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# Protective Effects of *Stevia rebaudiana* Leaf Extract on Cardio-Renal and Vascular Dysfunction in L-NAME-Induced Hypertensive Rats via Reduction of Oxidative Stress and Inflammation

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**Abstract**

Background: Hypertension (HTN) is a major contributor to cardiovascular and renal disorders, with oxidative stress and endothelial dysfunction as key mechanisms. Objective: *Stevia rebaudiana (S. rebaudiana),* a plant with antioxidant and anti-inflammatory features, was evaluated for its protective effects in an L-NAME–induced hypertensive rat model.Brief Methods: Thirty-two male Sprague–Dawley rats were randomly assigned to four groups (n = 8): Control (standard diet), L-NAME (40 mg/kg/day), L-NAME + High Dose *S. rebaudiana* (500 mg/kg/day), and L-NAME + Low Dose *S. rebaudiana* (250 mg/kg/day). Phytochemical profiling was performed via HPLC and LC–MS, and antioxidant activity was assessed. Blood pressure, oxidative stress markers (malondialdehyde (MDA), reduced glutathione (GSH)), nitric oxide levels, ACE activity, inflammatory cytokines (IL-6, TNF-α), and apoptotic markers (caspase-3, Bcl-2) were measured, alongside histopathological examination of heart, aorta, and kidney tissues. Key Results: *S. rebaudiana* treatment produced dose-dependent reductions in blood pressure, restored nitric oxide levels, decreased MDA, increased GSH, downregulated IL-6, TNF-α, and caspase-3, and upregulated Bcl-2. Histology confirmed preservation of tissue structure.Concise conclusion: *S. rebaudiana* provides cardio-renal protection by modulating oxidative stress, inflammation, and apoptosis, supporting its potential as a natural therapy for hypertension-related complications.

**Keywords:** Hypertension, L-NAME, Stevia rebaudiana, Oxidative stress, Inflammation, Apoptosis.

## 1. Introduction

Hypertension (HTN) is a prevalent chronic disorder that significantly increases the risk of stroke, diabetes mellitus (DM), renal impairment, cardiovascular diseases (CVDs), and premature mortality [1]. Recent estimates from the World Health Organization (WHO) signify that more than 1.28 billion people globally suffer from HTN [2]. Its pathogenesis is complex and multifactorial, characterized by vascular abnormalities, heightened oxidative stress, persistent low-grade inflammation, and dysregulation of nitric oxide (NO) signaling [3, 4]. These pathways contribute not only to elevated blood pressure but also to progressive o rgan damage. NO plays a fundamental role as a vasodilatory molecule, essential for maintaining vascular tone and endothelial integrity. Suppression of NO synthesis with pharmacological inhibitors such as Nω-nitro-L-arginine methyl ester (L-NAME), a non-selective NO synthase (NOS) blocker, reproduces many of the hemodynamic and pathological alterations observed in human HTN, making it a reliable experimental mode [5]. Prolonged L-NAME administration in animal models results in sustained HTN, endothelial dysfunction, heightened oxidative stress, and inflammatory cell infiltration in target organs [6, 7]. Thus, L-NAME-induced NO deficiency represents a well-established and widely employed model for investigating HTN pathogenesis and related complications.

Interest is increasing in natural compounds with potential antihypertensive features, particularly those capable of modulating oxidative stress and inflammatory pathways. *Stevia rebaudiana (S. rebaudiana*) is traditionally employed as a non-caloric sweetener owing to its high content of steviol glycosides [8]. In addition to its sweetening function, *S. rebaudiana* demonstrates a diverse spectrum of biological effects, notably including antihypertensive, antioxidant, anti-inflammatory, and vasodilatory activities, as evidenced in clinical and preclinical settings [9, 10]. These protective mechanisms are thought to be mediated through

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enhanced bioavailability of NO, efficient scavenging of reactive oxygen species (ROS), and downregulation of key pro-inflammatory mediators like tumor necrosis factor-alpha (TNF-α), Interleukin 1 beta (IL-), and Interleukin -6 ( IL-6) [11-13]. Despite its exhibited antihypertensive potential, the protective impacts of *S. rebaudiana* in NO-deficient HTN induced by L-NAME has not been fully elucidated. This research was designed to assess the protective impacts of S. rebaudiana in a rat model of L-NAME-induced HTN. Also, assess its influence on blood pressure and its capacity to mitigate oxidative stress, inflammatory responses, and apoptotic signaling in male Sprague–Dawley rats.

## 2. Materials and Methods

### *2.1. Experimental Animals*

Male Sprague–Dawley rats (n=32), aged 6–7 weeks and weighing 200–220 g, were used in this study. Before experimentation, all animals underwent a two-week acclimatization period under controlled housing conditions. Rats were kept in individual stainless-steel cages with a regulated environment consisting of a 12 h light/dark cycle, ambient temperature of 24 ± 1 °C, and relative humidity maintained at 52 ± 12%. Standard laboratory chow and water were available ad libitum. The animals were supplied by the Laboratory Animal Research Center, Faculty of Veterinary Medicine, Benha University, Egypt. All procedures were carried out in accordance with Institutional Animal Care and Use Committee (IACUC) guidelines and approved by the Research Ethics Committee (Approval Code: BUFVTM12-10-24).

### *2.2. Chemicals*

L-NAME (98% purity; Sigma-Aldrich, USA) was freshly prepared prior to administration and delivered orally at 40 mg/kg, three times/week for six weeks [14, 15]. Folin–Ciocalteu reagent (FCR), Trolox, ABTS, and DPPH were also obtained from Sigma-Aldrich to ensure consistency in reagent quality. Gallic acid (98% purity; Acros Organics, Belgium) served as the reference standard in phenolic content assays. All other chemicals and solvents were of analytical grade and sourced from Fisher Scientific, UK[16].

### *2.3. Extraction of S. rebaudiana*

Leaves of *S. rebaudiana* were obtained from Al-Harraz Co. for Agriculture Seeds, Herbs, and Medicinal Plants, a company located in Cairo, Egypt. To ensure the authenticity of the plant material, the collected leaves were examined and formally identified by specialists at the Faculty of Agriculture, Benha University, where taxonomic verification was done. The study received approval from the Ethical Committee and was conducted in compliance with laboratory animal ethical guidelines. After confirmation of the species, the leaves were subjected to drying under natural conditions of circulating air, avoiding excessive heat or direct sunlight, in order to preserve the stability and biological activity of the phytochemical constituents. Once completely dried, the leaves were ground into a fine and uniform powder via a mechanical pulverizer, making them suitable for the extraction process.

The extraction of bioactive compounds from the powdered material was done via 70% ethanol as the solvent. This procedure followed conventional methods that rely on solvent penetration and diffusion to efficiently release the phytoconstituents from the plant matrix. The ethanolic mixture was subsequently passed through successive stages of filtration, beginning with coarse filtration and followed by fine filtration via Whatman No. 54 filter paper, which ensured the removal of all particulate matter. The clarified extract obtained at the end of this process was collected, carefully preserved, and stored under appropriate conditions until it was required for subsequent pharmacological assessments [16].

### *2.4. Phytochemical Screening*

The phytochemical profile of the extract was systematically assessed to identify the presence of major secondary metabolites. Carbohydrates and glycosides were detected via the α-naphthol–sulfuric acid reagent [17]. Tannins were identified following the method described by Shellard and co-authors [18], while alkaloids were revealed through their reaction with Dragendorff's reagent [19]. whereas alkaloids were identified via reaction with Dragendorff's reagent [20, 21]. Saponins were confirmed by the formation of persistent froth lasting over 30 minutes [18]. Additionally, sterols and triterpenes were characterized according to established analytical protocols [22].

### *2.5. Antioxidant Activity of S. rebaudiana (In Vitro Research)*

The antioxidant potential of the alcoholic extract was rigorously assessed via DPPH and ABTS radical scavenging assays across a concentration range of 0.075–10 µg/mL. Ascorbic acid and Trolox were employed as benchmark standards to provide reference activity. The extent of radical neutralization was determined spectrophotometrically by monitoring the diminish in absorbance, and the corresponding percentage inhibition was calculated in accordance with established, yet appropriately adapted, protocols [23].

### *2.6. Profiling of secondary metabolites from S. rebaudiana via two methods HPLC and LC-MS*

The bioactive constituents of *S. rebaudiana* were characterized following organic solvent extraction to enable detailed identification and separation of secondary metabolites.

#### *2.6.1. HPLC Conditions*

Chromatographic separation done through an Agilent 1260 HPLC system fitted with a Zorbax Eclipse Plus C8 column (4.6 × 250 mm, 5 µm). The column temperature was kept at 40 °C. Samples (5 µL) were injected into a mobile phase composed of acetonitrile with 0.05% trifluoroacetic acid (solvent B) and water (solvent A). The flow rate was set at 0.9 mL/min. Gradient elution proceeded as follows: 0–1 min, 82% A; 1–11 min, linear reduction to 75% A; 11–18 min, further decrease to 60% A; 18–22 min, re-equilibration to 82% A; 22–24 min, maintained at 82% A. Detection of compounds was carried out at 280 nm using a multi-wavelength detector [24].

#### *2.6.2. LC-MS Analysis*

Secondary metabolite profiling of *S. rebaudiana* was performed via liquid chromatography–mass spectrometry (LC–MS) on an Esquire 3000 ion-trap system. The instrument was fitted with an electrospray ionization (ESI) source and operated in both negative and positive ionization modes to allow detection of a wide range of compound classes. Mass spectra were collected across an m/z window of 150–3000 at a scanning speed of 13,000 m/z/second. Ion transmission was optimized by setting Skimmer 1 voltage to −10 V, while the nebulizer was maintained at 30 psi. The drying gas was delivered at 9 L/min with a temperature of 310 °C [25, 26].

### *2.7. Experimental Design (In Vivo Study)*

A total of 32 healthy adult rats were randomly and blindly allocated into four experimental groups, each consisting of eight animals, to evaluate the pharmacological impacts of *S. rebaudiana* extract in a model of L-NAME–induced hypertension. The experimental groups were defined as follows:**(Normal control):** Rats in this group were maintained on a standard laboratory diet and provided with no pharmacological intervention over the 28-day study period. **(L-NAME):** Animals were administered L-NAME orally at a dose of 40 mg/kg/day for 28 days to induce hypertensive conditions [15]. **(L-NAME + High-Dose *S. rebaudiana*):** In addition to receiving L-NAME (40 mg/kg/day, orally), these rats were administered a high dose of *S. rebaudiana* extract (500 mg/kg/day, orally) for the same duration [27]. **(L-NAME + Low-Dose *S. rebaudiana*):** Rats in this group provided with the same L-NAME regimen (40 mg/kg/day, orally) combined with a lower dose of *S. rebaudiana* extract (250 mg/kg/day, orally) over 28 days [27].

#### *2.7.1. Monitoring of Systolic and diastolic blood pressure*

BP measurements were done via a BP-2010 AUL non-invasive monitoring system, specifically designed for rats [28]. Prior to measurement, the system's airtightness was carefully verified, and the incubator temperature was maintained at 37°C to ensure physiological stability. Each rat was gently placed into a restraining bag within the monitoring chamber and allowed to acclimate for 15 minutes. Tail-cuff sensors were then positioned at the base of the tail, and BP readings were recorded once a stable signal was achieved. Systolic and diastolic pressures were measured accurately, with the cuff volume adjusted proportionally at 1 mL/100 g of body weight to optimize measurement precision.

#### *2.7.2. Blood samples and tissue specimens*

Animals were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg)[29, 30]. Blood was collected via the retro-orbital plexus, and serum separated by centrifugation at 3000 rpm for 15 min at 4 °C, then stored at −20 °C for further assays.

Afterward, rats were euthanized according to ethical guidelines. Heart, aorta, and kidney tissues were excised and divided into two portions: one portion was rinsed, homogenized, and stored at −80 °C for ELISA and flow cytometry, while the other portion was fixed in 10% formalin for histology and immunohistochemistry

#### *2.7.3. Biochemical analysis*

##### *2.7.3.1. Determination of malondialdehyde (MDA)*

Lipid peroxidation was quantified via the thiobarbituric acid reactive substances (TBARS) assay, which measures malondialdehyde (MDA) as an index of oxidative stress. Briefly, 20 µL of serum was combined with 500 µL of 42 mM sulfuric acid, followed by 125 µL of phosphotungstic acid (PTA). The mixture was vortexed thoroughly and centrifuged at 13,000 × g for 5 minutes at room temperature to precipitate proteins. The resulting pellet was resuspended in 200 µL of distilled water containing butylated hydroxytoluene (BHT) to inhibit further lipid oxidation. Subsequently, 600 µL of TBA solution was added, and the reaction mixture was incubated at 95°C for 1 hour, then cooled on ice for 15 minutes. A 200 µL aliquot was transferred to a 96-well plate, and absorbance was measured at 532 nm via a microplate reader. MDA concentrations were calculated from a standard curve generated via known MDA standards [31, 32].

Antioxidant capacity was assessed by measuring reduced glutathione (GSH) via Ellman's reagent (5,5′-dithiobis-(2-nitrobenzoic acid), DTNB) assay. In this procedure, 100 µL of serum was deproteinized with 100 µL of 10% trichloroacetic acid (TCA) and centrifuged at 10,000 × g for 10 minutes to remove precipitated proteins. The supernatant was then mixed with 800 µL of 0.1 M phosphate buffer (pH 7.4), followed by the addition of 100 µL of 10 mM DTNB. After incubating for 10 minutes at room temperature, absorbance was recorded at 412 nm. GSH concentrations were determined via a standard curve prepared from known GSH standards [33], providing a quantitative measure of antioxidant status.

##### *2.7.3.2. Determination of Serum NO*

Serum NO titres were estimated via the Griess reaction, which indirectly quantifies NO by measuring its stable metabolite, nitrite. In this procedure, 100 µL of serum was deproteinized with an equal volume of 10% TCA and centrifuged at 10,000 × g

for 10 minutes to remove precipitated proteins. The resulting supernatant was incubated with nitrate reductase and NADH for 30 minutes at room temperature to enzymatically reduce nitrate to nitrite. Subsequently, 100 µL of sulfanilamide was added and incubated for 10 minutes, followed by 100 µL of N-(1-naphthyl) ethylenediamine dihydrochloride (NED) for an additional 10 minutes. Absorbance was measured at 540 nm, and NO concentrations were determined via a nitrite standard curve [33].

#### *2.7.3.3. Determination of Angiotensin-Converting Enzyme (ACE) Activity*

ACE activity was assessed via a colorimetric assay based on the enzymatic hydrolysis of hippuryl-histidyl-leucine (HHL). Upon cleavage by ACE, hippuric acid is liberated and subsequently extracted. The concentration of released hippuric acid was quantified spectrophotometrically at 228 nm, and ACE activity was calculated according to the method of Cushman and Cheung [34].

#### *2.7.3.4. Determination of IL-6 and TNF-α*

Proinflammatory cytokine, including IL-6 and TNF-α, were quantified via commercially available ELISA kits (CUSABIO, Cat. No: CSB-E04640r and CSB-E11987r, China) following the manufacturer's instructions. Absorbance readings were employed to calculate cytokine concentrations based on standard curves supplied with the kits [35, 36].

#### *2.7.3.5. Flow cytometry analysis of apoptosis markers*

Flow cytometry was utilized to assess apoptotic activity in tissue samples and cultured cells, employing the FITC Caspase-3 Apoptosis Detection Kit I (Catalog No. 560901) in combination with propidium iodide (PI) staining. This approach enabled simultaneous quantification of apoptotic and necrotic cells, providing a comprehensive evaluation of cell death pathways. Tissue samples were initially incubated with diluted enzymes in phosphate-buffered saline (PBS) to facilitate dissociation into single-cell suspensions. Following enzymatic treatment, samples were filtered and centrifuged to remove clumps and debris, then resuspended in staining buffer for viability assessment and accurate cell counting prior to flow cytometric analysis [37, 38].
In a complementary in vitro assay, apoptotic and non-apoptotic populations were distinguished in Jurkat cells (human T-cell leukemia line, ATCC TIB-152) based on active caspase-3 expression. Cells were either maintained under control conditions or treated with 4 µM camptothecin for 4h to induce apoptosis pharmacologically. Post-treatment, cells were washed with PBS, fixed, and permeabilized via the Cytofix/Cytoperm™ Kit (Catalog No. 554714) for 20 minutes at ambient temperature. Following centrifugation, cells were washed with Perm/Wash™ buffer and stained with a fluorescein isothiocyanate (FITC)-conjugated rabbit monoclonal antibody specific to active caspase-3 (clone C92-605). Samples were then washed again, resuspended in Perm/Wash™ buffer, and subjected to flow cytometric analysis. Data exhibited minimal caspase-3 activity in untreated control cells (M1), whereas over one-third of camptothecin-treated cells exhibited strong positivity for active caspase-3 (M2), confirming effective apoptosis induction.

For the assessment of anti-apoptotic signaling, Bcl-2 protein expression was assessed via a monoclonal antibody (Bcl-2-100, Catalog No. 13-8800) at a concentration of 0.5 mg/mL. This antibody was raised against a synthetic peptide corresponding to amino acid residues 41–54 of the human Bcl-2 protein, allowing for precise detection of anti-apoptotic protein titres. Together, these flow cytometry analyses provided quantitative insights into the balance of pro- and anti-apoptotic markers in both tissue and cellular models, enabling a comprehensive understanding of the regulatory mechanisms of cell survival and death.

### *2.8. Histopathological and immunohistochemical research:*

Heart, aorta, and kidney tissue samples were fixed in 10 % buffered neutral formalin for at least 72h before being prepared for paraffin blocks for histological evaluation. Following that, a sequence of histological techniques was done on the tissues, considering clearing, dehydration, infiltration, and embedding inside blocks of paraffin. Histochemical staining with eosin and hematoxylin (H&E) was done on slices of the tissues that were 5 µm thick [39].

Tissue sections embedded in paraffin were first dewaxed and rehydrated through a descending ethanol series. Endogenous peroxidase activity was quenched by incubating the sections in 3% hydrogen peroxide for 30 minutes. Antigen retrieval was performed using microwave heating for 10 minutes. To prevent non-specific binding, the sections were rinsed in PBS and treated with 5% bovine serum at room temperature. Sections were then incubated overnight at 4 °C in a humidified chamber with the primary antibody (anti- iNOS (diluted 1:200, Abcam, Cambridge, MA, USA). After three washes with PBS (5 minutes each), hematoxylin was applied for counterstaining. Immunoreactivity was visualized with 0.06% 3,3′-diaminobenzidine (DAB). The slides were subsequently dehydrated through graded ethanol solutions (70%, 90%, 100%; 2 minutes each) [40] s, cleared in xylene, and examined under an Olympus BX50 light microscope. Images were captured using an Olympus digital camera [41].

### *2.9. Statistical analysis*

Statistical analysis was performed using a Two-way ANOVA with SPSS version 27 (IBM Corp., 2013). Data were treated as a complete randomization design according to **Steel et al. [42]**. Multiple comparisons were conducted using the Duncan's multiple range test. A significance level of $p < 0.05$ was considered statistically significant.

## 3. Results and Discussion

### *3.1. Phytochemical screening*

Preliminary phytochemical evaluation of the plant extracts, summarized in **Table 1**, indicated the presence of several bioactive compounds, including carbohydrates, tannins, flavonoids, triterpenoids or steroids, alkaloids, and saponins. Volatile oils were not detected in any of the eight tested samples. Specifically, *S. rebaudiana* extracts were found to contain triterpenes, alkaloids, and tannins, corroborating findings from previous phytochemical analyses of this species.

**Table 1: Phytochemical screening of *stevia rebaudiana***

| Groups | *Stevia rebaudiana* |
|---|---|
| Volatile Oils | - |
| Carbohydrate | +++ |
| Tannins | +++ |
| Flavonoids, NaOH | +++ |
| Flavonoids (Shinoda test) | +++ |
| Saponin | +++ |
| Sterol and / or triterpenes | +++ |
| Coumarins | +++ |
| Alkaloids | ++ |

**++: High amount; +: Low amount; -: Absent**

### *3.2. Antioxidant Features*

*S. rebaudiana* extracts antioxidant activity was assessed via DPPH and $ABTS^{+}$ assays, alongside standard antioxidants (vitamin C and *Trolox*) at varying concentrations. At higher concentrations, all tested samples achieved 100% free radical scavenging activity, demonstrating strong antioxidant potential. However, at lower concentrations, a gradual decline in activity was observed. Both assays confirmed the antioxidant features of *S. rebaudiana*, though its activity was slightly lower than that of the standard antioxidants at specific concentrations. The IC50 values derived from these assays suggest that *S. rebaudiana* possesses a moderate antioxidant capacity, with efficacy comparable to Trolox but lower than vitamin C. (**Figure 1**).

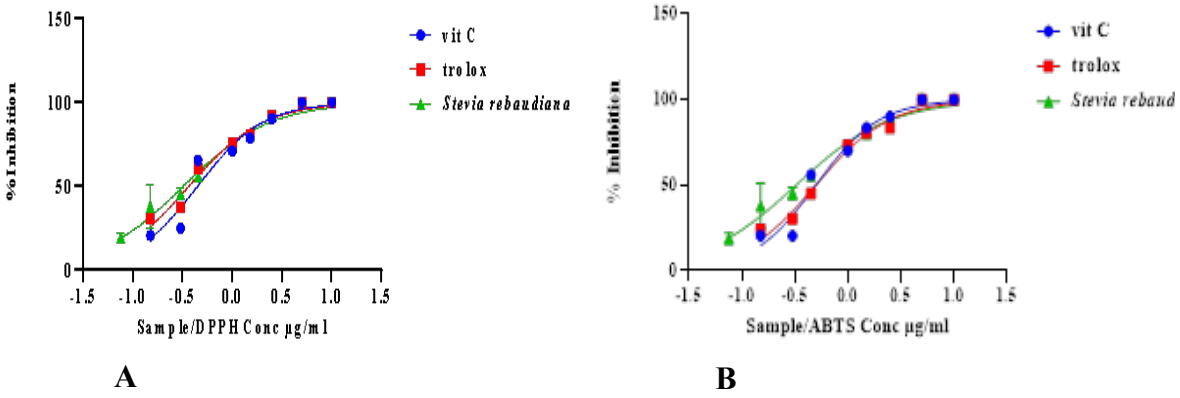


**Figure 1: In vitro DPPH and ABTS radical scavenging assays of *Stevia rebaudiana***
**The data are presented as the means ± SEM. The antioxidant activity of *S. rebaudiana* was evaluated via (A): DPPH and (B): ABTS radical scavenging assays. The radical scavenging activity was expressed in terms of the IC50 value.**

### *3.3. HPLC results*

- **Quantification of flavonoid and phenolic compounds in *S. rebaudiana* extract**

The analysis of phenolic and flavonoid compounds in *S. rebaudiana* extract revealed a complex profile of bioactive constituents, with their concentrations (µg/g) detailed in **(Figure 2)**.

Phenolic Acids: The extract exhibited a high concentration of chlorogenic acid (38,007.64 µg/g) and rosmarinic acid (35,410.81 µg/g), which are likely the primary contributors to its antioxidant potential. Other notable phenolic acids include ferulic acid (9,750.30 µg/g), gallic acid (3,656.10 µg/g), coumaric acid (656.73 µg/g), caffeic acid (372.72 µg/g), and syringic acid (79.33 µg/g), all of which contribute to the extract's bioactivity.

Flavonoids: Among flavonoids, naringenin (4,779.40 µg/g) was the most abundant, followed by rutin (2,657.99 µg/g). Other flavonoids, including quercetin (31.08 µg/g), kaempferol (43.78 µg/g), and hesperetin (41.45 µg/g), were present in relatively lower amounts.

Other Bioactive Compounds: Additional compounds identified in the extract included ellagic acid (249.38 µg/g), vanillin (1,241.95 µg/g), methyl gallate (28.61 µg/g), and daidzein (60.94 µg/g). Cinnamic acid was detected in trace amounts (5.81 µg/g), while catechin was not detected.

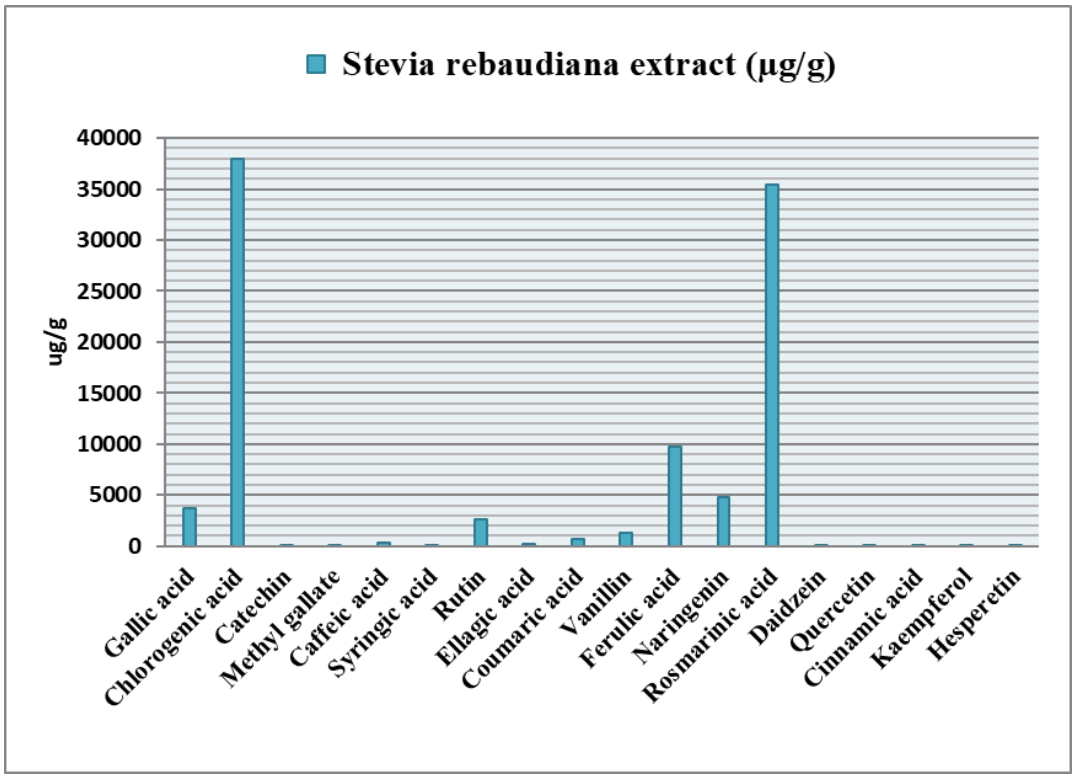


**Figure 2: Quantification of phenolic and flavonoid compounds in *Stevia rebaudiana* extract**

***3.4. LC-MS metabolite profiling of S. rebaudiana***

*S. rebaudiana* extract LC-MS analysis identified 33 metabolites, including phenolic acids, flavonoids, glycosides, and other bioactive compounds. Their retention times (RT), molecular weights, mass fragments, and molecular formulas are summarized in **Table 2**. The aforementioned metabolites contribute to the diverse pharmacological features of *S. rebaudiana*, highlighting its potential applications in nutraceuticals, pharmaceuticals, and functional foods.

Phenolic Acids: Several phenolic acids were detected, including vanillin (RT 1.24 min, m/z 151), coumaric acid (RT 3.17 min, m/z 163), cinnamic acid (RT 4.80 min, m/z 147), caffeic acid (RT 7.33 min, m/z 179), syringic acid (RT 13.41 min, m/z 197), ferulic acid (RT 18.89 min, m/z 193), and rosmarinic acid (RT 17.89 min, m/z 359). These compounds are well known for their antioxidant and anti-inflammatory features, which contribute significantly to the plant's therapeutic effects.

Flavonoids: The extract contained a variety of flavonoids, including hesperetin (RT 7.74 min, m/z 301), rutin (RT 8.35 min, m/z 609), quercetin (RT 19.50 min, m/z 301), naringenin (RT 10.33 min, m/z 271), and kaempferol (RT 18.51 min, m/z 285). The aforementioned metabolites exhibit antioxidant, cardioprotective, and anti-cancer activities, reinforcing *S. rebaudiana*'s potential as a functional food ingredient.

Glycosides and Diterpenoids: Several steviol glycosides, which contribute to the natural sweetness of *S. rebaudiana*, were identified. These included rebaudioside A (RT 9.86 min, m/z 965), rebaudioside C (RT 10.46 min, m/z 949), and stevioside (RT 12.27 min, m/z 803). These compounds have attracted interest as natural sugar substitutes, making *S. rebaudiana* an essential component in the food industry.

Other Bioactive Compounds: In addition to phenolic acids and flavonoids, other biologically active compounds were detected, like quinic acid (RT 2.42 min, m/z 191) and ellagic acid (RT 20.34 min, m/z 301). These compounds are recognized for their antioxidant and health-promoting features, further supporting the plant's use in health-related applications.

**Table 2: LC-MS-identified metabolites in stevia rebaudiana extract**

| No. | RT (min) | Metabolite Name | m/z [M-H]⁻ | Mass Fragments | Molecular Formula | Reference |
|---|---|---|---|---|---|---|
| **1** | 1.24 | Vanillin | 151 | 134 | $C_8H_8O_3$ | HPLC standard |
| **2** | 2.42 | Quinic acid | 191 | 108, 100 | $C_7H_{12}O_6$ | MS-Dial |
| **3** | 3.17 | Coumaric acid | 163 | 134, 101 | $C_9H_8O_3$ | HPLC standard |
| **4** | 4.80 | Cinnamic acid | 147 | 134 | $C_9H_8O_2$ | HPLC standard |
| **5** | 5.39 | 4-Caffeoylquinic acid | 353 | 179, 191, 173, 108 | $C_{16}H_{18}O_9$ | [46] |
| **6** | 5.61 | 5- acid | 353 | 179, 191, 173, 135 | $C_{16}H_{18}O_9$ | [46] |
| **7** | 7.33 | Caffeic acid | 179 | 135, 116 | $C_9H_8O_4$ | [46] |
| **8** | 7.74 | Hesperetin | 301 | 286, 257, 242, 164, 151 | $C_{16}H_{14}O_6$ | HPLC standard |
| **9** | 8.35 | Rutin | 609 | 447, 301, 151 | $C_{27}H_{30}O_{16}$ | Knapsack |
| **10** | 9.02Caffeoylquinic | Kaempferol monoglycoside | 417 | 343, 285, 209, 161 | $C_{20}H_{18}O_{10}$ | [46] |
| **11** | 9.86 | Rebaudioside A | 965 | 803, 641 | $C_{44}H_{70}O_{23}$ | [46] |
| **12** | 10.33 | Naringenin | 271 | 151, 119 | $C_{15}H_{12}O_5$ | HPLC standard |
| **13** | 12.27 | Stevioside | 803 | 641, 595, 359 | $C_{38}H_{60}O_{18}$ | [46] |
| **14** | 17.89 | Rosmarinic acid | 359 | 329, 314, 285 | $C_{18}H_{16}O_8$ | HPLC standard |
| **15** | 18.51 | Kaempferol | 285 | 151, 133 | $C_{15}H_{10}O_6$ | MS-Dial |
| **16** | 19.50 | Quercetin | 301 | 286, 179, 151, 135 | $C_{15}H_{10}O_7$ | HPLC standard |
| **17** | 20.34 | Ellagic acid | 301 | 284, 179, 151 | $C_{14}H_6O_8$ | HPLC standard |
| **18** | 28.94 | 1,5-Dicaffeoilquinic acid | 515 | 353, 191, 179, 173, 135 | $C_{25}H_{24}O_{12}$ | [46] |

**# Ref. mean identification compare to literature and MS-Dial and Knapsack database.**

### *3.5. Effect of oral administration of S. rebaudiana on the systolic and diastolic blood pressure against L-NAME-induced hypertensive rats*

The L-NAME caused a significant elevation ($p$ <0.05) in Both systolic and diastolic blood pressure titres by 150% and 140%, respectively, as opposed to the normal diet, The elevated systolic and diastolic blood pressure titres were notably diminished ($p$ <0.05) in the *S. rebaudiana* (250 mg/kg) groups by 91%, as opposed to rats in the L-NAME group. Moreover, treatment with *S. rebaudiana* (500 mg/kg) notably diminished ($p$ <0.05) by 74% and 81%, respectively, indicating a dose-dependent antihypertensive effect (p<0.05) **(Table 3)**.

### *3.6. Modulation of Serum NO by Oral S. rebaudiana in Rats with L-NAME–Induced Hypertension*

L-NAME-induced hypertensive rats *exhibit*ed markedly suppressed serum NO titres by 59% as opposed to normal diet animals (p<0.05), consistent with L-NAME's mechanism of inhibiting endothelial NOS (eNOS). However, Treatment with *S. rebaudiana* at both 500 mg/kg and 250 mg/kg *exhibit*ed a significant elevation (p <0.05) by 137% and 126% in NO titres, respectively, as opposed to rats in the L-NAME group, suggesting partial restoration of NO bioavailability **(Table 3).**

### *3.7. Modulation of ACE by Oral S. rebaudiana in Rats with L-NAME–Induced Hypertension*

Serum ACE activity was significantly elevated in the L-NAME group by 538% when as opposed to normotensive controls (p<0.05), reflecting activation of the renin-angiotensin system (RAS). Treatment with *S. rebaudiana* at 500 mg/kg and 250 mg/kg led to a significant reduction in ACE activity titres by 13.8% and 11.5% versus the L-NAME group (p<0.05), indicating suppression of RAS overactivity **(Table 3).**

### *3.8. Modulation of Glutathione by Oral S. rebaudiana in Rats Exposed to L-NAME–Induced HTN*

A notable depletion of hepatic reduced GSH content was observed in the L-NAME group by 57.8% as opposed to the control group (p<0.05), indicative of oxidative stress. Conversely, administration of *S. rebaudiana* at both 500 mg/kg and 250 mg/kg resulted in a significant elevation in GSH titres by 145% and 116% relative to L-NAME-treated rats (p<0.05), suggesting enhanced antioxidant defense **(Table 3)**.

### *3.9. Modulation of MDA* by *Oral S. rebaudiana in Rats Exposed to L-NAME–Induced HTN*

Administration of L-NAME significantly elevated serum MDA titres, a key marker of lipid peroxidation and oxidative stress, in hypertensive rats by 164% as opposed to controls ($p<0.05$). Furthermore, the serum level of MDA depleted significantly by 67% and 75%, respectively, in rats fed *S. rebaudiana* at both 500 mg/kg and 250 mg/kg as opposed to the L-NAME group, indicating attenuation of oxidative stress induced by L-NAME **(Table 3)**.

**Table 3: Effects of oral administration of stevia rebaudiana extract on systolic, diastolic blood pressure, NOx, ACE, GSH and MDA values in hypertensive rats received L-NAME**

| | Systolic Blood Pressure (mmHg) | Diastolic Blood Pressure (mmHg) | NOx (µmol/l) | ACE activity (ng/mL) | GSH (µmol/l) | MDA (nmol/l) |
|---|---|---|---|---|---|---|
| **Control** | 112.5 ± 1.764 | 80.52 ± 1.202 | 76.67 ± 0.8819 | 1.80 ± 0.05774 | 7.60 ± 0.1155 | 82.67 ± 1.453 |
| **L-NAME (40 mg/kg)** | 169 .58 ± 3.180* | 113.0 ± 2.603* | 45.33 ± 0.8819* | 9.70 ± 0.1155* | 4.40 ± 0.1155* | 136 ± 1.155* |
| **L-NAME (40 mg/kg) + *Stevia Rebaundia* (500mg/kg)** | 125.7 ±1.764*@ | 91.33 ± 0.8819*@ | 62.00± 1.155*@ | 1.34 ± 0.02082*@ | 6.40 ± 0.1155@ | 91.33 ± 0.8819*@ |
| **L- NAME (40 mg/kg) + *Stevia Rebaundia* (250mg/kg)** | 154.3 ±0.8819*@ | 103.0 ± 1.155*@ | 57.00 ±1.155 *@ | 1.118 ± 0.009701*@ | 5.118 ±0.009701*@ | 102.0 ± 1.155*@ |

Hypertension was induced by daily intake of L-NAME (40 mg/kg) for 28 days. Rats were received two doses from *S. Rebaundia* (high dose; 500 mg /kg and low dose; 250 mg /kg) concurrent with L-NAME for 28 days.. After the last doses of the drugs, systolic and diastolic blood pressure was monitored. Furthermore, the levels of NOx, ACE activity, GSH and MDA were measured in the serum samples from different experimental groups. Results are expressed as means ± SEM (n=8). * Significant difference from normal group ($p<0.05$). @ Significant difference from L-NAME group ($p<0.05$).

### *3.10. Modulation of TNF-α and Il-6 by Oral S. rebaudiana in Rats Exposed to L-NAME–Induced HTN*

Administration of L-NAME (40 mg/kg) resulted in a significant elevation ($p<0.05$) in the level of TNF-α and il-6 in the heart, aorta, and kidney by (1261%,968%,1058%), (1897%, 1379%,1428 %), respectively, when contrasted with those in normal rats. Additionally, when contrasted with L-NAME, *S. rebaudiana* (250mg/kg) significantly ($p<0.05$) reduced the titres of TNF-α and IL-6 in the heart, aorta, and kidney by (69%,60%,39%), 60%, 55%,56 %),respectively, Furthermore treatment with *S. rebaudiana* (500mg/kg) achieved a marked significantly ($p<0.05$) reduction in the level of TNF-α and IL-6 in the heart, aorta, and kidney by (25%,18%,25%), (22%, 13%, 25%), respectively, in a dose-dependent manner when contrasted with L-NAME group. **(Table 4)**.

**Table 4: Effects of oral administration of stevia rebaudiana on TNF-α and iL-6 in hypertensive rats: cardiac and renal tissues**

| Organ | Control | L-NAME (40 mg/kg) | L- NAME (40 mg/kg) + *Stevia Rebaundia* (500mg/kg | L-NAME (40 mg/kg) + *Stevia Rebaundia* (250mg/kg)) |
|---|---|---|---|---|
| | | | **TNF-α (Pg /mg)** | |
| **Heart** | 87.99± 1.453 | 1109.12±1.698* | 276.54±1.890*@ | 769.09±2.055*@ |
| **Aorta** | 102.25±1.181 | 989.98±2.639* | 176.09±1.518*@ | 598.11±2.341*@ |
| **Kidney** | 41.11±1.732 | 434.43±1.738* | 109.11±1.192*@ | 169.98±2.956*@ |
| | | | **iL-6 (Pg /mg)** | |
| **Heart** | 68.98±1.447 | 1328±10.59* | 301.11±2.025*@ | 804.48±1.739*@ |
| **Aorta** | 80.88±2.902 | 1115.2±2.609* | 148.98±2.304*@ | 611.24±2.980*@ |
| **Kidney** | 27.87±1.723 | 398.09±2.357* | 98.98±2.598*@ | 222.24±1.086*@ |

Rats were induced Hypertensive by daily intake of L-NAME (40 mg/kg) for 28 days. Rats were recived two doses *S. Rebaundia* (high dose ; 500 mg /kg and low dose ; 250 mg /kg) concurrently with L-NAME for 28 days. After the last doses of the drugs, TNF-α and il-6 valus were evaluated in the heart, aorta and kidneys of different experimental groups. The data are presented as the means ± SEM (8 rats) * significant difference from normal group $p<0.05$. @ Significant difference from L-NAME group $p<0.05$.

***3.11. Modulation of Caspase-3 Activity in the Heart and Kidneys of Hypertensive Rats Following S. rebaudiana Treatment***

Apoptosis Analysis **(Table 5)**: Caspase-3, Flow Cytometry). L-NAME treatment led to a marked increase in apoptotic cell populations in cardiac, aortic, and renal tissues ($p<0.05$), by 546%, 569% and 329%, respectively, when contrasted with the group fed a normal diet indicative of significant cellular damage, meanwhile treatment with *S. rebaudiana* (250mg/kg mg / kg ) led to a significant reduction by 62%, 74% and 79%, respectively, ($p<0.05$) in in cardiac, aortic, and renal tissues Caspase-3 expression when contrasted with that in the L-NAME group. Furthermore *S. rebaudiana* (500mg/kg) led to marked significant reduction by 46.6%, 39% and 65%, ($p<0.05$) in cardiac, aortic, and renal tissues Caspase-3 expression when contrasted with that in the L-NAME group.

**Table 5: Effect of oral administration of stevia rebaundia on caspase-3 : cardiac and renal levels in hypertensive rats**

| Group | Organ | | |
|---|---|---|---|
| | **Aorta (%)** | **Heart (%)** | Kidney (%) |
| **Normal** | 12.40±0.54 | 14.27±0.72 | 14.60±1.20 |
| **L-NAME (40 mg/kg)** | 70.65±1.35* | 77.95±1.63* | 48.17±1.17* |
| **L- NAME (40 mg/kg) + *stevia rebaundia* (500mg/kg)** | 27.55±1.05*@ | 36.40±1.11*@ | 31.60±0.57*@ |
| **L- NAME (40 mg/kg) + *stevia rebaundia* (250mg/kg** | 52.28±1.54*@ | 48.5±1.04*@ | 38.12±0.62*@ |

Hypertension was induced by daily intake of L-NAME (40 mg/kg) for 28 days. Rats were received two doses from *S. Rebaundia* (high dose 500 mg/kg , low dose; 250 mg/kg) concurrently with L-NAME for 28 days. After the last doses of the drugs, Caspase-3 was valuated in the heart, aorta and kidneys from different experimental groups. Results are expressed as means ± SEM (n=8). ).* Significant difference from normal group( $p<0.05$). @ Significant Difference from L-Name group ($p<0.05$).

***3.12. Modulation of Bcl-2 Expression in the Heart and Kidneys of Hypertensive Rats Following S. rebaudiana Treatment***

Anti-Apoptotic Impacts (**Table 6**): BCL-2, Flow Cytometry**),** L-NAME treatment was associated with a diminish in cardiac, aortic, and renal tissues BCL-2 expression by 29%, 36% and 18%, respectively, an essential anti-apoptotic protein, indicating increased susceptibility to apoptosis ($P<0.05$). *S. rebaudiana* (250mg/kg) treatment restored BCL-2 titres by 194%, 135% and 346%, respectively, in cardiac, aortic, and renal tissues when contrasted with the L-NAME group, Furthermore *S. rebaudiana* (500mg/kg) providing stronger protection via restoring BCL-2 titres by 325%, 220% and 196%, ($p<0.05$), respectively, in cardiac, aortic, and renal tissues when contrasted with the L-NAME group.These observations suggest that *S. rebaudiana* enhances cell survival signaling, which may contribute to its overall protective effects.

**Table 6: Effect of oral administration of stevia rebaundia on BcL-2: cardiac and renal levels in hypertensive rats**

| Group | Organ | | |
|---|---|---|---|
| | **Aorta (%)** | **Heart (%)** | Kidney (%) |
| **Normal** | 94.27±0.44 | 80.35±1.24 | 86.83±1.02 |
| **L-NAME (40 mg/kg)** | 34.52±0.80* | 23.23±0.63* | 15.47±0.34* |
| **L- NAME (40 mg/kg) + *stevia rebaundia* (500mg/kg)** | 75.18±1.00*@ | 75.58±1.22*@ | 30.33±0.21*@ |
| **L- NAME (40 mg/kg) + *stevia rebaundia* (250mg/kg** | 46.68±0.88*@ | 45.15±0.80*@ | 53.53±0.49*@ |

Hypertension was induced by daily intake of L-NAME (40 mg/kg) for 28 days. Rats were received two doses from *S. Rebaundia* (high dose 500 mg/kg , low dose; 250 mg/kg) concurrently with L-NAME for 28 days. After the last doses of the drugs, BcL-2 was valuated in the heart, aorta and kidneys from different experimental groups. Results are expressed as means ± SEM (n=8). ).* Significant difference from normal group ($p<0.05$). @ Significant Difference from L-Name group ($p<0.05$)

***3.13. Histopathological results***

***3.13.1. -Histopathology of the heart***

The control group *exhibit*ed intact myocardial fibers with normal structure. Moreover, the cardiac tissue of L-NAME-exposed rats exhibited loss of cellular morphology, inflammatory cell clusters, and widening of myofibrils. The L-NAME and *S. rebaudiana* at 500 mg/kg group maintained normal myocardial structure, while the L-NAME and *S. rebaudiana* at 250 mg/kg group displayed slight interstitial space with minimal inflammatory cells. **(Figure 3).**

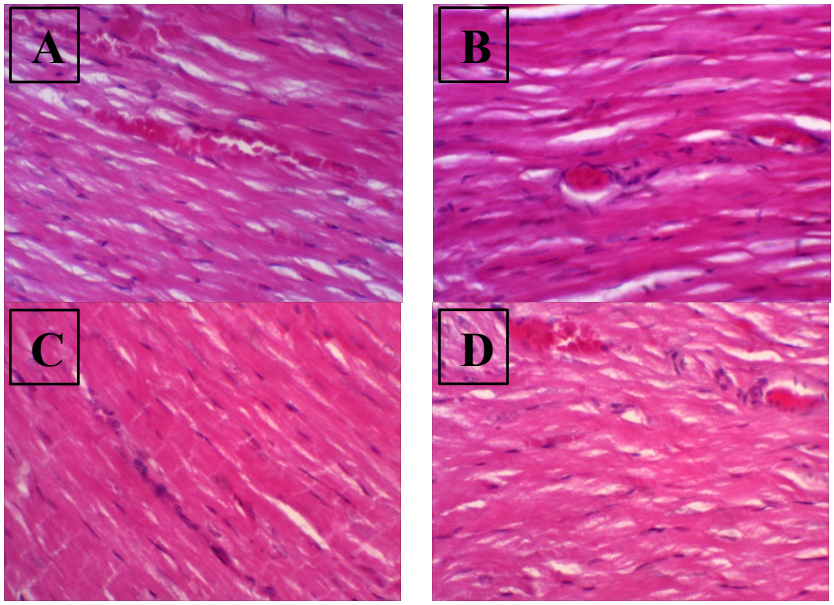


**Figure 3 : Photomicrograph of paraffin-embedded heart sections from control and experimental groups (H&E stain). (A) Control group showing intact and normally stained myocardial fibers (M). (B) Positive control group displaying loss of normal cellular morphology, widening of myofibrils, and small clusters of inflammatory cells (black arrow). (C) High-dose Stevia-treated group showing preserved cardiac fiber structure, size, and configuration similar to control. (D) Low-dose Stevia-treated group exhibiting mild interstitial space with a few inflammatory cells (black arrow).**

### *3.13.2. Histopathology of Aorta*

In the normal control group, normal aortic structure was observed with intact layers. Meanwhile, the Aorta of L-NAME-exposed rats *exhibit*ed thickening of the tunica media and smooth muscle cell proliferation. The L-NAME and *S. rebaudiana* at 500 mg/kg group maintained normal structure. The L-NAME and *S. rebaudiana* at 250 mg/kg group group *exhibit*ed slight thickening of the tunica media with preserved adventitia (**Figure 4).**

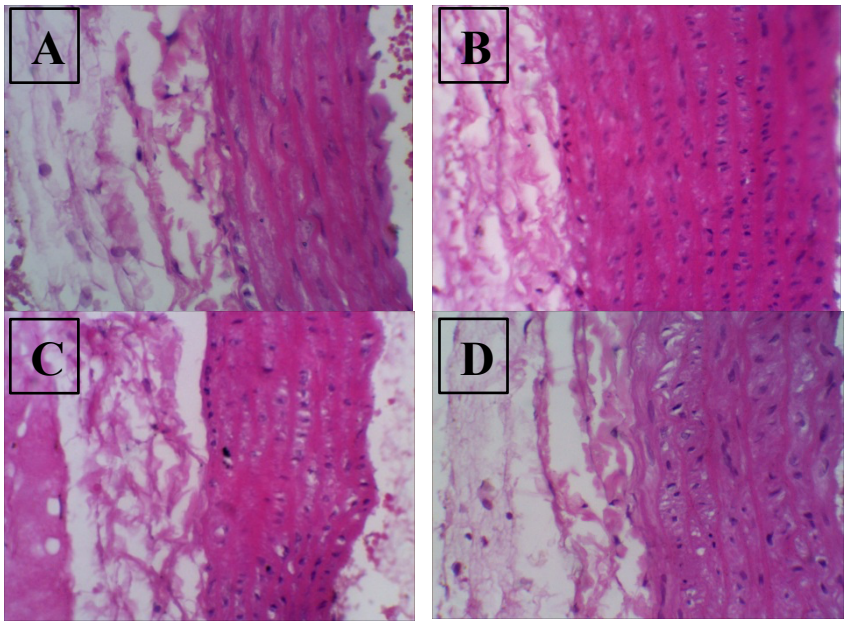


**Figure 4 : Photomicrograph of paraffin-embedded aortic sections from control and experimental groups (H&E stain). (A) Control group showing normal histological architecture with intact tunica adventitia (TA), fibroblasts (F), tunica intima (TI), and tunica media (TM) containing normal smooth muscle cells (Sc). (B) Positive control group exhibiting thickening of the tunica media (TM), increased smooth muscle cell proliferation (S), and alteration of the tunica intima (TI). (C) High-dose Stevia-treated group showing preserved aortic structure with no changes in layer thickness, resembling the control group. (D) Low-dose Stevia-treated group showing slight thickening of the tunica media (TM) with intact tunica adventitia (TA).**

#### *3.13.3. Histopathology of Kidney*

The normal control group displayed normal renal structures. Meanwhile, Kidney of L- NAME exposed rats Note marked glomerular hypertrophy, collapse, inflammatory infiltrates, and tubular fibrosis. The L-NAME and *S. rebaudiana* at 500 mg/kg group *exhibit*ed minor changes with slight collapse of proximal tubules, while the L-NAME and *S. rebaudiana* at 250 mg/kg group *exhibit*ed improved renal architecture and differentiation of glomerular shapes **(Figure 5)**

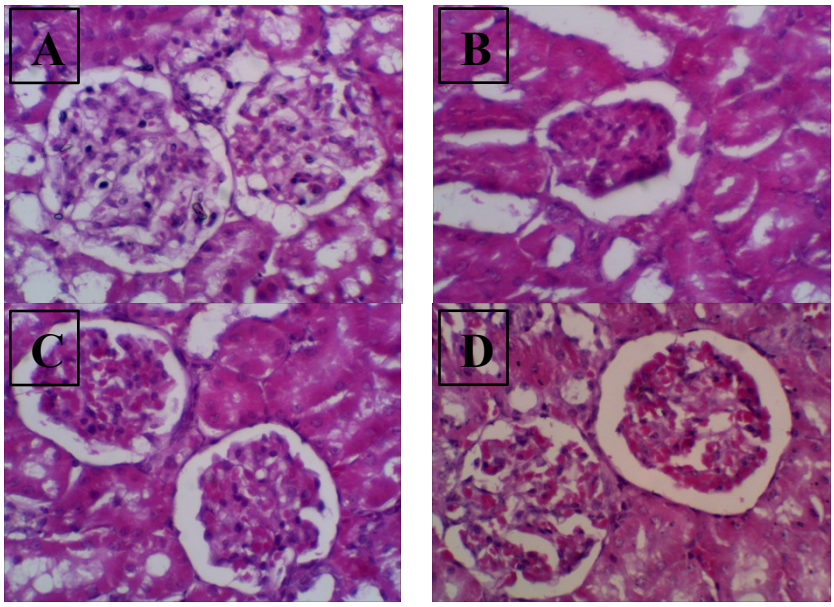


**Figure 5 : Photomicrograph of paraffin-embedded kidney sections from control and experimental groups (H&E stain). (A) Control group showing normal renal architecture with well-defined glomerulus (G), glomerular capsule (c), capsular space (S), vascular pole (P), and intact proximal (PCT) and distal convoluted tubules (DCT). (B) Positive control group exhibiting marked glomerular hypertrophy and collapse (G*), prominent inflammatory infiltration (white arrow), tubulointerstitial fibrosis (#), and infiltration of blood cells (black arrow). (C) High-dose Stevia-treated group displaying renal tissue similar to the control with only minimal PCT collapse. (D) Low-dose Stevia-treated group showing improved renal architecture with clear differentiation between glomerular structures.**

### *3.14. Immunohistochemical results*

#### *3.14.1. i-Nos immunoreactivity in the heart*

The normal control group exhibited no i-NOS immunoreactivity. Moreover, the cardiac tissue of L-NAME-exposed rats *exhibit*ed strong i-NOS expression in cardiomyocytes. The L-NAME and *S. rebaudiana* at 500 mg/kg group *exhibit*ed minimal I-NOS staining, similar to the control, while the L-NAME and *S. rebaudiana* at 250 mg/kg group *exhibit*ed the presence of fine iNOS-positive stained cells. (**Figure 6).**

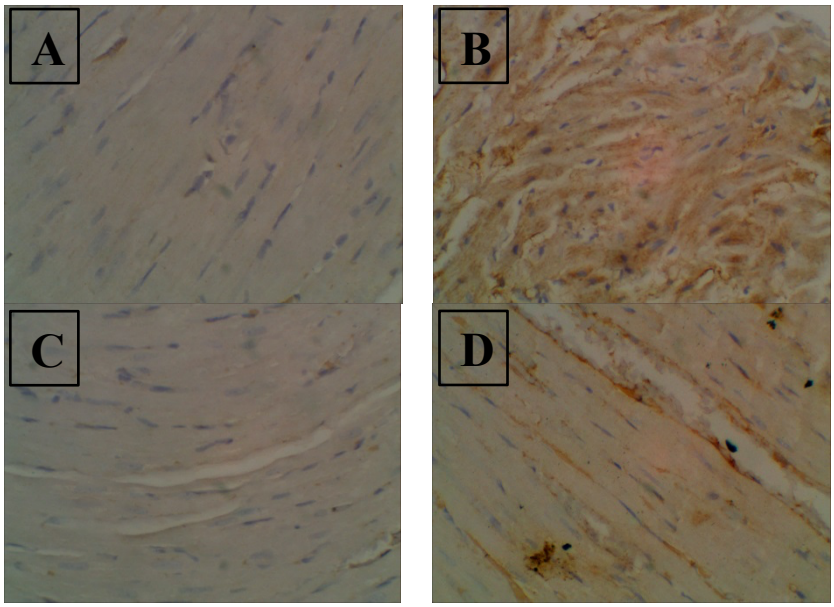


**Figure 6 : Representative photomicrograph of iNOS immunoreactivity in cardiac tissue of control and experimental groups. (A) Control group cardiomyocytes showing negative immunoreactivity for iNOS. (B) Positive control group displaying strong positive iNOS immunoreactivity (brown staining, arrow), indicating inflammation. (C) High-dose Stevia-treated group showing minimal iNOS-immunolabeled cells, resembling the control group. (D) Low-dose Stevia-treated group showing the presence of fine iNOS-positive stained cells.**

***3.14.2. i-Nos immunoreactivity in the aorta***

In the normal control group, no i-NOS expression was detected. Meanwhile, the Aorta of L-NAME-exposed rats *exhibit*ed significant i-NOS staining in the tunica adventitia. The L-NAME and *S. rebaudiana* at 500 mg/kg group *exhibit*ed absence of iNOS-immunolabeled cells, similar to the control group, while the L-NAME and *S. rebaudiana* at 250 mg/kg group *exhibit*ed mild iNOS immunos[illegible] tissu[illegible]

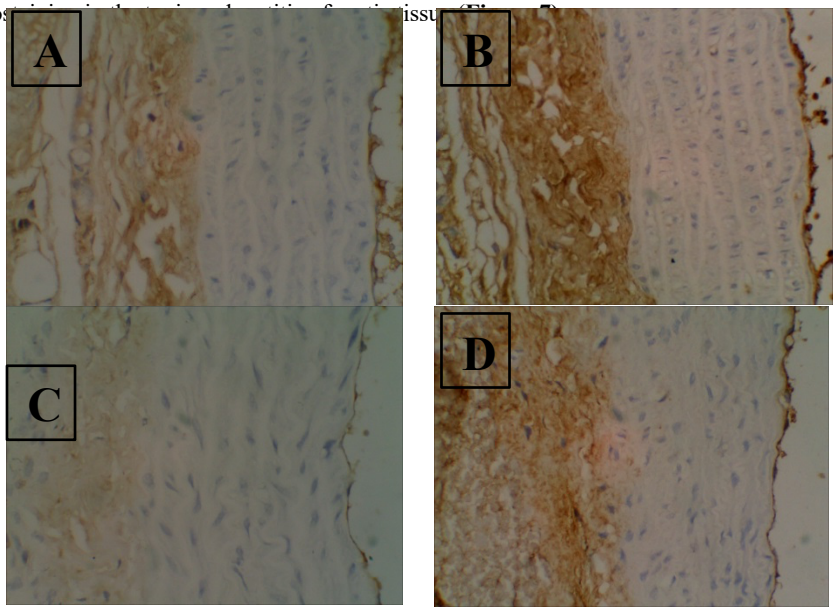


**Figure 7 : Representative photomicrograph of iNOS immunoreactivity in aortic tissue of control and experimental groups. (A) Control group showing no iNOS immunostaining in tunica adventitia cells. (B) Positive control group exhibiting a marked increase in iNOS expression (brown staining, arrow) in tunica adventitia, indicating inflammation. (C) High-dose Stevia-treated group showing absence of iNOS-immunolabeled cells, similar to the control group. (D) Low-dose Stevia-treated group showing mild iNOS immunostaining in the tunica adventitia of aortic tissue.**

***3.14.3. i-Nos immunoreactivity in the kidney***

The normal control group displayed no i-NOS immune staining in cortical tubular cells. Meanwhile, Kidney of L- NAME exposed rats noted marked intense i-NOS staining, indicating renal injury. The L-NAME and *S. rebaudiana* at 500 mg/kg group exhibited negligible iNOS-positive cells, reflecting tissue amelioration while the L-NAME and *S. rebaudiana* at 250 mg/kg group *exhibit*ed reduced i-NOS immune labeling in the cortex, indicative of improved renal tissue (**Figure 8).**

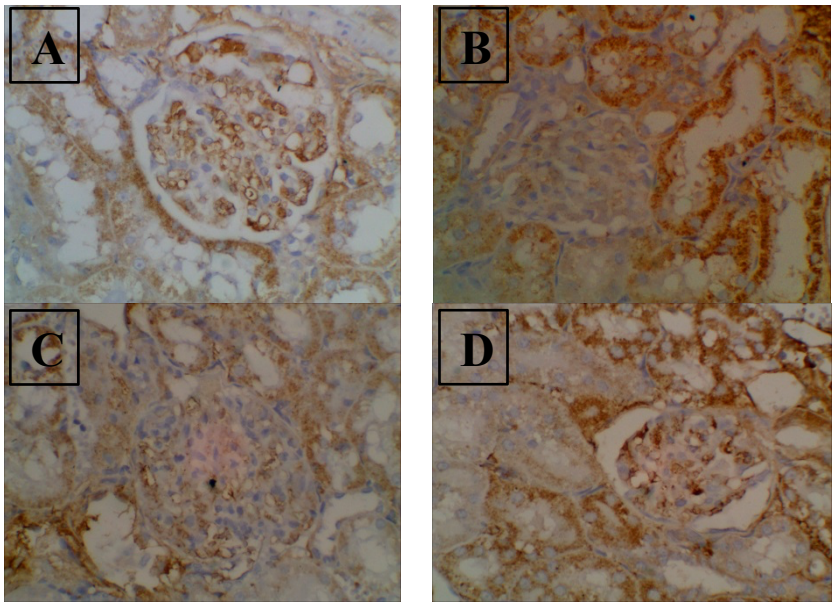


**Figure 8: Representative photomicrograph of iNOS immunoreactivity in renal tissue of control and experimental groups. (A) Control group showing no iNOS expression in cortical tubular cells. (B) Positive control group displaying strong iNOS expression (brown staining, arrow) in tubular cells surrounding the glomeruli, indicating renal injury. (C) High-dose Stevia-treated group showing negligible iNOS-positive cells, reflecting tissue amelioration. (D) Low-dose Stevia-treated group showing markedly reduced iNOS immunolabeling in the cortex, approaching levels seen in the control group.**

On a global scale, HTN stands as the predominant chronic non-communicable disease managed in medical practice, representing a significant public health challenge. It is widely recognized as a principal risk factor for life-threatening complications, including cerebrovascular accidents, myocardial infarction, chronic kidney disease, and premature mortality [43]. Despite advances in therapeutic strategies, the pathophysiology of hypertension involves complex interactions among vascular, renal, and neurohormonal systems, emphasizing the need for experimental models to elucidate underlying mechanisms and test potential interventions.

L-NAME has been extensively utilized as a pharmacological tool to investigate endothelial dysfunction and hypertension. L-NAME acts as a non-selective inhibitor of NOS, the enzyme responsible for converting L-arginine to NOs, a key mediator of vascular tone. Inhibition of NOS by L-NAME or related compounds like L-NMMA results in reduced NO bioavailability, leading to impaired endothelium-dependent vasodilation and heightened vascular resistance. Experimental experiments exhibited that blockade of NO synthesis in isolated vascular preparations promotes sustained vasoconstriction, while systemic administration in vivo elevates arterial blood pressure and reduces organ perfusion [44].

In Our Research, HTN was induced in adult rats via daily oral L-NAME at a dose of 40 mg/kg for 28 consecutive days. This regimen effectively suppressed NO production, resulting in marked increases in systolic and diastolic blood pressure, accompanied by heightened ACE activity. These alterations in vascular homeostasis align with prior experiments [45, 46], and establish a pathological milieu that predisposes cardiovascular, renal, and vascular tissues to injury.

Biochemical analysis of hypertensive animals revealed pronounced oxidative stress, as indicated by significantly elevated MDA level and diminished GSH content. The increased MDA reflects enhanced lipid peroxidation, while reduced GSH suggests a compromised antioxidant defense system. Such imbalances between ROS production and antioxidant capacity are widely reported as central mechanisms contributing to hypertension-induced tissue damage [47-50]. Concomitantly, hypertensive rats exhibited marked elevations in pro-inflammatory cytokines, including TNF-α and IL-6, in cardiac, aortic, and renal tissues. This pro-inflammatory milieu promotes endothelial dysfunction, vascular remodeling, and renal injury, and serves as a key driver of hypertension-associated complications, corroborating previous observations in experimental and clinical experiments [51-53].

The cardiovascular protective impacts of *S. rebaudiana* extract are largely attributable to its high content of bioactive plant compounds, including phenolic acids like chlorogenic and rosmarinic acids, as well as flavonoids like naringenin and rutin. These constituents are believed to act in concert to confer antioxidant, anti-inflammatory, and cytoprotective effects. In vitro evaluation of the extract's free radical scavenging capacity via DPPH and ABTS assays exhibited a clear dose-dependent antioxidant activity, confirming its strong radical-neutralizing potential.

Chemical profiling through HPLC and LC–MS revealed that the extract is particularly enriched with phenolic and flavonoid compounds, consistent with its potent biological activity. These phytochemicals have been extensively reported to mitigate oxidative stress, suppress inflammatory signaling, and inhibit apoptotic pathways [54-56]

In vivo, oral supplementation with *S. rebaudiana* extract at 250 mg/kg and 500 mg/kg significantly mitigated L-NAME–induced hypertension. The 500 mg/kg dose was especially potent, restoring systolic and diastolic pressures closer to baseline values. These outcomes align with earlier observations indicating that stevioside intake reduces blood pressure in both clinical and experimental models of mild HTN [57].

Beyond blood pressure regulation, treatment with *S. rebaudiana* restored serum NO titres and suppressed ACE activity. Concurrently, oxidative stress markers were ameliorated, with significant diminishs in MDA and increases in GSH, reflecting enhanced antioxidant defense. These observations are in agreement with prior experiments demonstrating that *S. rebaudiana* reduces lipid peroxidation, replenishes antioxidant reserves, and improves vascular redox status [53-56] who improved that *S. rebaudiana* enhance the antioxidant defense system by scavenging free radicals, thereby reducing lipid peroxidation and restoring GSH level. Moreover, they facilitate the recovery of endothelial function by increasing NO bioavailability and downregulating ACE activity, suggesting that the *S. rebaudiana* improves endothelial function and counteracts the deleterious activation of the renin–angiotensin system.

Beyond its capacity to mitigate oxidative stress, *S. rebaudiana* extract exerted pronounced anti-inflammatory features. The titres of TNF-α and IL-6 in the cardiac, aortic, and renal tissues were notably diminished in the Stevia-treated groups. This decrease in inflammatory mediators is likely attributable to the of NF-κB signaling suppression by the bioactive constituents of the extract, an effect observed in previous experiments on natural antioxidants [58], which advocates *S. rebaudiana* extract as a promising natural supplement for improving endothelial function, a key factor linked to adverse cardiovascular outcomes by reducing oxidative stress and inflammatory cytokines,

Furthermore, directly combating oxidative stress and inflammation, Our observations improved that *S. rebaudiana's* phytochemicals appear to exert anti-apoptotic impacts by modulating critical cell survival pathways. Flow cytometry analysis revealed that L-NAME-induced apoptosis evident by increased caspase-3 activity and diminished BCL-2 expression was markedly attenuated after treatment with *S. rebaudiana's* extract. The restoration of BCL-2 level, along with the reduction in caspase-3 activity, underscores the extract's ability to promote cell survival and preserve tissue integrity in the context of hypertensive injury. This coordinated regulation of apoptosis and inflammation highlights the therapeutic potential of *S. rebaudiana* for protecting against multifactorial damage in HTN- induced cardiorenal dysfunction. these observations was in agreement with the results from [53-56]

Histopathological evaluations provided supportive proof for the biochemical analyses and validated the present results. Through histological observation, cardiac sections from L-NAME-treated rats exhibited disorganized myofibrils with inflammatory infiltrates, while aortic tissues exhibited thickening of the tunica media and proliferation of smooth muscle cells. Renal tissues were characterized by glomerular hypertrophy, tubular fibrosis, and interstitial inflammation. In contrast, *S. rebaudiana* treatments at 500 and 250mg/kg dosage markedly improved tissue architecture demonstrating preservation of myocardial fibers, normalization of aortic wall structure, and restoration of renal morphology. Immunohistochemical analysis for iNOS corroborated these improvements by exhibiting a significant reduction in iNOS expression in tissues from the Stevia-treated groups as opposed to the untreated hypertensive controls [49, 52, 59, 60] were in accordance with our research.

## 4. Conclusions

The current research demonstrates that S. rebaudiana exerts significant antihypertensive, antioxidant, anti-inflammatory, and anti-apoptotic impacts in an L-NAME-induced hypertensive rat model. These benefits are likely mediated by the rich content of phenolic acids and flavonoids, which act synergistically to enhance NO availability while minimizing oxidative damage, and modulate inflammatory and apoptotic signaling cascades. Given the multifaceted protection observed with marked improvements in hemodynamic parameters, redox status, inflammatory profiles, tissue architecture, and cellular apoptosis *S. rebaudiana* exhibits promise as a natural therapeutic agent for the management of hypertension and related cardiorenal complications. Further investigations are advised to elucidate the precise molecular mechanisms and to assess the clinical applicability of *S. rebaudiana*- derived compounds.

**Conflicts of interest**

"There are no conflicts to declare".

**Formatting of funding sources**

Not applicable.

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Not applicable.

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