

Effects of β -blockers on hemorheological parameters in experimental arterial hypertension

Nana Kajaia^{1#}, Maia Mantskava^{1#}, Luiza Gabunia^{2#}, George Ormotsadze¹, Lela Chkitaui³, Elene Gogichaishvili⁴, Sophio Kalmakhelidze^{1*}, Tamar V Sanikidze¹

1. Department of Physics, Biophysics, Biomechanics and Informative Technologies, Tbilisi State Medical University, Georgia

2. Department of Clinical Pharmacology, Tbilisi State Medical University, Georgia

3. Medicine, European University, Tbilisi, Georgia

4. Ivane Beritashvili Centre of Experimental Biomedicine, Tbilisi, Georgia

ABSTRACT

Background: Arterial hypertension is a multifactorial disorder involving elevated blood pressure, microcirculatory and hemorheological disturbances, endothelial dysfunction, and oxidative stress, which contribute to increased peripheral resistance and disease progression. In this study, we aimed to evaluate the effects of β -blockers on hemorheological parameters, nitric oxide bioavailability, and oxidative stress in experimental arterial hypertension.

Methods: Experimental hypertension was induced in male Wistar rats using N^G-nitro-L-arginine methyl ester (L-NAME, 40 mg/kg/day). Animals were divided into control, hypertensive, and treatment groups receiving nebivolol, propranolol, metoprolol, or carvedilol. Blood pressure, hemorheological parameters, nitric oxide levels, and total antioxidant activity were evaluated.

Results: L-NAME administration significantly increased arterial pressure, erythrocyte aggregation, and plasma viscosity, accompanied by reduced NO levels and decreased antioxidant capacity ($p < 0.01$). β -blocker treatment significantly reduced arterial pressure in all groups. Under the present experimental conditions, nebivolol was associated with the greatest improvement in NOx levels, antioxidant activity, and erythrocyte aggregation. However, because equipotent β -blocker dosing was not established, direct comparisons of efficacy among agents should be interpreted cautiously. Carvedilol demonstrated moderate effects, while propranolol and metoprolol showed less improvement in parameters. β -blocker treatment was associated with simultaneous reductions in pulse arterial pressure and erythrocyte aggregation; however, no formal correlation analysis was performed.

Conclusions: Experimental hypertension is associated with disturbances in the microcirculation–hemorheology–oxidative stress axis. β -blockers partially reverse these alterations, with nebivolol being associated with more pronounced improvement under the conditions of the present study, likely related, at least in part, to its nitric oxide-mediated and antioxidant properties.

Keywords: arterial hypertension, erythrocyte aggregation, hemorheology, oxidative stress, β -blockers

Received: 5 May 2026; Accepted: 22 June 2026; Published: 28 June 2026.

INTRODUCTION

Arterial hypertension remains one of the most prevalent and clinically significant cardiovascular disorders worldwide and is a major contributor to global morbidity and mortality. It affects approximately 30–45% of the adult population, and its prevalence continues to rise due to population aging, sedentary lifestyles, and metabolic risk factors. [1].

Hypertension is a complex, multifactorial disease involving interactions between genetic, environmental, neurohumoral, and immune mechanisms. Importantly, growing evidence suggests that microcirculatory dys-

function plays a central role in both the development and progression of hypertension [2, 3]. Structural and functional alterations in small vessels - such as capillary rarefaction, impaired autoregulation, and increased flow heterogeneity- lead to elevated peripheral resistance and reduced tissue perfusion. These microvascular changes may precede the onset of clinically detectable hypertension, indicating that microcirculation is not only a target but also a contributing factor in disease pathogenesis.

A critical mechanism underlying microvascular dysfunction is oxidative stress, which has emerged as a key contributor to hypertension [4]. Excessive generation of

* Corresponding author: Sophio Kalmakhelidze, Department of Physics, Biophysics, Biomechanics and Informative Technologies, Tbilisi State Medical University, Georgia. E-mail: s.kalmakhelidze@tsmu.edu
These authors contributed equally to this work and share first authorship

reactive oxygen species (ROS) reduces nitric oxide (NO) bioavailability, promotes endothelial dysfunction, and enhances vascular tone and stiffness. In the microcirculation, oxidative stress disrupts normal blood flow by impairing endothelial signalling and altering the rheological behavior of blood cells, thereby contributing to increased vascular resistance and tissue hypoxia.

Blood rheology represents an essential determinant of microcirculatory flow and plays a significant role in blood pressure regulation. Hemorheological parameters—including plasma viscosity, hematocrit, erythrocyte deformability, and erythrocyte aggregation—directly influence blood fluidity, particularly in capillary networks where flow is highly sensitive to cellular mechanics [5, 6, 7]. Even minor disturbances in these parameters may markedly impair microcirculatory perfusion and oxygen delivery.

Hemorheological abnormalities are closely linked to oxidative stress-induced damage. Lipid peroxidation and protein modification of erythrocyte membranes reduce their deformability and increase aggregation, thereby elevating blood viscosity and impairing capillary flow [4, 8]. In parallel, oxidative stress decreases NO bioavailability, which weakens its anti-aggregatory and vasodilatory effects, further exacerbating microcirculatory dysfunction and promoting erythrocyte–endothelial interactions [9].

In addition to vascular dysfunction, chronic nitric oxide depletion may alter autonomic cardiovascular regulation. Although acute nitric oxide synthase inhibition is commonly associated with reflex bradycardia secondary to baroreceptor activation, prolonged NO deficiency has been shown to impair baroreflex sensitivity, increase sympathetic nervous system activity, and promote endothelial dysfunction and vascular remodelling [10–12]. These chronic neurohumoral adaptations may contribute to altered heart rate regulation and further aggravate microcirculatory dysfunction in chronic hypertension models.

β -adrenoceptor blockers, beyond their established antihypertensive effects, have been shown to modulate oxidative stress and hemorheological disturbances. Experimental and clinical studies indicate that certain β -blockers exert antioxidant effects, improve nitric oxide bioavailability [13, 14], and may influence erythrocyte membrane properties, which contribute to better microcirculatory flow and decreased blood viscosity [8, 15]. However, these effects are not uniform across all β -blockers and depend on their pharmacological profile, highlighting the need for comparative evaluation.

Thus, the interaction between oxidative stress and microcirculatory impairment constitutes a key pathogenic axis in hypertension. These mechanisms not only increase peripheral resistance but also play a crucial role in the development of target organ damage by limiting tissue perfusion and oxygenation.

Given the multifactorial nature of hypertension, effective treatment strategies should extend beyond blood pressure reduction and target underlying microvascular and rheological disturbances. β -adrenoceptor blockers remain an important class of antihypertensive agents. Notably, third-generation β -blockers such as nebivolol and carvedilol possess additional vasodilatory and antioxidant properties, including the ability to enhance NO bioavailability, which may improve endothelial function and microcirculation [13–15].

Therefore, the present study aimed to investigate the response of the rheological system to the treatment of experimental arterial hypertension with different β -blockers—nebivolol, propranolol, metoprolol, and carvedilol—with particular emphasis on their effects on microcirculation-related hemorheological parameters.

METHODS

Animals and experimental design

The study was conducted on male Wistar rats weighing 200–250 g. Animals were randomly divided into seven groups ($n = 10$ per group, including the control group). Male animals were used to avoid potential sex-related variability in blood pressure regulation and hemorheological parameters. Rats were obtained from a certified breeding facility and allowed to acclimatize to laboratory conditions for 7 days before the start of the experimental procedures.

Animals were housed in standard polypropylene cages (4–5 rats per cage) under controlled environmental conditions: temperature $22 \pm 2^\circ\text{C}$, relative humidity 50–60%, and a 12 h light/12 h dark cycle. Standard laboratory chow and tap water were provided ad libitum throughout the experiment.

All experimental procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals, complied with the principles of the 3Rs (Replacement, Reduction, Refinement), and were approved by the Ethics Committee of Tbilisi State Medical University (approval date: July 26, 2022).

Induction of experimental arterial hypertension

Experimental arterial hypertension was induced by chronic inhibition of nitric oxide synthase using N^G-nitro-L-arginine methyl ester (L-NAME, Sigma-Aldrich). L-NAME was administered intraperitoneally at a dose of 40 mg/kg/day, freshly dissolved in physiological saline.

The intraperitoneal administration ensured accurate dosing, controlled systemic exposure and minimised variability in intake associated with drinking water administration. While oral administration is more commonly used, the intraperitoneal route was selected to ensure

consistent intake and absorption. This method has been effectively utilized in experimental models of NO-deficient hypertension, resulting in a stable and reproducible elevation of blood pressure [8, 9].

Beta-blocker administration

β-blockers (nebivolol, propranolol, metoprolol, and carvedilol) were administered once daily by oral gavage at the same time each day. β-blockers (nebivolol, propranolol, metoprolol, and carvedilol) were administered once daily by oral gavage at the same time each day. To minimize potential stress-related confounding effects associated with repeated oral gavage, animals in the control and untreated hypertensive groups received sham gavage with an equivalent volume of vehicle under the same handling conditions and schedule as the treatment groups. All procedures were performed by experienced personnel under standardized experimental conditions.

In treatment groups, L-NAME administration was continued throughout the entire 8-week experimental period, while β-blockers were administered concomitantly during weeks 5–8. Drug doses were chosen based on published experimental studies that demonstrate anti-hypertensive efficacy without toxic effects [16, 17, 18]. The selected doses were not meant to establish equipotent antihypertensive effects among β-blockers; rather, they were based on previously reported experimental protocols and the pharmacological characteristics of each agent, including receptor selectivity, pharmacokinetics, and pleiotropic properties.

It is important to note that different doses were utilized for each β-blocker due to variations in pharmacokinetics and receptor selectivity. Therefore, direct quantitative comparison between drugs should be interpreted with caution [15, 18, 19]. A detailed description of each experimental group is provided in Table 1.

Animals were randomly allocated to experimental groups using a simple randomization procedure before the start of treatment. Investigators responsible for

hemorheological, biochemical, and blood pressure measurements were blinded to group allocation during data collection and analysis. Drug preparation and administration were performed by a separate investigator not involved in outcome assessment. Data analysis was conducted using coded samples, and group identities were disclosed only after completion of statistical analysis.

Blood pressure measurements

Systolic arterial pressure (SAP), diastolic arterial pressure (DAP), and pulse arterial pressure (PAP) were measured daily using a non-invasive tail-cuff plethysmography system (PhysioSuite, Kent Scientific). Pulse arterial pressure (PAP) was calculated as: $PAP = SAP - DAP$. Mean arterial pressure (MAP) was calculated as: $MAP = DAP + 1/3 (SAP - DAP)$. Before measurements, rats were habituated to the procedure to minimize stress-induced variability.

Hemorheological measurements

At the end of the 4th and 8th weeks, animals were anaesthetized using an approved protocol (ketamine 80 mg/kg and xylazine 10 mg/kg, intraperitoneally). Euthanasia was performed by exsanguination via abdominal aorta puncture under deep anaesthesia, followed by cervical dislocation to ensure death. Blood samples were collected immediately via cardiac puncture into anticoagulant-containing tubes.

Measurements were performed in a blinded manner with investigators blinded to treatment group allocation throughout outcome assessment, and each parameter was measured in triplicate, with the mean value used for analysis. The coefficient of variation was maintained <5%.

Erythrocyte aggregation index (EAI)

Aggregation was evaluated by determining the proportion of aggregated erythrocytes relative to non-aggregated cells and expressed as a percentage under standardized microscopic conditions, as previously described in hemorheological studies [3, 20].

Table 1. Experimental groups and treatment protocols

Group name (n=10 per group)	Treatment protocol	Parameters assessed
Control-intact (I)	Without treatment	
Hypertension rats (II)	Daily L-NAME for 4 weeks (40 mg/kg)	
Hypertension rats (III)	Daily L-NAME for 8 weeks (40 mg/kg)	
Treatment of hypertension in rats (IV)	Daily L-NAME for 8 weeks (40 mg/kg/day) + nebivolol (0.5 mg/kg/day) during weeks 5–8	RBC aggregation, RBC deformation, Plasma Viscosity
Treatment of hypertension in rats (V)	Daily L-NAME for 8 weeks (40 mg/kg/day) + propranolol (0.1 mg/kg/day) during weeks 5–8	
Treatment of hypertension in rats (VI)	Daily L-NAME for 8 weeks (40 mg/kg/day) + metoprolol (3 mg/kg/day) during weeks 5–8	
Treatment of hypertension in rats (VII)	Daily L-NAME for 8 weeks (40 mg/kg/day) + carvedilol (2 mg/kg/day) during weeks 5–8	

Erythrocyte deformation index (EDI)

Erythrocyte deformability was assessed using a filtration method, based on the ability of red blood cells to pass through microporous filters under standardized pressure conditions according to established experimental protocols [21].

Plasma viscosity

Plasma viscosity was measured using a rotational viscometer at different shear rates under standardised temperature conditions in accordance with standard hemorheological measurement techniques [20].

Hematocrit (Hct)

Hematocrit was determined by standard microcentrifugation and expressed as a percentage of packed RBCs.

Biochemical analysis

Determination of Nitric Oxide Metabolites (NOx) in Blood

Nitric oxide metabolites (NOx; nitrite and nitrate) in blood samples were determined using a modified Griess method [22]. This method indirectly assesses nitric oxide production by quantifying its stable metabolites, nitrite (NO_2^-) and nitrate (NO_3^-). The first step involved deproteinization of serum samples. To achieve this, 100 μL of blood serum was mixed with an equal volume of 0.3 M sodium hydroxide (NaOH) and incubated at room temperature for 5 minutes to ensure thorough mixing. Following this, 100 μL of a 5% zinc sulfate (ZnSO_4) solution was added to the mixture. After additional vigorous mixing, the solution was incubated at room temperature for another 5 minutes.

Next, the mixture was centrifuged at 3,000 rpm for 15 minutes at 4 °C to separate the components, and 100 μL of the clear supernatant was carefully collected. This aliquot was then combined with 200 μL of freshly prepared Griess reagent. The Griess reagent consisted of 0.25% vanadium chloride, 0.1% sulfanilamide, and 0.05% N-(1-naphthyl)-ethylenediamine (NED) (Sigma-Aldrich), all dissolved in 0.5 M hydrochloric acid (HCl). A reagent blank was prepared similarly, using 100 μL of distilled water in place of the blood serum sample.

The resulting mixture was incubated at 37 °C for 30 minutes, after which the absorbance was measured at 540 nm using a microplate reader (Multiskan GO, Thermo Fisher Scientific, Finland). A standard calibration curve was generated using sodium nitrite (NaNO_2) at concentrations ranging from 1 to 100 μM .

Oxidative stress intensity

The intensity of oxidative stress in blood samples was evaluated using an integrative indicator of AA, which primarily reflects the activity of low-molecular-weight non-enzymatic antioxidants found in serum. It is important to note that since serum samples were deproteinized before analysis, the values obtained mainly reflect the antioxidant capacity of these low-molecular-weight

non-enzymatic compounds rather than the complete antioxidant capacity of serum. Consequently, these values do not fully represent specific enzymatic antioxidant systems such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), which are more accurate indicators of oxidative stress [4]. Assessment of AA for low-molecular-weight non-enzymatic antioxidants is commonly used in experimental studies as a global marker of redox status and oxidative balance [20].

The total activity of low-molecular-weight non-enzymatic antioxidant compounds was determined using the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging assay [23]. Blood serum samples (1 mL) were deproteinized by adding 3 mL of acetonitrile and were then centrifuged at 9,500 g for 10 minutes at 4°C. The supernatant (1 mL) was collected and mixed with 3 mL of DPPH solution, and the absorbance was measured at 515 nm.

Statistical analysis

Data are presented as mean $\pm$ SEM (Standard Error of the Mean). Statistical analyses were performed using GraphPad Prism version 10.0 (GraphPad Software, San Diego, CA, USA).

Before inferential analysis, data distribution was evaluated using the Shapiro–Wilk normality test, while homogeneity of variance was assessed using Levene’s test. Because the sample size in each experimental group was relatively small ($n = 10$), the results of formal normality testing were interpreted cautiously. However, visual inspection of residual distribution and variance patterns demonstrated no major deviations from parametric assumptions. In addition, selected parameters were verified using non-parametric analysis (Kruskal–Wallis test followed by Dunn’s post hoc multiple-comparison test), which produced results consistent with the parametric analysis. Therefore, parametric statistical methods were considered appropriate for the primary analysis.

Comparisons among experimental groups for arterial pressure parameters (SAP, DAP, PAP, MAP, and heart rate), hemorheological indices (EAI, EDI, plasma viscosity, hematocrit), nitric oxide metabolite levels (NOx), and antioxidant activity (AA) were performed using one-way analysis of variance (ANOVA), followed by Tukey’s honestly significant difference (HSD) post hoc multiple-comparison test.

Adjustment for multiple comparisons was performed using the Tukey correction within each parameter separately rather than across all study variables, thereby controlling the family-wise type I error rate for intergroup comparisons within each dataset.

The sample size was estimated using power analysis based on the primary endpoint, erythrocyte aggregation index (EAI), using preliminary experimental data obtained in pilot observations. Power analysis assumed an effect size of Cohen’s $f = 0.55$, variance estimates cor-

responding to approximately 10–12% variability of the mean EAI value, a significance level of $\alpha = 0.05$, and statistical power of 80%. The calculated minimum sample size was 8 animals per group; therefore, 10 animals per group were included to compensate for potential biological variability and experimental loss.

Because the Results section reports threshold significance values ($p<0.05$ and $p<0.01$) rather than exact p-values, the previous statement indicating that exact p-values were calculated and reported was removed for consistency with the presented data.

Effect size estimates and confidence intervals were not included in the present analysis because the study was designed primarily as an exploratory experimental investigation rather than a quantitative efficacy comparison study. Statistical significance was accepted at $p<0.05$.

RESULTS

Changes in arterial pressure

Arterial pressure was measured in all experimental groups. Baseline SAP values were comparable across all groups before treatment (Table 2), indicating no initial differences between groups. In the control (intact) group (Group I), SAP, DAP, and PAP were 120 ± 2.5 mmHg, 80 ± 2.5 mmHg, and 40 ± 2.0 mmHg, respectively. In rats with L-NAME-induced arterial hypertension, a pronounced and statistically significant increase in arterial pressure was observed. After 4 weeks of L-NAME administration (Group II), SAP increased to 175 ± 2.5 mmHg and DAP to 110 ± 2.3 mmHg, resulting in an elevated PAP of 65 ± 2.0 mmHg ($p<0.01$ vs control).

Prolongation of L-NAME administration to 8 weeks (Group III) resulted in a further increase in arterial pressure, with SAP reaching 180 ± 3.0 mmHg and DAP 115 ± 2.8 mmHg, confirming progression and stability of the hypertensive model ($p<0.01$ vs control). MAP followed a similar pattern, increasing from 93 ± 2.0 mmHg in control animals to 132 ± 2.3 mmHg in Group II and 137 ± 2.6 mmHg in Group III ($p<0.01$).

Heart rate was also elevated in hypertensive animals (390–395 bpm) compared with controls (360 ± 15 bpm). Although sympathetic nervous system activity was not

directly measured in the present study, previous experimental studies have demonstrated that chronic L-NAME-induced hypertension is associated with impaired baroreflex regulation and increased sympathetic activation [10–12]. Therefore, the observed increase in heart rate may reflect compensatory autonomic and hemodynamic adaptations associated with sustained nitric oxide deficiency and hypertension.

Treatment with β -adrenoceptor blockers during the final 4 weeks resulted in significant reductions in arterial pressure compared with untreated hypertensive rats (Group III). Nebivolol (Group IV) produced near-complete normalisation of arterial pressure (SAP: 122 ± 1.5 mmHg; DAP: 80 ± 1.9 mmHg; MAP: 94 ± 1.7 mmHg; $p<0.01$ vs Group III), with values approaching those of the control group. Propranolol (Group V), metoprolol (Group VI), and carvedilol (Group VII) also significantly reduced SAP and DAP ($p<0.01$ vs Group III), although values remained higher than in the control group. Under the present experimental conditions, the antihypertensive effect was most pronounced for nebivolol, whereas carvedilol demonstrated comparatively weaker effects. All β -blockers reduced heart rate compared with hypertensive animals, consistent with their pharmacological effects (Table 2).

Hemorheological parameters, blood serum nitric oxide content, and antioxidant activity

Hematocrit (Hct) values did not differ significantly among experimental groups (Table 3), indicating that neither L-NAME-induced hypertension nor β -blocker treatment substantially affected red blood cell concentration. Therefore, the observed changes in blood rheology cannot be attributed to alterations in hematocrit. In contrast, significant changes were observed in plasma viscosity and erythrocyte aggregation.

Plasma viscosity increased significantly in hypertensive animals, from 1.30 ± 0.02 cP in the control group (Group I) to 1.39 ± 0.02 cP after 8 weeks (Group III) of L-NAME administration ($p<0.01$ vs control). This reflects altered rheological behaviour under experimental conditions; overall, viscosity remained elevated relative to control when disease progression is considered.

Treatment with β -blockers resulted in partial normalisation of plasma viscosity. Nebivolol (Group IV) produced the

Table 2. Arterial pressure parameters in experimental groups (mean $\pm$ standard error of the mean)

Parameter	Group I Control	Group II L-NAME 4 wk	Group III L-NAME 8 wk	Group IV Nebivolol	Group V Propranolol	Group VI Metoprolol	Group VII Carvedilol
Baseline SAP (mmHg)	120 ± 2.5	121 ± 2.0	119 ± 2.3	120 ± 2.2	121 ± 2.1	120 ± 2.4	119 ± 2.3
SAP (mmHg)	120 ± 2.5	$175 \pm 2.5^{**}$	$180 \pm 3.0^{**}$	$122 \pm 1.5^{##}$	$135 \pm 1.5^{##}$	$140 \pm 0.5^{##}$	$150 \pm 4.0^{##}$
DAP (mmHg)	80 ± 2.5	$110 \pm 2.3^{**}$	$115 \pm 2.8^{**}$	$80 \pm 1.9^{##}$	$85 \pm 1.5^{##}$	$87 \pm 1.3^{##}$	$95 \pm 3.0^{##}$
PAP (mmHg)	40 ± 2.0	$65 \pm 2.0^{**}$	65 ± 2.2	$42 \pm 1.8^{##}$	$50 \pm 2.0^{##}$	$53 \pm 1.5^{##}$	$55 \pm 2.5^{##}$
MAP (mmHg)	93 ± 2.0	$132 \pm 2.3^{**}$	$137 \pm 2.6^{**}$	$94 \pm 1.7^{##}$	$102 \pm 1.8^{##}$	$105 \pm 1.5^{##}$	$113 \pm 2.8^{##}$
Heart rate (bpm)	360 ± 15	390 ± 18	395 ± 20	$340 \pm 12^{##}$	$345 \pm 14^{##}$	$350 \pm 13^{##}$	$355 \pm 16^{##}$

Statistical comparisons were performed using one-way ANOVA with Tukey's honest significant difference post hoc multiple-comparison test. ****** $p<0.01$ vs control group (Group I); **##** $p<0.01$ vs hypertensive group (Group III).

most pronounced reduction, restoring viscosity values close to control levels ($p < 0.01$ vs Group III), whereas propranolol, metoprolol, and carvedilol showed moderate effects.

Erythrocyte aggregation index (EAI) increased from $42 \pm 3\%$ in the control group to $52 \pm 4\%$ (Group II) and $60 \pm 5\%$ (Group III). All β -blockers significantly reduced EAI compared with the hypertensive group ($p < 0.01$ vs Group III). Nebivolol restored aggregation values to near-control levels, while propranolol, metoprolol, and carvedilol showed progressively weaker effects.

Erythrocyte deformability index (EDI) showed a significant decrease in hypertensive animals, from 2.2 ± 0.01 in control to 2.1 ± 0.01 (Group II) and 2.05 ± 0.02 (Group III) ($p < 0.01$ vs control). Treatment with β -blockers did not result in statistically significant improvement in EDI compared with hypertensive animals, although a slight trend toward normalisation was observed. Although numerical improvement in EDI values was observed in β -blocker-treated groups, post hoc analysis demonstrated that these differences did not reach statistical significance after correction for multiple comparisons.

Chronic administration of L-NAME led to a marked reduction in circulating nitric oxide metabolites (NOx). After 4 weeks of treatment (Group II), blood NOx levels declined from $2.31 \pm 0.3 \mu\text{M}$ in control animals (Group I) to $1.62 \pm 0.3 \mu\text{M}$ ($p < 0.01$ vs control), corresponding to an approximately 30% decrease. Prolongation of L-NAME exposure to 8 weeks (Group III) resulted in a further decrease in NOx concentration to $1.30 \pm 0.3 \mu\text{M}$ ($p < 0.01$ vs control and Group II), indicating a sustained suppression of endogenous NO production (Table 3).

Treatment with β -adrenoceptor blockers during the final 4 weeks resulted in partial restoration of NOx levels. Nebivolol (Group IV) demonstrated the most pronounced effect, increasing NOx concentration to $2.21 \pm 0.3 \mu\text{M}$ ($p < 0.01$ vs Group III), approaching control values.

Carvedilol (Group VII) produced a moderate increase ($1.83 \pm 0.3 \mu\text{M}$; $p < 0.01$ vs Group III), whereas propranolol (Group V) and metoprolol (Group VI) showed only slight improvements ($1.63 \pm 0.3 \mu\text{M}$ and $1.65 \pm 0.3 \mu\text{M}$, respectively; $p < 0.05$ vs Group III).

Under the present experimental conditions, nebivolol was associated with greater improvement in NOx levels than propranolol and metoprolol; however, direct comparative interpretation should be made with caution because equipotent antihypertensive dosing was not established. These data demonstrate that prolonged NOS inhibition results in a sustained reduction of circulating NO, whereas β -blockers- particularly nebivolol- restore NO bioavailability.

L-NAME administration was also associated with a significant decline in systemic antioxidant capacity. After 4 weeks (Group II), low-molecular-weight compounds antioxidant activity decreased from $25.32 \pm 0.36\%$ in control animals to $18.5 \pm 0.32\%$ ($p < 0.01$ vs control), representing an approximately 27% reduction. Further continuation of L-NAME treatment to 8 weeks (Group III) led to an additional decrease in AA of low-molecular-weight compounds to $16.2 \pm 0.35\%$ ($p < 0.01$ vs control and Group II), indicating progressive oxidative stress (Table 3).

β -blocker therapy resulted in variable restoration of antioxidant capacity. Nebivolol (Group IV) produced the most substantial improvement, increasing AA of low-molecular-weight compounds to $22.6 \pm 0.33\%$ ($p < 0.01$ vs Group III), approaching control values. Carvedilol (Group VII) showed moderate improvement ($20.3 \pm 0.36\%$; $p < 0.01$ vs Group III), while propranolol (Group V) and metoprolol (Group VI) resulted in modest increases ($19.3 \pm 0.38\%$ and $18.9 \pm 0.37\%$, respectively; $p < 0.05$ vs Group III). Comparison among β -blockers demonstrated that nebivolol was associated with a more pronounced restoration of antioxidant parameters under the present experimental conditions.

Table 3. Hemorheological parameters in experimental groups (mean $\pm$ standard error of the mean)

Group	Description	Hct (%)	EAI (%)	EDI	Plasma Viscosity (cP)	NOx (μM)	AA (%)
Group I	Control (untreated)	42 ± 3	42 ± 3	2.20 ± 0.01	1.30 ± 0.02	2.31 ± 0.3	25.32 ± 0.36
Group II	L-NAME (4 weeks, untreated)	43 ± 4	$52 \pm 4^{**}$	2.10 ± 0.01	1.33 ± 0.03	$1.62 \pm 0.3^{**}$	$18.5 \pm 0.35^{**}$
Group III	L-NAME (8 weeks, untreated)	45 ± 4	$60 \pm 5^{**}$	$2.05 \pm 0.02^{**}$	$1.39 \pm 0.03^{**}$	$1.30 \pm 0.3^{**}$	$16.2 \pm 0.35^{**}$
Group IV	L-NAME (8 weeks) + nebivolol (weeks 5–8)	43 ± 3	$46 \pm 3^{##}$	2.15 ± 0.02	$1.32 \pm 0.02^{##}$	$2.21 \pm 0.3^{##}$	$22.6 \pm 0.33^{##}$
Group V	L-NAME (8 weeks) + propranolol (weeks 5–8)	43 ± 4	$50 \pm 4^{##}$	2.11 ± 0.02	$1.34 \pm 0.03^{##}$	$1.63 \pm 0.3^{\#}$	$19.3 \pm 0.38^{\#}$
Group VI	L-NAME (8 weeks) + metoprolol (weeks 5–8)	43 ± 3	$48 \pm 3^{##}$	2.12 ± 0.02	$1.33 \pm 0.03^{##}$	$1.65 \pm 0.3^{\#}$	$18.9 \pm 0.37^{\#}$
Group VII	L-NAME (8 weeks) + carvedilol (weeks 5–8)	42 ± 3	$45 \pm 3^{##}$	2.14 ± 0.02	$1.31 \pm 0.02^{##}$	$1.83 \pm 0.3^{##}$	$20.3 \pm 0.36^{##}$

Statistical comparisons were performed using one-way ANOVA followed by Tukey's honestly significant difference (HSD) post hoc multiple-comparison test. $^{**} p < 0.01$ vs control group (Group I); $^{\#} p < 0.05$ vs hypertensive group (Group III); $^{##} p < 0.01$ vs hypertensive group (Group III).

Overall, these findings indicate that prolonged inhibition of nitric oxide synthesis leads to increased oxidative stress and decreased NO metabolite content, while β -blockers—particularly nebivolol—partially reverse these alterations.

DISCUSSION

The present study investigated the effects of β -blocker therapy on hemorheological, microcirculatory, and oxidative stress parameters in experimental L-NAME-induced arterial hypertension. The findings demonstrate that chronic nitric oxide deficiency induces complex disturbances involving vascular function, hemorheology, and redox balance, while β -blocker treatment partially attenuates these alterations with varying efficacy among agents.

Microcirculatory function is strongly dependent on the rheological properties of blood, particularly erythrocyte deformability and plasma viscosity [3, 5]. In the present study, L-NAME-induced hypertension was associated with increased plasma viscosity and enhanced erythrocyte aggregation, supporting the concept that hypertension is not solely a hemodynamic disorder but also a disease of impaired microcirculation. These hemorheological disturbances may contribute directly to increased peripheral vascular resistance and reduced tissue perfusion [7]. Therefore, the observed hemorheological alterations likely represent active contributors to hypertension pathogenesis rather than merely secondary consequences of elevated blood pressure.

Although acute nitric oxide synthase inhibition is commonly associated with reflex bradycardia mediated by baroreceptor activation, chronic NO deficiency may produce more complex autonomic adaptations. Previous studies have shown that prolonged inhibition of NO synthesis impairs baroreflex sensitivity, increases sympathetic nervous system activity, and promotes endothelial dysfunction and vascular remodelling [13–15]. These chronic compensatory mechanisms may explain the elevated heart rate observed in hypertensive animals during prolonged L-NAME administration. Because sympathetic nervous system activity was not directly assessed in the present study, conclusions regarding the precise mechanisms underlying heart rate elevation should be interpreted cautiously. Nevertheless, previous studies support the contribution of sympathetic activation to chronic L-NAME-induced hypertension.

Although equipotent antihypertensive dosing was not established, all β -blockers produced a broadly comparable reduction in heart rate relative to untreated hypertensive animals (340–355 bpm versus 395 bpm in Group III), suggesting effective β -adrenergic receptor blockade in all treatment groups. Nevertheless, heart-rate reduction represents only an indirect pharmacodynamic indicator and does not establish equivalent degrees of β -blockade

among agents. Therefore, direct quantitative comparisons of efficacy should be interpreted cautiously.

A central mechanistic finding of the present study is the association between oxidative stress and impaired nitric oxide signalling. Chronic inhibition of NO synthesis significantly reduced NOx levels and antioxidant activity, indicating substantial redox imbalance. Oxidative stress is known to impair endothelial function, promote vascular dysfunction, and alter erythrocyte membrane properties [4, 9]. The observed increase in erythrocyte aggregation and plasma viscosity may therefore result from oxidative damage to erythrocyte membranes, including lipid peroxidation and cytoskeletal alterations, leading to reduced membrane flexibility and enhanced cell–cell interactions [4,8]. Simultaneously, reduced NO bioavailability weakens its vasodilatory and anti-aggregatory effects, thereby further aggravating microcirculatory impairment [9].

The observed association between oxidative stress, erythrocyte aggregation, and impaired nitric oxide bioavailability further supports the concept that endothelial dysfunction and altered blood rheology interact synergistically during hypertension progression. Oxidative membrane injury may promote erythrocyte rigidity and aggregation, thereby impairing capillary perfusion and tissue oxygenation. These mechanisms may contribute not only to elevated vascular resistance but also to hypertension-associated target organ damage.

An important methodological consideration should be acknowledged regarding antioxidant assessment. Because serum samples were deproteinized with acetonitrile before DPPH analysis, the obtained values predominantly reflect low-molecular-weight non-enzymatic antioxidants rather than total serum antioxidant capacity. Consequently, the measured antioxidant activity does not fully characterise enzymatic antioxidant defence systems such as superoxide dismutase, catalase, and glutathione peroxidase.

Interestingly, erythrocyte deformability showed only minor alterations compared with the more pronounced changes in erythrocyte aggregation and plasma viscosity. This finding suggests that aggregation-related parameters may represent a more sensitive indicator of early hemorheological impairment in NO-deficient hypertension, which is consistent with previous reports emphasising the dominant role of aggregation dynamics in microvascular flow disturbance [3, 20]. All tested β -adrenoceptor blockers reduced arterial pressure; however, their effects on oxidative stress and hemorheological parameters differed substantially. Under the present experimental conditions, nebivolol was associated with greater improvement in arterial pressure, erythrocyte aggregation, plasma viscosity, NOx levels, and antioxidant activity. However, because equipotent β -blocker dosing was not established, these findings should not be interpreted as definitive evidence of pharmacological superiority.

Carvedilol was also associated with beneficial effects, which may be attributed to its antioxidant activity and additional α 1-adrenergic blocking properties [21]. In contrast, propranolol and metoprolol were associated with smaller changes in the measured parameters under the present experimental conditions, suggesting that conventional β -blockade without additional pleiotropic mechanisms may exert limited influence on microcirculatory dysfunction [12].

Both pulse arterial pressure and erythrocyte aggregation decreased following β -blocker treatment. However, because no formal correlation analysis was performed, the present data do not allow conclusions regarding a direct relationship between these variables. Future studies incorporating correlation and regression analyses are required to clarify whether improvements in macrohemodynamic parameters are associated with restoration of microcirculatory function.

β -adrenoceptor blockers demonstrated significant but heterogeneous effects on both vascular and hemorheological parameters, supporting the study hypothesis. While all agents reduced arterial pressure, their effects on microcirculatory-related parameters differed substantially. The beneficial effects of β -blockers observed in the present study may involve several interconnected mechanisms, including:

- reduction of reactive oxygen species (ROS) production,
- restoration of nitric oxide bioavailability,
- stabilisation of erythrocyte membrane structure,
- improvement of erythrocyte–endothelial interactions,
- and enhancement of microcirculatory blood fluidity [4, 10].

Collectively, these findings emphasise the importance of the “oxidative stress–microcirculation–hemorheology” axis in hypertension pathogenesis and support its potential as a therapeutic target.

An additional important limitation relates to the pathophysiological characteristics of the L-NAME model itself. Because hypertension in this model is induced primarily through chronic nitric oxide synthase inhibition and reduced NO bioavailability, the experimental design inherently favours interventions capable of restoring nitric oxide signalling. Consequently, the greater improvements observed with nebivolol may be partially attributable to its NO-mediated vasodilatory properties in a model specifically characterised by NO deficiency. Therefore, caution is required when extrapolating these findings to other forms of hypertension in which nitric oxide deficiency is not the dominant pathogenic mechanism, including spontaneously hypertensive rat (SHR), angiotensin II-induced, or DOCA-salt hypertension models. Future studies using multiple experimental models are needed to determine whether similar differences among β -blockers persist independently of NO-deficient pathophysiology.

The present findings should also be interpreted in the context of current clinical evidence regarding β -blocker therapy in hypertension. Contemporary European Society of Hypertension (ESH) and European Society of Cardiology (ESC) guidelines recognise β -blockers as established antihypertensive agents, particularly in patients with specific cardiovascular comorbidities such as ischemic heart disease, heart failure, arrhythmias, and hyperadrenergic states, although they are no longer universally preferred as first-line therapy for uncomplicated hypertension. Clinical studies and meta-analyses have demonstrated that third-generation β -blockers, particularly nebivolol and carvedilol, may exert additional pleiotropic effects beyond blood pressure reduction, including improvement of endothelial function, enhancement of nitric oxide bioavailability, reduction of oxidative stress, and favourable effects on vascular stiffness and microcirculatory function. Several clinical hemorheological studies in hypertensive patients have also reported associations between increased plasma viscosity, erythrocyte aggregation, impaired microcirculatory flow, and cardiovascular risk. However, despite these mechanistic and physiological observations, direct evidence linking improvement of hemorheological parameters to superior long-term cardiovascular outcomes remains limited. Therefore, the translational significance of the present experimental findings should be interpreted cautiously, and further clinical investigations are required to determine whether modulation of microcirculatory and hemorheological abnormalities provides measurable prognostic benefit in human hypertension.

The present findings may also have clinical implications. The present experimental findings suggest that, in addition to blood pressure reduction, microcirculatory and oxidative stress-related mechanisms may represent potentially relevant therapeutic targets in hypertension; however, confirmation in clinical studies is required before definitive therapeutic conclusions can be drawn. In this context, β -blockers with pleiotropic properties, particularly nebivolol, may provide additional benefits in preventing microvascular dysfunction and hypertension-associated target organ damage.

Several limitations of the present study should be acknowledged. First, equipotent antihypertensive dosing among the tested β -blockers was not established. The selected doses were based on previously published experimental studies and pharmacological characteristics of each agent, including receptor selectivity, pharmacokinetics, and pleiotropic properties. Therefore, direct quantitative comparisons between β -blockers should be interpreted cautiously. In particular, the relatively low propranolol dose used in the present study may limit definitive conclusions regarding comparative efficacy among agents.

Second, antioxidant assessment primarily reflected low-molecular-weight non-enzymatic antioxidant activity and therefore did not fully characterise enzymatic antioxidant defence systems.

Finally, due to the experimental design, the present findings demonstrate associations between oxidative stress, hemorheological alterations, and hypertension-related changes but do not establish direct causal relationships.

An additional important limitation of the present study relates to the specific pathophysiological characteristics of the L-NAME-induced hypertension model. Since L-NAME induces hypertension primarily through chronic inhibition of nitric oxide synthase and suppression of NO bioavailability, the experimental design inherently favours agents capable of restoring NO signalling. Consequently, the more pronounced beneficial effects observed with nebivolol may be partially related to the NO-dependent nature of the model itself, because nebivolol possesses NO-mediated vasodilatory properties in addition to β 1-selective blockade. Therefore, caution is required when extrapolating these findings to other forms of hypertension in which the underlying mechanisms are less dependent on nitric oxide deficiency, such as salt-sensitive, renin-angiotensin-aldosterone-driven, genetic, or neurogenic hypertension models. Future studies should evaluate the comparative effects of β -blockers in multiple experimental models of hypertension to determine whether the superiority of nebivolol persists independently of NO-deficient pathophysiology.

CONCLUSIONS

L-NAME-induced hypertension is associated with microcirculatory dysfunction, impaired hemorheology, and increased oxidative stress, characterised by elevated erythrocyte aggregation, increased plasma viscosity, and reduced nitric oxide bioavailability as reflected by decreased NOx levels. β -blocker therapy partially attenuated these alterations, with nebivolol being associated with more pronounced improvements under the specific conditions of the NO-deficient L-NAME hypertension model, likely related, at least in part, to its nitric oxide-mediated and antioxidant properties within these experimental conditions.

These findings highlight the importance of targeting the “oxidative stress–microcirculation–hemorheology” axis in pathologies such as arterial hypertension and suggest that β -blockers with pleiotropic effects may provide additional therapeutic benefits beyond blood pressure control, particularly in improving microcirculatory function and vascular redox balance.

ABBREVIATIONS

AA – antioxidant activity
ANOVA – analysis of variance
CAT – catalase
DAP – diastolic arterial pressure
DPPH – 2-diphenyl-1-picryl-hydrazyl
EAI – erythrocyte aggregation index
EDI – erythrocyte deformability index
GPx – glutathione peroxidase
HCl – hydrochloric acid
Hct – hematocrit
L-NAME – N(ω)-nitro-L-arginine methyl ester
MAP – mean arterial pressure
NED – N-(1-naphthyl)-ethylenediamine
NO – nitric oxide
PAP – pulse arterial pressure
ROS – reactive oxygen species
SAP – systolic arterial pressure
SEM – standard error of the mean
SOD – superoxide dismutase

ACKNOWLEDGEMENT

None.

AUTHORS' CONTRIBUTION

NK – data curation, investigation, validation, writing (original draft)
MM – supervision, project administration, writing (review & editing)
LG – methodology, investigation, writing (review & editing)
GO – data interpretation, statistical analysis
EG – data acquisition, interpretation
SK – visualization, formal analysis
LC – methodology, validation
TS – supervision, funding acquisition, project administration
All authors have read and approved the final version of the manuscript.

CONFLICT OF INTEREST

None to declare.

Publisher's Note: The Editorial Office stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2026 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

REFERENCES

1. Kreutz R, Brunström M, Burnier M, et al. 2024 European Society of Hypertension clinical practice guidelines for the management of arterial hypertension. *Eur J Intern Med.* 2024;126:1-15. DOI:10.1016/j.ejim.2024.05.033
2. Pinho D, Carvalho V, Gonçalves IM, Teixeira S, Lima R. Visualization and measurements of blood cells flowing in microfluidic systems and blood rheology: a personalized medicine perspective. *J Pers Med.* 2020;10(4):249. DOI:10.3390/jpm10040249
3. Mchedlishvili G. Hemorheological changes in microcirculation: mechanisms and measurement techniques. *Indian J Exp Biol.* 2007;45(1):32-40.
4. Franco C, Sciatti E, Favero G, et al. Essential hypertension and oxidative stress. *Int J Mol Sci.* 2022;23:14489. DOI:10.3390/ijms232214489
5. Chien S. Blood rheology in myocardial infarction and hypertension. *Biorheology.* 1986;23(6):633-653. DOI:10.3233/BIR-1986-23614
6. Zannad F, Stoltz JF. Blood rheology in arterial hypertension. *J Hypertens Suppl.* 1992;10(5):S69-S78. DOI:10.1097/00004872-199207005-00010
7. Pirrelli A. Arterial hypertension and hemorheology: what is the relationship? *Clin Hemorheol Microcirc.* 1999;21(3-4):157-160.
8. Bor-Küçükataş M, Yalçın O, Gökalp O, et al. RBC rheological alterations in NO-deficient hypertension. *Clin Hemorheol Microcirc.* 2000;22(4):267-275.
9. Kakabadze K, Megreladze I, Khvichia N, et al. Role of nitric oxide in experimental hypertension. *Cardiol Res.* 2021;12(1):16-24. DOI:10.14740/cr1172
10. Sander M, Hansen PG, Victor RG. Sympathetically mediated hypertension caused by chronic inhibition of nitric oxide. *Hypertension.* 1995;26(4):691-695. DOI:10.1161/01.HYP.26.4.691
11. Scrogin KE, Hatton DC, Chi Y, Luft FC. Chronic nitric oxide inhibition with L-NAME: effects on autonomic control of the cardiovascular system. *Am J Physiol.* 1998;274(2):R367-R374. DOI:10.1152/ajpregu.1998.274.2.R367
12. Zanzinger J. Role of nitric oxide in the neural control of cardiovascular function. *Cardiovasc Res.* 1999;43(3):639-649. DOI:10.1016/S0008-6363(99)00085-1
13. Silva IVG, de Figueiredo RC, Rios DRA. Effects of antihypertensive drugs on endothelial function. *Int J Mol Sci.* 2019;20(14):3458. DOI:10.3390/ijms20143458
14. Kadi A, Moby V, de Isla N, et al. Nebivolol-induced NO production in endothelial cells. *Biomed Mater Eng.* 2008;18(4-5):279-286. DOI:10.3233/BME-2008-0543
15. Wiysonge CS, Bradley HA, Volmink J, et al. Beta-blockers for hypertension. *Cochrane Database Syst Rev.* 2017;1:CD002003. DOI:10.1002/14651858.CD002003.pub5
16. Mayer B, Holmer SR, Hengstenberg C, Lieb W, Pfeifer M, Schunkert H. Functional improvement in heart failure patients treated with beta-blockers is associated with a decline of cytokine levels. *Int J Cardiol.* 2005;103(2):182-186. DOI:10.1016/j.ijcard.2004.08.053
17. Ulleryd MA, Bernberg E, Yang LJ, Bergström GM, Johansson ME. Metoprolol reduces proinflammatory cytokines and atherosclerosis in ApoE^{-/-} mice. *Biomed Res Int.* 2014;2014:548783. DOI:10.1155/2014/548783
18. Hanif N, Zamir A, Imran I, et al. Clinical pharmacokinetics of nebivolol: a systematic review. *Drug Metab Rev.* 2023;55(4):428-440. DOI:10.1080/03602532.2023.2271195
19. Frishman WH. Beta-adrenergic blockers. *Circulation.* 2003;107(18):e117-e119. DOI:10.1161/01.CIR.0000070983.15903.A2
20. Baskurt OK, Meiselman HJ. Blood rheology and hemodynamics. *Semin Thromb Hemost.* 2003;29(5):435-450. DOI:10.1055/s-2003-44551
21. Baskurt OK, Hardeman MR, Rampling MW, Meiselman HJ. *Handbook of Hemorheology and Hemodynamics.* Amsterdam: IOS Press; 2009.
22. Miranda KM, Espey MG, Wink DA. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide.* 2001;5(1):62-71. DOI:10.1006/niox.2000.0319
23. Chrzczanowicz J, Gawron A, Zwolinska A, de Graft-Johnson J, Krajewski W, Krol M, et al. Simple method for determining human serum 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity-possible application in clinical studies on dietary antioxidants. *Clin Chem Lab Med.* 2008;46(3):342-349. DOI:10.1515/CCLM.2008.062
24. Feuerstein GZ, Ruffolo RR Jr. Carvedilol, a novel antihypertensive agent with antioxidant activity. *Eur Heart J.* 1995;16(Suppl F):38-42. DOI:10.1093/eurheartj/16.suppl_F38