International Journal of Medical Laboratory 2025;12(3):222-235.

International Journal of Medical Laboratory
Shahid Sadoughi University of Medical Sciences

Original Article

N-Acetylcysteine Attenuates Social Isolation-Induced Behavioral Alterations and Liver Injury Through Modulation of Oxidative Stress and Inflammatory Cytokines in NMRI Mice

Javad Asgharzadeh [1] Ph.D., Leila Derakhshan [1] Ph.D., Seyedeh Ommolbanin Ghasemian [2*] Ph.D.

[1] Department of Veterinary, Sho.C., Islamic Azad University, Shoushtar, Iran
[2] Department of Veterinary, Be.C., Islamic Azad University, Behbahan, Iran

Article history
Received: 21 Jan 2026
Accepted: 2 May 2026
Available online: 22 Nov 2026

Keywords
Inflammation
Liver
N-acetylcysteine
Oxidative stress
Social isolation stress

CC BY-NC 4.0

© Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License, which permits any non-commercial use, sharing, adaptation, distribution, and reproduction in any medium or format, provided that appropriate credit is given to the original author(s) and the source.

A B S T R A C T

Introduction: Social isolation stress (SIS) induces persistent behavioral disturbances and hepatic dysfunction mediated by oxidative, nitrosative, and inflammatory mechanisms. N-acetylcysteine (NAC), a glutathione precursor with antioxidant and anti-inflammatory properties, may counteract these stress-induced alterations. This study examined the effects of early-life SIS on behavioral and hepatic changes in NMRI mice and evaluated NAC's protective role.

Materials and Methods: Forty-eight NMRI mice (24 males and 24 females; n=8 per group) were randomly allocated to six groups: control, SIS + normal saline, and SIS + NAC (150 mg/kg/day, IP) for each sex. Social isolation was applied for 4 weeks after weaning. Behavioral assessments included the elevated plus maze, marble burying, three-chamber social interaction, and resident–intruder tests. Hepatic and serum total antioxidant capacity, malondialdehyde, nitrite levels, catalase activity, and hepatic interleukin 1β (*IL-1β*) and tumor necrosis factor α (*TNF-α*) mRNA expression were measured.

Results: SIS significantly reduced open-arm entries (≈50%) and time spent the open arms (≈45%) in the elevated plus maze ($p<0.01$), increased marble burying ($p<0.001$), and elevated aggression ($p<0.001$). Hepatically, SIS decreased total antioxidant capacity (≈40%) and catalase (≈55%), while increasing malondialdehyde (≈60%), nitrite (≈40%), *IL-1β* (≈1.8-fold), and *TNF-α* (≈1.7-fold) ($p<0.01$–0.001). NAC significantly improved behavioral indices, restored total antioxidant capacity and catalase, reduced malondialdehyde and nitrite ($p<0.05$), and downregulated *IL-1β* and *TNF-α* toward control values. No significant sex differences were observed.

Conclusion: Early-life social isolation induced behavioral abnormalities and hepatic oxidative/nitrosative stress with inflammatory activation. NAC markedly attenuated these alterations. This can support its protective role in ceasing stress-induced liver–brain axis dysfunction; however, further clinical studies are required.

* **Corresponding Author:** Department of Veterinary, Be.C., Islamic Azad University, Behbahan, Iran. **Email:** Ommolbanin.Ghasemian@iau.ac.ir, **Tel/Fax**: +989166711485/+986152870105-9

[Downloaded from ijml.ssu.ac.ir on 2026-11-24]

Introduction

Psychological stress, particularly early-life social separation and social isolation, is increasingly recognized as a major public health concern with adverse consequences that extend beyond the central nervous system to peripheral organs, including the liver [1]. Accumulating evidence supports a bidirectional relationship between hepatic physiology and brain function, often described as the liver–brain axis, through which metabolic, inflammatory, and neuroendocrine signals can influence behavior and emotional regulation [2].

Chronic social isolation can persistently activate the hypothalamic–pituitary–adrenal axis and increase hepatic vulnerability by enhancing oxidative and inflammatory pathways. In this context, increased reactive oxygen species generation and compromised antioxidant defenses contribute to lipid peroxidation and accumulation of malondialdehyde in the liver tissue [3]. In parallel, dysregulation of nitric oxide signaling, often reflected by altered nitrite levels, may further amplify oxidative injury and inflammatory responses [4].

Inflammation is another key component of stress-associated hepatic injury. Upregulation of pro-inflammatory mediators, such as interleukin (IL)1β, IL-6, and tumor necrosis factor α (TNF-α) can aggravate local tissue damage and may also affect brain function via systemic circulation, thereby linking hepatic pathology to behavioral outcomes [5]. Consistent with this view, early-life stress paradigms have been shown to produce persistent anxiety-related and social behavioral abnormalities, which are frequently accompanied by immune and redox imbalances [6].

Accordingly, behavioral consequences of early-life social stress are commonly evaluated using validated paradigms, including the three-chamber social interaction test, elevated plus maze (EPM), and marble burying test, which assess social behavior, anxiety-like behavior, and repetitive/compulsive-like responses, respectively [7, 8].

N-acetylcysteine (NAC), a precursor of glutathione, has well-established antioxidant and anti-inflammatory properties and has shown protective effects in experimental models characterized by oxidative injury and inflammatory activation [9, 10]. By enhancing endogenous antioxidant capacity and modulating inflammatory signaling, NAC may provide a dual benefit by attenuating hepatic oxidative damage while also improving stress-related behavioral disturbances. Previous studies have demonstrated that NAC exhibits neuroprotective effects against lipopolysaccharide-induced cognitive deficits and brain oxidative damage [11]. Additionally, the hepatoprotective effects of NAC have been confirmed in various models of liver injury, including diabetic conditions [12].

Although previous studies have separately examined the effects of social isolation stress, NAC treatment, or hepatic injury, a few works have simultaneously investigated the potential protective role of NAC against hepatic oxidative and inflammatory alterations induced by social isolation stress in association with behavioral disturbances. In particular, the interaction

[Downloaded from ijml.ssu.ac.ir on 2026-11-24]

between stress-induced hepatic dysfunction and behavioral outcomes within the framework of the liver–brain axis remains insufficiently explored. Recent evidence has highlighted that unpredictable chronic mild stress can serve as a reliable model for studying depression-induced neuroinflammation, and has further emphased the need for a comprehensive investigation of stress-related disorders [13]. Moreover, psychosocial stress has been implicated in immune and metabolic dysregulation leading to liver disease [14].

Therefore, the present study investigated whether NAC administration can mitigate behavioral abnormalities and hepatic oxidative/ inflammatory alterations induced by social isolation stress in NMRI mice. Given the significance of the liver–brain axis and the existing research gap regarding the prophylactic role of NAC in mitigating stress-induced hepatic injury in offspring, this study was designed to investigate the impact of social isolation stress on hepatic health and to evaluate the therapeutic potential of NAC through modulation of the nitric oxide system and inflammatory cytokines (IL-1β, IL-6, TNF-α).

Materials and Methods

Study design and experimental animals

This experimental study was conducted using NMRI mice. A total of 10 adult male and 10 adult female mice (8–10 weeks old) were obtained from the Pasteur Institute of Iran, Tehran, for breeding purposes. Animals were housed under standard laboratory conditions, including a 12:12 h light/dark cycle, an ambient temperature of 22 ± 1°C, and relative humidity of 55 ± 5%, with free access to standard pellet chow and water.

Experimental grouping and randomization

Offspring entered the study after weaning at the end of the third postnatal week. In total, 48 pups (24 males and 24 females) were randomly assigned to six experimental groups (n = 8 per group):

Group A (Female Isolation): social isolation + normal saline (vehicle)

Group B (Female Control): group-housed + normal saline

Group C (Female Isolation + NAC): social isolation + N-acetylcysteine (150 mg/kg)

Group D (Male Isolation): social isolation + normal saline (vehicle)

Group E (Male Control): group-housed + normal saline

Group F (Male Isolation + NAC): social isolation + N-acetylcysteine (150 mg/kg)

For clarity, animals in the NAC-treated groups were also exposed to social isolation stress and received NAC treatment during the experimental period. The primary objective of the present study was to examine the protective effects of NAC under social isolation stress conditions rather than to assess its basal effects in non-stressed animals; therefore, a Control + NAC group was not included in the experimental design. The sample size (n = 8 per group) was determined based on previous similar experimental studies and was sufficient to detect statistically significant differences with an effect size comparable to those reported earlier [4, 6].

Induction of the social isolation stress model

Mice assigned to the isolation groups were housed individually in polycarbonate cages for

[Downloaded from ijml.ssu.ac.ir on 2026-11-24]

four weeks. To minimize environmental and social stimuli, the cage walls were covered with a dark opaque material. Animal handling was restricted to once per week, for bedding replacement, and was performed by the same experimenter throughout the study for reducing variability associated with external stressors [4, 11]. Previous research has showed that social isolation stress induces hepatic hypertrophy in C57BL/6J mice. This can highlight the relevance of this model for studying stress-induced liver alterations [15].

Pharmacological intervention

NAC (Sigma-Aldrich, St. Louis, MO, USA; Cat. No. A9165) was freshly dissolved in 0.9% normal saline and administered intraperitoneally at a dose of 150 mg/kg/day using a 29-gauge insulin syringe (BD, USA) at an injection volume of 10 mL/kg for 4 consecutive weeks [12]. The efficacy of NAC in attenuating oxidative stress and liver injury has been previously reported in rat models [16]. Moreover, NAC has shown therapeutic potential in psychiatric disorders through its antioxidant and glutamatergic modulatory effects [17].

Behavioral assessments in adulthood

All behavioral tests were conducted at a fixed time of day by an investigator blinded to group allocation.

Three-chamber social interaction test

Social behavior was assessed using a three-chamber apparatus (Arya Teb, Iran). Following a five-min habituation period, a stimulus mouse (Stranger 1) was placed inside a wire cage, and the test was continued for 10 min [7]. Behavioral parameters were analyzed using ANY-maze software (version 6.3).

Elevated plus maze

The apparatus consisted of two open arms and two closed arms, elevated 50 cm above the floor. Each mouse was allowed to explore the maze for 5 min [13]. The recorded parameters included the time spent in and the number of entries into the open and closed arms, as measured by the tracking software.

Marble burying test

A total of 18 glass marbles were evenly placed on the bedding surface. After 30 min, marbles that were covered by more than two-thirds of their surface with bedding were considered buried [8].

Sample collection and euthanasia

Animals were deeply anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) (Alfasan, Woerden, the Netherlands). Blood samples were collected by cardiac puncture. Using a Hettich centrifuge (Germany), serum was separated by centrifugation at 3000 × g for 10 min at 4°C, and stored at −80°C until analysis. Liver tissue was rapidly excised, snap-frozen, and stored at −80°C for subsequent assays.

Resident–intruder test

Aggressive behavior was evaluated using the resident–intruder paradigm. Each experimental mouse (resident) was placed in its home cage, and an unfamiliar age- and sex-matched mouse (intruder) was introduced for 5 min. The latency to the first attack, number of aggressive episodes, and total duration of aggressive behavior were recorded by an observer blinded to group allocation. Social isolation-induced anxiety-like behavior and associated neurochemical changes have been well-documented in rodents, validating the behavioral paradigms employed in this study [18].

[Downloaded from ijml.ssu.ac.ir on 2026-11-24]

Gene expression analysis by real time-quantitative polymerase chain reaction (RT-qPCR) RNA extraction

Total RNA was extracted, using TRIzol reagent (Thermo Fisher Scientific, USA; Cat. No. 15596026), and homogenized with an IKA T10 homogenizer (Germany). RNA purity and concentration were confirmed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA) [14].

cDNA synthesis and RT-PCR

Complementary DNA (cDNA) was synthesized using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA), and according to the manufacturer's instructions. RT-qPCR was performed using SYBR Green Master Mix (Ampliqon, Denmark) on an ABI StepOnePlus Real-Time PCR System (Applied Biosystems, USA). Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method, with beta-2 microglobulin used as the reference gene [19]. To evaluate gene expression levels, primer sequences were designed, as shown in Table 1.

PCR amplification was performed under the following cycling conditions: initial denaturation at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s. Melt curve analysis was carried out to confirm amplification specificity. Primer amplification efficiency ranged between 90% and 110%, and amplicon sizes were between 100 and 150 bp.

Biochemical assays

Total antioxidant capacity in liver and serum samples was assessed using the ferric reducing antioxidant power assay with a commercial reagent kit (ZellBio GmbH, Germany; Cat. No. ZB ferric reducing antioxidant power 96A), and according to the manufacturer's protocol. Absorbance was read at 593 nm using a BioTek microplate reader/spectrophotometer (BioTek Instruments, Winooski, VT, USA).

Lipid peroxidation was evaluated by measuring malondialdehyde levels, using a commercial TBARS/ malondialdehyde (MDA) assay kit (ZellBio GmbH, Germany; Cat. No. ZB malondialdehyde 96A), and according to the manufacturer's instructions. Absorbance was recorded at 535 nm using the same instrument.

Catalase activity was measured in liver homogenates, using a commercial catalase activity assay kit (ZellBio GmbH, Germany; Cat. No. ZB CAT 96A), and following the manufacturer's instructions, with absorbance read at 240 nm. Total protein concentration was determined using a Bradford protein assay kit.

Nitric oxide production was estimated indirectly by measuring nitrite levels, using Griess reagent, and absorbance was measured at 540 nm, using the BioTek spectrophotometric system. Nitric oxide has been shown to contribute to stress-induced depression- and anxiety-like behaviors, which can support the relevance of measuring this parameter in the present study [20].

Statistical analysis

Data was analyzed using GraphPad Prism version 8. Results are presented as the mean ± standard error of the mean. To evaluate the independent effects of sex and treatment, as well as their interaction, two-way analysis of variance (two-way ANOVA) was performed, followed by Tukey's post hoc test. A value of $p < 0.05$ was considered statistically significant.

[Downloaded from ijml.ssu.ac.ir on 2026-11-24]

Table 1. Primer sequences used for quantitative real-time PCR (qRT-PCR)

No.	Gene	Forward primer (5′→3′)	Reverse primer (5′→3′)
1	*TNF-α*	TCTTCTCATTCCTGCTTGTGGC	TGATCTGAGTGTGAGGGTCTGG
2	*IL-6*	TGCCTTCTTGGGACTGATGTTG	TGTTGTGGGTGGTATCCTCTGT
3	*IL-1β*	GAAATGCCACCTTTTGACAGTG	TGGATGCTCTCATCAGGACAG
4	*Beta-2 microglobulin*	CGTGATCTTTCTGGTGCTTGTC	GGAAGTTGGGCTTCCCATTCT

Results

As shown in Figure 1, social isolation stress significantly affected behavioral indices in the EPM test. Animals subjected to social isolation stress and receiving normal saline exhibited a significant reduction in the number of entries into the open arms compared with the control group receiving normal saline ($p < 0.01$). Similarly, the time spent in the open arms was significantly decreased in socially isolated animals compared with controls ($p < 0.01$).

Administration of NAC to socially isolated animals significantly increased the number of entries into the open arms compared with the social isolation group receiving normal saline ($p < 0.05$). NAC treatment also significantly increased the time spent in the open arms ($p < 0.05$).

When behavioral responses were compared between sexes, both male and female animals showed similar patterns across the social isolation and NAC treatment groups, although females tended to exhibit slightly greater sensitivity to social isolation stress. Figure 2 illustrates the effects of NAC on compulsive-like and social interaction behaviors across the experimental groups. In the marble burying test, the duration of compulsive-like behaviors was significantly increased in socially isolated animals receiving normal saline compared with control animals ($p < 0.001$). NAC administration significantly reduced the duration of these behaviors compared with the social isolation group receiving normal saline ($p < 0.01$).

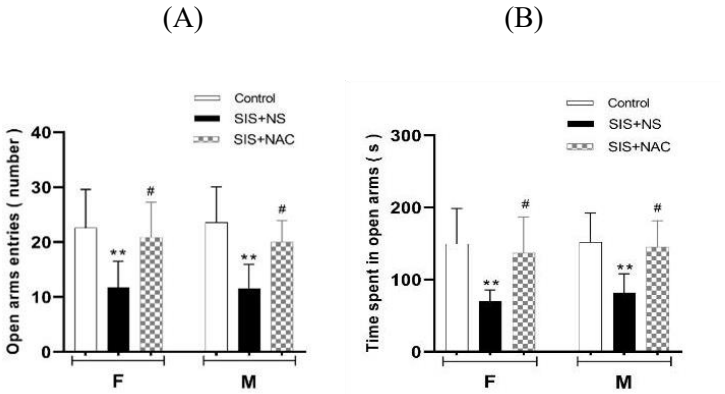

Fig. 1. Effect of NAC on behavioral indices in the Elevated Plus Maze test across experimental groups
(A) Number of entries into open arms; (B) Time spent in the open arms
NS = Normal saline; SIS = Social isolation stress; NAC = N-acetylcysteine; F = Female; M = Male
$^{**}p < 0.01$ compared with the control group; $^{\#}p < 0.05$ compared with the SIS + NS group.

[Downloaded from ijml.ssu.ac.ir on 2026-11-24]

In the three-chamber social interaction test, socially isolated animals showed a significant reduction in the duration of social interaction and the social preference index compared with control animals ($p < 0.001$ for both). NAC treatment significantly increased the duration of social interaction and the social preference index compared with the social isolation group receiving normal saline ($p < 0.001$ for both).

The behavioral responses showed comparable patterns in male and female animals across the experimental groups.

Effect of NAC on aggressive behaviors

The duration of aggressive behavior in the resident–intruder test was significantly altered across the experimental groups following NAC administration (Fig. 3). Socially isolated animals receiving normal saline exhibited a significantly longer duration of aggressive behavior compared with the control group ($p < 0.001$). NAC treatment significantly reduced the duration of aggressive behavior in socially isolated animals compared with the social isolation group receiving normal saline ($p < 0.01$ and $p < 0.05$).

Similar patterns of aggressive behavior were observed in male and female animals across the experimental groups.

Effect of NAC on oxidative stress markers and antioxidant capacity

The levels of oxidative stress markers and antioxidant capacity across the experimental groups are presented in Figure 4. Hepatic antioxidant capacity was significantly reduced in socially isolated animals receiving normal saline compared with control animals ($p < 0.001$, $p < 0.01$). Serum antioxidant capacity was also significantly reduced in the social isolation group compared with controls ($p < 0.001$).

NAC treatment significantly increased antioxidant capacity in the liver ($p < 0.05$) and serum ($p < 0.05$) compared with the social isolation group receiving normal saline.

Hepatic MDA levels were significantly elevated in socially isolated animals compared with controls ($p < 0.001$). Serum MDA levels were also significantly increased in the social isolation group ($p < 0.001$, $p < 0.01$). Administration of NAC significantly reduced MDA levels in both liver and serum compared with the social isolation group receiving normal saline ($p < 0.05$).

Effect of NAC on nitrite levels and catalase activity

Figure 5 illustrates the effects of NAC on nitrite levels and catalase activity across the experimental groups. Hepatic nitrite levels were significantly increased in socially isolated animals receiving normal saline compared with the control group receiving normal saline ($p < 0.01$). Serum nitrite levels were also significantly elevated in socially isolated animals ($p < 0.001$, $p < 0.01$).

NAC administration significantly reduced nitrite levels in both liver and serum compared with socially isolated animals receiving normal saline ($p < 0.05$). Hepatic catalase activity was significantly decreased in the social isolation stress group compared with the control group ($p < 0.001$). NAC treatment significantly increased hepatic catalase activity in socially isolated animals ($p < 0.001$ and $p < 0.01$).

[Downloaded from ijml.ssu.ac.ir on 2026-11-24]

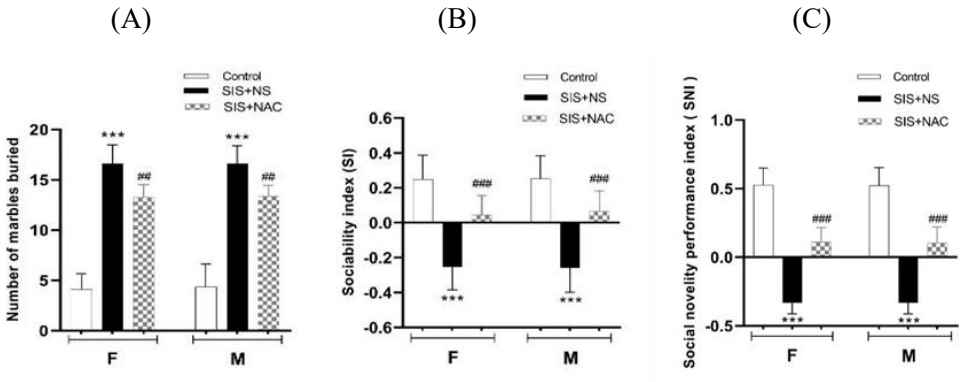

Fig. 2. Effect of NAC on compulsive-like and social interaction behaviors across experimental groups
(A) Compulsive-like behaviors in the marble burying test; (B) Duration of social interaction in the three-chamber test; (C) Social preference index
NS = Normal saline; SIS = Social isolation stress; NAC = N-acetylcysteine; F = Female ;M = Male
$^{***}p < 0.001$ vs. the control group; $^{\#\#}p < 0.01$ vs. the SIS + NS group.

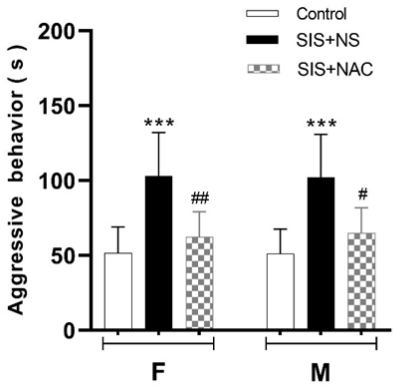

Fig. 3. The effect of NAC on the duration of aggressive behaviors in the resident-intruder test across experimental groups
NS = Normal saline; SIS = Social isolation stress; NAC = N-acetylcysteine; F = Female; M = Male
$^{***}p < 0.001$ vs. the control group; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ vs. the SIS + NS group.

Effect of NAC on inflammatory gene expression in liver tissue

Figure 6 illustrates the effects of NAC on inflammatory gene expression in liver tissue across the experimental groups. Expression of *IL-1β* was significantly elevated in socially isolated animals receiving normal saline compared with controls ($p < 0.001$). NAC treatment significantly reduced *IL-1β* expression in socially isolated animals compared with the social isolation group receiving normal saline ($p < 0.001$). *IL-6* gene expression was significantly increased in socially isolated animals compared with controls ($p < 0.001$, $p < 0.01$). NAC treatment significantly reduced *IL-6* expression compared with the social isolation group receiving normal saline ($p < 0.01$).

TNF-α expression was significantly increased in the social isolation group compared with controls ($p < 0.001$, $p < 0.01$). NAC administration significantly reduced *TNF-α* expression in socially isolated animals compared with the social isolation group receiving normal saline ($p < 0.01$).

[Downloaded from ijml.ssu.ac.ir on 2026-11-24]

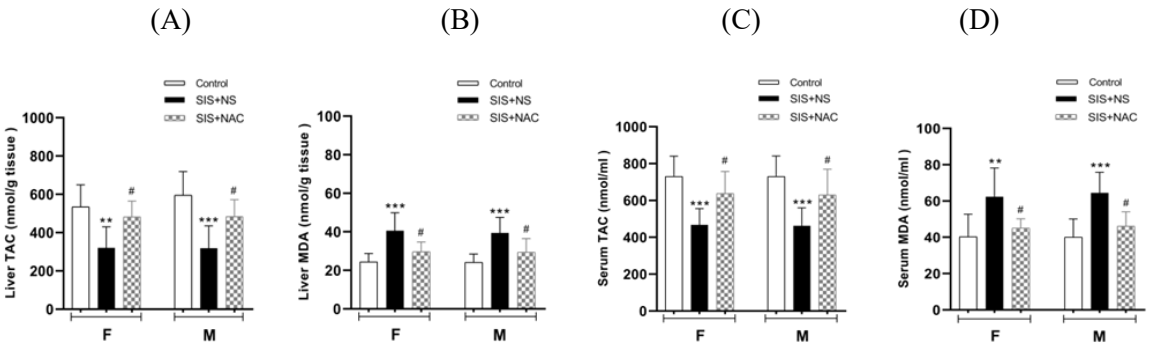

Fig. 4. The effect of NAC on oxidative stress markers and antioxidant capacity across experimental groups
(A) Hepatic antioxidant capacity; (B) Serum antioxidant capacity; (C) Hepatic malondialdehyde levels; (D) Serum malondialdehyde levels
NS = Normal saline; SIS = social isolation stress; NAC = N-acetylcysteine; F = Female; M = Male; MDA= Malondialdehyde.
$^{***}p < 0.001$ and $^{**}p < 0.01$ vs. the control group; $^{\#}p < 0.05$ vs. the SIS + NS group.

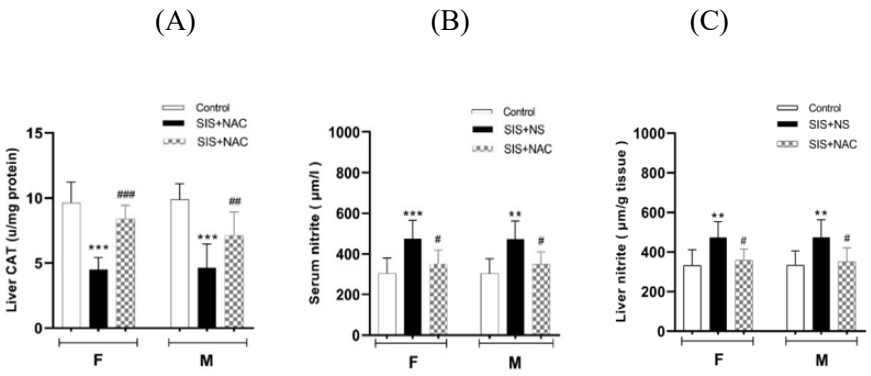

Fig. 5. The effect of NAC on nitrite levels and catalase activity across experimental groups
(A) Hepatic nitrite levels; (B) Serum nitrite levels; (C) Hepatic catalase activity
NS = Normal saline; SIS = Social isolation stress; NAC = N-acetylcysteine; F = Female; M = Male
$^{***}p < 0.001$ vs. the control group; $^{\#\#\#}p < 0.001$ and $^{\#\#}p < 0.01$ vs. the SIS + NS group.

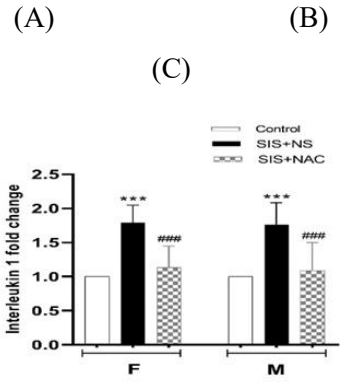

Fig. 6. The effect of NAC on inflammatory gene expression in the liver tissue across experimental groups
(A) *IL-1β* gene expression; (B) *IL-6* gene expression; (C) *TNF-α* gene expression

NS = Normal saline; SIS = Social isolation stress; NAC = N-acetylcysteine; F = Female; M = Male
$^{***}p < 0.001$ and $^{**}p < 0.01$ vs. the control

).

emical alterations

As summarized in Table 2, social isolation stress (SIS) significantly decreased the number of open arm entries and the time spent in open arms in the

[Downloaded from ijml.ssu.ac.ir on 2026-11-24]

EPM test in both male and female mice ($p < 0.01$ for both). In liver tissue, SIS significantly decreased total total antioxidant capacity (TAC) and increased MDA and nitrite levels compared with the control group ($p < 0.01$ and $p < 0.001$). NAC administration significantly increased TAC and reduced MDA and nitrite levels compared with the SIS+NS group ($p < 0.05$). NAC-treated mice also showed increased open arm entries and time spent in open arms compared with the SIS+NS group. No significant sex-based differences were observed in the measured parameters or in the response to NAC treatment.

Hepatic enzymatic antioxidant defense and inflammatory markers

As presented in Table 3, SIS significantly decreased hepatic CAT activity in both male and female mice compared with the control group ($p < 0.001$). SIS also significantly increased the expression of the pro-inflammatory cytokine genes IL-1 and TNF-α in liver tissue compared with controls ($p < 0.01$ and $p < 0.001$, respectively). NAC treatment significantly increased hepatic CAT activity and reduced the mRNA expression of IL-1 and TNF-α in both male and female mice compared with the SIS+NS group ($p < 0.01$ and $p < 0.001$, respectively).

Table 2. The effects of SIS and NAC treatment on anxiety-like behaviors and hepatic oxidative/nitrergic stress markers in male and female mice

Group	Sex	Open Arm Entries (Number)	Time Spent in Open Arms (s)	TAC (nmol/g tissue)	MDA (nmol/g tissue)	Nitrite (µM/g tissue)
Control	Female	22.6±7.1	151.2±48.5	535.4±115.2	24.5±4.2	335.2±78.4
	Male	23.8±6.4	154.5±40.2	595.8±125.4	24.1±4.5	332.8±75.1
SIS + NS	Female	11.8±4.8**	72.4±15.3**	318.2±112.5**	40.2±9.8***	474.5±82.3**
	Male	11.5±4.5**	83.2±26.8**	316.5±118.6***	39.5±8.2***	472.1±91.6**
SIS + NAC	Female	21.1±6.3	140.2±47.9	482.7±82.4	29.8±5.1	358.4±58.2
	Male	20.3±3.7	146.8±36.5	485.3±88.5	29.3±7.4	352.7±69.4

Data are expressed as Mean ± SEM (n=8 per group).
SIS= Social isolation stress; NS= Normal saline; NAC= N-acetylcysteine; TAC= Total antioxidant capacity; MDA= Malondialdehyde.
**$p < 0.01$, ***$p < 0.001$ vs. Control; $p < 0.05$ vs. SIS+NS.

Table 3. The effects of SIS and NAC treatment on hepatic enzymatic antioxidant activity and pro-inflammatory cytokine expression in male and female mice

Group	Sex	Liver CAT (U/mg protein)	IL-1 mRNA Expression (Fold Change)	TNF-α mRNA Expression (Fold Change)
Control	Female	9.6±1.6	1.00±0.00	1.00±0.00
	Male	9.9±1.2	1.00±0.00	1.00±0.00
SIS + NS	Female	4.4±0.9***	1.82±0.25***	1.81±0.42***
	Male	4.6±1.8***	1.78±0.31***	1.72±0.38**
SIS + NAC	Female	8.4±1.0	1.15±0.28	1.15±0.31
	Male	7.1±1.9	1.10±0.38	1.08±0.29

Data are presented as Mean ± SEM (n=8).
SIS= Social isolation stress; CAT= Catalase; IL-1= Interleukin-1; TNF-α= Tumor necrosis factor-alpha. NS= Normal saline; NAC= N-acetylcysteine
**$p < 0.01$, ***$p < 0.001$ vs. Control; $p < 0.05$ vs. SIS+NS

No significant differences were observed between male and female mice in baseline hepatic parameters, SIS-induced changes, or responses to NAC treatment. In summary, social isolation

[Downloaded from ijml.ssu.ac.ir on 2026-11-24]

stress induced a broad range of behavioral and biochemical disturbances, including increased anxiety-like, compulsive, and aggressive behaviors, reduced social interaction and antioxidant capacity, increased lipid peroxidation and nitric oxide metabolites, decreased catalase activity,

and upregulated inflammatory gene expression in liver tissue. NAC administration substantially attenuated many of these alterations, demonstrating protective effects across behavioral, oxidative, and inflammatory domains.

Discussion

This study examined whether NAC can attenuate behavioral disturbances and hepatic alterations induced by SIS in NMRI mice. The main findings indicate that chronic social isolation elicited a broad spectrum of behavioral abnormalities, manifested as increased anxiety-like and repetitive/compulsive-like behaviors and impaired social interaction, along with marked hepatic biochemical and molecular alterations consistent with oxidative/nitrosative stress and inflammatory activation. NAC treatment substantially reversed these changes, supporting its protective role under stress-related conditions.

The behavioral impact of social isolation is consistent with evidence that early-life or prolonged social stress disrupts neurodevelopmental trajectories and predisposes individuals to persistent affective and social deficits [6]. In the present work, SIS reduced open-arm exploration in the EPM and increased repetitive behavior in the marble-burying assay. NAC improved performance across behavioral domains. These findings align with prior reports indicating that NAC mitigates anxiety-like and depressive-like behaviors in rodent stress models by regulating glutamatergic transmission and enhancing antioxidant defenses [5, 10, 16]. The efficacy of NAC in improving social behavior and cognition has been further demonstrated in animal models of schizophrenia, reinforcing its potential as a broad-spectrum behavioral modulator [21].

A growing body of research highlights a bidirectional relationship between stress physiology and liver function [2]. One mechanistic pathway linking SIS to hepatic dysfunction is oxidative stress: excessive free-radical generation, combined with inadequate antioxidant capacity promotes lipid peroxidation and hepatocellular injury [9]. Recent evidence indicates that social isolation suppresses Nrf2 signaling and activates NF-κB-dependent inflammatory pathways in the liver, thereby contributing to oxidative damage [22]. Consistent with these findings, SIS in the present study reduced antioxidant indices and catalase activity while increasing malondialdehyde levels. As a glutathione precursor, NAC may enhance Nrf2 nuclear translocation and restore phase II antioxidant enzyme activity, thereby explaining the improved redox balance observed in this study. The protective effects of NAC against oxidative stress-induced liver injury have been well-documented in various experimental models [23, 24]. A systematic review has also confirmed the efficacy of NAC in improving liver function across multiple clinical and preclinical settings [25].

Nitrosative imbalance likely contributed to hepatic alterations following SIS. Elevatednitrite

[Downloaded from ijml.ssu.ac.ir on 2026-11-24]

levels indicate increased nitric oxide turnover, which is typically associated with iNOS induction during inflammation. NAC significantly reduced nitrite concentrations in both serum and liver, suggesting modulation of iNOS-related signaling. This findig is consistent with studies examining NAC's capacity to attenuate nitrosative stress under chronic stress conditions [16, 18]. Nitric oxide has been shown to contribute to stress-induced depression- and anxiety-like behaviors, further underscoring the relevance of nitrosative pathways in stress-related pathology [26].

Inflammation was another prominent feature of SIS-induced hepatic pathology. SIS upregulated the expression of *IL-1β*, *IL-6*, and *TNF-α* genes in the liver, reflecting activation of the NF-κB pathway. Stress-related reactive oxygen species act as secondary messengers that promote NF-κB nuclear translocation and inflammatory cytokine production. NAC downregulated these markers, consistent with its established role in inhibiting NF-κB activation and suppressing cytokine transcription [5, 16]. Recent studies have also highlighted the importance of liver–brain inflammatory crosstalk in chronic psychosocial stress, indicating that hepatic cytokines may influence central neuroinflammation [19].

Peripheral inflammation and the liver–brain axis are increasingly recognized as critical pathways in stress-related disorders [27]. Moreover, maternal separation stress has been shown to trigger neuro-immune responses associated with autistim-like behaviors, supporting the broader implications of early-life stress for inflammatory pathways [28]. These findings indicate that NAC exerts protective effects across both hepatic and neurobehavioral domains by modulating redox and inflammatory pathways.

Collectively, these findings indicated that NAC mitigates SIS-induced behavioral and hepatic impairments primarily by strengthening antioxidant defenses through Nrf2 activation, inhibiting NF-κB-mediated inflammation, and reducing iNOS-related nitrosative stress, situated within the broader framework of the liver–brain axis. The previously reported high risk of drug toxicity associated with social isolation stress, due to liver dysfunction further underscores the clinical relevance of these findings [29, 30].

Conclusion

The present study demonstrated that early-life social isolation induces persistent behavioral abnormalities and biochemical hepatic alterations through the activation of oxidative, nitrosative, and pro-inflammatory pathways. Treatment with NAC markedly decreased these adverse effects by enhancing antioxidant defense mechanisms and modulating the expression of inflammatory genes. Although these findings provide important insights into the protective role of NAC within the liver-brain axis in a rodent model, caution is warranted when extrapolating them to clinical settings. While NAC represents a promising candidate, further clinical investigations are needed to confirm the translational validity of these findings and evaluate the potential efficacy of NAC as an adjunctive strategy for managing social stress-related disorders in humans. The current status and prospects of NAC in the treatment of

[Downloaded from ijml.ssu.ac.ir on 2026-11-24]

psychiatric disorders have been comprehensively reviewed, supporting its therapeutic potential.

Despite these encouraging findings, several limitations should be acknowledged. First, protein-level analyses were not conducted to validate the gene expression data. Accordingly, measuring cytokine and signaling protein levels using techniques such as Western blotting would strengthen the mechanistic interpretation of the results. Second, specific intracellular pathways that may mediate NAC's protective effects, such as the Nrf2/HO-1 axis, were not examined. Future studies should investigate these molecular signaling pathways in greater detail at the protein level. In addition, the long-term consequences of NAC treatment, including its potential epigenetic effects and transgenerational impact, warrant further investigation.

Ethical Considerations

The study protocol was approved by the Biomedical Research Ethics Committee of Islamic Azad University, Ahvaz Branch, under ethics approval code IR.IAU.AHVAZ.REC.1402.045.

Funding Statement

This research did not receive any specific grant from funding agencies in the public, commercial, or non-profit sectors.

Conflict of Interest

The authors declared no conflict of interest.

Acknowledgment

The authors would like to thank the Avin-Bonyan-Gene Research Center (affiliated with Shahid Chamran University of Ahvaz) for their extensive technical support during the experimental procedures. This manuscript benefited from AI-assisted refinement and formatting support, including language polishing and abbreviation standardization.

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

Authors' Contributions

J.A and L.D: Study conception and design, data interpretation, and drafting the manuscript.
S.O.G= Data collection, screening, and critical revision. All authors reviewed and approved the final manuscript.

References

[1]. National Academies of Sciences, Engineering, and Medicine. Social isolation and loneliness in older adults: opportunities for the health care system. Washington (DC): National Academies Press; 2020.
[2]. Nguyen HH, Swain MG. Avenues within the gut-liver-brain axis linking chronic liver disease and symptoms. Front Neurosci. 2023; 17: 1171253.
[3]. Bove M, Lama A, Schiavone S, Pirozzi C, Tucci P, Sikora V, et al. Social isolation triggers oxidative status and impairs systemic and hepatic insulin sensitivity in normoglycemic rats. Biomed Pharmacother. 2022;149:112820.
[4]. Amiri S, Haj-Mirzaian A, Amini-Khoei H, Razmi A, Shirzadian A, Rahimi-Balaei M, et al. Protective effects of gabapentin against the seizure susceptibility and comorbid behavioral alterations in the early socially isolated mice. Eur J Pharmacol. 2015;766:39-45.
[5]. Murakami S, Imamura M, Uchida T, Suehiro Y, Namba M, Fujii Y, et al. Serum interleukin-6 level predicts the prognosis for patients with alcohol-related acute-on-chronic liver failure. Hepatol Int. 2023;17(5):1225-1232.
[6]. Malcon LMC, Wearick-Silva LE, Zaparte A, Orso R, Luft C, Tractenberg SG, et al. Maternal separation induces long-term oxidative stress alterations and increases anxiety-like behavior of male Balb/cJ mice. Exp Brain Res. 2020;238(9):2097-2107.
[7]. Kaidanovich-Beilin O, Lipina T, Vukobradovic I, Roder J, Woodgett JR. Assessment of social interaction behaviors. J Vis Exp. 2011;(48):2473.
[8]. Angoa-Pérez M, Kane MJ, Briggs DI, Francescutti DM, Kuhn DM. Marble burying and nestlet shredding as tests of repetitive, compulsive-like behaviors in mice. J Vis Exp. 2013;(82):50978.
[9]. Halliwell B. Free radicals and antioxidants: updating a personal view. Nutr Rev. 2012;70(5):257-65.
[10]. Réus GZ, Fries GR, Stertz L, Badawy M, Passos IC, Barichello T, et al. The role of inflammation and microglial activation in the

[Downloaded from ijml.ssu.ac.ir on 2026-11-24]

pathophysiology of psychiatric disorders. Neuroscience. 2015;300:141-54.
[11]. Song H, Fang F, Arnberg FK, Mataix-Cols D, Fernández de la Cruz L, Almqvist C, et al. Stress related disorders and subsequent risk of life threatening infections: population based sibling controlled cohort study. BMJ. 2021;373:n746.
[12]. Wright DJ, Renoir T, Smith ZM, Frazier AE, Francis PS, Thorburn DR, et al. N-acetylcysteine improves mitochondrial function and ameliorates behavioral deficits in the R6/1 mouse model of Huntington's disease. Transl Psychiatry. 2015;5(1):e492.
[13]. Treit D, Ménard J, Royan C. Anxiogenic stimuli in the elevated plus-maze. Pharmacol Biochem Behav. 1993;44(2):463-69.
[14]. Mouhid L, Gómez de Cedrón M, Quijada-Freire A, Fernández-Marcos PJ, Reglero G, Fornari T, et al. Yarrow supercritical extract ameliorates the metabolic stress in a model of obesity induced by high-fat diet. Nutrients. 2020;12(1):72.
[15]. Gałecki P, Gałecka E, Maes M, Chamielec M, Orzechowska A, Bobińska K, et al. The expression of genes encoding for COX-2, MPO, iNOS, and sPLA2-IIA in patients with recurrent depressive disorder. J Affect Disord. 2012;138(3):360-66.
[16]. Minarini A, Ferrari S, Galletti M, Giambalvo N, Perrone D, Rioli G, et al. N-acetylcysteine in the treatment of psychiatric disorders: current status and future prospects. Expert Opin Drug Metab Toxicol. 2017;13(3):279-92.
[17]. Zlatković J, Filipović D. Chronic social isolation induces NF-κB activation and upregulation of iNOS protein expression in rat prefrontal cortex. Neurochem Int. 2013;63(3):172-179.
[18]. Wegener G, Volke V. Nitric oxide synthase inhibitors as antidepressants. Pharmaceuticals (Basel). 2010;3(1):273-99.
[19]. Lee SS, Yoo YC. NOX-NOS crosstalk in the liver-brain axis: novel insights for redox regulation and neurodegenerative diseases. Redox Biol. 2025;86:103807.
[20]. Chen H, Ma N, Song X, Wei G, Zhang H, Liu J, et al. Protective effects of N-acetylcysteine on lipopolysaccharide-induced respiratory inflammation and oxidative stress. Antioxidants (Basel). 2022;11(5):879.
[21]. Erdogan MM, Erdogan MA, Koc S, Yalcin A, Turk A, Yetkin EA. Hepatoprotective effects of N-acetylcysteine on liver injury by irisin upregulation and oxidative stress reduction in diabetic rats. Egypt Liver J. 2023;13(1):33.
[22]. Farooq RK, Isingrini E, Tanti A, Le Guisquet AM, Arlicot N, Minier F, et al. Is unpredictable chronic mild stress (UCMS) a reliable model to study depression-induced neuroinflammation? Behav Brain Res. 2012;231(1):130-137.
[23]. Vere CC, Streba CT, Streba LM, Ionescu AG, Sima F. Psychosocial stress and liver disease status. World J Gastroenterol. 2009;15(24):2980-2986.
[24]. Sakakibara H, Suzuki A, Kobayashi A, Motoyama K, Matsui A, Sayama K, et al. Social isolation stress induces hepatic hypertrophy in C57BL/6J mice. J Toxicol Sci. 2012;37(5):1071-1076.
[25]. Minarini A, Ferrari S, Galletti M, Giambalvo N, Perrone D, Rioli G, et al. N-acetylcysteine in the treatment of psychiatric disorders: current status and future prospects. Expert Opin Drug Metab Toxicol. 2017;13(3):279-292.
[26]. Dimonte S, Sikora V, Bove M, Morgese MG, Tucci P, Schiavone S, et al. Social isolation from early life induces anxiety-like behaviors in adult rats: relation to neuroendocrine and neurochemical dysfunctions. Biomed Pharmacother. 2023;158:114181.
[27]. Rogóż Z, Kamińska K, Lech MA, Lorenc-Koci E. N-acetylcysteine and aripiprazole improve social behavior and cognition and modulate brain BDNF levels in a rat model of schizophrenia. Int J Mol Sci. 2022;23(4):2125.
[28]. Cai Z, Lou Q, Wang F, Li E, Sun J, Fang H, et al. N-acetylcysteine protects against liver injure induced by carbon tetrachloride via activation of the Nrf2/HO-1 pathway. Int J Clin Exp Pathol. 2015;8(7):8655-8662.
[29]. Thong-Ngam D, Samuhasaneeto S, Kulaputana O, Klaikeaw N, Navasumrit P. N-acetylcysteine attenuates oxidative stress and liver injury in rats. World J Gastroenterol. 2007;13(38):5127-5132.
[30]. Nikbaf-Shandiz M, et al. The efficacy of N-acetylcysteine in improving liver function: a systematic review and meta-analysis of controlled clinical trials. PharmaNutrition. 2023;24:100343.

[Downloaded from ijml.ssu.ac.ir on 2026-11-24]