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Ameliorative Effect of Hydroethanolic Leaf Extract of *Byrsocarpus coccineus* in Alcohol- and Sucrose-Induced Hypertension in Rats

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ABSTRACT

Hypertension remains a major health problem worldwide considering the prevalence of morbidity and mortality. Plants remain a reliable source of efficacious and better tolerated drugs and botanicals. This study was designed to investigate the effect of the chemo-profiled hydroethanolic leaf extract of *Byrsocarpus coccineus* in ethanol- and sucrose-induced hypertension. Groups of rats were treated orally (p.o.) with distilled water (10 ml/kg), ethanol (35%; 3 g/kg), sucrose (5-7%), and *B. coccineus* (100, 200, and 400 mg/kg), and nifedipine together with ethanol and sucrose separately for 8 weeks. At the end of the treatment period, blood pressure and heart rate of rats were determined. Blood was collected for serum biochemical parameters and lipid profile assessment, and the liver, aorta, kidney, and heart were harvested for estimation of *in vivo* antioxidants and malondialdehyde (MDA). Results obtained in this study showed that *B. coccineus* at the various doses administered reduced the systolic, diastolic, and arterial blood pressure elevated by ethanol and sucrose. Also, the extract reversed the reduction in catalase (CAT), reduced glutathione (GSH), glutathione peroxidase (GPx), and superoxide dismutase (SOD) induced by ethanol and sucrose. The level of MDA was reduced compared to the ethanol- and sucrose-induced hypertensive group. With respect to lipid profile, administration of *B. coccineus* at the various doses reduced the levels of triglycerides, low-density lipoprotein (LDL), cholesterol, and atherogenic indices, compared to the ethanol and sucrose groups. In conclusion the hydroethanolic leaf extract of *B. coccineus* exerted significant antihypertensive effect and this is probably related to the antioxidant property and improvement of lipid profile observed in this study.

Key words: Alcohol, Antioxidant, *Byrsocarpus coccineus*, Connaraceae, Hypertension, Sucrose

INTRODUCTION

Hypertension is one of the primary risk factors for cardiovascular diseases, including cerebrovascular accident or stroke,[1] and a major cause of disability and death.[2] The leading causes of mortality worldwide are ischemic heart disease and cerebrovascular accident or stroke,[3] and suboptimal blood pressure (BP) control has been identified as the third ranked factor for disability-adjusted life years.[4] According to Pater,[5] changes in definition and classification of BP levels make hypertension the most commonly

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diagnosed condition in primary and secondary healthcare systems and projects the entity at the first place in terms of workload and prescribing cost. As of the year 2000, 972 million people were living with hypertension worldwide,[6] with the overall prevalence being 26.4% of the world's population (26.6% male and 26.1% female).[7] It is estimated that this number will rise to 1.56 billion by the year 2025.[6] According to Kearney *et al*.,[7] the estimated number of hypertensives in developing countries outweighed that of developed countries by almost twofold (639 million in developing countries vs. 333 million in developed countries). Chockalingam *et al*.[6] reported that about two-thirds of hypertensives live in low- and middle-income countries, resulting in a huge economic burden. Despite the disturbing facts, studies have shown that early diagnosis and treatment of hypertension leads to better quality of life and increased longevity.[8] Conventional drugs used in the treatment of hypertension, either to reduce cardiac output or lower peripheral resistance, exhibit deleterious side effects including dry cough, hyperkalemia, angioneurotic edema [angiotensin converting enzyme (ACE) inhibitors], symptomatic hypotension, angio-edema (angiotensin II receptor blockers), central nervous system (CNS) side effects, hypotension, sexual dysfunction, disturbance of lipid metabolism, rebound hypertension (β-blockers), ankle swelling, headaches, flushing (calcium channel blockers), hypokalemia, hyperuricemia, and hyperglycemia (thiazide diuretics).[9] The search for new drugs, especially from natural products and majorly plants, is geared toward the development of more efficacious and better tolerated drugs.

Byrsocarpus coccineus Schum. and Thonn. (Connaraceae) is a shrub or liane of savanna thickets found across West Africa, which grows in the wild and is cultivated.[10] The plant, commonly called "crimson thyme," is known locally in Nigeria as "Tsaamiyar-kasa" (Hausa), "Oke-abolo" (Igbo), "Onyankpe-chi" (Idoma), Anune-chigh (Tiv), and "Orikoteni" (Yoruba). Preparations of the leaves, roots, and whole plant are used for the treatment of earache, jaundice, and venereal diseases, as a sedative,[11] and for treating diarrhea, inflammation,[12] urogenital diseases,[13] and urinary problems.[10] Extracts of the plant have been reported to possess molluscidal,[14] uterotonic,[15] *in vitro* antioxidant,[16] antimicrobial,[17,18] anti-inflammatory,[19] hepaprotective and *in vivo* antioxidant,[20] and *in vitro* biological[21] activities.

This study was designed to investigate the effect of the chemo-profiled hydroethanolic leaf extract of *B. coccineus* on alcohol- and sucrose-induced hypertension in rats based on the reported antioxidant activity of the extract and the involvement of oxidative stress in the pathogenesis of hypertension.

MATERIALS AND METHODS

Plant material and extraction

The fresh leaves of *B. coccineus* were purchased from Oja-Oba Market in Mushin LGA of Lagos State, Nigeria, in April 2011. Botanical identification and authentication was done by Prof. J. D. Olowokudejo of the Department of Botany and Microbiology, Faculty of Science, University of Lagos, Lagos, Nigeria and Mr. T. K. Odewo, a former Senior Superintendent of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. Voucher specimen (FHI 106623) was deposited in the herbarium of the institute.

The pulverized air-dried leaves of *B. coccineus* were macerated in hydroethanol solution (1:1; 100 g/1500 ml). The liquid was decanted after 48 h and the filtrate was concentrated to dryness at 40°C under reduced pressure, giving a dark brown solid with a yield of 8.67%. The brownish dried extract was weighed and stored in an air-tight sample bottle in the refrigerator at 4°C. The extract was reconstituted in distilled water to achieve the desired working concentrations just before administration to experimental animals.

Experimental animals

Albino mice and rats of either sex, weighing on average 15 g and 150 g, respectively, were obtained from the Laboratory Animal Centre of the College of Medicine, University of Lagos. The experimental animals were kept in well-ventilated hygienic compartments maintained under standard environmental conditions (23°C-25ºC, 12 h/12 h light/dark cycle) and were acclimatized for 2 weeks before the start of experimental procedures. The animals were fed with standard rodent diet (Livestock Feed PLC, Lagos, Nigeria) and water *ad libitum*. The experimental procedures adopted were in accordance with the provisions of the Experimentation Ethics Committee on Animal Use of the College of Medicine, University of Lagos and the United States National Academy of Sciences Guide for the Care and Use of Laboratory Animals.[22]

Phytochemical screening and high-performance liquid chromatography fingerprint analysis

Phytochemical screening, consisting of simple chemical tests to detect the presence of phytochemicals in the hydroethanolic leaf extract of *B. coccineus*, was carried out according to the methods of Edeoga *et al*.[23]

Compositional analysis of the extract was done by high-performance liquid chromatography (HPLC) using flavonoids as markers. A solution of 22.8 mg/2 ml MeOH: H_2O (1:1) extract was prepared, filtered through a 0.45-μm membrane filter, and injected into the HPLC column (RP-18, 5 μm). The injected volume was separately 20 and 40 μl (to give two separate HPLC chromatograms of the extract), flow rate was 0.8 ml/min, mobile gradient was 0.05% trifluoroacetic acid (TFA) in acetonitrile (ACN):0.05% TFA in water (gradient), and the run time was 70 min. The detection wavelength was 210-400 nm and the column temperature was 28°C-35ºC.

The Fourier transform infrared (FTIR) spectra were studied under IR region 4000-400 cm^{-1} (using KBr) for the identification of functional groups in different classes of compounds present in the samples.

Acute toxicity study

A set of mice were divided into five groups of five animals each. The animals were fasted (food was withheld, but not water) for 12 h prior to the commencement of the experiment. The mice were treated with graded doses (50, 100, 200, 400, and 800 mg/kg) of the extract by intraperitoneal (i.p.) administration. Another set of animals was treated with graded doses of the extract up to 10 g/kg in divided doses by oral (p.o.) route. The control group received distilled water (10 ml/kg). All experimental animals were closely observed for manifestation of toxic symptoms and behav-

ioral changes for 2 h post-administration. Mortality in each group within 24 h was recorded and the surviving mice were observed for a further 14 days for signs of delayed toxicity. The LD_{50} was estimated using the log dose-probit analysis method.[24]

Alcohol- and sucrose-induced hypertension

Rats were divided into 11 groups of six animals each and were treated p.o. as outlined below:

- Group 1: Distilled water 10 ml/kg (normal control)
- Group 2: 35% ethanol (3 g/kg/day; hypertensive control for the ethanol model)
- Group 3: 35% ethanol (3 g/kg/day) + *B. coccineus* 100 mg/kg
- Group 4: 35% ethanol (3 g/kg/day) + *B. coccineus* 200 mg/kg
- Group 5: 35% ethanol (3 g/kg/day) + *B. coccineus* 400 mg/kg
- Group 6: 35% ethanol (3 g/kg/day) + nifedipine 10 mg/kg (standard group for the ethanol model)
- Group 7: 5% sucrose for 4 weeks, 6% for the next 2 weeks, and 7% for the last 2 weeks (hypertensive control for the sucrose model)
- Group 8: Sucrose (5-7%) + *B. coccineus* 100 mg/kg
- Group 9: Sucrose (5-7%) + *B. coccineus* 200 mg/kg
- Group 10: Sucrose (5-7%) + *B. coccineus* 400 mg/kg
- Group 11: Sucrose (5-7%) + nifedipine 10 mg/kg (standard group for the sucrose model). Treatments were administered for 8 weeks as outlined above and the animals were weighed every week.[25] At the end of the treatment period of 8 weeks, rats were weighed and anesthetized by i.p. injection of urethane (1.5 g/kg). The trachea was exposed and cannulated to ensure free and normal respiration. The femoral artery was exposed by dissection, and cannulated and flushed with 1 ml of heparinized saline. The cannulated femoral artery was used for the recording of BP and heart rate (HR) by connecting the arterial cannula to a calibrated Statham strain-gauge transducer connected to a polygraph (Grass Polygraph E and M, Harvard Physiograph). After the determination of BP and HR, blood was withdrawn from each animal into plain sample bottles by cardiac puncture. Blood samples were centrifuged at 3000 rpm for 5 min and the serum was collected using sterile pipettes. Serum levels of total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides (TG), urea, bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were estimated using Roche and Cobas commercial kits and Roche/Hitachi 904 automated analyzer. After blood collection, the animals were sacrificed by cervical dislocation, dissected, and the heart, aorta, liver, and kidneys of each rat were harvested, homogenized, and appropriately treated for the determination of the levels of tissue protein,[26] catalase (CAT), reduced glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GPx), and malondialdehyde (MDA).[27]

Atherogenic indices were calculated using the method of Ikewuchi:[28]

Cardiac risk ratio (CRR) = total cholesterol ÷ HDL cholesterol

Atherogenic coefficient (AC) = (total cholesterol − HDL cholesterol) ÷ HDL cholesterol

Atherogenic index (AI) = log (TG ÷ HDL cholesterol)

Statistical analysis

Results are expressed as Mean ± standard error of mean (SEM) ($n = 6$). The data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test using GraphPad Prism 5 software (GraphPad Software Inc., La Jolla, CA, USA). Results were considered significant when $P < 0.05$.

RESULTS

Phytochemical screening and HPLC fingerprint analysis

Preliminary phytochemical analysis revealed the presence of alkaloids, tannins, saponins, and cardiac glycosides.

The fingerprint chromatogram of the hydroethanolic extract of *B. coccineus* was established with reference to chlorogenic acid, daidzin, rutin, and quercetin [Figure 1a]. As shown in Figure 1b and c, the presence of chlorogenic acid, rutin, and quercetin was established in the extract. The relative amount of the flavonoids in *B. coccineus* was 1081.50, 1202.93, and 83.11 ng for chlorogenic acid, rutin and quercetin, respectively (sample concentration: 22.8 mg/2 ml, injection volume: 40 µl).

The strong absorption band observed at 3500-3000 cm^{-1} may be due to the presence of bonded O–H and/or N–H of alcohol or amine/amide, respectively [Figure 2].

In general, the extract is suggested to contain alcohols, amines/amide, as well as compounds with conjugated double bond such as flavonoids, terpenes, or coumarins.

Acute toxicity

The hydroethanolic leaf extract of *B. coccineus* did not produce any mortality when administered p.o. up to 10 g/kg. No visible signs of delayed toxicity and mortality were observed when the animals were monitored for a further 14 days. In respect of the i.p. route, no deaths were recorded at the lowest dose of 50 mg/kg while mortality was 100% at the highest dose of 800 mg/kg. At the higher doses of 400 mg/kg and 800 mg/kg, animals manifested piloerection, sedation, and writhing. The LD_{50} for the i.p. route was estimated to be 288.40 mg/kg.

Alcohol- and sucrose-induced hypertension

Effect of B. coccineus on BP and HR

Eight weeks of ethanol administration (35%; 3 g/kg/day, p.o.) caused significant increase in systolic BP (37.56%, $P < 0.001$), diastolic BP (51.52%, $P < 0.001$), and HR (20%, $P < 0.05$), compared to normal control. *B. coccineus* at different doses caused significant reduction in systolic and diastolic BP relative to the ethanol hypertensive control group. The dose of 100 mg/kg produced 47.79, 58.61, 50.63, and 22.86% reduction in systolic BP, diastolic BP ($P < 0.001$), mean arterial pressure (MAP), and HR ($P < 0.01$), respectively, compared to the ethanol hypertensive control group. Reduction in these values was significantly higher ($P < 0.001$) than the 20.56% ($P < 0.001$) and 23.34% ($P < 0.01$) reduction elicited by nifedipine for systolic and diastolic BP, respectively [Table 1].

With regard to the sucrose-induced hypertension model, 8 weeks of sucrose administration (5-7% w/v in drinking water) also caused significant increase in systolic (44.48%, $P < 0.05$) and

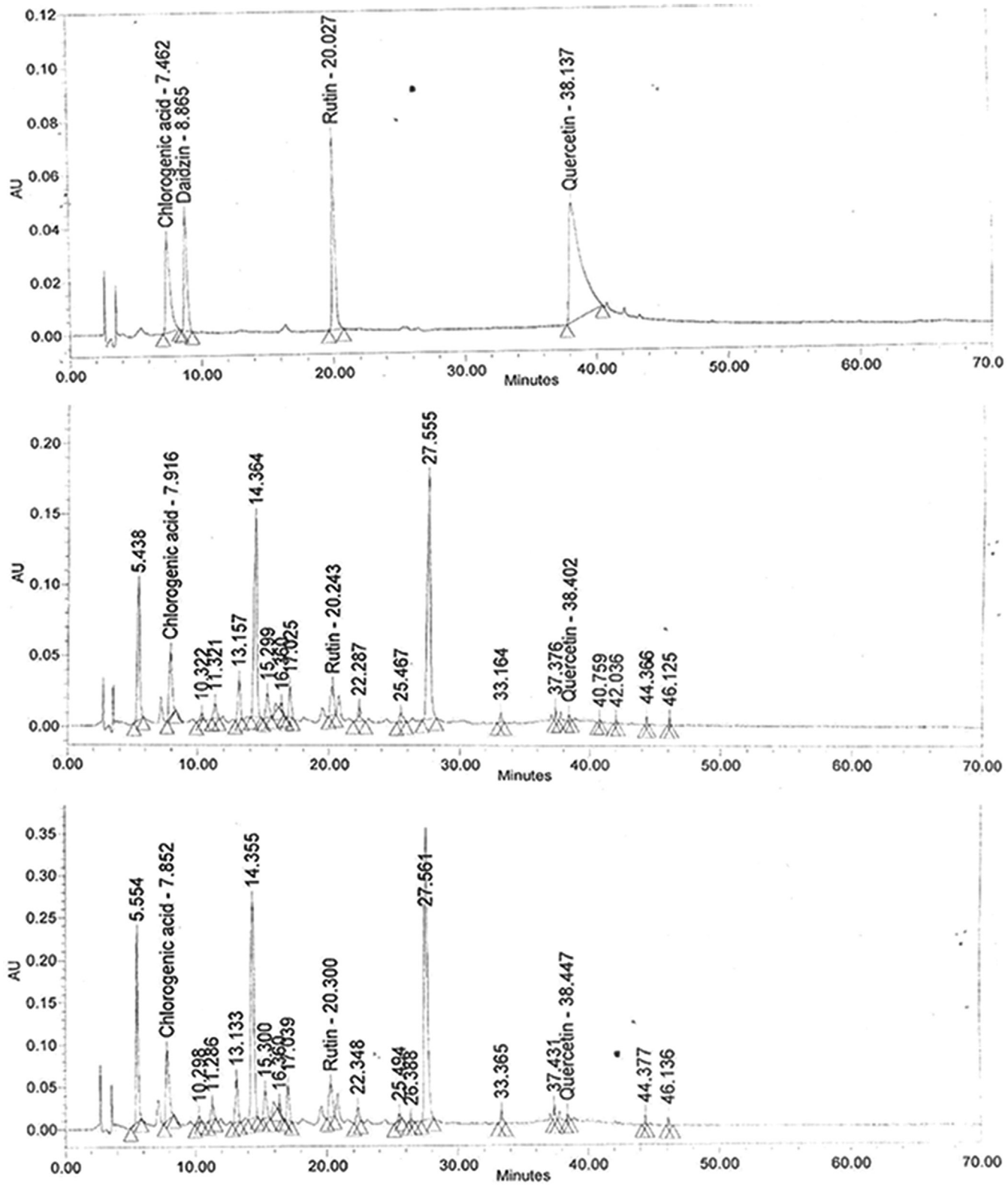

Figure 1. (a) HPLC chromatogram of standard flavonoids; hydroethanolic extract of *B. coccineus* (b) 20 µl and (c) 40 µl injection volumes

diastolic (52.90%, $P < 0.001$) BP, compared to the normal control group. The extract at the various doses caused significant reduction in systolic BP, diastolic BP, and MAP, compared to the sucrose hypertensive control group. Pronounced effect was observed at the dose of 100 mg/kg for systolic BP (42.71%, $P < 0.001$), diastolic BP (47.06%, $P < 0.001$), and MAP (42.78%, $P < 0.01$), compared to the sucrose hypertensive control group. The effect of the extract at the dose of 100 mg/kg was comparable and not significantly

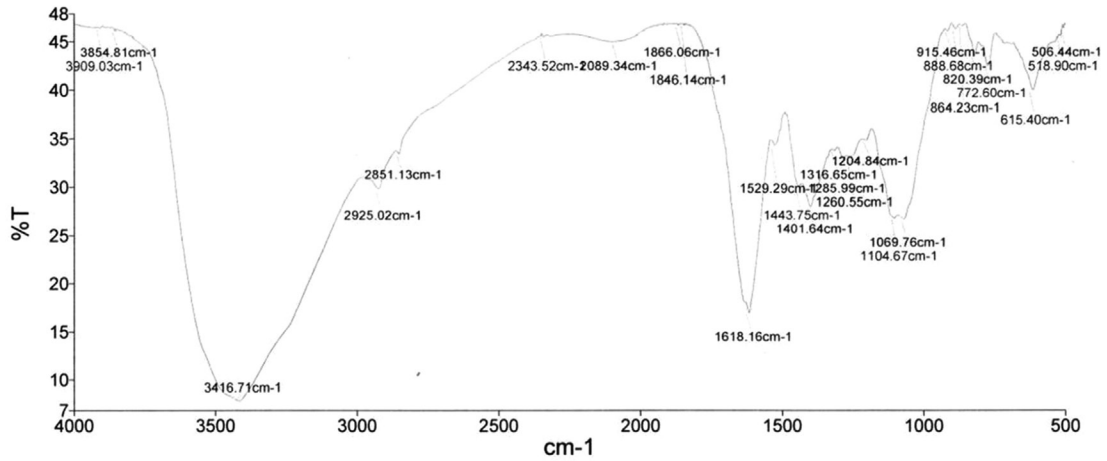

Figure 2. FTIR spectra of hydroethanolic extract of *B. coccineus* ran under IR region 4000-400 cm−1 (using KBr)

different ($P > 0.05$) from the effect of nifedipine which elicited 33.93, 45.80 ($P < 0.001$), and 41.50% ($P < 0.01$) reduction in systolic BP, diastolic BP, and MAP, respectively, compared to the sucrose hypertensive control group [Table 1].

Effect of B. coccineus on antioxidant indices and MDA level in liver, kidney, heart, and aorta tissues

In the liver tissues, administration of ethanol (35%; 3 g/kg/day) for 8 weeks caused significant reduction ($P < 0.01$, 0.001) in the levels of CAT, GSH, SOD, and GPx and significant increase ($P < 0.001$) in the level of MDA compared to the normal control group. *B. coccineus*, at doses of 100, 200, and 400 mg/kg, and nifedipine reversed the effect of ethanol administration by causing significant increase ($P < 0.05$, 0.01, and 0.001, respectively) in the levels of CAT, GSH, SOD, GPx, and protein and significant reduction ($P < 0.05$, 0.01, and 0.001) in the level of MDA relative to the ethanol hypertensive group. Pronounced effects of the extract were observed at the dose of 400 mg/kg, except in the case of GSH and protein in which marked increments were observed at doses of 100 and 200 mg/kg, respectively. These effects were comparable and not significantly different ($P > 0.05$) from the effects of nifedipine [Table 2]. Sucrose (5-7% w/v), administered for 8 weeks, caused significant reduction ($P < 0.05$, 0.01) in the levels of CAT, GSH, and GPx and significant increase ($P < 0.001$) in the level of MDA in liver tissues compared to the normal control group. The extract at the dose of 100 mg/kg significantly reduced ($P < 0.001$) the level of MDA and increased ($P < 0.001$) the level of protein relative to the sucrose group, while at the dose of 200 mg/kg, the extract significantly reversed the effect of sub-chronic administration of sucrose by increasing the levels of CAT, GPx, and protein ($P < 0.05$, 0.01) and reducing the level of MDA ($P < 0.001$) compared to the sucrose group. At the dose of 400 mg/kg, the extract significantly increased the level of CAT ($P < 0.01$) and reduced the level of MDA ($P < 0.001$) relative to the sucrose group, as was also observed with nifedipine which further significantly increased the level of protein ($P < 0.05$). These effects were comparable and not significantly different ($P > 0.05$) from the effects of nifedipine, as was the case in the ethanol model [Table 2].

With regard to kidney tissues, administration of ethanol caused significant reduction ($P < 0.001$) in the levels of CAT, SOD, and GPx and increase in the level of MDA ($P < 0.001$) compared to the normal control group. *B. coccineus* (100, 200, and 400 mg/kg) reversed the diminution in parameters produced by ethanol by causing significant increase ($P < 0.05$, 0.01, and 0.001, respectively) in the levels of CAT, GSH, SOD, GPx, and protein and reduction in the level of MDA ($P < 0.001$) compared to the ethanol hypertensive group. The extract at the dose of 100 mg/kg additionally caused significant increase ($P < 0.05$) in the level of protein compared to the ethanol group. Nifedipine also elicited significant increase ($P < 0.01$, 0.001) in the levels of CAT, GSH, SOD, and GPx and reduction in the level of MDA ($P < 0.001$) relative to the ethanol hypertensive control group. Marked effects were generally observed with the extract at the highest dose of 400 mg/kg and these effects were comparable and not significantly different ($P > 0.05$) from those of nifedipine [Table 3]. As shown in Table 3, administration of sucrose elicited significant reduction ($P < 0.05$, 0.001) in the levels of CAT, GSH, SOD, and GPx and increase in the level of MDA ($P < 0.001$) compared to the normal control group. *B. coccineus* at the dose of 100 mg/kg produced significant increase in the levels of CAT and GPx ($P < 0.05$ and 0.001, respectively) and reduction in the level of MDA ($P < 0.001$), while at the dose of 200 mg/kg, the extract caused significant increase in the levels of GPx and protein ($P < 0.001$) and reduction in the level of MDA ($P < 0.001$) compared to the ethanol group. At the dose of 400 mg/kg, the extract significantly increased the level of GPx ($P < 0.001$) and reduced the level of MDA ($P < 0.001$) relative to the ethanol group. Nifedipine significantly increased the levels of CAT, SOD, and GPx ($P < 0.05$, 0.001) and reduced the level of MDA ($P < 0.001$) compared to the ethanol hypertensive group. Pronounced effects were produced on CAT and MDA at the dose of 100 mg/kg, with these effects being significantly less ($P < 0.001$) and comparable ($P > 0.05$) relative to nifedipine, respectively. With respect to GPx and protein, pronounced effects comparable and not significantly different from those of nifedipine were produced at a dose of 200 mg/kg of the extract.

With respect to the heart tissues, significant reduction ($P < 0.001$) was observed in the levels of CAT, GSH, GPx, and

Table 1. Effect of *B. coccineus hydroethanolic* leaf extract (BC) on blood pressure and heart rate in ethanol- and sucrose-induced hypertension in rats

Parameters	Normal control	Ethanol 3 g/kg/day (%)	BC 100 mg/kg+ Ethanol (%)	BC 200 mg/kg+ Ethanol (%)	BC 400 mg/kg+ Ethanol (%)	Nifedipine 10 mg/kg+ Ethanol (%)	Sucrose 5-7% w/v (%)	BC 100 mg/kg+ Sucrose (%)	BC 200 mg/kg+ Sucrose (%)	BC 400 mg/kg+ Sucrose (%)	Nifedipine 10 mg/kg+ Sucrose (%)
SBP (mmHg)	96.90±4.06	133.30±5.64^{c} (37.56)	69.60±4.10$^{b,\gamma,***}$ (47.79)	90.50±4.56$^{\gamma}$ (32.11)	116.80±3.45^{a} (12.38)	105.90±5.86$^{\gamma}$ (20.56)	140.00±3.96^{a} (44.48)	80.20±2.80$^{\gamma}$ (42.71)	82.70±4.60$^{\gamma}$ (40.93)	89.40±1.20$^{\gamma}$ (36.14)	92.50±7.57$^{\gamma}$ (33.93)
DBP (mmHg)	72.40±4.19	109.70±4.96^{c} (51.52)	45.40±3.46$^{b,\gamma,***}$ (58.61)	68.90±5.22$^{\gamma}$ (37.19)	99.10±3.74^{b} (9.66)	84.10±6.47$^{\beta}$ (23.34)	110.70±3.46^{c} (52.90)	58.60±3.33$^{\gamma}$ (47.06)	60.00±4.98$^{\gamma}$ (45.80)	68.20±1.31$^{\gamma}$ (38.39)	60.00±7.65$^{\gamma}$ (45.80)
MAP (mmHg)	96.22±9.73	108.30±11.48 (12.55)	53.47±7.81$^{a,\beta}$ (50.63)	76.10±10.37 (29.73)	98.00±9.68 (9.51)	85.17±14.89 (21.36)	115.00±8.14 (19.52)	65.80±5.42$^{\beta}$ (42.78)	67.50±10.28$^{\beta}$ (41.30)	75.47±2.40$^{\alpha}$ (34.37)	67.28±9.47$^{\beta}$ (41.50)
HR (beat/min.)	350.00±18.44	420.00±0.00^{a} (20.00)	324.00±14.70$^{\beta}$ (22.86)	360.00±0.00 (14.29)	372.00±22.45 (11.43)	372.00±22.45 (11.43)	400.00±12.65 (14.29)	372.00±12.00 (7.00)	360.00±18.97 (10.00)	324.00±30.59 (19.00)	340.00±25.30 (15.00)

Values are mean±S.E.M. (n=5-6). ^{a}P<0.05, ^{b}P<0.01, ^{c}P<0.001 vs. normal control; $^{\alpha}P$<0.05, $^{\beta}P$<0.01, $^{\gamma}P$<0.001 vs. ethanol/sucrose; ***P<0.001 vs. nifedipine (One-way ANOVA followed by Tukey's multiple comparison test)

Table 2. Effect of *B. coccineus hydroethanolic* leaf extract (BC) on liver level of antioxidants and malondialdehyde in ethanol- and sucrose-induced hypertension in rats

Group	Ethanol model						Sucrose model					
	CAT (U/mg protein)	GSH (U/mg protein)	SOD (U/mg protein)	GPx (U/mg protein)	MDA (U/mg protein)	Protein (mg)	CAT (U/mg protein)	GSH (U/mg protein)	SOD (U/mg protein)	GPx (U/mg protein)	MDA (U/mg protein)	Protein (mg)
Normal control	30.59±0.77	1.26±0.30	4.77±0.14	1.57±0.12	0.008±0.001	43.46±1.33	30.59±0.77	1.59±0.49	4.77±0.14	1.57±0.12	0.008±0.001	43.46±1.33
Ethanol/Sucrose	9.56±1.46b	0.24±0.06^{b}	0.89±0.17^{c}	0.38±0.12^{c}	0.420±0.104^{c}	29.41±2.22	11.10±1.73^{b}	0.18±0.01^{a}	2.81±0.09	0.52±0.19^{b}	0.205±0.036^{c}	30.29±0.59
BC 100 mg/kg+Toxicant	20.87±4.73	1.62±0.31$^{\gamma}$	3.03±0.82*	1.01±0.20$^{\alpha}$	0.016±0.004$^{\gamma}$	55.74±7.39$^{\gamma}$	24.56±3.86	0.85±0.15	3.63±0.74	1.14±0.26	0.008±0.001$^{\gamma}$	66.16±8.22$^{a,\gamma}$
BC 200 mg/kg+Toxicant	20.03±4.26	1.48±0.04$^{\gamma}$	2.76±0.63**	1.06±0.19$^{\alpha}$	0.014±0.001$^{\gamma}$	61.45±4.28$^{\gamma,a}$	28.82±4.15$^{\beta}$	0.55±0.03	4.47±0.56	1.35±0.13$^{\alpha}$	0.013±0.001$^{\gamma}$	60.45±6.89$^{\beta}$
BC 400 mg/kg+Toxicant	34.39±1.19$^{\gamma}$	1.35±0.01$^{\beta}$	4.36±0.08$^{\gamma}$	1.18±0.06$^{\beta}$	0.012±0.001$^{\gamma}$	46.82±0.67$^{\alpha}$	28.84±1.70$^{\beta}$	1.23±0.46	3.56±0.74	1.18±0.26	0.014±0.001$^{\gamma}$	48.44±2.33
Nifedipine 10 mg/kg+Toxicant	35.64±0.04$^{\gamma}$	1.44±0.04$^{\gamma}$	5.65±0.71$^{\gamma}$	1.33±0.04$^{\gamma}$	0.012±0.001$^{\gamma}$	48.82±0.37$^{\beta}$	28.62±4.66$^{\beta}$	1.37±0.34	4.84±0.81	1.13±0.06	0.009±0.003$^{\gamma}$	52.10±4.61$^{\alpha}$

Values are mean±S.E.M. (n=5). ^{a}P<0.05, ^{b}P<0.01, ^{c}P<0.001 vs. control; $^{\alpha}P$<0.05, $^{\beta}P$<0.01, $^{\gamma}P$<0.001 vs. ethanol/sucrose; *P<0.05, **P<0.01 vs. nifedipine (One-way ANOVA followed by Tukey's multiple comparison test), CAT: Catalase; GSH: Reduced glutathione; SOD: Superoxide dismutase; GPx: Glutathione peroxidase; MDA: Malondialdehyde

Table 3. Effect of *B. coccineus hydroethanolic* leaf extract (BC) on kidney level of antioxidants and malondialdehyde in ethanol- and sucrose-induced hypertension in rats

Group	Ethanol model						Sucrose model					
	CAT (U/mg protein)	GSH (U/mg protein)	SOD (U/mg protein)	GPx (U/mg protein)	MDA (U/mg protein)	Protein (mg)	CAT (U/mg protein)	GSH (U/mg protein)	SOD (U/mg protein)	GPx (U/mg protein)	MDA (U/mg protein)	Protein (mg)
Normal control	33.09±2.21	0.84±0.21	4.04±0.66	1.52±0.10	0.006±0.001	46.41±1.08	33.09±2.21	1.68±0.44	4.04±0.68	1.52±0.10	0.006±0.001	46.41±1.07
Ethanol/Sucrose	6.36±1.11^{c}	0.14±0.06	0.76±0.11^{c}	0.06±0.01^{c}	0.074±0.008^{c}	33.86±1.20	9.95±0.07^{c}	0.04±0.01^{a}	0.68±0.08^{a}	0.60±0.09^{c}	0.048±0.003^{c}	32.74±0.57
BC 100 mg/kg+Toxicant	24.67±2.64$^{\gamma,*}$	0.48±0.14	3.72±0.42$^{\gamma}$	1.36±0.08$^{\gamma}$	0.015±0.002$^{\gamma}$	56.19±6.53$^{\alpha}$	23.77±2.93$^{\alpha,***}$	1.36±0.43	3.06±0.72	1.44±0.04$^{\gamma}$	0.011±0.002$^{\gamma}$	45.97±0.84
BC 200 mg/kg+Toxicant	21.37±2.49$^{a,\beta,**}$	1.17±0.34$^{\beta}$	1.73±0.25^{a,**}	1.38±0.06$^{\gamma}$	0.044±0.019^{a}	46.47±8.12	17.76±3.59^{b,***}	0.82±0.22	2.43±0.58	1.57±0.07$^{\gamma}$	0.017±0.001$^{b,\gamma,*}$	82.70±13.12$^{b,\gamma}$
BC 400 mg/kg+Toxicant	37.91±3.13$^{\gamma}$	1.16±0.01$^{\beta}$	4.95±0.72$^{\gamma}$	1.39±0.08$^{\gamma}$	0.013±0.001$^{\gamma}$	45.40±0.44	20.07±3.82^{a,***}	1.13±0.33	3.07±0.61	1.39±0.09$^{\gamma}$	0.018±0.002$^{b,\gamma,*}$	52.82±2.60
Nifedipine 10 mg/kg+Toxicant	37.76±3.81$^{\gamma}$	1.10±0.02$^{\beta}$	4.32±0.12$^{\gamma}$	1.70±0.22$^{\gamma}$	0.012±0.001$^{\gamma}$	41.69±0.22	43.32±3.18$^{\gamma}$	0.60±0.27	4.04±1.04$^{\alpha}$	1.58±0.04$^{\gamma}$	0.007±0.001$^{\gamma}$	57.58±9.74

Values are mean±S.E.M. (n=5). ^{a}P<0.05, ^{b}P<0.01, ^{c}P<0.001 vs. control; $^{\alpha}P$<0.05, $^{\beta}P$<0.01, $^{\gamma}P$<0.001 vs. ethanol/sucrose; *P<0.05, **P<0.01 vs. nifedipine (One-way ANOVA followed by Tukey's multiple comparison test), CAT: Catalase; GSH: Reduced glutathione; SOD: Superoxide dismutase; GPx: Glutathione peroxidase; MDA: Malondialdehyde

protein and increase was observed in the level of MDA ($P < 0.001$) in the ethanol hypertensive control group compared to the normal control rats. *B. coccineus* at doses of 100, 200, and 400 mg/kg and nifedipine elicited significant reduction in MDA and increase in GSH and protein levels ($P < 0.001$) compared to the ethanol group. In addition, the extract at the dose of 100 mg/kg significantly increased the level of SOD ($P < 0.001$) while there was a significant increase in the level of CAT ($P < 0.001$) at the dose of 400 mg/kg, compared to the ethanol group. Peak effects of the extract were produced at the dose of 100 mg/kg with respect to SOD, MDA, and protein and at the dose of 400 mg/kg for CAT and GSH. These effects of the extract were comparable and not significantly different ($P > 0.05$) from those of nifedipine, except in the case of SOD which was significantly higher ($P < 0.001$) [Table 4]. As presented in Table 4, sucrose significantly reduced ($P < 0.05$, 0.001) the levels of CAT, GSH, SOD, GPx, and protein and increased the level of MDA ($P < 0.001$) relative to the normal control group. *B. coccineus* at doses of 100, 200, and 400 mg/kg and nifedipine significantly increased ($P < 0.01$, 0.001) the levels of GPx and protein and reduced the level of MDA ($P < 0.01$, 0.001) compared to the sucrose hypertensive group. In addition, the extract at the dose of 400 mg/kg significantly increased the level of GSH ($P < 0.05$) while nifedipine further significantly increased the level of CAT, GSH, and SOD ($P < 0.05$, 0.001) relative to the sucrose group. Pronounced effects were produced in the levels of MDA and protein at the extract dose of 200 mg/kg and in the levels of GSH and GPx at the extract dose of 400 mg/kg. The effect of the extract was significantly higher on the level of MDA ($P < 0.01$) and significantly lower ($P < 0.001$) on the level of GPx compared to nifedipine.

Considering the aorta, ethanol elicited significant ($P < 0.01$, 0.001) reduction in the levels of CAT, SOD, and protein while causing increase in MDA compared to the normal control group. *B. coccineus* (100, 200, and 400 mg/kg) and nifedipine reversed the effect of ethanol by causing significant increase ($P < 0.05$, 0.01, 0.001) in the levels of GSH, CAT, SOD, GPx and protein while reducing MDA compared to the ethanol hypertensive control group. Marked effect was obtained with the extract at the dose of 200 mg/kg for CAT, GSH, and SOD, and at the dose 400 mg/kg for GPx, MDA, and protein. The most pronounced effects of the extract were comparable and not significantly different ($P > 0.05$) from those of nifedipine, except for the effect of *B. coccineus* at 200 mg/kg on GSH and at 400 mg/kg on GPx which were significantly higher ($P < 0.01$, 0.001) than the values for nifedipine [Table 5]. Sucrose administration caused significant ($P < 0.001$) reduction in the levels of CAT, SOD, GPx, and protein, while causing increase in MDA compared to the normal control group. *B. coccineus* at doses of 100, 200, and 400 mg/kg caused significant increase ($P < 0.05$, 0.001) in the levels of GSH, GPx, protein, and CAT and reduction in the level MDA compared to the sucrose hypertensive group. Nifedipine caused significant increase ($P < 0.001$) in the levels of GPx and protein and reduction in the level of MDA compared to the sucrose hypertensive group. Marked effects of the extract were observed at 400 mg/kg for CAT and GPx, at 200 mg/kg for GSH and protein, and at 100 mg/kg for MDA. The most pronounced effects of the extract were comparable and not significantly different ($P > 0.05$) from the effects of nifedipine, except at the dose of 400 mg/kg for GPx which was significantly higher ($P < 0.001$) [Table 5].

Effect of B. coccineus on serum biochemical parameters

Eight weeks of administration of ethanol caused significant increase ($P < 0.01$, 0.001) in the levels of albumin, total cholesterol, and LDL compared to the normal control group. *B. coccineus* caused significant reduction in the levels of albumin (100 mg/kg, $P < 0.05$), total cholesterol (100-400 mg/kg, $P < 0.01$, 0.001), LDL (100-400 mg/kg, $P < 0.001$), TG (100 mg/kg, $P < 0.05$), and urea (100 and 200 mg/kg, $P < 0.05$) compared to the ethanol hypertensive group. Nifedipine elicited significant reduction in the levels of albumin ($P < 0.05$), total cholesterol ($P < 0.05$), HDL ($P < 0.05$), and LDL ($P < 0.001$) and increase in AST ($P < 0.05$) compared to the ethanol hypertensive group. The values for albumin (100-400 mg/kg, $P < 0.001$) and TG (100 mg/kg, $P < 0.05$) were significantly lower than the corresponding values for nifedipine [Table 6].

As shown in Table 7, sucrose administration caused significant ($P < 0.05$, 0.01, 0.001) increase in albumin, total cholesterol, LDL, and TG and reduction in protein compared to the normal control group. The extract elicited significant reduction in the levels of total cholesterol (100-400 mg/kg, $P < 0.001$), LDL (100-200 mg/kg, $P < 0.05$), and TG (100-200 mg/kg, $P < 0.05$, 0.01) and increase in protein (100-400 mg/kg, $P < 0.001$) compared to the sucrose hypertensive group. Nifedipine also reversed the effects of sucrose administration by causing significant reduction in the levels of total cholesterol ($P < 0.01$), LDL ($P < 0.05$), and TG ($P < 0.05$) and increase in protein ($P < 0.001$) relative to the sucrose hypertensive group. A significant increase ($P < 0.05$) in the level of AST was observed with nifedipine. The value for albumin at the extract dose of 400 mg/kg was significantly lower ($P < 0.05$), while that for protein at the extract dose of 100 mg/kg was significantly higher ($P < 0.05$) relative to the corresponding values for nifedipine.

Effect of B. coccineus on atherogenic indices

Ethanol increased the values of CRR, AC, and AI (3.19, 2.19, and −0.10, respectively) compared to the normal control group (2.42, 1.42, and − 0.18, respectively). *B. coccineus* at doses of 100, 200, and 400 mg/kg reversed the ethanol-induced increase in atherogenic indices except in respect of AI at the extract dose of 200 mg/kg. Pronounced effect was produced at the lowest dose of 100 mg/kg with values of 1.50, 0.50, and −0.52 for CRR, AC, and AI, respectively. Nifedipine did not reduce the values of the atherogenic indices relative to the ethanol hypertensive group [Figure 3].

Sucrose also increased the values of CRR, AC, and AI (3.87, 2.87, and 0.16, respectively) compared to the normal control group (2.42, 1.42, and −0.18, respectively). The extract at doses of 100 and 200 mg/kg reduced the atherogenic indices values, relative to the sucrose group. The values for CRR, AC, and AI at the dose of 100 mg/kg were 1.48, 0.48, and −0.33, respectively. *B. coccineus* at the dose of 400 mg/kg did not reduce the value of the athero-

Table 4. Effect of *B. coccineus hydroethanolic* leaf extract (BC) on heart level of antioxidants and malondialdehyde in ethanol- and sucrose-induced hypertension in rats

Group	Ethanol model						Sucrose model					
	CAT (U/mg protein)	GSH (U/mg protein)	SOD (U/mg protein)	GPx (U/mg protein)	MDA (U/mg protein)	Protein (mg)	CAT (U/mg protein)	GSH (U/mg protein)	SOD (U/mg protein)	GPx (U/mg protein)	MDA (U/mg protein)	Protein (mg)
Normal control	91.27±5.87	1.48±0.03	13.92±0.78	4.32±0.26	0.006±0.002	45.74±1.27	91.27±5.87	1.48±0.37	13.92±0.78	4.32±0.26	0.006±0.002	45.74±1.27
Ethanol/Sucrose	8.79±0.41$^{c,\gamma}$	0.13±0.05^{c}	1.10±0.13	0.18±0.02^{c}	0.438±0.084^{c}	29.21±2.24^{c}	7.76±1.68^{c}	0.27±0.01^{a}	0.48±0.07^{c}	0.33±0.10^{c}	0.106±0.002^{c}	21.16±3.14^{c}
BC 100 mg/kg+Toxicant	20.65±1.91^{c,***}	1.37±0.11$^{\gamma}$	68.44±8.65$^{c,\gamma,***}$	2.01±0.82^{a}	0.014±0.003$^{\gamma}$	51.28±2.19$^{\gamma}$	34.60±10.56^{c,*}	0.72±0.12	5.68±1.89^{a,*}	1.89±0.18$^{c,\gamma,***}$	0.022±0.001$^{\gamma,*}$	47.47±6.02$^{\gamma}$
BC 200 mg/kg+Toxicant	28.65±8.49^{c,**}	1.11±0.12$^{a,\gamma,*}$	4.36±1.78	2.11±0.68	0.020±0.006$^{\gamma}$	44.21±0.48$^{\gamma}$	35.71±9.33^{c,*}	1.15±0.19	6.78±2.37^{a}	1.57±0.21$^{c,\beta,***}$	0.011±0.001$^{\gamma,**}$	59.40±5.86$^{\gamma}$
BC 400 mg/kg+Toxicant	51.83±3.83$^{c,\gamma}$	1.53±0.03$^{\gamma}$	8.26±0.69	1.18±0.02^{b,*}	0.040±0.026$^{\gamma}$	42.32±0.26$^{\gamma}$	37.55±8.13^{c,*}	1.51±0.39$^{\alpha}$	7.42±2.35	1.81±0.19$^{c,\gamma,***}$	0.016±0.001$^{\gamma,**}$	43.41±0.88$^{\beta}$
Nifedipine 10 mg/kg+Toxicant	59.69±5.89^{b}	1.45±0.03γ	15.37±1.29	3.94±0.68$^{\gamma}$	0.011±0.002$^{\gamma}$	45.02±1.16$^{\gamma}$	68.73±3.42$^{\gamma}$	1.58±0.28$^{\alpha}$	13.22±0.05$^{\gamma}$	3.71±0.32$^{\gamma}$	0.061±0.020$^{c,\beta}$	47.51±0.82$^{\gamma}$

Values are mean±S.E.M. (n=5). ^{a}P<0.05, ^{b}P<0.01, ^{c}P<0.001 vs. control; $^{\alpha}P$<0.05, $^{\beta}P$<0.01, $^{\gamma}P$<0.001 vs. ethanol/sucrose; *P<0.05, **P<0.01 vs. nifedipine (One-way ANOVA followed by Tukey's multiple comparison test), CAT: Catalase; GSH: Reduced glutathione; SOD: Superoxide dismutase; GPx: Glutathione peroxidase; MDA: Malondialdehyde

Table 5. Effect of *B. coccineus hydroethanolic* leaf extract (BC) on aorta level of antioxidants and malondialdehyde in ethanol- and sucrose-induced hypertension in rats

Group	Ethanol model						Sucrose model					
	CAT (U/mg protein)	GSH (U/mg protein)	SOD (U/mg protein)	GPx (U/mg protein)	MDA (U/mg protein)	Protein (mg)	CAT (U/mg protein)	GSH (U/mg protein)	SOD (U/mg protein)	GPx (U/mg protein)	MDA (U/mg protein)	Protein (mg)
Normal control	234.86±19.78	1.94±0.61	47.18±1.82	13.08±0.23	0.025±0.004	4.52±0.05	234.86±19.78	2.28±0.56	47.18±1.82	13.08±0.24	0.025±0.004	4.52±0.05
Ethanol/Sucrose	78.69±5.42^{b}	0.31±0.13	14.88±1.16^{b}	3.46±1.14	0.513±0.098^{c}	1.22±0.24c	83.88±0.49^{c}	0.07±0.00	14.64±0.22^{c}	1.72±0.05^{c}	0.853±0.043^{c}	2.03±0.27^{c}
BC 100 mg/kg+Toxicant	193.43±15.17	26.97±10.80$^{b,\beta,**}$	29.32±2.20	11.03±1.20	0.081±0.025$^{\gamma}$	2.54±0.62^{a,*}	165.01±14.75	7.61±2.26$^{\alpha}$	30.17±1.39	12.24±0.66$^{\gamma}$	0.047±0.016$^{\gamma}$	5.30±0.21^{c}
BC 200 mg/kg+Toxicant	326.33±42.90$^{\gamma}$	27.96±3.22$^{b,\beta,**}$	61.25±6.51$^{\beta}$	30.97±5.82$^{b,\gamma,**}$	0.116±0.032$^{\gamma}$	2.29±0.62^{b,**}	130.13±8.37^{a}	8.07±2.131$^{\alpha}$	28.03±0.57	15.25±0.95$^{\gamma}$	0.065±0.018$^{\gamma}$	5.75±0.47$^{c,\gamma}$
BC 400 mg/kg+Toxicant	249.68±37.06$^{\beta}$	23.51±2.95$^{a,\alpha,*}$	45.41±8.92$^{\beta}$	41.40±2.63$^{c,\gamma,***}$	0.028±0.002$^{\gamma}$	4.48±0.13$^{\gamma}$	195.02±44.84$^{\alpha}$	7.55±1.79$^{\alpha}$	34.42±5.06	21.88±1.73$^{c,\gamma,***}$	0.121±0.036$^{\gamma}$	5.55±0.39$^{a,\gamma}$
Nifedipine 10 mg/kg+Toxicant	219.37±34.99$^{\alpha}$	1.38±0.02	48.79±4.31$^{\gamma}$	15.30±1.59	0.023±0.006$^{\gamma}$	4.36±0.19$^{\gamma}$	142.61±7.21^{a}	3.56±1.92	32.84±3.02	15.04±1.17$^{\gamma}$	0.067±0.030$^{\gamma}$	5.03±0.39$^{\gamma}$

Values are mean±S.E.M. (n=5). ^{a}P<0.05, ^{b}P<0.01, ^{c}P<0.001 vs. control; $^{\alpha}P$<0.05, $^{\beta}P$<0.01, $^{\gamma}P$<0.001 vs. ethanol/sucrose; *P<0.05, **P<0.01 vs. nifedipine (One-way ANOVA followed by Tukey's multiple comparison test), CAT: Catalase; GSH: Reduced glutathione; SOD: Superoxide dismutase; GPx: Glutathione peroxidase; MDA: Malondialdehyde

Table 6. Effect of *B. coccineus hydroethanolic* leaf extract (BC) on serum biochemical parameters in ethanol-induced hypertension in rats

Group	ALT (u/L)	Albumin (g/L)	ALP (u/L)	Cholesterol (mol/L)	AST (u/L)	Creatinine (µmol/L)	HDL (mol/L)	LDL (mol/L)	TG (mol/L)	Protein (g/L)	Urea (mmol/L)
Normal control	63.54±25.75	7.84±0.96	130.38±21.22	1.74±0.16	200.10±42.57	44.37±2.84	0.72±0.12	0.22±0.04	0.48±0.09	56.52±3.70	7.84±0.97
Ethanol (35%; 3 g/kg/day)	71.32±12.99	55.00±3.46^{c}	93.54±1.47	4.02±0.27^{b}	68.02±14.14	39.64±9.42	1.26±0.24	1.12±0.24^{c}	1.00±0.19	23.46±6.60	13.96±6.34
B. coccineus 100 mg/kg+Ethanol	29.26±6.62	16.64±5.55$^{\alpha,***}$	81.60±17.62	1.20±0.19$^{\gamma}$	113.30±35.69	25.23±6.35	0.80±0.11	0.28±0.06$^{\gamma}$	0.24±0.05$^{\alpha,*}$	42.54±9.14	3.28±0.80$^{\alpha}$
B. coccineus 200 mg/kg+Ethanol	135.10±52.28	23.80±5.11***	113.60±22.02	1.79±0.79$^{\beta}$	232.00±56.26	52.05±13.33	0.61±0.06*	0.26±0.04$^{\gamma}$	0.70±0.23	41.98±8.69	4.16±0.67$^{\alpha}$
B. coccineus 400 mg/kg+Ethanol	57.58±8.19	23.44±4.78***	128.30±28.61	1.69±0.22$^{\beta}$	208.90±27.18	37.62±10.08	0.92±0.14	0.32±0.06$^{\gamma}$	0.52±0.11	47.64±6.76	22.10±9.09
Nifedipine 10 mg/kg+Ethanol	51.72±5.11	35.98±1.61$^{c,\alpha}$	155.80±21.73	2.06±0.12$^{\alpha}$	348.02±104.20$^{\alpha}$	20.66±0.26	0.54±0.10$^{\alpha}$	0.26±0.02$^{\gamma}$	0.94±0.05	36.70±0.80	28.71±6.60

Values are mean±S.E.M. (n=5). ^{a}P<0.05, ^{b}P<0.01, ^{c}P<0.001 vs. control; $^{\alpha}P$<0.05, $^{\beta}P$<0.01, $^{\gamma}P$<0.001 vs. ethanol/sucrose; *P<0.05, **P<0.01 vs. nifedipine (One-way ANOVA followed by Tukey's multiple comparison test), ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, AST: Aspartate aminotransferase, HDL: High density lipoprotein, LDL: Low density lipoprotein, TG: Triglycerides

Table 7. Effect of *B. coccineus hydroethanolic* leaf extract (BC) on serum biochemical parameters in sucrose-induced hypertension in rats

Group	ALT (u/L)	Albumin (g/L)	ALP (u/L)	Cholesterol (mol/L)	AST (u/L)	Creatinine (µmol/L)	HDL (mol/L)	LDL (mol/L)	TG (mol/L)	Protein (g/L)	Urea (mmol/L)
Normal control	63.54±25.75	7.84±0.96	130.38±21.22	1.74±0.16	200.10±42.57	44.37±2.84	0.72±0.12	0.22±0.04	0.48±0.09	56.52±3.70	7.84±0.97
Sucrose (5-7% w/v)	79.62±22.70	28.54±2.71^{b}	158.32±31.61	3.56±0.49^{c}	208.20±10.92	58.56±3.81	0.92±0.09	0.87±0.29^{a}	1.32±0.25^{b}	19.26±4.40^{c}	4.08±1.23
B. coccineus 100 mg/kg+Sucrose	44.66±5.23	32.88±1.34^{c}	123.78±14.09	1.60±0.12$^{\gamma}$	208.60±21.16	49.37±2.84	1.08±0.08	0.24±0.05$^{\alpha}$	0.50±0.89$^{\beta}$	58.71±1.34$^{\gamma,*}$	6.10±0.24
B. coccineus 200 mg/kg+Sucrose	39.26±1.46	29.80±1.38^{c}	154.76±5.58	1.76±0.07$^{\gamma}$	200.50±16.40	34.97±5.34	1.08±0.07	0.30±0.03$^{\alpha}$	0.60±0.09$^{\alpha}$	56.84±2.40$^{\gamma}$	9.54±1.76
B. coccineus 400 mg/kg+Sucrose	38.90±6.76	25.22±2.06^{b,*}	116.06±33.06	1.28±0.14$^{\gamma}$	290.60±69.86	43.91±8.21	0.18±0.15	0.36±0.05	0.56±0.09	52.94±1.92$^{\gamma}$	7.78±3.20
Nifedipine 10 mg/kg+Sucrose	85.20±18.15	40.38±6.78	145.54±19.35	1.86±0.35$^{\beta}$	285.00±115.70$^{\alpha}$	40.58±11.11	1.04±0.19	0.24±0.60$^{\alpha}$	0.74±0.15$^{\alpha}$	44.40±19.26$^{\gamma}$	17.92±8.19

Values are mean±S.E.M. (n=5). ^{a}P<0.05, ^{b}P<0.01, ^{c}P<0.001 vs. control; $^{\alpha}P$<0.05, $^{\beta}P$<0.01, $^{\gamma}P$<0.001 vs. sucrose; *P<0.05, **P<0.01 vs. nifedipine (One-way ANOVA followed by Tukey's multiple comparison test), ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, AST: Aspartate aminotransferase, HDL: High density lipoprotein, LDL: Low density lipoprotein, TG: Triglycerides

genic indices compared to the sucrose group. The standard drug, nifedipine, reversed sucrose-induced increment in the values of CRR, AC, and AI (1.79, 0.79, and −0.15, respectively), but to a lesser extent compared to the effect of the extract at the dose of 100 mg/kg [Figure 4].

DISCUSSION

According to the WHO, hypertension is the leading cause of cardiovascular mortality worldwide.[3,8] With epidemiological studies revealing that hypertension is a "silent killer" with no warning signs[29-31] and many developing countries transiting from infectious to degenerative chronic diseases,[32] the prevalence of hypertension is on the increase. As a clinical and major public health problem[2] which affects about one-quarter of the world's adult population, hypertension as a modifiable, preventable, and controllable risk factor has reached epidemic proportions.[6] The prevention of potential end-organ damage including myocardial infarction, kidney failure, and cerebrovascular stroke[8] would depend on early diagnosis and timely appropriate interventions. According to Touyz *et al*.,[33] risk factors for hypertension include dietary habits, such as high sodium or low potassium intake, high alcohol consumption, low levels of physical activity, and overweight.

Figure 4. Effect of *B. coccineus* hydroethanolic leaf extract (BC) on atherogenic indices derived from the mean values of relevant parameters in sucrose-induced hypertension in rats

Figure 3. Effect of *B. coccineus* hydroethanolic leaf extract (BC) on atherogenic indices derived from the mean values of relevant parameters in ethanol-induced hypertension in rats

In this study, hypertension was induced in rats by chronic administration of ethanol and sucrose. High consumption of salt, alcohol, and sugar has been shown to induce cardiovascular dysfunction, and therefore are the major contributory factors to hypertension.[25,34] Estruch and Coca[35] reported that ethanol has complex direct vascular effects, including basal constriction and prominent elastic lamellae fragmentation, which affect vessels' elasticity, and that endothelial dysfunction due to ethanol may induce changes in the relaxant capacity of endothelium and decrease the release of nitric oxide. Vasdev *et al*.[36] demonstrated that intracellular Ca^{2+} in vascular smooth muscle preparation increases with ethanol, even in the presence of Ca^{2+} channel blockade. Alcohol reduces the level of ionized magnesium which causes the blood vessels to relax, whereas calcium ion has the opposite effect.[25]

Sucrose-induced hypertension, caused due to chronic ingestion of large amounts of the sugar, is associated with increased peripheral sympathetic activity,[25] and the cause–effect relationship between insulin perturbations and many hormonal disturbances, capable of elevating BP, has been demonstrated.[34] The presence of high concentrations of sucrose in the renal tubule causes kidney lesions, and sucrose produces an accumulation of lipid in the aorta leading to enhanced atherogenicity.[25] High sucrose ingestion increases blood platelet stickiness that may predispose to coronary thrombosis, and healthy subjects show a significant increase in the level of TG after intake of a high sucrose or fructose diet.[34]

High consumption of alcohol and sugar is associated with oxidative stress and increased level of free radicals, which have been reported to play an important role in the pathogenesis of arterial hypertension.[37] Therefore, the inhibition of lipid peroxidation induced cellular and tissue oxidative damage by antioxidants is beneficial in the treatment of associated conditions, including diabetes and hypertension.

In this study, the administration of ethanol and sucrose to experimental rats for 8 weeks caused significant increase in systolic and diastolic BP, MAP, HR, and serum levels of albumin, cholesterol, LDL, and TG, and reduction in the levels of enzymatic and non-enzymatic antioxidants in the liver, kidney, heart, and aorta, compared to the normal control group.

Higher doses of ethanol induce hypertension by depleting antioxidants and increasing oxidative tissue injury in rats, while chronic consumption of sucrose rapidly raises insulinemia which leads to insulin resistance and consequently hypertension.[25,38,39] Regarding the effect on HR, Bunag *et al*.[38] also previously reported hypertension associated with tachycardia in sucrose hypertensive group of animals. However, in contrast to the observation in this study, Resstel *et al*.[40] and Bilanda *et al*.[25] reported hypertension induced by chronic ethanol administration with no change in the HR of rats. The concurrent administration of *B. coccineus* with ethanol and sucrose in this study significantly prevented the rise in systolic and diastolic BP, MAP, and HR observed in hypertensive rats. With respect to lipid profile, in earlier studies, alcohol and sucrose feeding was found to significantly increase total cholesterol, TG, and AI.[25,41] According to Stevens *et al*.,[42] dyslipidemia enhances vascular resistance and leads to an increase of BP. In this study, *B. coccineus* intervention reversed the elevation in total cholesterol, LDL, and TG observed in the ethanol and sucrose hypertensive groups, suggesting improvement in lipid profile. According to Martirosyan *et al*.[43] and Ikewuchi,[28] atherogenic indices are strong indicators of the risk of heart disease, and the risk of developing cardiovascular disease increases with increases in the values of these indices and vice versa. In this study, *B. coccinues*, especially at the lowest dose of 100 mg/kg, considerably reduced the value of the atherogenic indices (CRR, AC, and AI) relative to the ethanol and sucrose hypertensive groups. Nifedipine elicited a similar trend of effect in the sucrose model, but the effect of the extract at the dose of 100 mg/kg was more pronounced.

Development of cardiovascular dysfunction in alcohol- and sucrose-induced hypertension has been linked with increase in reactive oxygen species (ROS) and alteration of antioxidant defense status,[44] and oxidative stress induces tissue damage leading to impairment of kidney and liver functions.[25] As observed in this study, *B. coccineus* administration significantly reversed reduction in the levels of enzymatic and non-enzymatic antioxidants in liver, kidney, heart, and aorta induced by ethanol and sucrose. This suggests that enhancement of *in vivo* antioxidant activity is one of the possible mechanisms for the antihypertensive property observed with the hydroethanolic leaf extract of *B. coccineus*. This finding is supported by the previous report of hepatoprotective activity associated with the *in vivo* antioxidant activities of the extract.[20]

The phytochemical screening of the hydroethanolic leaf extract of *B. coccineus* in this study revealed the presence of alkaloids, tannins, saponins, and cardiac glycosides. It has been shown that the biological activities of alkaloids include hypolipidemic and hypoglycemic activities,[45,46] and saponins have been reported to assist in the prevention of cardiovascular diseases by lowering plasma cholesterol concentrations through the excretion of cholesterol directly or indirectly as bile acids.[28,47,48] HPLC fingerprint analysis revealed the presence of chlorogenic acid, rutin, and quercetin. Suzuki *et al*.[49] reported that chlorogenic acid attenuates hypertension and improves the endothelial function in spontaneously hypertensive rats. In the study, dietary 5-caffeoylquinic acid reduced oxidative stress and improved nitric oxide bioavailability by inhibiting excessive production of ROS in the vasculature. These actions resulted in the attenuation of endothelial dysfunction, vascular hypertrophy, and hypertension in spontaneously hypertensive rats. In a study by Panchal *et al*.,[50] rutin ameliorated metabolic changes including abdominal fat pads and glucose tolerance, changes in hepatic and cardiovascular structure and function, oxidative stress and inflammation in the liver and heart, and expression of liver markers induced by high-carbohydrate, high-fat diet. Based on the results obtained in the study, the authors suggested a non-nutritive role for rutin to attenuate chronic changes in metabolic syndrome (obesity, diabetes, and hypertension). Egert *et al*.[51] investigated the effects of quercetin supplementation on BP, lipid metabolism, markers of oxidative stress, inflammation, and body composition in an at-risk population of 93 overweight or obese subjects aged 25-65 years with metabolic syndrome traits. Findings in the study revealed that quercetin reduced systolic BP and plasma oxidized LDL concentrations in overweight subjects with a high cardiovascular disease risk phenotype, suggesting that quercetin may provide protection against cardiovascular disease. The presence of these phenolic compounds and other bioactive

phytocomponents in *B. coccineus* extract may be responsible for the observed biological effects in this study. Further studies are required to isolate, identify, and characterize the specific principles and the precise mechanisms of action responsible for the antihypertensive activity produced by the hydroethanolic leaf extract of *B. coccineus* in this study.

With regard to acute toxicity, *B. coccineus* did not induce lethality in mice when administered p.o. up to 10 g/kg. It can, therefore, be said to be safe when administered orally, according to the assertion of Clarke and Clarke,[52] that a substance that does not produce lethality up to 10 g/kg orally is relatively non-toxic. This fact is corroborated by the fact that there were no mortality and visible signs of delayed toxicity when animals were observed for a further 14 days. However, the LD_{50} administered intraperitoneally was estimated to be 288.40 mg/kg.

CONCLUSION

The results obtained in this study suggest that the hydroethanolic leaf extract of *B. coccineus* possesses antihypertensive activity possibly due to antioxidant activity and improvement in lipid profile. The observed biological actions may be due to the presence of phenolic compounds and other phytocomponents identified in the plant extract. Further studies are required to isolate, identify, and characterize the active phytoconstituents and determine the precise mechanism(s) of action.

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