ACTIVATION OF NRF2/HO-1 SIGNALING MEDIATES THE PROTECTIVE EFFECTS OF HIBISCUS SABDARIFFA AGAINST RESTRAINT STRESS–INDUCED OXIDATIVE DAMAGE IN FEMALE WISTAR RATS

ABSTRACT

Chronic psychological stress disrupts redox homeostasis, leading to oxidative damage, inflammation, and organ dysfunction. Females may be particularly vulnerable due to stress-related hormonal modulation of antioxidant signaling pathways. *Hibiscus sabdariffa* (HS) possesses documented antioxidant properties; however, its redox-regulated molecular mechanisms under stress conditions remain incompletely understood.
This study investigated whether activation of the nuclear factor erythroid 2-related factor 2/heme oxygenase-1 (Nrf2/HO-1) pathway underlies the protective effects of HS in restraint stress–exposed female Wistar rats. Thirty-six rats were allocated to six groups: non-stressed control, stress control, stress + HS (250 or 500 mg/kg), and HS alone (250 or 500 mg/kg). Restraint stress was applied for 6 h/day for 14 days. Oxidative stress biomarkers, antioxidant enzyme activities, gene and protein expression of Nrf2, HO-1, TNF-α, and iNOS, and histopathological alterations in the brain, liver, and kidney were assessed.

Restraint stress markedly increased lipid peroxidation and suppressed endogenous antioxidant defenses, accompanied by downregulation of Nrf2 and HO-1 and upregulation of TNF-α and iNOS. HS treatment dose-dependently restored antioxidant enzyme activities, reduced oxidative damage, activated Nrf2/HO-1 signaling, and attenuated inflammatory gene and protein expression. High-dose HS (500 mg/kg) normalized redox and inflammatory markers to near-control levels and conferred significant histological protection across examined tissues.

These findings demonstrate that HS mitigates stress-induced oxidative injury through activation of Nrf2-dependent antioxidant signaling and suppression of inflammatory mediators. The study provides mechanistic evidence supporting HS as a low-cost, accessible redox-modulating therapeutic candidate for stress-related oxidative disorders, particularly in females.

Keywords: *Hibiscus sabdariffa*; oxidative stress; Nrf2/HO-1; restraint stress; inflammation; antioxidant enzymes.

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INTRODUCTION

Chronic psychological stress is a major contributor to the development of oxidative stress–related disorders, including neurodegenerative diseases, metabolic dysfunction, cardiovascular pathology, and reproductive impairment [1]. Prolonged stress exposure disrupts cellular redox homeostasis by promoting excessive generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), overwhelming endogenous antioxidant defense systems and leading to lipid peroxidation, protein oxidation, DNA damage, and inflammatory activation [2,3,4]. These redox disturbances play a central role in stress-induced tissue injury and organ dysfunction.
Emerging evidence indicates that females may exhibit heightened vulnerability to stress-induced oxidative damage due to sex-specific neuroendocrine and hormonal modulation of antioxidant signaling pathways [5,6]. Fluctuations in estrogen and glucocorticoids under chronic stress conditions can influence mitochondrial function, redox-sensitive transcription factors, and inflammatory mediators, thereby exacerbating oxidative and inflammatory responses [7,8]. Despite increasing recognition of sex differences in stress biology, mechanistic studies investigating redox regulation under stress conditions in females remain limited.

At the molecular level, the nuclear factor erythroid 2–related factor 2 (Nrf2) signaling pathway is a master regulator of cellular antioxidant and cytoprotective responses [9,10,11]. Under basal conditions, Nrf2 is sequestered in the cytoplasm by Kelch-like ECH-associated protein 1 (Keap1) and targeted for proteasomal degradation [12,13]. Oxidative or electrophilic stress disrupts this interaction, allowing Nrf2 to translocate into the nucleus, where it binds antioxidant response elements (AREs) and induces transcription of phase II detoxifying and antioxidant enzymes, including heme oxygenase-1 (HO-1), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) [14,15,16]. Impairment of Nrf2 signaling has been implicated in stress-induced oxidative injury, neuroinflammation, and systemic inflammatory disorders [17,18,19]. In parallel, chronic stress activates pro-inflammatory signaling cascades characterized by increased expression of tumor necrosis factor-α (TNF-α), inducible nitric oxide synthase (iNOS), and excessive nitric oxide production, further amplifying oxidative damage through nitrosative stress mechanisms [20]. The reciprocal regulation between Nrf2-mediated antioxidant defense and inflammatory signaling highlights the importance of redox-inflammatory crosstalk in stress-related pathology [21].

Plant-derived polyphenols have attracted considerable attention as modulators of redox signaling pathways, particularly due to their ability to activate endogenous antioxidant defenses rather than acting solely as direct radical scavengers [22,23,24]. *Hibiscus sabdariffa* L. (Malvaceae) is a widely consumed medicinal plant rich in anthocyanins, flavonoids, phenolic acids, and organic acids [25,26]. Previous studies have documented its antioxidant, anti-inflammatory, antihypertensive, hepatoprotective, and neuroprotective properties. However, many of these studies remain largely descriptive, focusing on biochemical endpoints without clearly elucidating upstream molecular signaling mechanisms [27,28,29]. Recent experimental evidence suggests that *Hibiscus sabdariffa* may exert its protective effects through modulation of redox-sensitive transcription factors, including Nrf2. Nonetheless, whether activation of the Nrf2/HO-1 signaling axis underlies the protective actions of *Hibiscus sabdariffa* against chronic psychological stress particularly in female subjects—remains poorly defined. Moreover, comprehensive studies

integrating biochemical, molecular, and histopathological outcomes in stress-exposed female models are scarce.

Restraint stress is a well-established experimental paradigm that mimics chronic psychological stress and reliably induces oxidative stress, inflammation, and tissue injury across multiple organ systems, including the brain, liver, and kidney [30,31]. Investigating therapeutic interventions within this model provides valuable mechanistic insights into stress-related redox dysregulation.

Therefore, the present study was designed to investigate the molecular mechanisms underlying the therapeutic potential of *Hibiscus sabdariffa* in restraint stress–induced oxidative damage in female Wistar rats. We hypothesized that *Hibiscus sabdariffa* confers cytoprotection by activating the Nrf2/HO-1 antioxidant signaling pathway while suppressing pro-inflammatory mediators such as TNF-α and iNOS. To test this hypothesis, we evaluated oxidative stress biomarkers, antioxidant enzyme activities, gene and protein expression of key redox and inflammatory markers, and histopathological alterations in stress-vulnerable organs.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals and reagents used in this study were of analytical grade. Assay kits for malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were obtained from standard commercial suppliers. Primary antibodies for Nrf2, HO-1, TNF-α, iNOS, and β-actin were procured from reputable vendors, and all reagents for molecular analyses were prepared according to manufacturers' instructions.

Plant Material and Extract Preparation

Fresh calyces of *Hibiscus sabdariffa* L. were obtained from a local market and authenticated by a qualified botanist. A voucher specimen was deposited in the departmental herbarium for future reference. The calyces were air-dried at room temperature and pulverized into a fine powder. The powdered material was extracted using standard solvent extraction procedures. The resulting extract was filtered, concentrated under reduced pressure, and stored at 4 °C until use. Appropriate dilutions were prepared freshly prior to administration.

Experimental Animals

Adult female Wistar rats (180–220 g) were obtained from an accredited animal facility. Animals were housed under standard laboratory conditions (12 h light/12 h dark cycle, temperature 22 ± 2 °C, relative humidity 50–60%) with free access to standard rat chow and water. Animals were acclimatized for one week prior to the commencement of the experiment.

All experimental procedures were conducted in accordance with internationally accepted guidelines for the care and use of laboratory animals and were approved by the Institutional Animal Ethics Committee.

Experimental Design and Treatment Protocol

Thirty-six female Wistar rats were randomly assigned into six experimental groups (n = 6 per group):

Group I: Non-stressed control

Group II: Restraint stress control

Group III: Restraint stress + *Hibiscus sabdariffa* (250 mg/kg)

Group IV: Restraint stress + *Hibiscus sabdariffa* (500 mg/kg)

Group V: *Hibiscus sabdariffa* only (250 mg/kg)

Group VI: *Hibiscus sabdariffa* only (500 mg/kg)

Extract administration was performed orally once daily using an oral gavage for 14 consecutive days. Dose selection was based on previous experimental studies demonstrating biological efficacy and safety.

Induction of Restraint Stress

Restraint stress was induced using a well-established protocol. Animals in the stress groups were placed in ventilated restraint tubes for 6 h daily for 14 consecutive days. Non-stressed control animals were handled similarly but were not subjected to restraint. All stress procedures were conducted at the same time each day to minimize circadian variability.

Sample Collection

Twenty-four hours after the final stress session, animals were anesthetized, and blood samples were collected via cardiac puncture. Serum was separated by centrifugation and stored at −80 °C until biochemical analysis. Animals were subsequently sacrificed, and brain, liver, and kidney tissues were harvested, rinsed in ice-cold saline, and processed for biochemical, molecular, and histopathological analyses.

Assessment of Oxidative Stress Biomarkers

Serum levels of MDA were measured as an index of lipid peroxidation. Antioxidant enzyme activities, including SOD, CAT, and GPx, were determined spectrophotometrically using commercially available assay kits, following manufacturers' protocols. Enzyme activities were expressed per milligram of protein.

RNA Extraction and Quantitative Real-Time PCR

Total RNA was extracted from tissue samples using standard RNA isolation reagents. RNA purity and concentration were assessed spectrophotometrically. Complementary DNA (cDNA) was synthesized using a reverse transcription kit. Quantitative real-time PCR (qPCR) was performed to determine the mRNA expression levels of Nrf2, HO-1, TNF-α, and iNOS using gene-specific primers. β-actin was used as the housekeeping gene. Relative gene expression was calculated using the $2^{-}\Delta\Delta Ct$ method.

Protein Expression Analysis

Protein expression levels of Nrf2, HO-1, TNF-α, and iNOS were evaluated using Western blotting and/or enzyme-linked immunosorbent assay (ELISA), as appropriate. Equal amounts of protein were separated by SDS-PAGE, transferred to PVDF membranes, and incubated with specific primary antibodies followed by appropriate secondary antibodies. Protein bands were visualized and quantified using densitometric analysis, normalized to β-actin.

Histopathological Examination

Brain, liver, and kidney tissues were fixed in 10% buffered formalin, processed using standard histological techniques, and embedded in paraffin wax. Tissue sections (4–5 µm) were stained with hematoxylin and eosin (H&E) and examined under a light microscope by a blinded histopathologist for structural alterations and tissue integrity.

Statistical Analysis

Data were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using appropriate statistical software. Comparisons among groups were conducted using one-way analysis of variance (ANOVA) followed by post hoc multiple comparison tests. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Restraint Stress Induces Oxidative Damage and Suppresses Antioxidant Defense in Female Rats

Exposure of female Wistar rats to restraint stress for 14 days resulted in a marked disruption of redox homeostasis. Serum malondialdehyde (MDA) levels were significantly elevated in the stress control group compared with non-stressed controls ($p < 0.001$), indicating enhanced lipid peroxidation (Figure 1A). Concurrently, restraint stress significantly reduced the activities of key endogenous antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) ($p < 0.001$ for all; Figures 1B–D).

These findings confirm that chronic restraint stress induces pronounced oxidative stress in female rats, characterized by increased oxidative damage and impaired antioxidant capacity.

Hibiscus sabdariffa Restores Redox Balance in a Dose-Dependent Manner

Oral administration of *Hibiscus sabdariffa* (HS) significantly attenuated restraint stress–induced oxidative damage. Treatment with HS at both 250 mg/kg and 500 mg/kg resulted in a dose-dependent reduction in serum MDA levels compared with the stress control group ($p < 0.01$ and $p < 0.001$, respectively; Figure 1A). Notably, MDA levels in the high-dose HS group were comparable to those of non-stressed controls.

Similarly, HS treatment significantly restored SOD, CAT, and GPx activities in stressed rats (Figures 1B–D). The 500 mg/kg dose produced a more pronounced effect, normalizing antioxidant enzyme activities to near basal levels. Administration of HS alone in non-stressed animals did not produce adverse alterations in oxidative stress markers, indicating a favorable safety profile.

Hibiscus sabdariffa Activates Nrf2/HO-1 Antioxidant Signaling Under Stress Conditions

To elucidate the molecular mechanisms underlying the antioxidant effects of HS, the expression of Nrf2 and its downstream effector HO-1 was assessed at both mRNA and protein levels. Restraint stress significantly downregulated Nrf2 and HO-1 expression in brain, liver, and kidney tissues compared with non-stressed controls ($p < 0.01$; Figures 2A–D).

HS treatment markedly reversed stress-induced suppression of Nrf2 and HO-1 expression in a dose-dependent manner. High-dose HS (500 mg/kg) significantly upregulated Nrf2 and HO-1 expression relative to stress controls ($p < 0.001$), restoring levels comparable to non-stressed animals. These effects were consistently observed across all examined tissues.

These results indicate that HS exerts its antioxidant effects, at least in part, through activation of the Nrf2/HO-1 signaling pathway.

Hibiscus sabdariffa Suppresses Stress-Induced Pro-Inflammatory Gene and Protein Expression

Given the close interplay between oxidative stress and inflammation, the expression of key inflammatory mediators was evaluated. Restraint stress significantly upregulated tumor necrosis factor-α (TNF-α) and inducible nitric oxide synthase (iNOS) at both gene and protein levels ($p < 0.001$; Figures 3A–D), consistent with heightened inflammatory activation.

HS treatment significantly attenuated the stress-induced upregulation of TNF-α and iNOS. The effect was dose-dependent, with the 500 mg/kg HS group exhibiting near-normalization of inflammatory marker expression. HS-only groups did not show significant changes compared with non-stressed controls, further supporting the safety of the extract.

Histopathological Analysis Confirms Multi-Organ Protection by Hibiscus sabdariffa

Histopathological examination revealed marked structural alterations in the brain, liver, and kidney tissues of restraint-stressed rats. Observed changes included neuronal degeneration, hepatocellular distortion, sinusoidal congestion, glomerular shrinkage, and tubular epithelial damage (Figure 4).

In contrast, HS-treated stressed rats exhibited substantial preservation of tissue architecture. Low-dose HS partially ameliorated stress-induced damage, whereas high-dose HS conferred near-complete protection, with tissue morphology closely resembling that of non-stressed controls. No histological abnormalities were observed in HS-only groups.

These findings corroborate the biochemical and molecular data, demonstrating that HS provides robust structural protection against stress-induced oxidative injury.

Integrated Analysis Highlights Nrf2-Mediated Redox and Anti-Inflammatory Protection

Collectively, the results demonstrate that restraint stress induces oxidative damage through suppression of Nrf2-dependent antioxidant defenses and activation of inflammatory mediators. HS treatment restores redox homeostasis by activating the Nrf2/HO-1 pathway, enhancing endogenous antioxidant capacity, suppressing inflammatory signaling, and preserving tissue integrity across multiple organs.

DISCUSSION

The present study demonstrates that *Hibiscus sabdariffa* confers robust protection against restraint stress–induced oxidative damage in female Wistar rats through coordinated activation of antioxidant signaling and suppression of inflammatory pathways. Chronic restraint stress disrupted redox homeostasis, as evidenced by increased lipid peroxidation, impaired antioxidant enzyme activities, downregulation of the Nrf2/HO-1 axis, and heightened expression of pro-inflammatory mediators. Treatment with *Hibiscus sabdariffa* dose-dependently reversed these alterations, highlighting its capacity to modulate redox-sensitive molecular mechanisms rather than acting solely as a direct radical scavenger.

One of the central findings of this study is the stress-induced suppression of Nrf2 signaling observed across multiple organs. Nrf2 is widely recognized as a master regulator of cellular defense against oxidative and electrophilic stress. Although acute oxidative stimuli typically activate Nrf2, chronic psychological stress has been shown to paradoxically impair Nrf2 signaling through sustained glucocorticoid exposure, mitochondrial dysfunction, and Keap1-mediated degradation. The observed downregulation of Nrf2 and its downstream effector HO-1 in stressed female rats is consistent with this maladaptive redox response and provides mechanistic insight into stress-induced vulnerability to oxidative injury.

Activation of the Nrf2/HO-1 pathway by *Hibiscus sabdariffa* represents a key mechanistic advance of this study. Restoration of Nrf2 signaling was accompanied by normalization of endogenous antioxidant enzymes, including SOD, CAT, and GPx, indicating functional transcriptional activation of antioxidant response elements. These findings support the concept that HS acts as a redox signaling modulator, likely through its rich content of polyphenolic compounds capable of modifying Keap1 cysteine residues and facilitating Nrf2 nuclear translocation [32,33,34]. Such indirect antioxidant mechanisms are increasingly recognized as more biologically relevant than direct free radical scavenging in vivo [35]. The suppression of inflammatory mediators TNF-α and iNOS by HS further underscores the tight interplay between oxidative stress and inflammation. Excessive ROS and RNS production can activate pro-inflammatory transcription factors, while inflammatory cytokines further exacerbate oxidative

damage, creating a self-amplifying cycle. Activation of Nrf2 has been shown to antagonize inflammatory signaling by inhibiting NF-κB activation and reducing nitric oxide overproduction. The concurrent upregulation of Nrf2/HO-1 and downregulation of TNF-α and iNOS observed in this study suggests that HS disrupts this vicious cycle, thereby attenuating both oxidative and inflammatory components of stress-induced pathology. Importantly, the protective effects of HS extended beyond biochemical and molecular markers to preservation of tissue architecture in the brain, liver, and kidney. These organs are particularly susceptible to stress-induced oxidative injury due to their high metabolic activity and vulnerability to redox imbalance. The histopathological findings provide structural validation of the molecular data and strengthen the translational relevance of the study.

The use of a female-specific stress model represents a notable strength of this investigation. Females exhibit distinct neuroendocrine responses to stress, with estrogen and glucocorticoid signaling exerting complex effects on redox regulation [36]. By focusing on female rats, this study addresses an important gap in stress-redox research, which has historically been male-biased [37]. The findings suggest that HS may be Estrogen can both enhance antioxidant defenses and, under certain conditions, increase oxidative susceptibility particularly beneficial in mitigating stress-related oxidative disorders in females.

Despite its strengths, this study has some limitations. The precise upstream molecular interactions between HS phytochemicals and the Keap1–Nrf2 complex were not directly examined. Additionally, assessment of mitochondrial function and downstream Nrf2 target genes beyond HO-1 would provide further mechanistic depth. Future studies employing pharmacological or genetic inhibition of Nrf2 could help establish causality and further delineate the signaling pathways involved. From a translational perspective, the findings highlight *Hibiscus sabdariffa* as a low-cost, accessible, and culturally accepted nutraceutical with potential therapeutic relevance for stress-related oxidative disorders. Given the increasing global burden of chronic stress and its disproportionate impact on women, interventions that enhance endogenous antioxidant defenses through redox signaling modulation may offer significant public health benefits.

In conclusion, this study provides compelling mechanistic evidence that *Hibiscus sabdariffa* mitigates restraint stress–induced oxidative damage in female rats by activating the Nrf2/HO-1 antioxidant pathway, restoring redox balance, suppressing inflammatory mediators, and preserving tissue integrity. These findings advance our understanding of plant-derived redox modulators and support further investigation of *Hibiscus sabdariffa* as a therapeutic strategy for managing stress-associated oxidative pathologies.

Ethical Approval

All experimental protocols were approved by the Animal Research Ethics Committee of Usmanu Danfodiyo University, Sokoto, Nigeria (Approval number: PTAC/HS/(Ae)/OT/36-17).

Authors' Contributions

- **Aliyu Buhari:** Formal analysis (equal); investigation (equal); methodology (equal); writing – original draft (equal); visualization (equal); writing – review and editing (equal), Project administration (equal); supervision (equal).
- **Lawali Ibrahim**: Conceptualization (equal); writing – review and editing (equal).
- **Sani Suleman**: Visualization (equal); writing – review and editing (equal).
- **Shehu Ibrahim**: Validation (equal); visualization (equal); writing – review and editing (equal).
- **SulemanTijjani:** Conceptualization (equal); writing – review and editing (equal).
- **Bello Muhammad**: Visualization (equal); writing – review and editing (equal).

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Conflict of Interest Statement

The authors declare no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Figures

Figure 1. Fleshy calyces of *Hibiscus sabdariffa*. Representative photograph showing the dark-red calyces used for aqueous extraction. (Adapted from Aliyu et al., 2014).

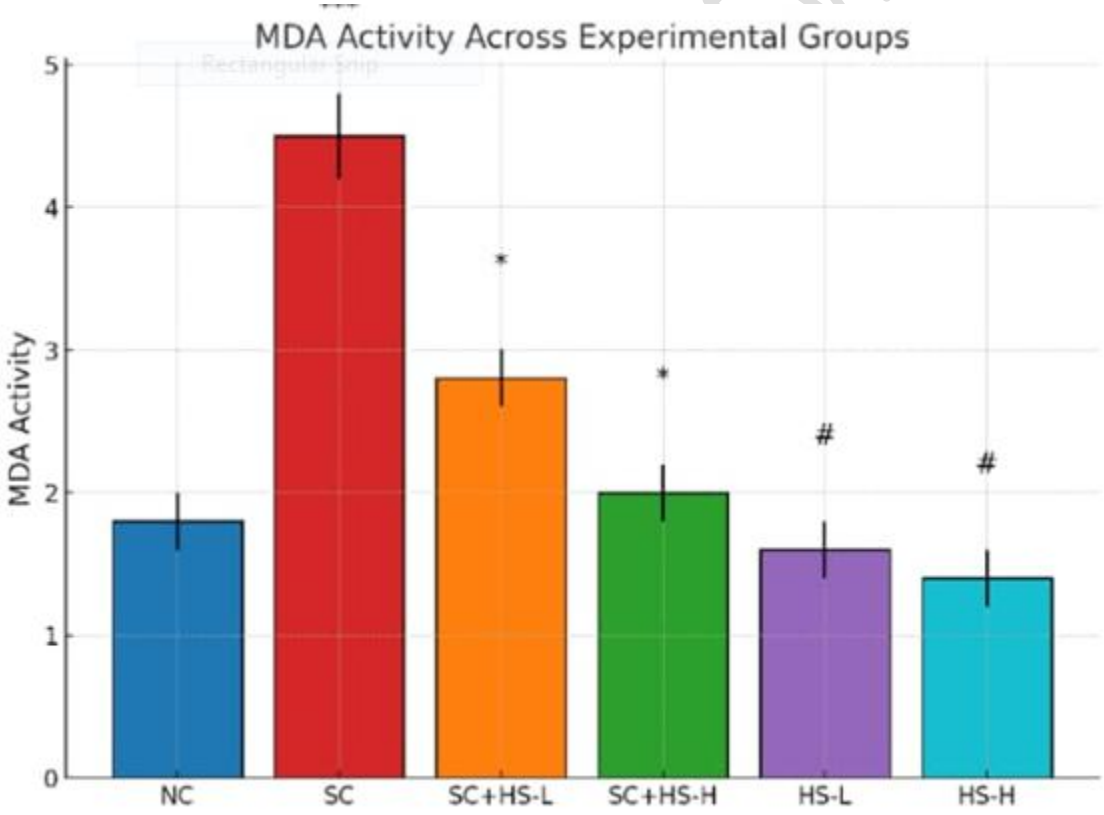

Figure 2. Serum malondialdehyde (MDA) levels across experimental groups. Restraint stress significantly increased MDA levels, whereas *H. sabdariffa* (HS) supplementation dose-dependently reduced lipid peroxidation. Data are presented as mean ± SEM (n = 6 per group). *p < 0.001 vs NC; #p < 0.05 vs SC. NC = non-stress control; SC = stress control; HS-L = HS 250 mg/kg; HS-H = HS 500 mg/kg.

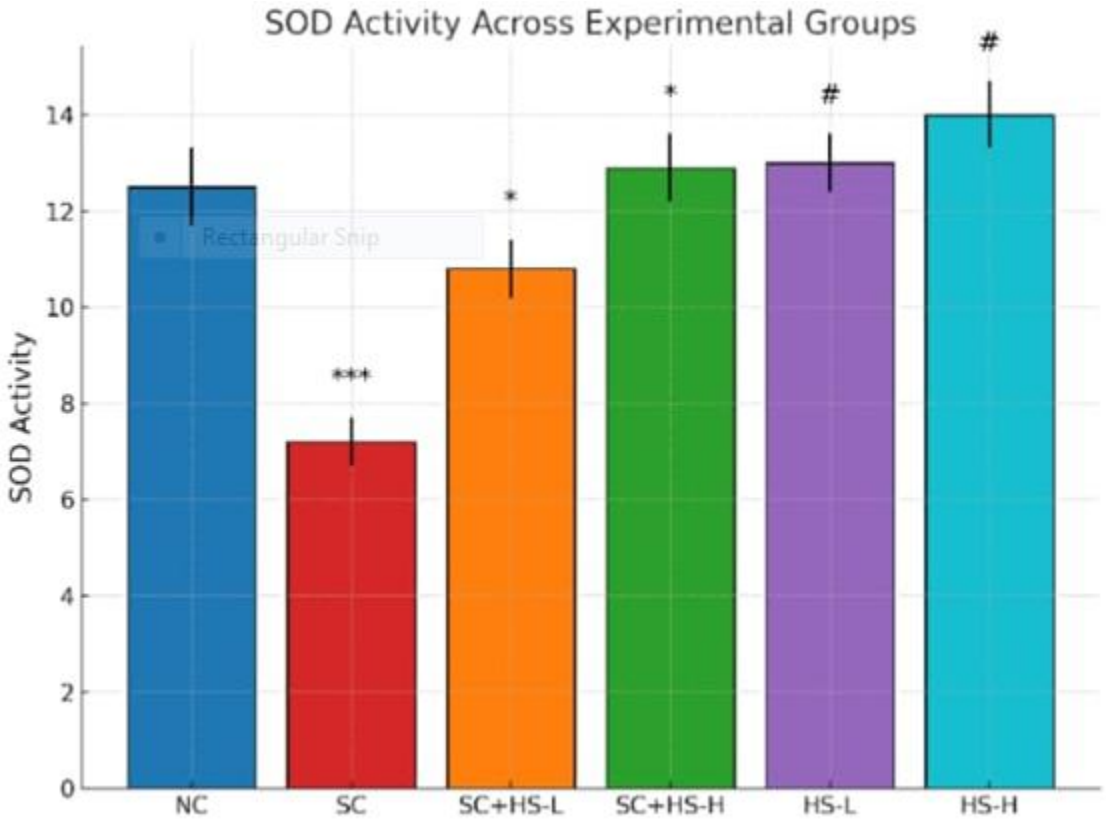

Figure 3. Superoxide dismutase (SOD) activity in serum. HS treatment restored SOD activity suppressed by restraint stress in a dose-dependent manner. Data are mean ± SEM (n = 6). *p < 0.001 vs NC; #p < 0.05 vs SC.

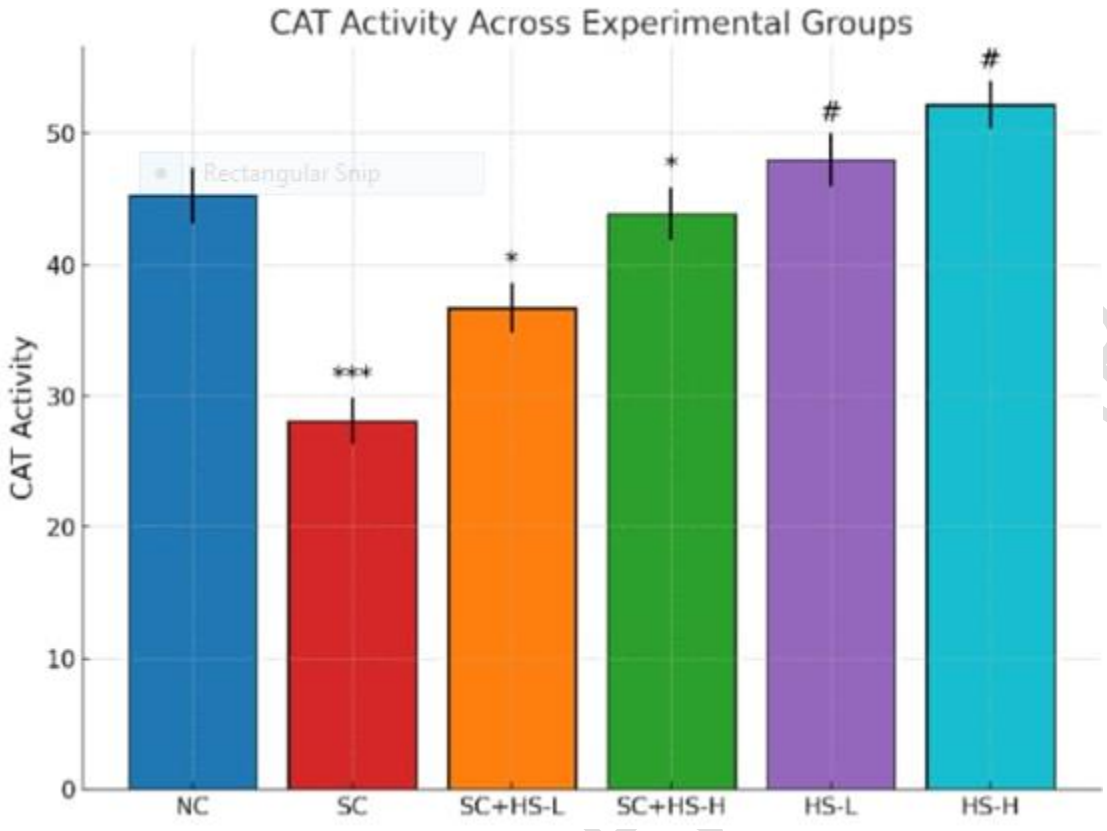

Figure 4. Catalase (CAT) activity in serum. HS supplementation improved CAT activity diminished by stress exposure. Data are mean ± SEM (n = 6). Statistical analysis: one-way ANOVA with Bonferroni post hoc test.

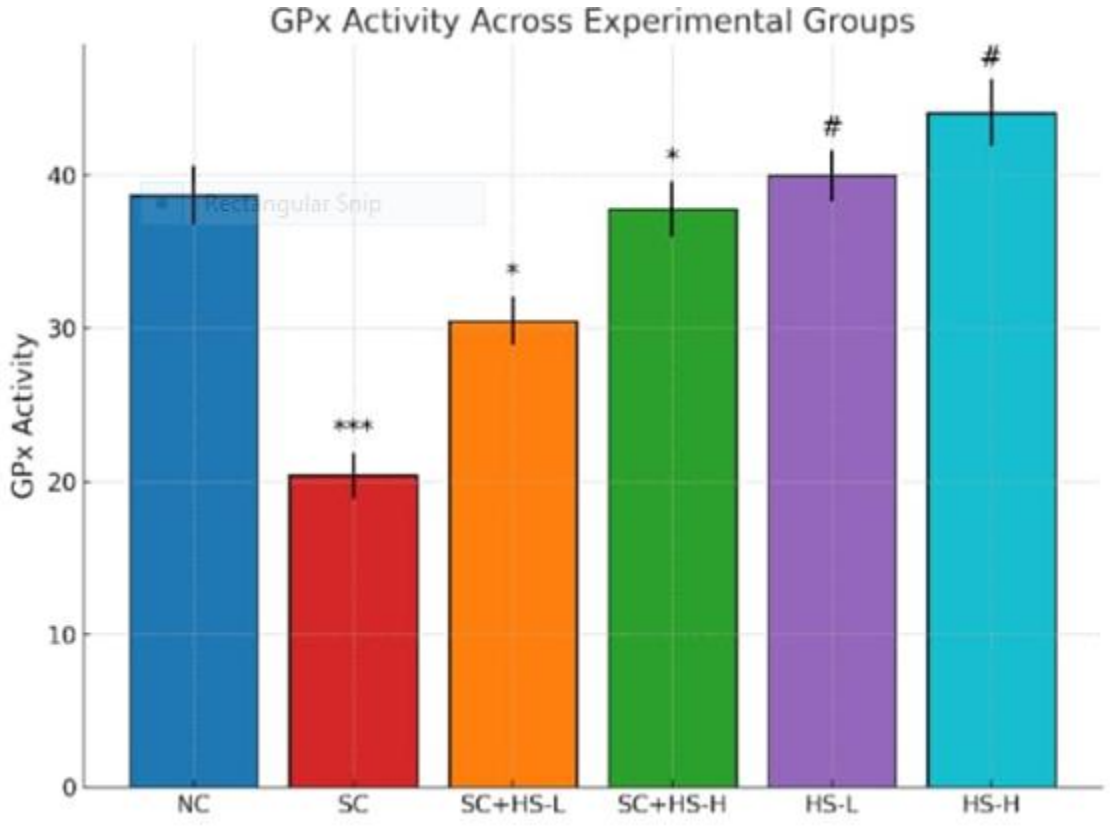

Figure 5. Glutathione peroxidase (GPx) activity in serum. HS restored GPx levels in stressed rats in a dose-dependent manner. Data are mean ± SEM (n = 6); *p < 0.001 vs NC; #p < 0.05 vs SC.

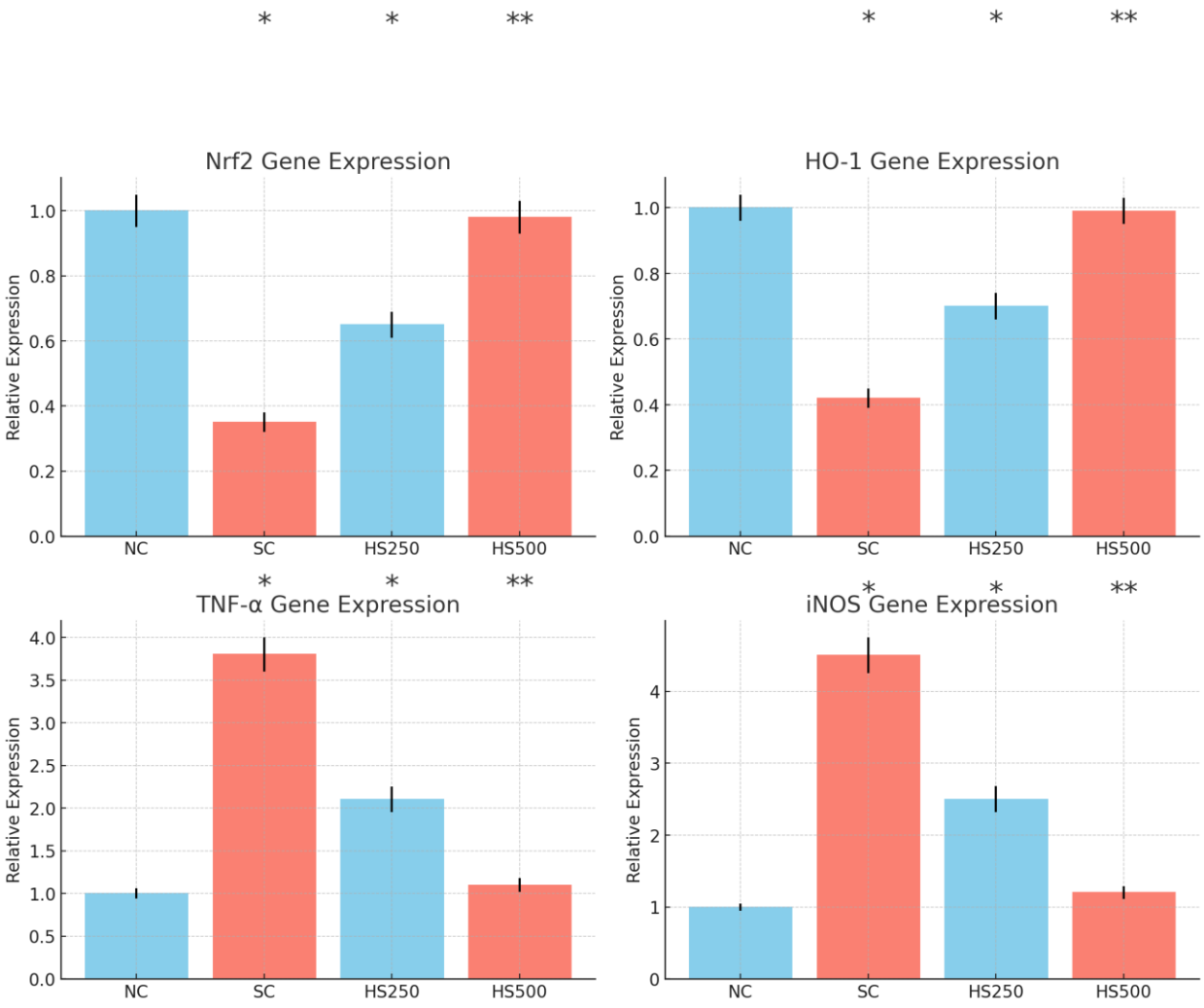

Figure 6. Relative gene expression of antioxidant (Nrf2, HO-1) and inflammatory (TNF-α, iNOS) markers in liver tissue. Restraint stress downregulated Nrf2/HO-1 and upregulated TNF-α/iNOS; HS treatment normalized these genes. Data normalized to NC (1.0) and expressed as mean ± SEM (n = 6). *p < 0.05, **p < 0.01.

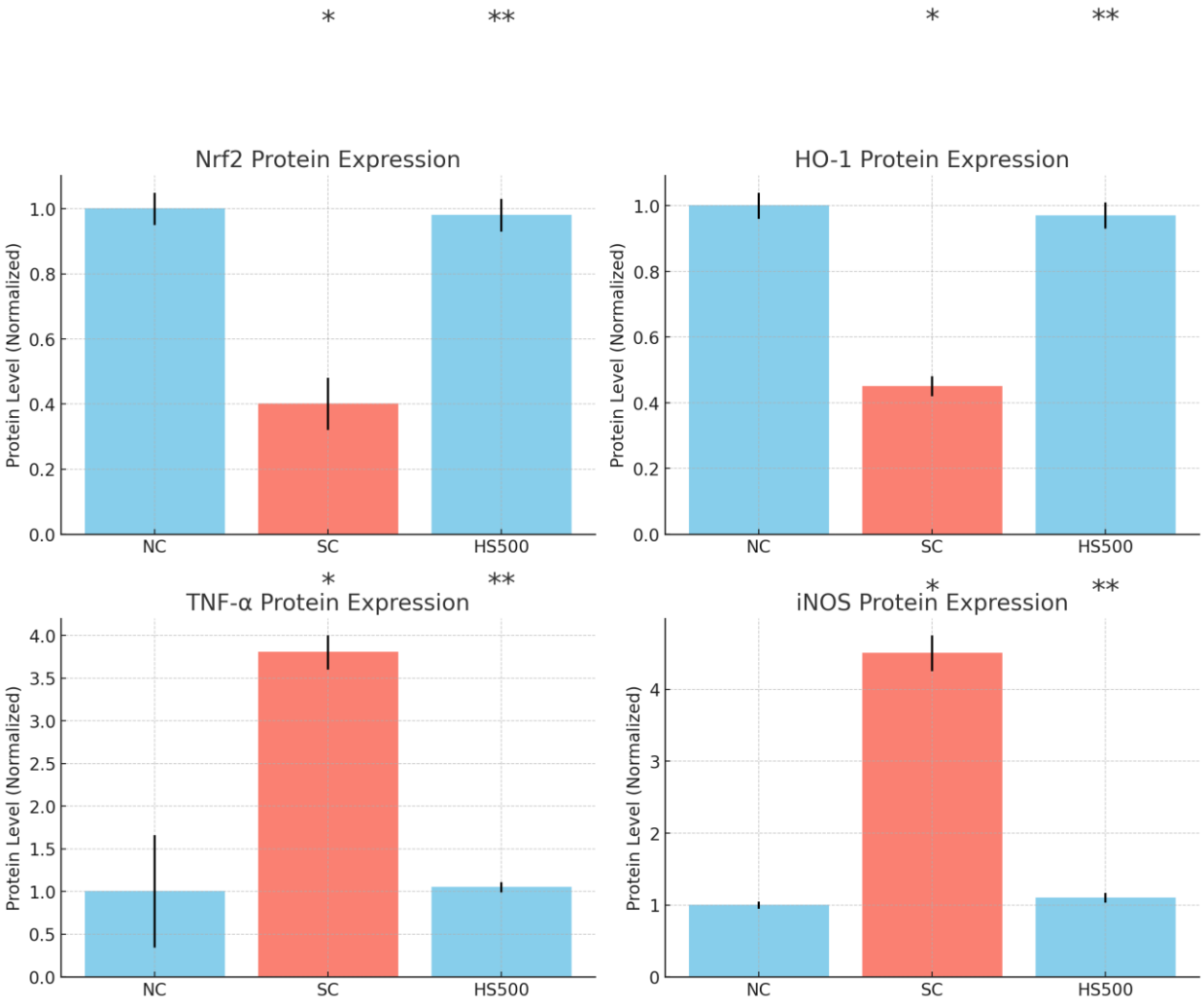

Figure 7. Protein expression of Nrf2, HO-1, TNF-α, and iNOS in liver tissue via Western blot. HS treatment restored antioxidant protein levels and suppressed inflammatory proteins. Representative blots are shown; densitometry values normalized to NC. Data are mean ± SEM (n = 6). *p < 0.05, **p < 0.01.

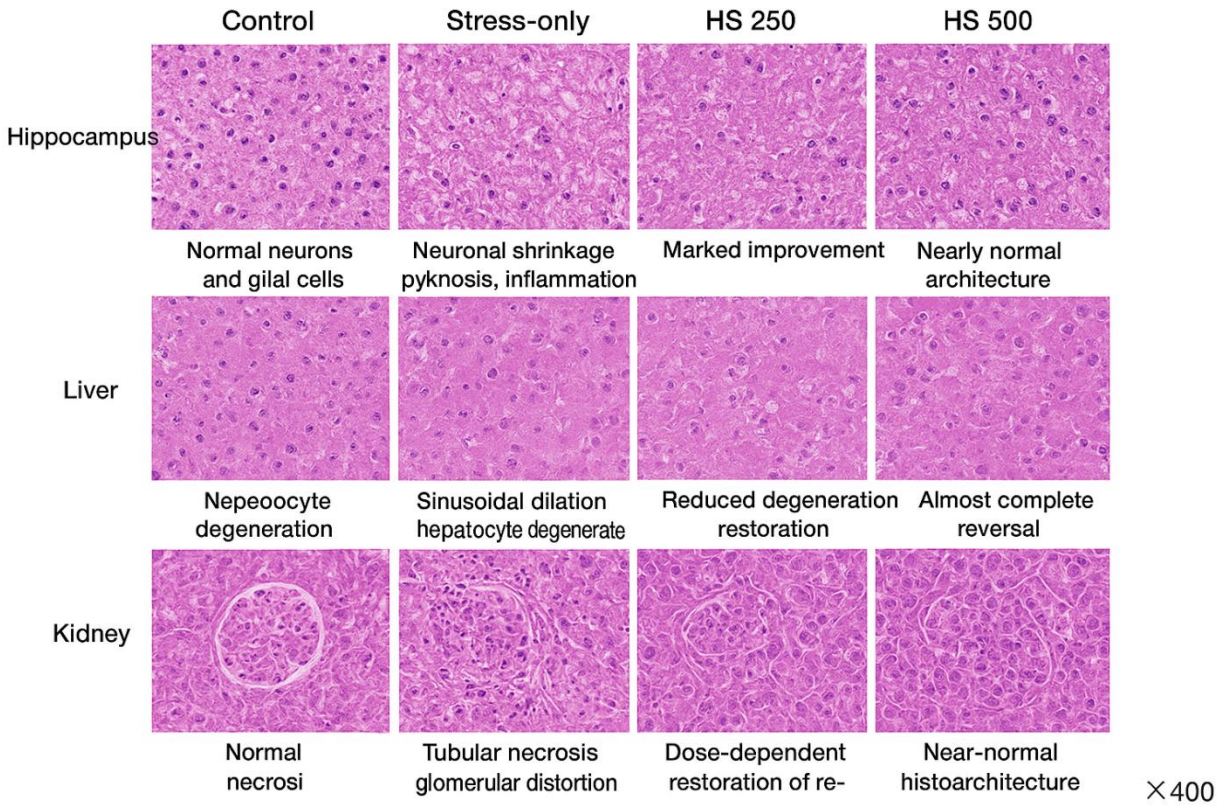

Plate 1. Histopathological assessment of hippocampus, liver, and kidney (H&E, ×400). (A) NC: normal architecture; (B) SC: tissue degeneration; (C) SC + HS 250 mg/kg: partial restoration; (D) SC + HS 500 mg/kg: near-complete restoration.

Tables

Table 1. Body weight changes across experimental groups.

Group	Initial Weight (g)	Final Weight (g)	Weight Gain (g)
NC	192.3 ± 3.4	218.5 ± 4.2	26.2 ± 2.5
SC	191.4 ± 4.1	198.7 ± 3.6	7.3 ± 1.8*
SC + HS 250 mg/kg	190.6 ± 3.9	210.4 ± 4.5	19.8 ± 2.1†
SC + HS 500 mg/kg	192.5 ± 4.2	216.9 ± 3.8	24.4 ± 2.3††
HS 250 mg/kg	193.8 ± 3.7	219.1 ± 4.0	25.3 ± 2.4

*Data are mean ± SEM (n = 6). *p < 0.01 vs NC; †p < 0.05 vs SC; ††p < 0.01 vs SC.*

Table 2. Serum oxidative stress biomarkers.

Group	MDA (nmol/mL)	SOD (U/mL)	CAT (U/mL)	GPx (U/mL)
NC	3.12 ± 0.19	12.4 ± 0.6	32.8 ± 1.4	39.5 ± 1.8
SC	8.41 ± 0.25*	5.9 ± 0.4*	18.2 ± 1.1*	21.4 ± 1.5*
SC + HS 250 mg/kg	5.02 ± 0.31†	10.1 ± 0.5†	26.4 ± 1.3†	31.7 ± 1.6†
SC + HS 500 mg/kg	3.45 ± 0.22††	12.2 ± 0.7††	30.9 ± 1.2††	37.6 ± 1.3††
HS 500 mg/kg	3.07 ± 0.18	12.9 ± 0.6	33.5 ± 1.3	40.1 ± 1.7

*Data are mean ± SEM (n = 6). *p < 0.001 vs NC; †p < 0.01 vs SC; ††p < 0.001 vs SC.*

Table 3. Gene expression analysis (qRT-PCR) in stress-exposed rats.

Gene	NC	SC	SC + HS 250 mg/kg	SC + HS 500 mg/kg	% Change vs NC (SC)
Nrf2	1.00 ± 0.05	0.35 ± 0.03	0.65 ± 0.04	0.98 ± 0.05	-65%
HO-1	1.00 ± 0.04	0.42 ± 0.03	0.70 ± 0.04	0.99 ± 0.04	-58%
TNF-α	1.00 ± 0.06	3.80 ± 0.20	2.10 ± 0.15	1.10 ± 0.08	+280%
iNOS	1.00 ± 0.05	4.50 ± 0.25	2.50 ± 0.18	1.20 ± 0.09	+350%

Table 4. Protein expression (Western blot / ELISA) in stress-exposed rats.

Protein	NC	SC	SC + HS 500 mg/kg	% Change vs NC (SC)
Nrf2	1.00 ± 0.05	0.40 ± 0.08	0.98 ± 0.05	-60%
HO-1	1.00 ± 0.04	0.45 ± 0.03	0.97 ± 0.04	-55%
TNF-α	1.00 ± 0.66	3.80 ± 0.20	1.05 ± 0.06	+280%
iNOS	1.00 ± 0.05	4.50 ± 0.25	1.10 ± 0.07	+350%