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ORIGINAL ARTICLE **OPEN ACCESS**

Effect of L-Citrulline as mono or combination therapy with β-blocker antihypertensive drug in experimental animals with adrenaline induced hypertension.

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ABSTRACT
L-Citrulline (CIT), is a natural antioxidant & nitric oxide modulator, providing beneficial cardiovascular properties. Present study investigated antihypertensive effects of the CIT alone or in combination with a Propranolol (PRL) in Wistar rats with adrenaline induced hypertension. In this 30-day study, hypertension was induced in male Wistar rats using adrenaline (0.5 mg/kg/day,i.p.) administration for initial 10 days. Thereafter for the next 20 days, hypertensive animals were treated with CIT (50 or 100 mg/kg,p.o.) alone dose or in combination with PRL (5 or 10 mg/kg,i.p) dose. Parameters evaluated were blood pressure, heart weight, vascular reactivity to sympathomimetic agents, acetylcholine-induced vasorelaxation in the isolated artery and oxidative stress in heart specimen. Findings showed that, CIT alone therapy significantly brought down the raised BP levels, improved the aorta relaxant responses, attenuated oxidative stress as compared to adrenaline hypertensive rats. In combination therapy, all doses significantly reversed the increased BP levels, increased body weight and reduced HW/BW ratio, reduced impaired responses to Ach, improved antioxidant parameters (except CIT-50 + PRL 5 dose) as compared to adrenaline hypertensive rats. However, in the combination therapy only CIT-100 mg/kg dose with PRL (5 or 10 mg/kg) significantly decreased the rise in BP in response to sympathomimetic agents administered. These findings are consistent with the conclusion that CIT is a promising treatment option in treating hypertension and due to synergistic effects with PRL, β- blocker dose can be reduced down to achieve the equivalent antihypertensive effect and to minimize adverse effects as produced by β- blocker monotherapy.
Keywords**:** L-Citrulline, Hypertension, Antihypertensive combination therapy, Blood pressure, β- blocker, Propranolol

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INTRODUCTION

Global statistics showed that 1 in 4 men and 1 in 5 women had hypertension, whereas fewer than 1 in 5 people with hypertension have the problem under control. For cases where monotherapy can't control hypertension, polytherapy involving either mix of calcium channel blockers, angiotensin receptor blockers (ARBs), diuretics, statins, or angiotensin-converting enzyme inhibitors (ACE-Is) is recommended in 'HEARTS' technical package treatment protocols. This protocol is now accepted and implemented by about Fifteen countries globally [1]. Previously an increase in the dose of one agent to its maximum potentially effective dose as long as side effects were tolerable were suggested. For most antihypertensives, the dose-response curves flatten out at high doses, whereas the appearance of side effects are usually dose-related [2]. Though polytherapy is being encouraged increasingly in routine clinical care to minimize dose related side effects and adverse reactions.Few of the polytherapy has been identified as inappropriate, for e.g., concurrent combination of ACE-Is and ARBs is reducing as proof on the likelihood of renal impairment [3]. Hence, the need for novel, effective and safe combination therapies for patients with hypertension who not responding monotherapy is well acknowledged. The L-Citrulline, a natural precursor of L-Arginine is gaining attention with number of evidences from preclinical and clinical studies, as it bypasses hepatic metabolism and it is not substrate for arginase, unlike L-Arginine [4]. Oral CIT supplementation increases L-Arginine synthesis and thereby NO biosynthesis, which in turn improves endothelial vasodilator function [5]. In spontaneous hypertension rodent model, CIT increased L-Arginine/asymmetrical dimethylarginine ratio and improved levels of NO [6,7]. In high cholesterol diet induced atherosclerosis model in rabbits, CIT preserves endothelial function by decreasing the

production of superoxide and associated oxygen-sensitive proteins ELK-1 and p-CREB [8]. Preclinical studies support the evidence to control hypertension after CIT administration. In Clinical trials on healthy, young subjects, CIT supplementation increases levels of L-Arginine, nitrate/nitrite, cGMP activities, vascular conductance, peripheral tissue oxygenation, and decreases systemic blood pressure [9–11]. However, no obvious side effects have seen after CIT supplementation [4,12–14]. Evidences supports CIT supplementation therapeutic benefits on cardiovascular system. To the best of our knowledge, we have not found any research assessing the role of CIT combination with marketed β-blocker antihypertensive agents like propranolol (PRL). Considering the fact, when smaller doses of medications with different mechanisms of action are combined, synergistic or additive effects on blood pressure are achieved, dose-dependent side effects are minimized [2] and the need for novel, efficacious and safe polytherapy in hypertension, the present study is devised. Present study assesses whether combination therapy of CIT with β- blocker in male Wistar rats with adrenaline induced hypertension is efficacious and allows use of lower doses to reduce the blood pressure level to goal.

MATERIAL AND METHODS

ANIMALS

The male Wistar rats (300 gm ± 20 gm) were purchased from the National Institute of Biosciences, Pune, India. After seven days acclimatization period rats were used in the present study and the experimental animal room was kept at a temperature of 22ºC (± 3ºC) with a relative humidity of 50–60%. Artificial 12 hours light and dark cycle was provided throughout the experiment. FThroughout the experiment, a simulated 12-hour light and dark cycle was present. A standard laboratory food and an endless supply of water were employed for feeding with three animals per cage. The research was done in compliance with the New Delhi-based Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) rules (India). The protocol for the study was approved by the Institutional Animal Ethical Committee of M.V.P.S College of Pharmacy, Nashik (IAEC/2017/08).

MATERIALS

Adrenaline (®) and pharmaceutical grade CIT were purchased from T. Walker's Healthcare and Nutrija Lifesciences, respectively, in India. The other chemicals used were analytical-grade and came from reputable suppliers.

DOSE SELECTION

Based on arginase inhibitory efficacy to reduce fructose-induced hypertension in the study by El-Bassossy et al., a low dose of 50 mg/kg of CIT was chosen [15]. The chosen two doses of 50 and 100 mg/kg were prepared in solution form by combining the right amount of CIT powder with distilled water, and they were then given orally.

EXPERIMENTAL DESIGN

Hypertension was induced in animals by administration of Adrenaline (0.5mg/kg, *i.p.*) consecutively for 10 days. After induction of hypertension, animals were assigned to the eleven treatment groups (n = 6) as follows-

Group I- Normal Control Group treated with Vehicle only,

Group II- First 10 days Normal saline i.p. afterwards CIT (50 mg/kg/p.o.) for 20 days,

Group III- First 10 days Normal saline i.p. afterwards CIT (100 mg/ kg/p.o.) for 20 days,

Group IV- Adrenaline (0.5mg/kg) for 10 days/i.p.,

Group V- Adrenaline (0.5mg/kg/i.p.) for 10 days thereafter CIT (50 mg/kg/p.o.) for 20 days,

Group VI- Adrenaline (0.5mg/kg/i.p.) for 10 days thereafter CIT (50 mg/kg/p.o.) + PRL (5 mg/ kg/i.p.) for 20 days,

Group VII- Adrenaline (0.5mg/kg/i.p.) for 10 days thereafter CIT (50 mg/kg/p.o.) + PRL (10 mg/ kg/i.p.) for 20 days,

Group VIII- Adrenaline (0.5mg/kg/i.p.) for 10 days thereafter CIT (100 mg/kg/p.o.) for 20 days,

Group IX- Adrenaline (0.5mg/kg/i.p.) for 10 days thereafter CIT (100 mg/kg/p.o.) + PRL (5 mg/ kg/i.p.) for 20 days,

Group X- Adrenaline (0.5mg/kg/i.p.) for 10 days thereafter CIT (100 mg/kg/p.o.) + PRL (10 mg/ kg/i.p.) for 20 days,

Group XI- Adrenaline (0.5mg/kg/i.p.) for 10 days thereafter PRL (10mg/ kg/i.p.) for 20 days after inducing hypertension.

DATACOLLECTION

Hemodynamic Assessment

In this assessment parameters measured were heart rate (HR), non-invasive blood pressures (NIBP), systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial blood pressure (MAP). NIBP measurements were done before the start, day 10, day 20 and at the end of experiment using tail

cuff method. To measure HR, SBP, DBP, and MAP at the end of the experiment right carotid artery of each animal was cannulated using pentobarbitone sodium (70 mg/kg, i.p.) anesthesia. Thereafter, right jugular vein was cannulated with catheter to record BP responses to ADR (1µg/kg/ml, i.v.), Nor-ADR (1µg/kg/ml, i.v.) and PE (1µg/kg/ml, i.v.). These hemodynamic parameters were recorded by eight- channel PowerLab (ADInstruments) running LabChart Pro 7 software (ADInstruments).

Sample collection

Rats were beheaded, the heart tissues were removed, and they were promptly rinsed in ice-cold saline after the blood pressure reading. Each heart was homogenized (10% w/v) with Tris-HCl/Phosphate buffer (0.1 M/ pH 7.4) following the weight measurement.

Ach induced vasorelaxation

Isolated artery approach was utilized to measure the effects of Ach on vasorelaxation [16]. The entire descending thoracic aorta, from the arch to the diaphragm, was isolated and put in a 37°C Krebs solution (95% oxygen and 5% carbon dioxide). Connective tissue and adhering fat removed from aorta. Aorta's connective tissue and fat sticking to it were removed. 3 mm long rings that have been created and mounted in an organ bath with 15 ml of krebs solution. The rings were suspended between two stainless steel hooks, one of which was attached to the end of a bathing tube and the other to the force transducer, in order to record contractions (PowerLab, AD Instruments).Prior to the experiment, the preparation was subjected to a 0.5 g resting tension and equilibrated in a 15 ml bathing solution for 90 to 120 min with a solution change every 15 min. The rings were subjected to 10-6M PE after equilibration. Acetylcholine was gradually introduced once the contractile response to PE reached a plateau, and vasorelaxation was seen.

Biochemical analyses

The heart tissues were used to measure oxidative stress parameters like catalase by Luck method [17], Reduced glutathione (GSH) by Ellman et al. method [18], Superoxide dismutase by Kono et al method [19], malondialdehyde (MDA) levels by Wills method [20], NO activity by Titheradge M. A. method [21].

Statistical analysis

The statistical analysis was conducted using Microsoft office (2019) Excel and version 9 of GraphPad Prism software. A two-way ANOVA followed by a Tuckey's post hoc test was carried out to statistically examine the effects of time and the treatment groups on the initial and subsequent NIBP readings. For additional parameters, a one-way ANOVA was employed, followed by a Tuckey's post hoc analysis. The mean and standard error of the mean were used to present all the data (SEM). When the confidence level reached 95%, groups were deemed to be significantly different.

RESULTS AND DISCUSSION

Multiple studies have confirmed that repeated ADR administration is associated with increased levels of blood pressure in Wistar rats. Use of ADR repetitive administration by i.p. route to develop hypertension in Wistar rats is well established murine experimental model to explore newer antihypertensive agents [22–27]. It is suggested after repetitive administration of ADR hypertensive state is set by the gradual uptake and accumulation of ADR in noradrenergic nerve terminals, from which it is subsequently released as a co-transmitter and mediates an auto-facilitatory positive feedback loop on. This results in to exaggerated noradrenergic transmission and surges activity in sympathetically innervated tissues and this is reflected as an increased blood pressure well beyond the period for which the plasma concentrations of ADR is elevated because of adaptive structural changes [27]. In our study, adrenaline induced amplification of sympathetic activity in Wistar rats has showed the following results-

Effects of CIT therapy alone or in combination with PRL on Hemodynamic Assessment

In all the ADR administered groups mean blood pressure level on the 10^{th} day was significant higher as compared to vehicle treated group and day zero for the same group. Though there is difference, no statistically significant difference found in between the mean blood pressure level recorded on day 10, 20 and 30 in only ADR treated group. On the contrary, all of the treatment groups except CIT (50) reported significantly low values ($p<0.001$) for mean blood pressure level recorded on the day 30 when compared to only ADR treated group (see Table 1). Heart Rate measurement (see Figure 1A) after right carotid artery cannulation showed significantly higher beats per minute in all the groups who received Adrenaline injections except group treated with CIT (100) + PRL (10) as compared to vehicle control group. There were variations in beats per minute in all the experimental treatment groups and comparator group but the difference did not reach significance when compared to only ADR treated group. Most of the study [22,28,29] results are in affirmation of our HR findings except study done by Tung et al which showed ADR group is not significantly different compared to normal control group [24]. In the only ADR treated group, the SBP, DBP and MAP assessment (see Figure 1C,D,E) on the last day of experiment by invasive technique, showed significant increment ($p<0.001$) when compared to vehicle

control group. In this context, when treatment groups compared to the only ADR treated group, all groups significantly decreased elevated levels of the SBP, DBP and MAP except the group treated with only CIT (50). Others studies have also demonstrated that NIBP, SBP, DBP, and MAP increases after the administration of the ADR in rats. [22,25,30] CIT has been shown to have anti-hypertensive effects in a number of preclinical models and human trials [7,11,15,31,32].

Effects of CIT therapy alone or in combination with PRL on heart weight index

At the end of one month, Adrenaline administration is associated with increased heart weight/ body weight ratio ($p< 0.001$, see Figure 1B) as compared to vehicle treated group. This increased index was significantly inhibited by CIT (50) + PRL (10) ($P< 0.01$), CIT (100) + PRL (5) ($P< 0.01$), CIT (100) + PRL (10) ($P< 0.001$), and PRL (10) ($P < 0.05$) treatment. The study done by Ara et al has also shown the increase In the heart weight after the administration of ADR. [26]

Effects of CIT therapy alone or in combination with PRL on Vascular reactivity

Similar to study by Nade et al [22], the vehicle group showed a normal vascular reactivity to catecholamine [Adr (1μg/kg), NA (1 μg/kg) and PE (1μg/kg)], whereas adrenaline treated group showed a significant ($p < 0.001$) exaggeration in mean change in BP to all the three catecholamine as compared to vehicle group (see Figure 2). As compared to adrenaline treated group only treatments with CIT (100) + PRL (5) and CIT (100) + PRL (10) showed a significant fall in mean change in BP to all the three catecholamine responses with $p<0.05$ and $p<0.005$ respectively. Although there was also a trend towards fall in mean change in BP to all the three catecholamine responses in other treated groups but this did not reach statistical significance.

Effects of CIT therapy alone or in combination with PRL on Acetylcholine-induced relaxation of rat aorta pre-contracted with phenylephrine (1×10^{-6} M)

Final relaxant response to cumulative doses of acetylcholine (10^{-7} M to10^{-3} M) on the aorta from vehicle group was considered 100%. This is regarded as normal endothelial function. The ADR treated group showed a significant ($p<0.001$) impairment of relaxation. Aortas from groups treated with CIT (50) + PRL (10), CIT (100), CIT (100) + PRL (5), CIT (100) + PRL (10) and PRL showed significant improvement in relaxant response with $p<0.05$, $p<0.01$, $p<0.005$, $p<0.001$ and $p<0.05$ respectively as compared to only ADR group. But this study did not demonstrate significant differences in relaxant response by the aortas from groups treated with CIT (50) & CIT (50) + PRL (5) compared to only ADR group (see Figure 3). Treatment group responses to rat artery relaxation and improved vascular reactivity point to the preservation of vascular endothelial function. Romero et al. shown through the use of arginase knockout diabetic mice that lower arginase expression enhances endothelium-dependent vasorelaxation [33]. In a different investigation, CIT (50 & 100 mg/kg) shown inhibitory effects on arginase expression, maintaining the normal endothelium vasculature in a vascular dysfunction caused by the metabolic syndrome [15]. Arginase inhibitors are beneficial by restoring typical vascular function in diabetes and hypertension, according to other studies [11]. Numerous investigations [11,34–38] have demonstrated that CIT supplementation enhances arginine and NO availability while decreasing arginase expression. Results from studies by us and others indicate that CIT controls endothelium ageing as a productive source of L-arginine and NO.

Effects of CIT therapy alone or in combination with PRL on Antioxidant assays

Using supernatant from the heart homogenates SOD, catalase, Lipid peroxidation, GSH and Nitric oxide assays were performed. Statistical analysis revealed mean SOD levels (see Figure 4A) in ADR treated group significantly lower ($p<0.001$) as compared to vehicle treated groups. This decrease in the ADR group significantly attenuated with treatment groups except CIT (50) only. Figure 4B shows the activity of catalase in rat heart samples. The activity of this enzymatic antioxidant was significantly reduced in only ADR treated hypertensive rats ($P<0.001$). Treatment with PRL and all alone and combination doses of CIT significantly restored the activity of catalase except the treatment dose with CIT (50) only. There was a maximum effect at the dose of 100 mg/kg of CIT in combination with PRL 10 mg/kg ($P<0.001$). The heart specimen level of MDA was significantly increased in ADR hypertensive rats ($P<0.001$). This marker of lipid peroxidation was considerably reduced ($P<0.001$) after administration of PRL and different CIT alone and combination treatment groups (see Figure 4C). Significant decrease in reduced glutathione concentration was detected in heart specimens of ADR hypertensive rats compared to the vehicle control group ($P<0.001$). CIT 100 mg/kg and all the combination administrations significantly ($P < 0.05$) improved the level of this non-enzymatic antioxidant (Figure 5D). The effect of CIT on level of nitric oxide was shown in Figure 5E. The level of this indicator of oxidative stress in ADR hypertensive group was lower than vehicle control group ($P<0.001$). All the CIT-treated animals alone or in combination with PRL (05 or 10 mg/kg) exhibited highly increment in nitric oxides concentration ($P < 0.05$). PRL alone also brought back this indicator ($P <0.001$).

Very few studies were found showing the impact of ADR administration on the oxidative stress parameters. Study by Amin et al. [39] showed increases in serum MDA level after ADR administration, while Ali et al. [40] showed reduced DPPH radical scavenging activity. These results support our identifications after ADR administration.

Antioxidant properties of CIT have been demonstrated to reduce blood pressure in hypertension [15,41,42]. The antioxidant activity of CIT is explained by NO-dependent and NO-independent pathways. CIT stimulates the action in the NO-dependent pathway by raising endothelial NOS, which reduces ROS formation [43]. In the second pathway, water is formed as a result of a decrease in the production of hydroxyl radicals via alpha-amino acids in their protonated NH_3 state [44].

After the rigorous examination, it was discovered that intervention of CIT along with PRL attenuated SBP, DBP, and MAP, improved HW/BW ratio, brought changes in vascular reactivity and Ach-induced vasorelaxation towards normal values, reduced oxidative stress and enhanced antioxidant defense in heart of ADR induced hypertensive rats. However, the performance of the CIT alone doses when compared to combination therapy is slightly lagging behind and somewhat more mixed with results. Though both the CIT's high and low alone doses showed non-significant improvements in vascular reactivity, treatment with higher dose being statistically more effective compared to low dose in the other measured parameters. Conclusively, the present study showed that administration of the CIT in combination with PRL could be a promising strategy in improving blood pressure control.

Results

Table1. Effect of CIT (50 or 100 mg/kg) administration with or without PRL (5 or 10 mg/kg) combination on non-invasive blood pressure in different groups of ADR-induced hypertensive rat model

Group no.	Non-Invasive Blood Pressure (mmHg) at the end of			
	Day 0	**Day 10**	**Day 20**	**Day 30**
I	108.89 ± 2.53	108.74 ± 2.10^{d,z}	110.21 ± 1.93^{d,y}	112.74 ± 3.03^{d}
II	112.12 ± 3.50	111.62 ± 4.14^{b,x}	112.70 ± 4.10^{b,w}	113.32 ± 4.78^{b}
III	109.07 ± 2.86	108.69 ± 1.31^{d,z}	109.06 ± 2.54^{d,y}	111.81 ± 3.42^{c}
IV	109.56 ± 2.64	150.15 ± 1.38^{u,*}	154.20 ± 1.38^{u,*,x}	156.30 ± 1.82^{u,*,z}
V	107.62 ± 2.29	151.56 ± 2.47^{u,*}	149.17 ± 2.49^{u,*}	141.97 ± 3.00$^{u,\#}$
VI	110.92 ± 2.77	149.98 ± 2.80^{u,*}	146.28 ± 3.07$^{u,\$}$	135.63 ± 3.85$^{u,\#,a}$
VII	111.43 ± 3.01	152.33 ± 3.09^{u,*}	138.57 ± 3.41$^{t,\#}$	122.48 ± 4.19^{r,b}
VIII	113.05 ± 2.13	154.96 ± 2.55^{u,*}	148.10 ± 2.68^{u,*}	135.30 ± 2.92$^{t,\#,b}$
IX	104.24 ± 2.61	148.48 ± 3.54$^{u,\$}$	136.24 ± 3.60$^{u,\#,a}$	125.64 ± 2.12^{t,d}
X	106.33 ± 3.59	152.30 ± 2.95^{u,*}	133.32 ± 4.05$^{t,@,a}$	124.73 ± 3.70^{r,b}
XI	108.89 ± 2.07	149.64 ± 1.97^{u,*}	137.41 ± 2.34$^{u,\$,b}$	126.61 ± 2.52^{t,c}

Data are shown as mean ± SEM (n=6 per group, Two-way ANOVA). Significant difference of respective groups for day 10, 20 and 30 compared to day zero is indicated by (r) $p<0.05$, (s) $p<0.01$, (t) $p<0.001$, and (u) $p<0.0001$. Significant difference from the Group I compared to the remaining groups is indicated by (@) $p<0.05$, (#) $p<0.01$, ($) $p<0.001$, and (*) $p<0.0001$. Significant difference from Group IV compared to the remaining groups is indicated by (a) $p<0.05$, (b) $p<0.01$, (c) $p<0.001$, and (d) $p<0.0001$. Significant difference from the Group XI compared to the remaining groups is indicated by (w) $p<0.05$, (x) $p<0.01$, (y) $p<0.001$, and (z) $p<0.0001$.

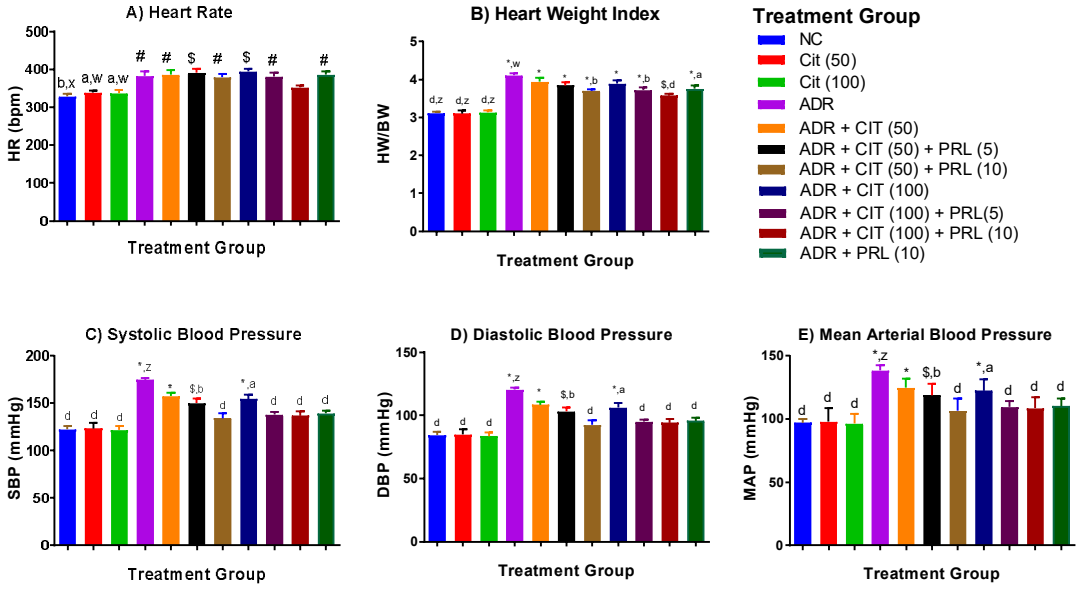

Figure 1. Effect of CIT (50 or 100 mg/kg) administration with or without PRL (5 OR 10 mg/kg) combination in different groups of ADR-induced hypertensive rat model on Heart rate, Heart weight index, and different parameters of invasive blood pressure (mmHg) measurements.
A: Heart weight/ body weight index, B: Heart rate, C: Systolic Blood pressure levels, D: Diastolic Blood pressure levels, E: Mean Arterial Blood pressure levels. Data are shown as mean ± SEM (n=6 per group). Significant differences from the Vehicle Control group [NC] are indicated by (@) p<0.05, (#) p<0.01, ($) p<0.005, and (*) p<0.001. Significant differences from only ADR-treated group are indicated by (a) p<0.05, (b) p<0.01, (c) p<0.005, and (d) p<0.001. Significant differences from only Standard control group [ADR + PRL (10)] are indicated by (w) p<0.05, (x) p<0.01, (y) p<0.005, and (z) p<0.001.

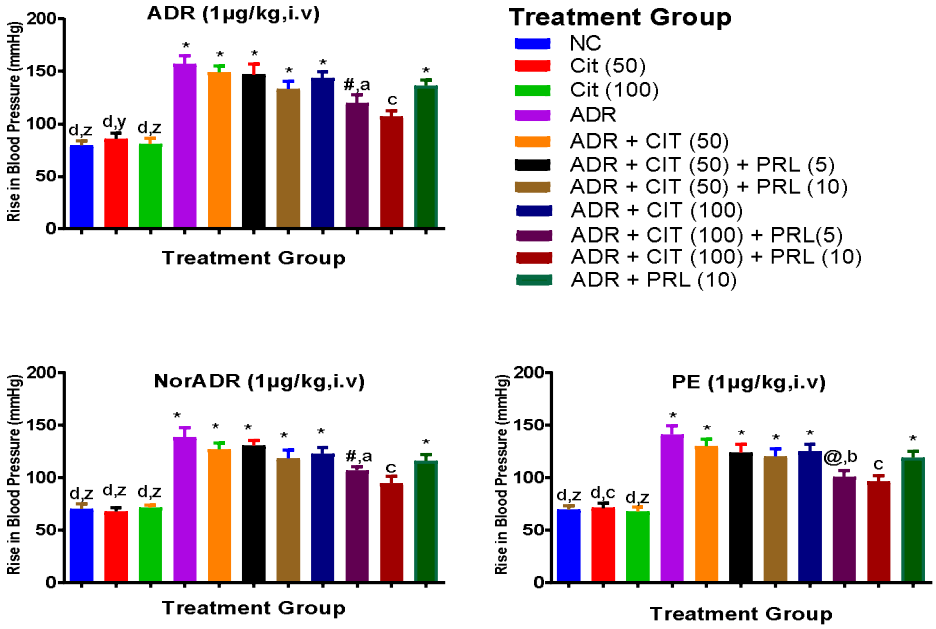

Figure 2. Effect of CIT (50 or 100 mg/kg) administration with or without PRL (5 OR 10 mg/kg) combination in different groups of ADR-induced hypertensive rat model on vascular reactivity after different sympathomimetic agent's administration.
A: BP responses to ADR (1µg/kg/ml, i.v), B: BP responses to Nor-ADR (1µg/kg/ml, i.v) and C: BP responses to PE (1µg/kg/ml, i.v). (A) Data are shown as mean± SEM (n=6 per group, One-way ANOVA). Significant differences from the Vehicle Control group [NC] are indicated by (@) p<0.05, (#) p<0.01, ($) p<0.005, and (*) p<0.001. Significant differences from only ADR-treated group are indicated by (a) p<0.05, (b) p<0.01, (c) p<0.005, and (d) p<0.001. Significant differences from only Standard control group [ADR + PRL (10)] are indicated by (w) p<0.05, (x) p<0.01, (y) p<0.005, and (z) p<0.001.

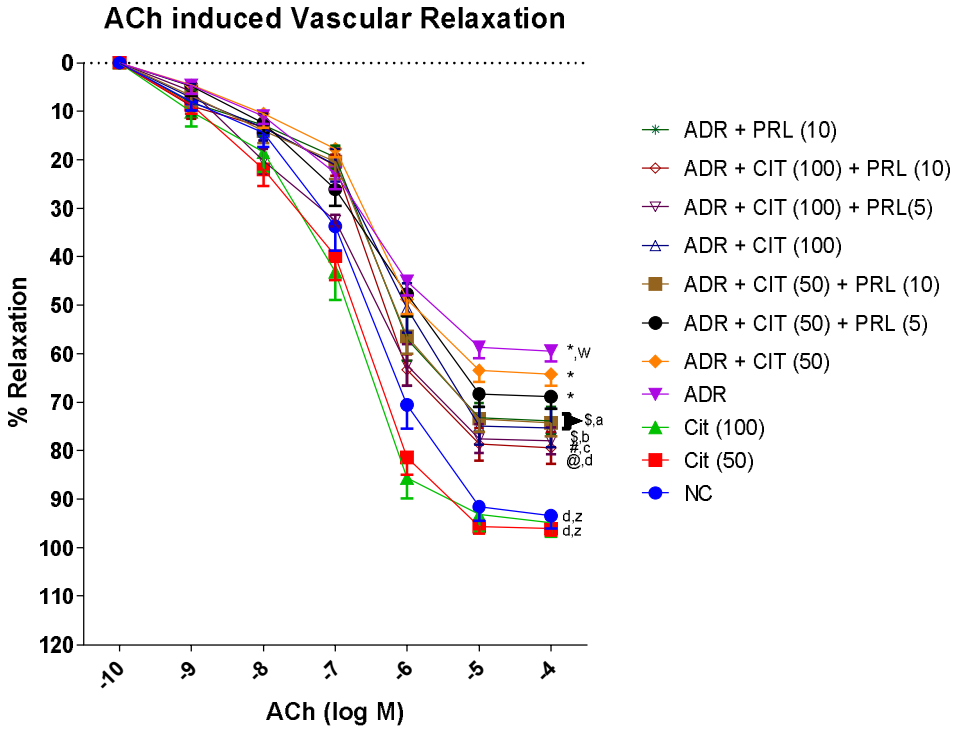

Figure 3. Effect of CIT (50 or 100 mg/kg) administration with or without PRL (5 OR 10 mg/kg) combination in different groups of ADR-induced hypertensive rat model on the mean (log M) Ach concentration-response curves in isolated rat artery.
Data are shown as mean± SEM (n=6 per group). Significant differences from the Vehicle Control group [NC] are indicated by (@) p<0.05, (#) p<0.01, ($) p<0.005, and (*) p<0.001. Significant differences from only ADR-treated group are indicated by (a) p<0.05, (b) p<0.01, (c) p<0.005, and (d) p<0.001. Significant differences from only Standard control group [ADR + PRL (10)] are indicated by (w) p<0.05, (x) p<0.01, (y) p<0.005, and (z) p<0.001.

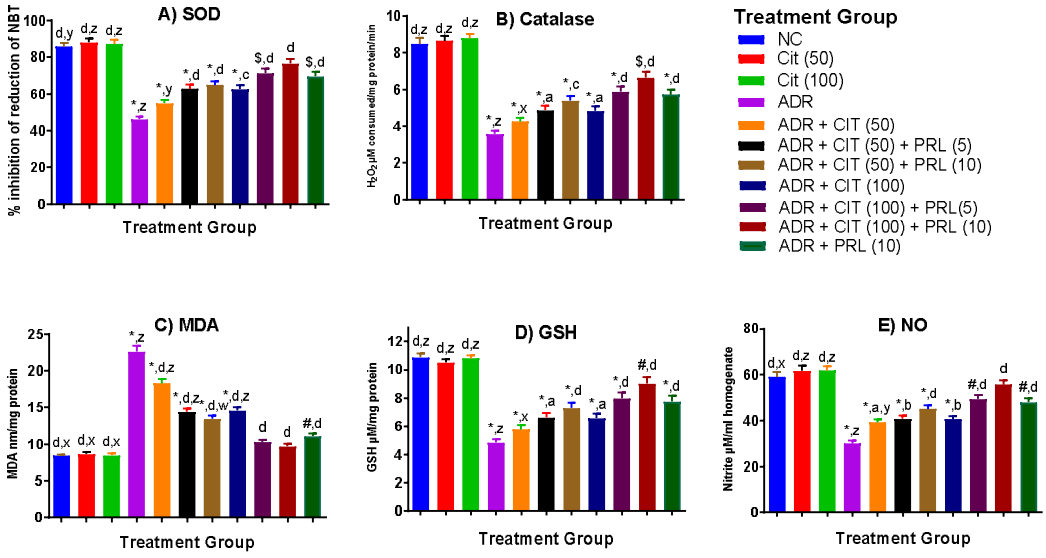

Figure 4. Effect of CIT (50 or 100 mg/kg) administration with or without PRL (5 or 10 mg/kg) combination in different groups of ADR-induced hypertensive rat model on levels of oxidative stress parameters in heart specimen.
A: SOD levels, B: Catalase, C: MDA levels, D: GSH levels, E: Nitric Oxide levels. Data are shown as mean± SEM (n=6 per group). Significant differences from the Vehicle Control group [NC] are indicated by (@) p<0.05, (#) p<0.01, ($) p<0.005, and (*) p<0.001. Significant differences from only ADR-treated group are indicated by (a) p<0.05, (b) p<0.01, (c) p<0.005, and (d) p<0.001. Significant differences from only Standard control group [ADR + PRL (10)] are indicated by (w) p<0.05, (x) p<0.01, (y) p<0.005, and (z) p<0.001.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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