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Cardiorenal Protective Effects of Allicin Derived from *Allium sativum* Against High-Salt Diet-Induced Hypertension in Sprague-Dawley Rats

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Abstract

Background: Cardiovascular diseases (CVDs) remain a leading cause of illness and death worldwide, and phytochemicals from medicinal plants have attracted growing attention as cardioprotective agents. This study investigated the cardio-renal protective effects of allicin, an organosulfur compound derived from *Allium sativum* (garlic), against hypertension and associated organ damage induced by a high-salt diet (HSD) in Sprague-Dawley (SD) rats.

Methods: Hypertension was induced in rats with L-NAME (16 mg/kg, i.p.) followed by an 8% high-salt diet. Allicin (16 mg/kg, i.p.) was administered for 21 days, with amlodipine (1 mg/kg) included as a positive control. Serum lipid profile, liver enzymes (ALT, AST), haematological indices, urinary electrolytes and diuretic activity, cardiac and renal histopathology, and the expression of IL-6, TNF-α, NOX, caspase-3, VCAM-1 and ET-1 were evaluated. Molecular docking was additionally performed to assess the binding of allicin to eNOS, IL-6 and TNF-α.

Results: HSD administration raised cholesterol, triglycerides and LDL, elevated ALT and AST, disturbed haematological parameters (haemoglobin, haematocrit, platelet count, RBC), and caused tubular dilation in the kidney and haemorrhage in the myocardium, alongside increased expression of IL-6, TNF-α, NOX, caspase-3, VCAM-1 and ET-1. Twenty-one days of allicin treatment reversed these biochemical and histopathological changes and lowered the inflammatory markers. Allicin also significantly increased urine output and urinary sodium excretion, indicating diuretic and natriuretic activity, while potassium loss was only mildly attenuated, suggesting a potassium-sparing effect. Molecular docking identified eNOS, IL-6 and TNF-α as plausible binding targets for allicin.

Conclusion: Allicin protects against HSD-induced cardiac and renal injury by reversing lipid, hepatic, haematological and histopathological abnormalities, suppressing inflammatory signalling, and preserving electrolyte balance. These findings support allicin as a promising candidate for further development as an adjunct therapy for hypertension-associated organ damage.

Keywords: Cardiovascular disease; Allicin; High-salt diet; Hypertension; Diuretic activity; Cardio-renal protection; Inflammatory biomarkers; Molecular docking

Introduction

Cardiovascular diseases (CVDs) are considered multifactorial conditions that especially affect the essential components of the circulatory system of the human body such as the heart, blood vessels, kidneys, and blood itself [1]. CVDs may be congenital or acquired over a person's lifespan, with atherosclerosis, rheumatic heart disease, hypertension, and vascular inflammation ranking among the most common acquired forms [2]. According to the World Health Organization (WHO), ischaemic heart disease (IHD) is one of the leading causes of death worldwide, accounting for almost one-third of all deaths worldwide [3]. As of 2023, there were 437 million (95% UI: 401 to 465 million) CVD DALYs globally, a 1.4-fold increase from the number in 1990 of 320 million (292 to 344 million) (Global, Regional, and National Burden of Cardiovascular Diseases and Risk Factors in 204 Countries and Territories, 2025) [4]. Cardiovascular disease (CVD) often coexists with metabolic disorders such as diabetes and kidney disease [5]. The heart and kidneys are both vital organs essential for maintaining life [6]. The kidneys, responsible for excreting exogenous substances like drugs and nutrients, are especially susceptible to injury from these agents [7]. Hypertension is the most common cardiovascular condition worldwide, affecting both industrialized and developing nations [8]. The higher the blood pressure, the greater the likelihood of myocardial infarction, heart failure, diabetes mellitus, and hypertensive renal disease [9,10,11]. Unhealthy dietary habits can result in both acute and chronic kidney damage—such as kidney stones, which are linked to high oxalate intake [12].

Research suggests that oxidative stress might lead to hypertension and associated consequences. ROS may inhibit eNOS activation and increase NO inactivation, leading to decreased levels of NO and hypertension [13,14]. Oxidative stress in the kidney is strongly linked to the development and progression of hypertension. ROS may improve afferent arteriolar tone and responsiveness by increasing tubular glomerular feedback and decreasing NO responses in the renal microvascular system, causing vascular smooth muscle cells to contract [15,16]. ROS reduces pO2 in the renal cortex, reducing the efficacy of Na+ transport [17,18]. Cardioprotective mechanisms encompass the range of approaches that help prevent pathological changes in the heart [19], blood vessels, and kidney [20]. Current therapeutic options include vasodilators to lower vascular resistance, adrenergic antagonists to dampen overactive sympathetic activity, and calcium channel antagonists. Unfortunately, each of these approaches carries drawbacks of its own, including cluster headaches with nitroglycerine, withdrawal effects and sexual dysfunction with β-adrenergic antagonists, and reflex tachycardia and hypotension with calcium channel blockers (CCBs) [21].

In the search for alternative therapeutic agents, many phytochemicals from medicinal plant sources have been reported to demonstrate cardioprotective properties [22]. Natural organosulfur compounds (OSCs), a class of bioactive molecules with both hydrophilic and lipophilic properties, are predominantly distributed in plant species belonging to the Cruciferae and Liliaceae families [23]. OSCs have demonstrated significant pharmacological potential, including cardio-renal protection, through multiple mechanisms such as lipid-lowering, antioxidant, and anti-inflammatory effects [24]. Other examples of organosulfur compounds include sulforaphane (SFN), allicin, diallyl disulfide (DADS), and diallyl trisulfide (DATS), to mention but a few [25].

Allicin (thio-2-propene-1-sulfinic acid S-allyl ester) is a thiosulfinate found in garlic and ramsons [26]. Garlic (*Allium sativum*), ramsons (Allium ursinum), and onion (*Allium cepa*) are sources of allicin with reported anti-oxidant and cardioprotective activities [27]. Allicin is one of the most important phytochemical constituents of these plants and has been reported to exhibit antioxidant [28,29], hypolipidemic [30], anti-inflammatory [31], and hepatoprotective [32] activity. Previous

studies have linked these activities to the management of cardiovascular disease, particularly hypertension. Preliminary studies by Sánchez-Gloria et al. (2022) and Deng et al. (2021) [31,33] have reported the potential cardioprotective response of allicin, but no detailed study has yet examined its effects on cardiac biomarkers, haematological parameters, lipid profile, and myocardial and renal histopathology. The present study was therefore designed to investigate the cardio-renal protective effects of allicin, to compare these effects with those of amlodipine, and to examine changes in electrolyte excretion and urine output following myocardial and renal injury induced by a high-salt diet in rats.

Materials and methods

Chemicals and reagents

Drugs used in this study were purchased from the source specified: pentothal sodium (Abbott Laboratories, Pakistan), amlodipine besylate, and Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME) (Arlington Hts, IL, USA.). All the chemicals and reagents used in this study were of analytical grade.

Animals Used

All in vivo studies were performed after the approval of the Institutional Review Board for Animal Studies (Study No ERB/FS/140226), University of Sargodha, Pakistan, and were carried out in accordance with the provided guidelines established by the Institute of Laboratory Animal Resources, National Research Council (NRC, 2011). Male Sprague Dawley rats (SD) (weighing approximately 200–350 g) were used in in vivo and ex vivo studies and were kept at the animal house at standard conditions.

Experimental Protocol

The rats were randomly divided into four (4) groups (n=5). Before the start of the experiment, the animals had been examined for any signs of disease (e.g., sneezing, watery nose, reduced eye and skin elasticity, and lethargy when walking). For this reason, we have selected the healthy animals. The SD rats were placed in metabolic cages for one week before the experiment to acclimate them to the environment.

Allicin Response against Hypertension Induced by High-Salt Diet

As previously described [34–36], hypertension-induced organ damage was established using deoxycorticosterone acetate, high-fructose diet (HFD), or a high-salt diet (8%) administered daily. In this protocol, rats first received L-NAME (16 mg/kg, i.p.) for 7 days; after a washout period, they were then given the high-salt diet orally together with allicin administered intraperitoneally. DMSO (1%) served as the vehicle for allicin, while amlodipine (1 mg/kg) was used as a positive standard for comparison. Group allocations (n = 5 per group) are shown in Table 1.

Table 1. Distribution of experiment rats in different groups for allicin cardioprotective study.

Sr.NO	Groups	Procedure
1.	Control	Normal Diet and Normal saline
2.	High-salt-alone-treated	L-NAME for 7 days and followed by wash out period and then fed with HSD(8% NaCl)
3.	Amlodipine + high-salt-treated	Amlodipine at 1mg/kg, i.p. for 21 days and also HSD
4.	Allicin + high-salt-treated	Allicin at 16 mg/kg, i.p. for 21 days and also HSD.

The body weights of rats were measured before and at the end of the experiment

Baseline Diuretic Activity

To establish the baseline diuretic activity in the 21-day experimental model, all animals were administered normal saline (10 mg/kg, orally) via gavage according to their body weight.

Immediately after administration, the animals were individually placed in metabolic cages for a period of 4 hours. Urine samples were collected at the end of this period, and total urine volume (mL) was measured. These values were considered the baseline urine output for each animal [37].

Acute Diuretic Activity

Acute diuretic activity was assessed during the fifth week, prior to sacrifice. All animals received normal saline (10 mg/kg, orally) via gavage based on their body weight and were individually housed in metabolic cages for 4 hours. Urine was collected and measured to determine the total urine volume (mL). The electrolyte analyzer was used for the electrolyte analysis (Na+, K+) [37]. The percentage of urinary excretion, diuretic action, and diuretic index were calculated for all 4 groups on the average urine production at the 4th hour.

$$Urinary\ Excretion\ (\%) = \frac{\text{Urine Volume (ml)}}{\text{Normal Saline (ml)}} \times 100$$

$$Diuretic\ Index = \frac{\text{Diuretic action of the test group}}{\text{Diuretic action of the control group}}$$

At the end of the experiment, rats were euthanized within 24 hours of their final injection of amlodipine or allicin, and blood samples were collected via cardiac puncture following anesthesia with diazepam and ketamine [38]. Additional blood samples were collected to analyze serum biomarkers of hypertension, including cholesterol, LDL, TG, HDL, AST, and ALT, while anticoagulated blood was used for a complete blood count of Hb, RBC, MCV, MCH, MCHC, WBC, Platelet and hematocrit [39,40]. Analyses were performed using standard assay kits on a Biolyzer 100 system. Hearts from each group were preserved in 10% formalin for histopathological study. Heart and kidney tissues were stained with haematoxylin and eosin (H&E) and examined under light microscopy (200×) for changes in the branched appearance of cardiac and renal muscle fibres, indicative of inflammation characterized by oedema and, ultimately, necrosis [41,42].

Real-Time PCR

Total RNA was isolated using TRIzol reagent (Tiangen Biotech (Beijing) Co., Ltd.) according to the manufacturer's protocols. Reverse transcription was performed using the RevertAid First Strand cDNA Synthesis Kit (K1622, Thermo) according to the manufacturer's instructions, while the real-time PCR was performed using Quantitative PCR with TB Green® Premix Ex Taq™ II (RR820A, Takara) and ABI Step one Plus sequence detection PCR system. The mRNA expression was calculated using the comparative cycle threshold (Ct) method, where the relative quantity of target transcript levels was determined by subtracting the Ct values of target genes from the Ct values of GAPDH [43]. The sequences of primers are listed in Table 2.

Table 2

List of primers

Target Gene	**Sense**	**Anti-sense**
ET-1	5'-ACTTACTTCCCACAAAGACCAC-3'	5'-GCTCGGAGTTCTTTGTCTGC-3'
VCAM-1	5′-AAGTGGAGGTCTACTCATTCC-3′	5′-GGTCAAAGGGGTACACATTAG-3'
NOX-2	5'-TGCAGACAAAATCAAAGAATGG-3'	5'-AACATGGGACCCACTATCCA-3'
IL-6	5'- GATACCACCCACAACAGA-3'	5'-ATACAATCAGAATTGCCAT-3'
TNF-α	5'-TCCATGGCCCAGACCCTC-3'	5'-TCCAGCTGCTCCTCCGCTTG-3'
Caspase-3	5′-ACTGGAATGTCAGCTCGCAA-3′	5′-TAACCGGGTGCGGTAGAGTA-3′
GAPDH	AGGTCGGTGTG AACGGATTTG	TGTAGACCATG TAGTTGAGGTCA

Molecular Docking

Molecular docking is a bioinformatics technique that models the interaction between two or more molecules to identify a stable complex, predicting the three-dimensional structure of a ligand-target complex from their binding properties. Molecular docking of allicin against the selected target proteins was performed accordingly.

Statistical Analysis

Data were analyzed using GraphPad Prism (version 10.4.2). One-way ANOVA followed by Tukey's post hoc test was used to compare group differences, with $p < 0.05$ considered statistically significant.

Results

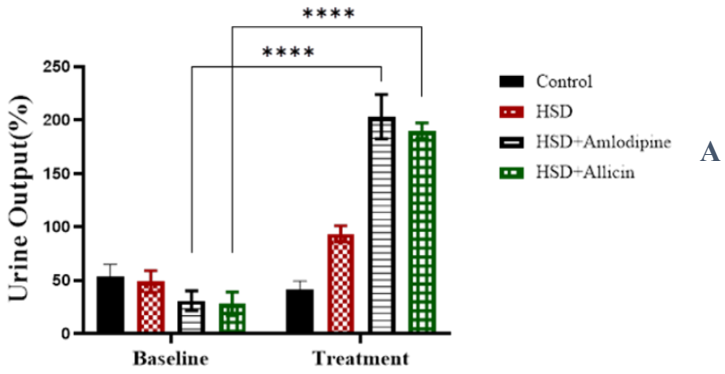
Acute Diuretic Effect

Baseline diuretic activity was measured after oral saline administration and a 4-hour metabolic-cage collection. The same procedure was repeated on day 21, before sacrifice, to assess the acute diuretic response.

Urine output in the Control and High-salt-alone-treated groups did not differ significantly from baseline to treatment (adjusted $p > 0.05$). However, compared with baseline, urine output in the Allicin + high-salt-treated group increased significantly (adjusted $p < 0.01$). In contrast, that of the Amlodipine + high-salt-treated showed a sharp increase (adjusted $p < 0.001$), indicating significant renal protection, as shown in **Figure 1**

Protective Response of Allicin against Alteration in Lipid Biomarkers

Relative to control, HSD-alone-treated rats showed a significant rise in serum total cholesterol, triglycerides, and LDL, alongside a significant reduction in HDL. Amlodipine (1 mg/kg) largely prevented these HSD-induced changes, and allicin (16 mg/kg) likewise restored the altered lipid profile toward control values.



A

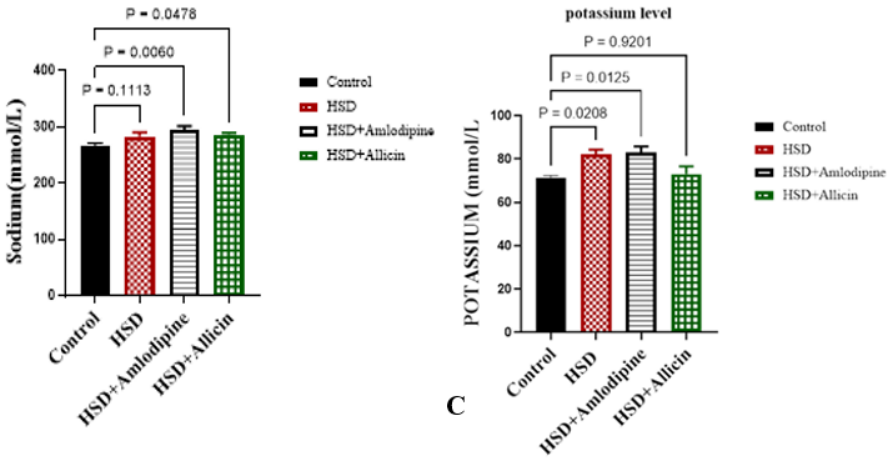


Figure 1. (A) This bar graph shows baseline versus treatment urine output across experimental groups. Values are expressed as mean ± SD (n = 5). Data were analyzed by two-way ANOVA followed by Bonferroni's post hoc test, where **p < 0.01 vs. baseline allicin urine volume and ****p < 0.001 vs. baseline amlodipine urine volume. **(B)** shows Urinary sodium concentration across experimental groups. Data presented as mean ± SD (n = 5). ***p < 0.001 vs. control; analyzed by one-way ANOVA with Tukey's test. **(C)** shows Urinary potassium concentration across experimental groups. Data presented as mean ± SD (n = 5). Analyzed by one-way ANOVA with Tukey's post hoc test.

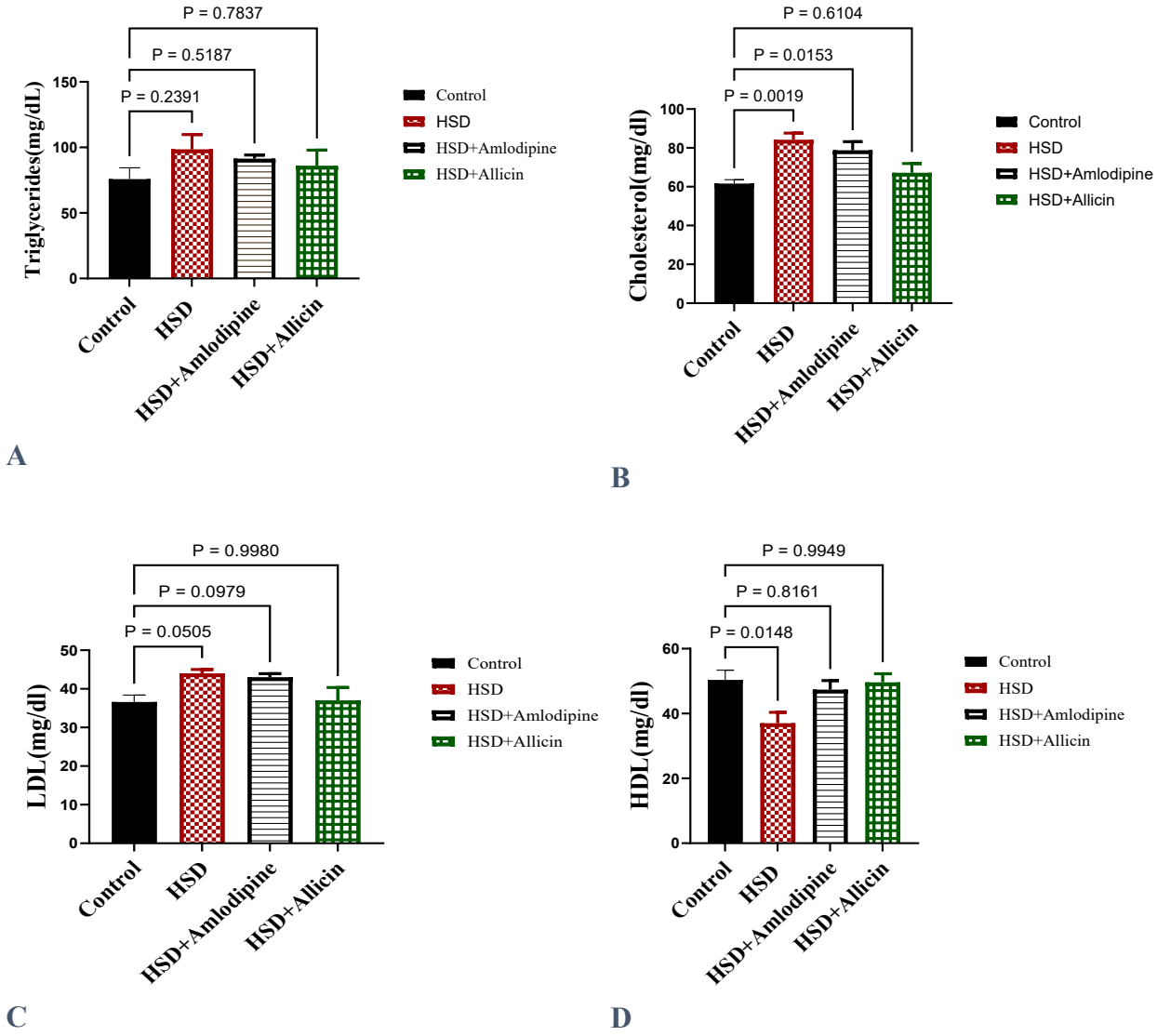


Figure 2. Levels of lipid biomarkers in the serum of control and treated groups. Effect of allicin and amlodipine on lipid markers in the serum of high-salt diet (HSD)-induced hypertensive rats: (a) total cholesterol, (b) triglycerides, (c) low-density lipoprotein (LDL), and (d) high-density lipoprotein (HDL). Results are expressed as mean ± SEM (n = 5). Analysis by one-way ANOVA with Tukey's multiple comparisons post hoc test.

Protective Response of Allicin against Alteration in Liver Biomarkers

Relative to control, HSD-alone-treated rats showed a significant rise in the serum hepatic markers AST and ALT. Amlodipine (1 mg/kg) largely prevented these HSD-induced changes, and allicin (16 mg/kg) likewise restored AST and ALT toward control values.

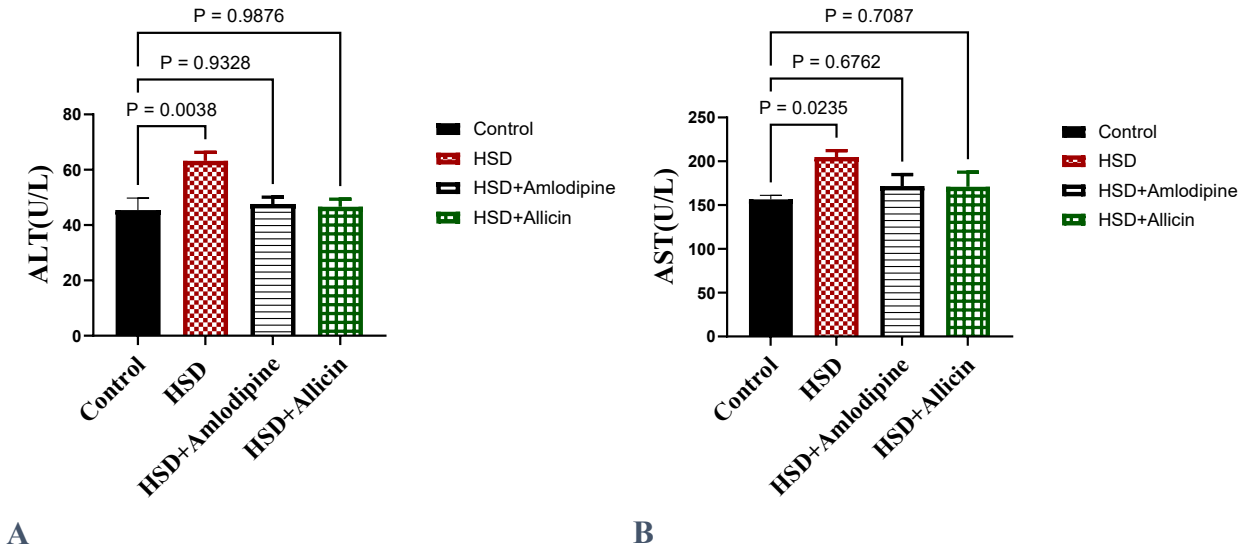
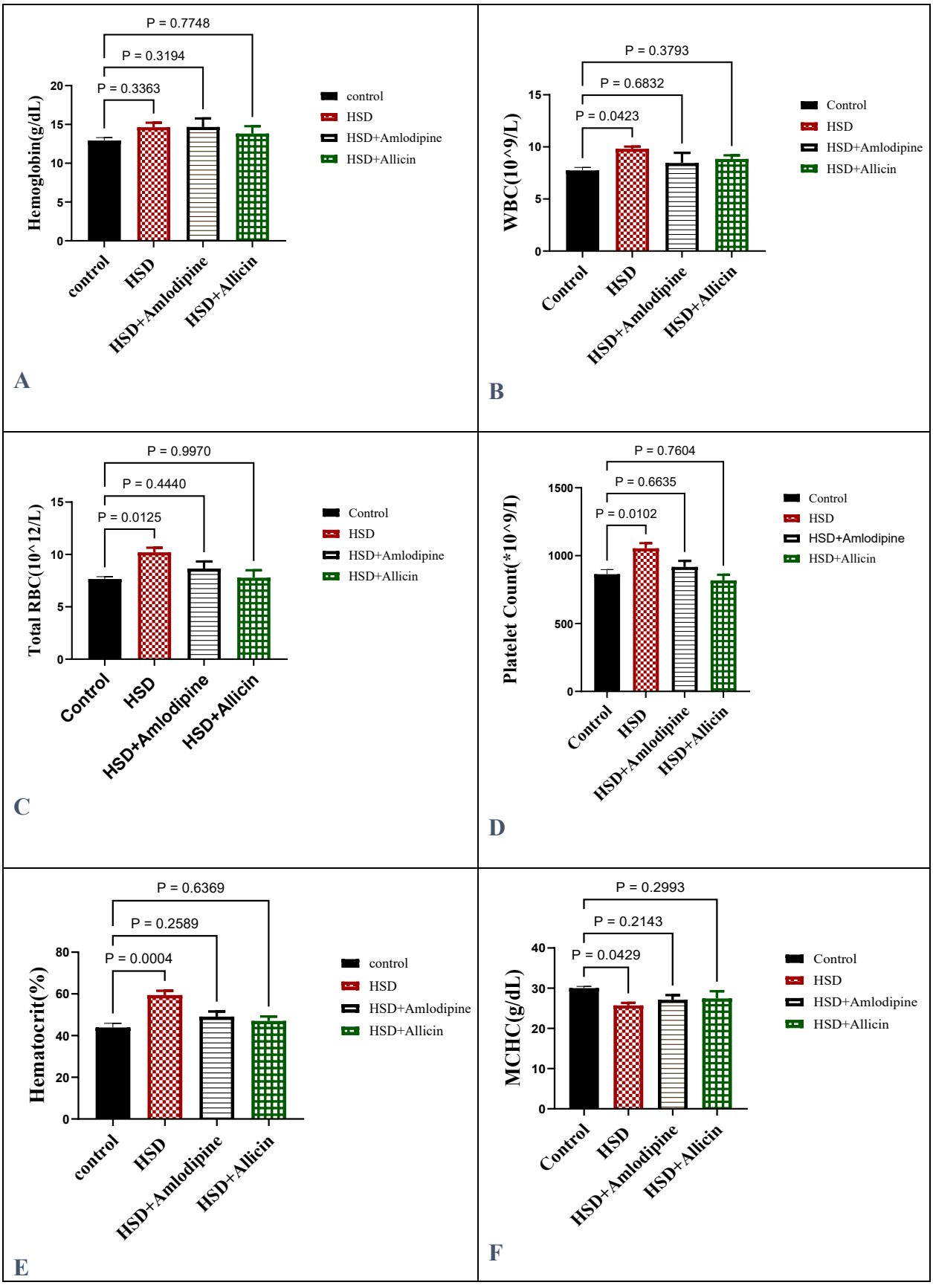


Figure 3. Levels of liver biomarkers in the serum of control and treated groups. Effect of allicin and amlodipine on hepatic markers in the serum of high-salt diet (HSD)-induced hypertensive rats: (**a**) aspartate transaminase (AST) and (**b**) alanine transaminase (ALT). Results are expressed as Mean ± SEM (n = 5). Analysis by 1-way ANOVA with a Tukey multiple comparisons post hoc test.

Protective Response of Allicin against Alteration in hematological Biomarkers

Relative to control, HSD-alone-treated rats showed a significant rise in the haematological parameters haematocrit, RBC count, haemoglobin, platelet count, and WBC count. Amlodipine (1 mg/kg) largely prevented these HSD-induced changes, and allicin (16 mg/kg) likewise restored the altered haematological indices toward control values.


P = 0.7748
P = 0.3194
P = 0.3363
Hemoglobin(g/dL)
control
HSD
HSD+Amlodipine
HSD+Allicin
A
P = 0.3793
P = 0.6832
P = 0.0423
WBC(10^9/L)
Control
HSD
HSD+Amlodipine
HSD+Allicin
B
P = 0.9970
P = 0.4440
P = 0.0125
Total RBC(10^12/L)
C
P = 0.7604
P = 0.6635
P = 0.0102
Platelet Count(*10^9/I)
D
P = 0.6369
P = 0.2589
P = 0.0004
Hematocrit(%)
E
P = 0.2993
P = 0.2143
P = 0.0429
MCHC(g/dL)
F

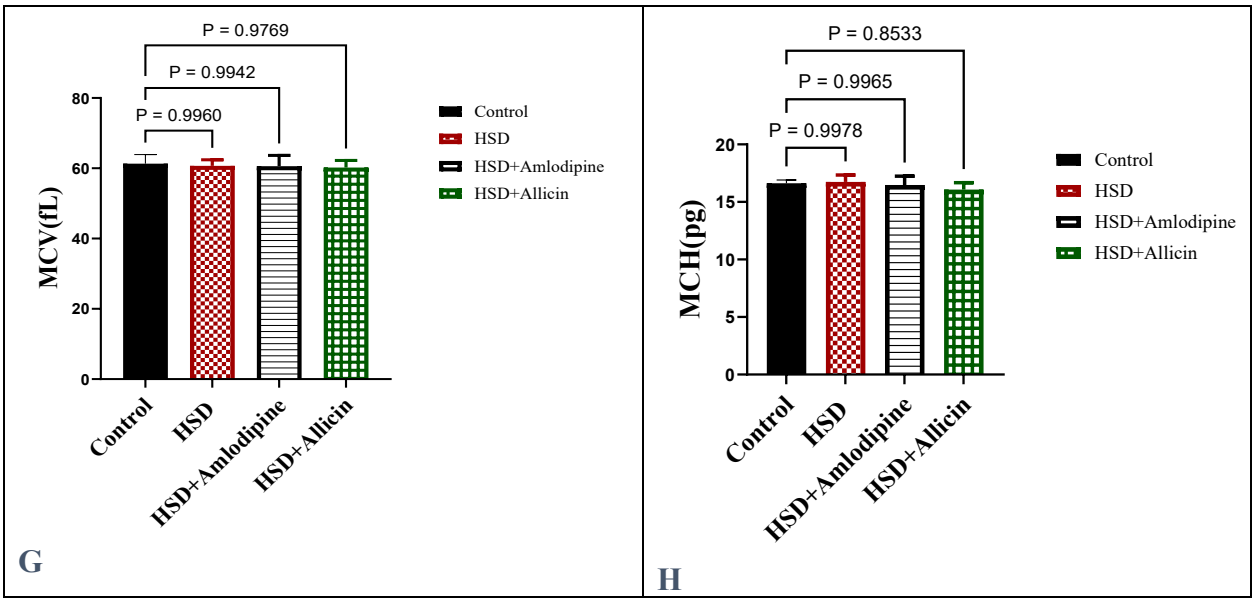
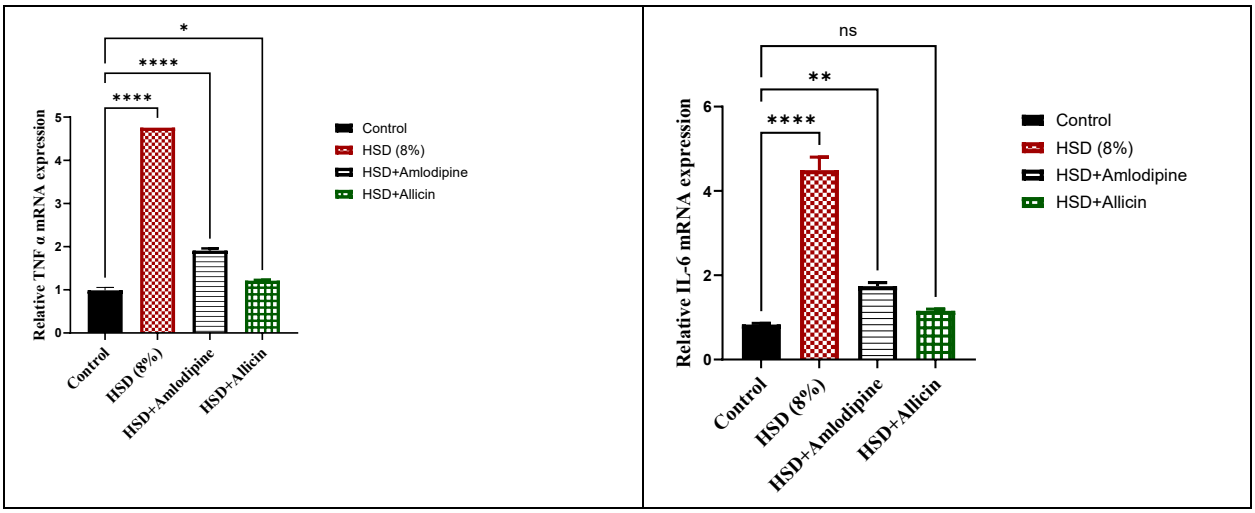


Figure 4. Levels of haematological biomarkers in the serum of control and treated groups. Effect of allicin and amlodipine on haematological parameters in serum of high-salt diet (HSD)-induced hypertensive rats; (a) haemoglobin (Hb), (b) white blood cell count (WBC), (c) red blood cell count (RBC), (d) platelet count, (e) haematocrit (HCT), (f) mean corpuscular haemoglobin concentration (MCHC), (g) mean corpuscular volume (MCV), and (h) mean corpuscular haemoglobin (MCH). Results are expressed as mean ± SEM (n = 5). Analysis by one-way ANOVA with Tukey's multiple comparisons post hoc test.

Effect of Allicin on Immune indicators in HSD induced hypertension

Relative to control, HSD-alone-treated rats showed a significant rise in the TNF-α IL-6, caspase-3 VCAM-1,, ET-1 and NOX. Amlodipine (1 mg/kg) largely prevented these HSD-induced changes, and allicin (16 mg/kg) likewise restored TNF-α IL-6, caspase-3 , VCAM-1, , ET-1 and NOX toward control values.



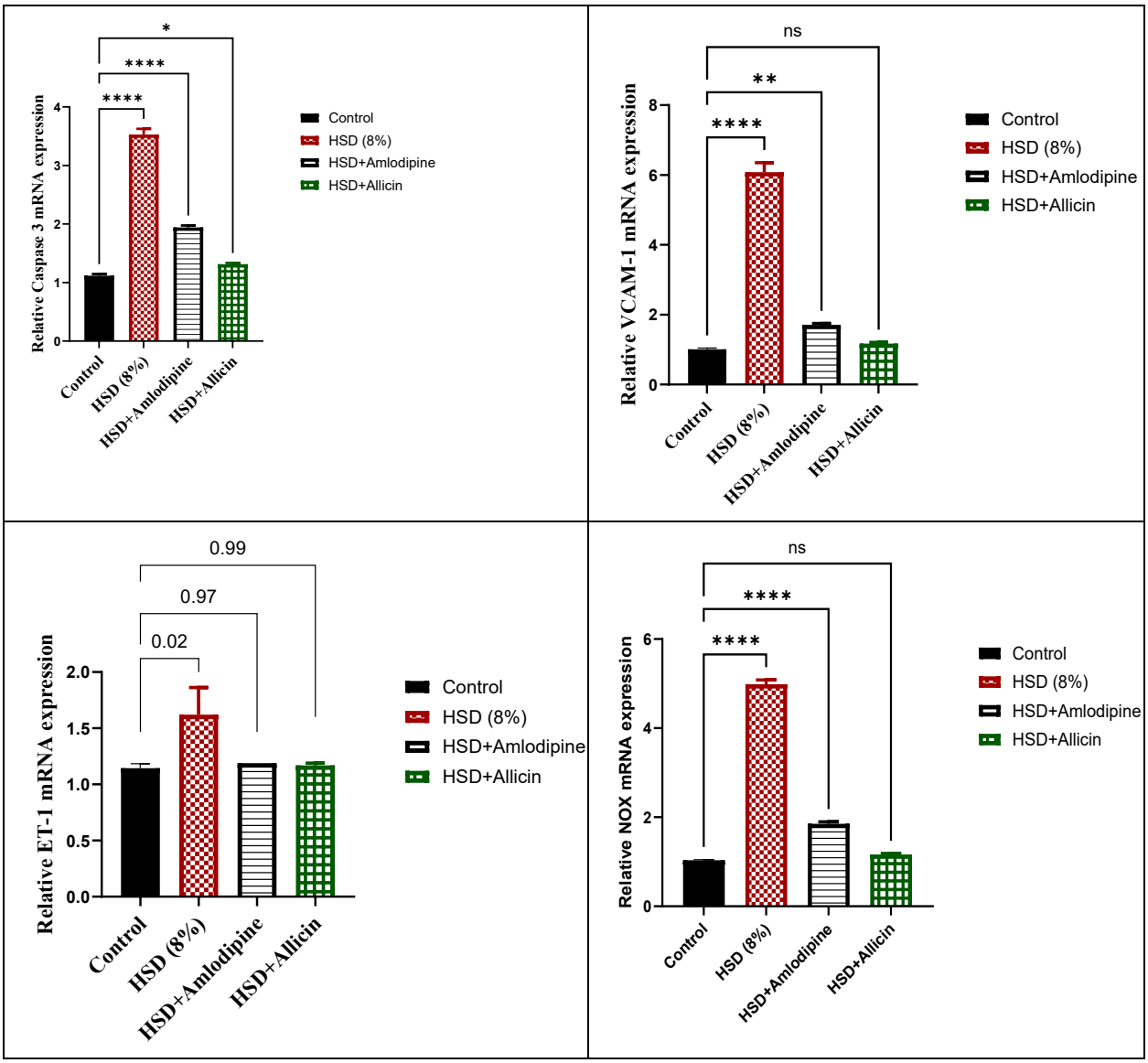


Figure 4.10. The mRNA relative expression levels by quantitative real-time polymerase chain reaction (qRT-PCR) analysis in HSD indued hypertension model (A) The mRNA relative level of TNF-α. (B) The mRNA relative level of IL-6. (C) The mRNA relative level of Caspase 3. (D) The mRNA relative level of VCAM-1 (E) The mRNA relative level of ET-1 (F) The mRNA relative level of NOX (n = 5). Where * $p < 0.05$ vs. control, **** $p < 0.0001$ vs. control

Allicin Prevent Histopathological Changes Induced by HSD

The myocardium of the control group showed normal striations with a branched appearance (Figure 5A), whereas HSD administration alone at a high dose produced interstitial widening and areas of haemorrhage (Figure 5B). Heart tissue from the amlodipine- (1 mg/kg; Figure 5C) and allicin- (16 mg/kg; Figure 5D) treated groups showed markedly less haemorrhage, with the myocardial architecture relatively well preserved and the least evidence of necrosis compared with the HSD-alone group.

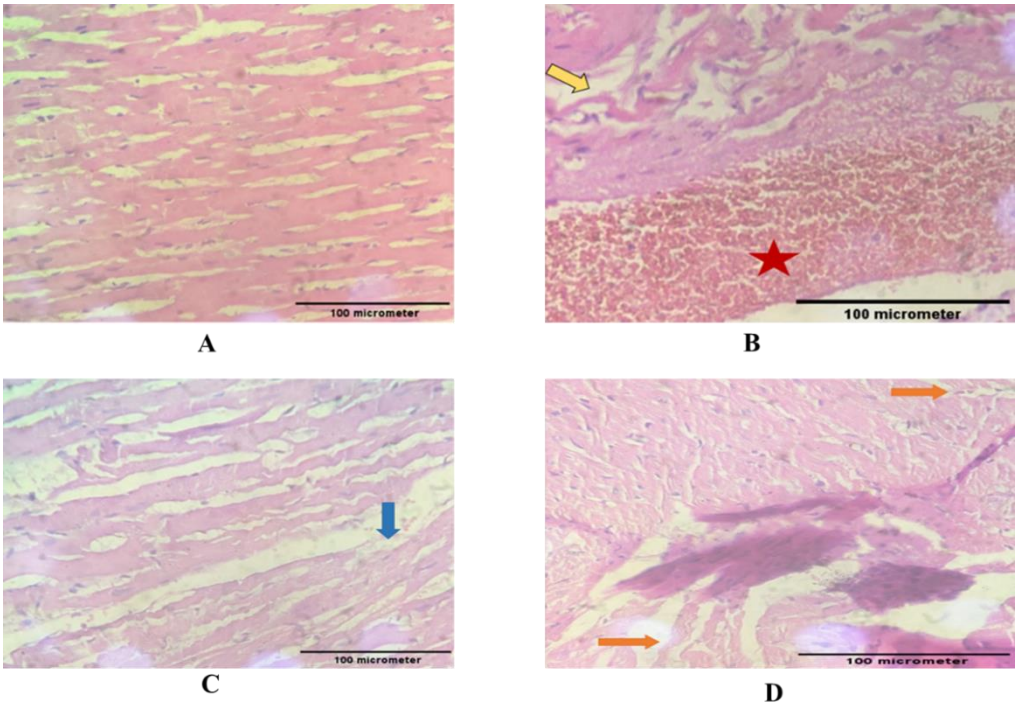
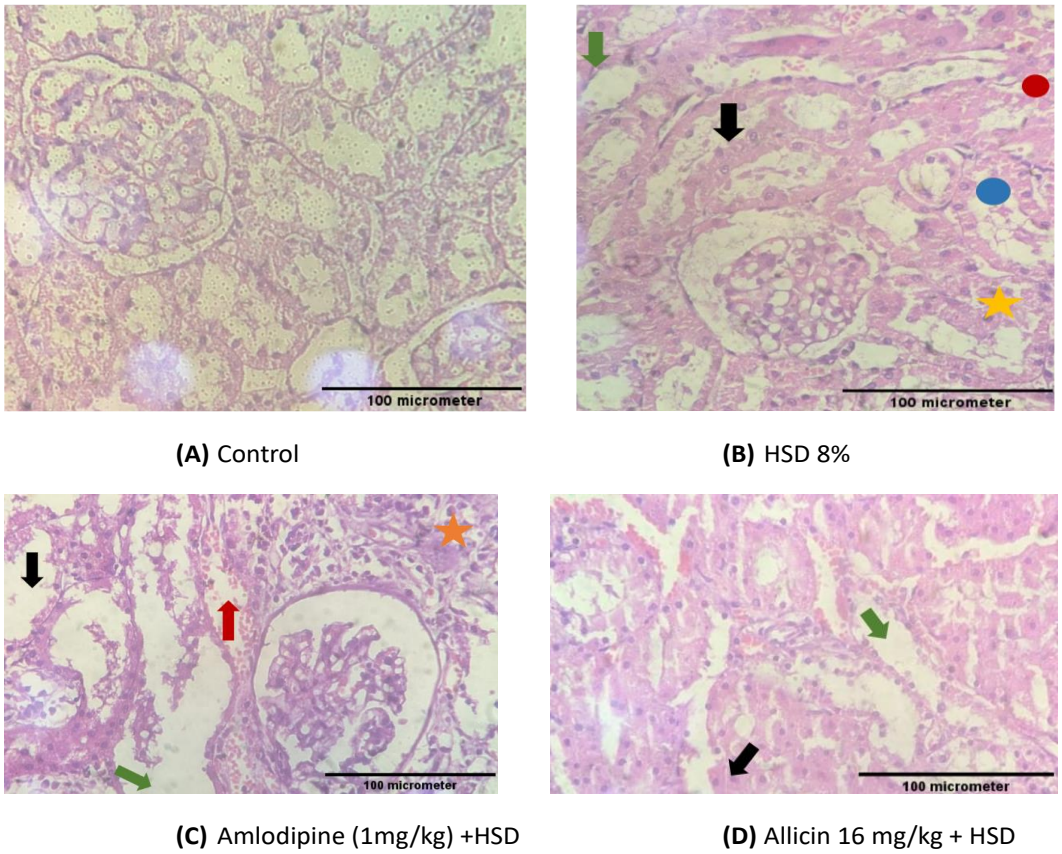


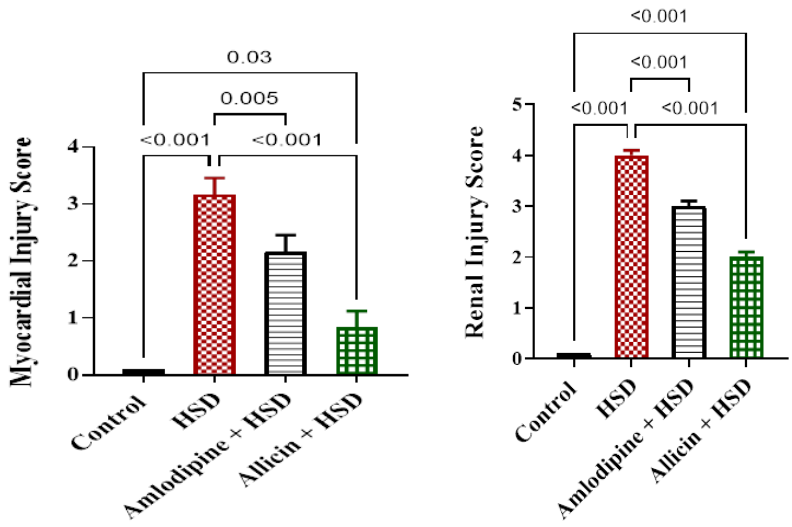
Figure 5 Effect of allicin on histopathological changes. Photomicrographs of heart tissue (H&E, 200×) from control and HSD-treated experimental rats. (A) Control group, showing myocardial fibres arranged in parallel bundles with preserved architecture and no evidence of necrosis, inflammation, oedema, haemorrhage, or fibrosis. (B) HSD-alone group, showing marked interstitial widening and areas of haemorrhage. (C) Amlodipine-treated group, showing moderate widening of the interstitial space with haemorrhage. (D) Allicin-treated group, showing mild widening of the interstitial space without haemorrhage.



(A) Control **(B)** HSD 8%

(C) Amlodipine (1mg/kg) +HSD **(D)** Allicin 16 mg/kg + HSD

Figure 6 Effect of allicin on histopathological changes. Photomicrographs of kidney tissue (H&E, 200×) from control and HSD-treated experimental rats. (A) Control group, showing normal renal histology with preserved architecture. (B) HSD-alone group, showing marked tubular epithelial vacuolar degeneration, tubular dilation, congestion, tubular epithelial degeneration, and interstitial oedema. (C) Amlodipine-treated group, showing moderate tubular dilation, tubular epithelial degeneration, interstitial mononuclear inflammatory infiltrate, and congested blood vessels. (D) Allicin-treated group, showing mild tubular dilation and tubular epithelial degeneration.



Molecular Docking of Core Targets

The absolute docking-score values for allicin against the targeted proteins were ranked as follows: eNOS (−4.44 kcal/mol > TNF-alpha (−4.28 kcal/mol) > IL-1b (−4.02 kcal/mol) — all close to the threshold of −4.5 kcal/mol, indicating moderate and comparable binding stability between allicin and the three receptors. Binding energies from the molecular docking analysis are summarized in Table 3

Table 3: The binding energy of molecular docking

No	Target	Compound	Binding energy (kcal/mol)	Dissociation constant (μM)
A	TNF-alpha	Allicin	−4.28	728.98
B	eNOS		−4.44	561.179
C	IL-1b		−4.02	1122.971904

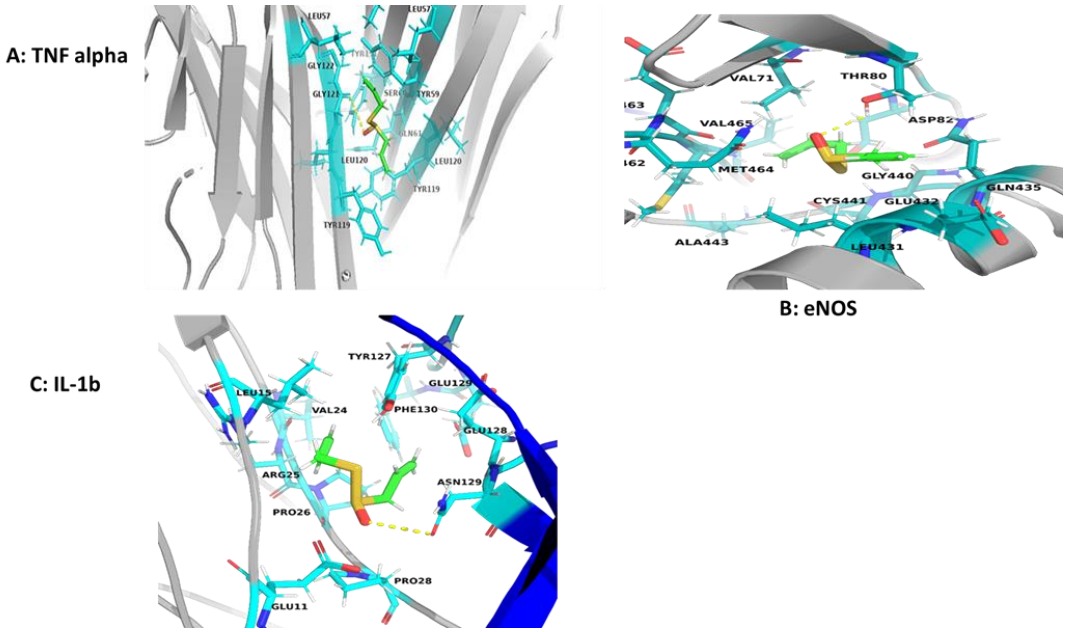


Figure 7 Molecular docking of allicin with its core target proteins. (A) TNF-alpha and Allicin molecular docking visualization. (B) eNOS and Allicin molecular docking visualization. (C) IL-1b and Allicin molecular docking visualization.

Discussion

Plant sources of allicin have been reported to exhibit antioxidant and cardioprotective activities [28,31]. We tested the protective response of allicin in an HSD-induced hypertension and organ-damage rat model to confirm its cardio-renal protective activity. High salt intake activates multiple key signalling pathways (NF-κB, JAK/STAT, MAPK and the NLRP3 inflammasome) in immune cells, such as antigen-presenting cells, macrophages and Th17 cells, triggering significant oxidative stress and inflammatory cascades. Specific mechanisms include high salt inducing immune cells to perceive sodium ions through the ENaC channel and NCX1, activating the SGK1/FOXO1 axis and NFAT5 to drive Th17/Treg imbalance and the release of pro-inflammatory factors such as IL-6, IL-17A, TNF-α, and IL-1β), while excessive ROS production and the resulting protein modifications create new antigens (e.g. IsoLG), and gut microbiota dysbiosis (e.g.

reduced Lactobacillus and elevated TMAO) amplifies systemic inflammation by reducing short-chain fatty acids (SCFAs) and increasing endotoxin release, thereby activating TLR4/NF-κB and other pathways [44]. The principal finding of this study is that allicin significantly attenuated the pathological changes in the heart and kidney induced by hypertension following submaximal high-salt administration. Administration of an 8% HSD produced pronounced stress in the renal and cardiac tissues of SD rats, resulting in inflammatory infiltration, oedema, elevated pathological biomarkers, and structural remodeling of the heart and kidney closely resembling the changes seen in hypertensive patients [45].

In our experiments, salt-sensitive male Sprague-Dawley rats pretreated with L-NAME became hypertensive within weeks of starting the 8% NaCl diet. By the fourth week of the salt-loading diet, the rats appeared visibly weak and were observed trembling. Following a two-week washout period, administration of allicin and amlodipine was then started in their respective groups. Unlike the untreated rats, those in the allicin-treated group appeared active and healthy, with the pre-treatment weakness reversed, indicating a protective effect of allicin. In this study, the allicin-treated group's urine output increased significantly over baseline, suggesting enhanced diuretic activity. Allicin's diuretic effect may help offset the maladaptive fluid and electrolyte retention associated with high-salt intake. Supporting this observation, Li et al. (2022) demonstrated that allicin reduces oxidative stress and apoptosis in ischaemia-reperfusion (I/R) injury, mechanisms that are closely associated with improved renal perfusion and tubular function and, ultimately, enhanced diuresis [46]. Moreover, Cui et al. (2020) reported that allicin-induced vasodilation, mediated through hydrogen sulfide and nitric oxide signalling pathways, contributes significantly to its diuretic and natriuretic actions [47].

Lipid profile parameters, including triglycerides, LDL, and total cholesterol, were significantly elevated in the HSD-alone group, while HDL was reduced relative to control. A high-salt diet raises serum cholesterol levels, potentially through activation of the hepatic enzyme CYP51, which plays a key role in hepatic cholesterol synthesis. In cholesterol-fed rabbits, amlodipine has been shown to reduce cardiac and blood oxidative stress and modify lipid levels [48]. In the present study, allicin administration significantly reduced the elevated lipid levels and increased HDL in rats.

Administration of the 8% high-salt diet increased AST and ALT levels, consistent with the findings of Olorunnisola (2021), who likewise reported significant elevations in AST and ALT activity in HSD-fed rats [49]. Elevated liver enzyme levels are commonly observed alongside increased blood pressure. Previous studies have found that the serum AST and ALT levels were notably higher in the hypertensive group than in the normotensive group [50]. Pretreatment with allicin (16 mg/kg, i.p.) significantly ($p < 0.05$) decreased the HSD-induced elevations in serum AST and ALT activity.

In our study, the HSD-induced hypertension organ-damage model exhibited pronounced deviations in haematological parameters, characterised by elevated haematocrit, RBC count, haemoglobin, platelet count, and WBC count, suggestive of systemic immune dysfunction and a polycythaemic response. Haemoglobin has a high affinity for nitric oxide (NO) [51], and NO scavenging by haemoglobin triggers systemic vasoconstriction [52,53], contributing to blood pressure elevation. A clinical study likewise found accelerated NO scavenging by haemoglobin to be a key factor in the development of hypertension in patients with polycythaemia vera [54]. Notably, treatment with allicin and amlodipine restored these altered haematological indices, normalizing leukocyte counts and haemoglobin levels. These findings highlight the therapeutic potential of this phytoconstituent in correcting peripheral hematological abnormalities associated with hypertension.

High salt diet administration has also been associated with an elevation in aortic tissue levels of IL-6, TNF-α, NOX, VCAM-1, ET-1 and caspase-3 mRNA expression, indicating the presence of inflammation, oxidative stress, endothelial activation and apoptosis. However, treatment with allicin and amlodipine significantly reduced the expression levels of IL-6, TNF-α, NOX, VCAM-

11, ET-1 and caspase-3. Previously in relevance of these findings a number of studies reported the similar effects in which allicin showed protective effect against hypertensive vascular remodeling by suppressing inflammatory jnk/NF-κB signalling and reducing IL-6 and TNF-α, while its antioxidant and vasodilatory properties may additionally counteract the NOX–ROS–ET-1 axis [54]. These findings is consistent with the molecular docking results, which demonstrated the potential binding affinity of allicin toward IL-6 and and TNF-α . Therefore, it is speculated that allicin reverses the NOX/ TNF-α,/IL-6 induced by HSD. So, does allicin reduce inflammation and oxidative stress.

Significant renal tissue injury was likewise observed in HSD-alone-treated rats, characterized by tubular epithelial vacuolar degeneration, tubular dilation, congestion, tubular epithelial degeneration, and interstitial oedema. Similarly, HSD-alone treatment produced significant cardiac tissue injury in the myocardium, characterized by widening of the interstitial space with haemorrhage and an irregular appearance of the cardiac muscle fibres. Allicin has been reported to inhibit inflammation by suppressing the expression of P38 and phospho-c-Jun N-terminal kinase (p-JNK) and by activating the Nrf2/ARE pathway, thereby attenuating the production of pro-inflammatory molecules [55]. Even at submaximal doses, high-salt intake can drive oxidative injury: Heimlich et al. reported that a 4% high-salt diet increased glomerular ROS production via endothelin-1 (ET-1)–ETA receptor activation [56]. The transcription factor Nrf2 and its partner Keap1 are key negative regulators of ROS production; in a related model, hepatic Nrf2 expression was found to be down-regulated following high-salt exposure while Keap1 expression was up-regulated, suggesting that excess ROS production under high-salt conditions may stem from reduced inhibitory Nrf2/Keap1 signalling [57]. In the present study, pre-administration of allicin and amlodipine countered the pathological changes initiated by HSD, consistent with previous reports that allicin exhibits significant free-radical-scavenging activity [58,59].

In summary, the data indicate that pre-administration of allicin significantly prevented the pathological changes induced by HSD. Allicin preserved myocardial integrity, as confirmed by histopathology, and maintained cardiac, haematological, and lipid biomarkers, as well as urinary electrolytes, within the normal range. Further studies are warranted to clarify the molecular mechanisms underlying these effects.

Conclusion

In conclusion, the findings of this study indicate that an 8% high-salt diet caused endothelial dysfunction, oxidative damage, and inflammatory activation leading to hypertension in rats, while treatment with allicin and amlodipine produced a marked reduction in blood pressure in salt-induced hypertensive rats. Allicin also produced a significant increase in urine volume and electrolyte excretion. This study further demonstrates that allicin improved the liver enzyme profile (AST and ALT), lipid profile (TG, TC, LDL, HDL), and blood cell parameters in HSD-treated rats. Allicin- and amlodipine-treated rats showed normal-appearing myocytes with no haemorrhage, and although kidney damage occurred in the HSD-alone group, allicin and amlodipine partially reduced the glomerular injury. In isolated rat aortic tissue, allicin and amlodipine restored TNF-α, IL-6, NOX, caspase, VCAM-1, and ET-1 to near-normal levels. Moreover, allicin demonstrated favourable binding affinities with eNOS and TNF-α, indicating its potential to inhibit inflammatory signalling pathways implicated in hypertension. Overall, the data suggest that allicin may offer combined cardiovascular and renal benefits in hypertensive patients at risk of organ damage while minimizing electrolyte disturbances, positioning it as a promising candidate for the development of new therapeutically useful compounds. Further studies are needed to investigate the pathways underlying the pharmacological effects described here.

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