldonk DP, Crouwel F, Seinen ML, Scheffer PG, Veldkamp AI, de Boer NK, et al. Exploring the role of oxidative stress and the effect of N-acetylcysteine in thiopurine-induced liver injury in inflammatory bowel disease: a randomized crossover pilot study. Basic Clin Pharmacol Toxicol 2024;134(4):507-18. doi:10.1111/bcpt.13978