ORIGINAL ARTICLE

Journal of Pre-Clinical and Clinical Research 2023, Vol 17, No 2, 79-84
www.jpccr.eu

BY-NC

Genistein increases blood pressure in pre-treated normotensive rats

Funmileyi Olubajo Awobajo[1,A-F], John Ihayi Ogbu[1,C-F]✉, Abdullahi Adeyinka Adejare[1,B-F], Grace Akpanika[2,C-E], Chiamaka Egede[1,B-E], Bukola Timothy[1,B-D]

[1] *University of Lagos, Nigeria*
[2] *University of Calabar, Nigeria*
A – Research concept and design, B – Collection and/or assembly of data, C – Data analysis and interpretation, D – Writing the article, E – Critical revision of the article, F – Final approval of the article

Funmileyi Olubajo Awobajo, John Ihayi Ogbu, Abdullahi Adeyinka Adejare, Grace Akpanika, Chiamaka Egede, Bukola Timothy. Genistein increases blood pressure in pre-treated ormotensive rats. J Pre-Clin Clin Res. 2023; 17(2): 79–84. doi: 10.26444/jpccr/166675

Abstract

Introduction and Objective. High blood pressure is an important risk factor for heart-related disease. Combining dietary adjustments with standard drugs in the therapy of heart diseases have gained attention. Genistein, a soy isoflavone, has many biological effects but its role in blood pressure is inconclusive. The aim of the study was to investigate the effect of genistein on blood pressure in normotensive rats pre-treated with standard drugs.

Materials and method. Twenty healthy adult male Sprague Dawley rats (body weight 160–180g) were administered with genistein, and SBP, DBP, PP, MABP and HR were recorded. Subsequently, the rats (5 each) were treated with Indomethacin, a non-steroidal, anti-inflammatory and a cyclooxygenase (COX-2) enzyme inhibitor (5 mg/kg subcutaneously), methylene blue,an inhibitor of nitric oxide synthase and guanylate cyclase (7.5 mg/kg, by infusion), nifedipine,a calcium channel blocker (0.75 mg/kg intravenously), and tetraethylammonium, a voltage-gated K+ channels blocker (60 mg/kg intravenously). Genistein was administered intravenously in graded doses after the administration of these blockers. SPB, DBP, PP and MABP were significantly increased following genistein administration only, as well as in the methylene blue and tetraethylammonium groups each.

Results. Higher doses of genistein significantly decreased SBP, DBP and PP after indomethacin treatment. Falling DBP and MABP were reversed in the nifedipine group.

Conclusions. Results suggests that genistein elevates blood pressure in experimental normotensive male rats. This has implications for alternative therapy in the management of hypertension, and implications for blood pressure in cardiovascular related diseases

Key words

genistein, hypertension, heart, isoflavones, oestrogen

INTRODUCTION

Blood pressure is an important parameter used in the assessment of the cardiovascular functions and health status in mammals [1]. Environmental factors can influence blood pressure and notable among such factors are stress, life style, drugs and diet [2]. Consumption of a diet rich in fat and energy without corresponding energy burn-out, will lead to obesity and consequently hypertension [3]. Previous studies have documented that people who are known to go on diet rich in legumes and soya meals were found to have consistently low blood pressure [4]. Genistein and daidzein are the major isoflavones constituents of soya, [5]. As a phytoestrogen, genistein has been found to be an oestrogen agonist, having a structural similarity to oestradiol with higher affinity for beta oestrogen receptor [6].

Genistein has drawn wide attention due to its potential health benefits in preventing chronic diseases such as cardiovascular diseases [7], obesity [8]. Genistein possesses beneficial effects on serum lipids [7], but its mechanism of action on the cardiovascular system are still not fully explained. This study was aimed at investigating the effect of genistein on blood pressure in normotensive male rats pre-treated with Indomethacin, Methylene Blue, Nifedipine and Tetraethylammonium.

✉ Address for correspondence: John Ihayi Ogbu, University of Lagos, College of Medicine, University of Lagos, Idi-Araba, 100254, Lagos, Nigeria
E-mail: ogbu11john@gmail.com

Received: 05.11.2022; accepted: 31.05.2023; first published: 16.06.2023

MATERIALS AND METHOD

Animals and preparation for blood pressure measurement. A total of 20 healthy adult male Sprague Dawley rats (body weight 160–180g) were used for the study, obtained from the animal house of the college of Medicine, University of Lagos. The rats were housed in a standard experimental animal laboratory and placed on phytoestrogen free diet and water *ad-libitum*. Room temperature was maintained at 28 ± 1 °C with the aid of an air conditioner throughout the period of the experiment. All procedures, including extract preparation and drug administration, as well as housing and euthanasia, were in accordance with the Helsinki guidelines for the care and use of experimental animals [8], as approved by the Institutional Health Research and Ethics Committee, College of Medicine, University of Lagos, Nigeria (Approval No. CM/HREC/010/16/064).

Animal grouping and experimental procedures. The animals were divided into 4 groups of 5 animals each. Group-1 served as the Indomethacin (5 mg/kg subcutaneously)

group, group-2 served as Methylene-blue (7.5 mg/kg, by intravenous) group, group-3 served as while group-4 served as the Nifedipine (0.75 mg/kg intravenous) group and Tetraethylammonium (60 mg/kg intravenous) group. The animals were fasted overnight prior to the day of the experiment. On that day, the rats were anesthetized with a solution of 25% (w/v) urethane and 1% (w/v) α-chloralose injected intraperitoneally at a dose of 5 ml/kg body weight. Loss of consciousness and pain sensation was confirmed in each rat with the loss of response to pricking the limb paw with a sharp needle, after which the rat was placed in a supine position and fastened onto a warm surgical dissecting table. The trachea was cannulated and trachea tube PE250 was inserted and tied with a sterilized tread to open up the airways, and to prevent possible congestion of the airway due to the effect of the anesthetic agent administered. The right femoral vein and arteries were carefully cannulated using a 10–15 cm cannula. The drugs and the graded concentrations of genistein used in this study were administered via the cannulated vein while the femoral artery was connected to the blood pressure transducer which was connected to the power lab acquisition system (ADInstruments, USA) to record the continuous blood pressure before and during the drug and genistein administration. The cannulation was flushed with heparinized saline to prevent blood clotting [9]. The whole set-up was allowed to stabilize for 30 minutes and the baseline blood pressure recording after administration of the graded doses of genistein had been recorded. Blood pressure parameters, including systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (PP) and heart rate (HR), were all recorded while the rate pressure product (RPP) was also calculated from the above parameters. A schematic diagram of the experimental design is provided in Figure 1.

Preparation and administration of Genistein and different drugs. Genistein was prepared from a stock solution of 0.025 mg/ml of distilled water and injected intravenously after being sonicated via the femoral vein in graded doses of 0.008, 0.016, 0.032, 0.064, 0.128, and 0.256 mg/kg body weight on the day of the experiment. These doses were less than doses of genistein used in the work of O'Connor et al., (2002) [10]. In the first part of the study, after the initial administration of genistein at different doses, basal blood pressure recordings were obtained and the blood pressure allowed to return to baseline and to stabilize. Thereafter, a one-time dose of the drug was administered either intravenously or subcutaneously, as applicable, and allowed to circulate for 60 seconds after which the graded concentration of genistein were re-administered and new blood pressure readings were recorded for each animal set-up. This procedure was repeated for each of the drugs in the different groups. The drugs administered include Indomethacin, a non-steroidal anti-inflammatory and a cyclooxygenase (COX-2) enzyme inhibitor (5 mg/kg subcutaneously), methylene blue, an inhibitor of nitric oxide synthase and guanylate cyclase (7.5 mg/kg, by infusion), nifedipine,a calcium channel blocker (0.75 mg/kg intravenously), and tetraethylammonium a voltage-gated K^+ channels blocker (60 mg/kg intravenously) [11–14]. Genistein was administered in graded doses of 0.008, 0.016, 0.032, 0.064, 0.128, and 0.256 mg/kg body weight intravenously [10], after the administration of the drugs to determine the effect and possible mechanism of actions of genistein on the blood pressure.

Statistical analysis and result presentation. Data acquired with the ADPower Lab acquisition system were subjected to one-way Analysis of Variance (ANOVA) and paired T-test using Graph pad software. Differences were considered significant when $P<0.05$. Results were presented as mean ± SEM. Tables and Figures were used for the presentation of all results.

RESULTS

Intravenous administration of graded doses of genistein. Blood pressure changes after administration of graded doses of genistein (0.008mg/kg, 0.016 mg/kg, 0.032 mg/kg, 0.064 mg/kg, 0.128 mg/kg, and 0.256 mg/kg) is shown in Table 1.

Intravenous administration of genistein at graded doses of 0.064 mg/kg, 0.128mg/kg and 0.256mg/kg significantly increased SBP ($p<0.05$), compared to baseline (Fig. 2, Plate A).

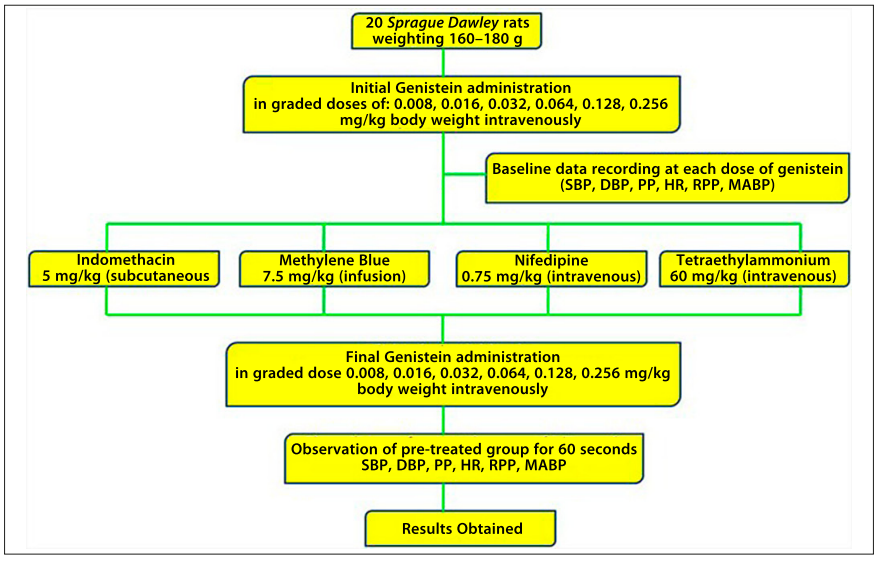

Figure 1. A Schematic diagram of the Experimental design

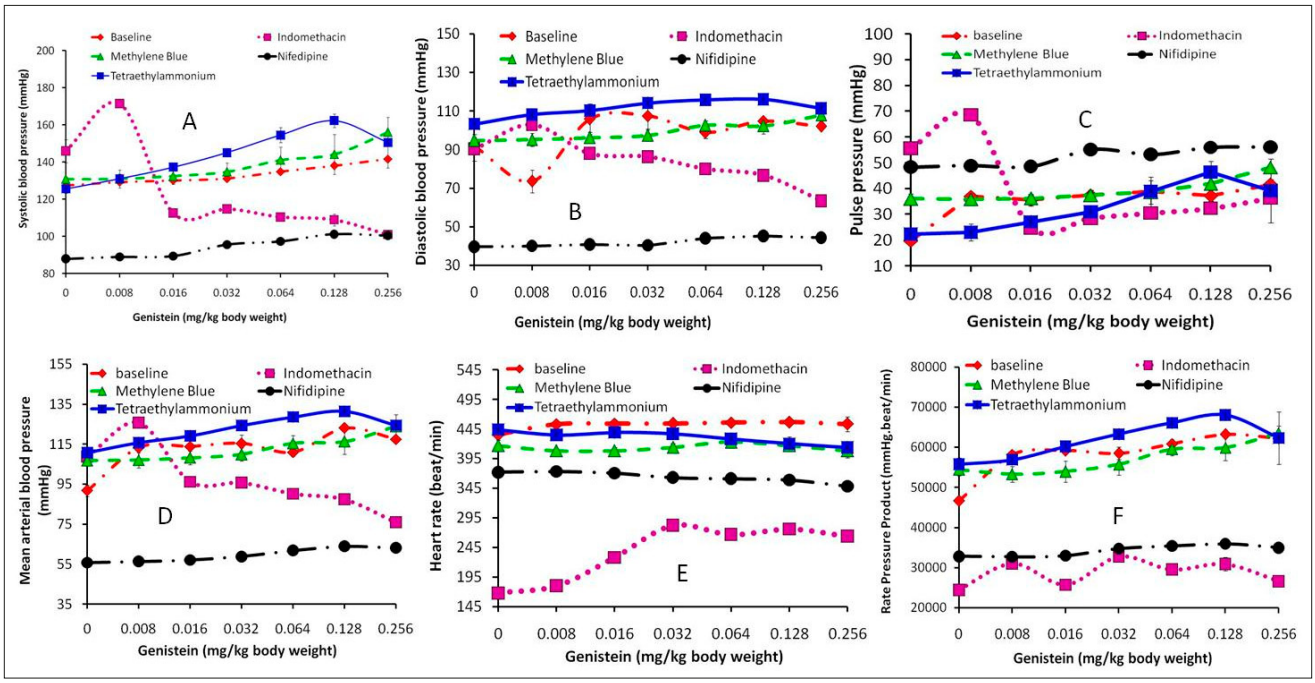

Figure 2:Comparison of blood pressure parameters in response to intravenous administration of graded concentration of genistein after the administration of indomethacin, methylene blue, nifedipine, and tetraethylammonium separately Plate A-Systolic blood pressure; Plate B-Diastolic pressure; Place C-Pulse pressure; Plate D-Mean arterial pressure; Plate E-Heart rate; Plate F- Rate pressure product

PP recorded a significant increase following administration of graded doses of 0.016mg/kg, 0.032mg/kg, 0.128mg/kg and 0.256mg/kg, in comparison with that of 0.08mg/kg of genistein ($p<0.05$) (Fig. 2, Plate C). There was a significant rise in MABP when the graded doses were compared to baseline ($p<0.05$) (Fig. 2, Plate D). Graded doses of 0.008mg/kg, 0.016mg/kg,

Table 1. Blood pressure responses to intravenous administration of graded concentration of genistein

Parameters	Genistein concentration (mg/kg body wt.) n=4						
	0.000	0.008	0.016	0.032	0.064	0.128	0.256
SBP (mmHg)	127.33 ± 1.25	129.18 ± 0.09	130.07 ± 0.16	131.08 ± 0.47	134.83 ± 0.23	138.03 ± 0.97	141.62 ± 1.41
DBP (mmHg)	91.60 ± 4.06	73.57 ± 5.81	105.76 ± 7.83	107.48 ± 6.58	98.89 ± 3.04^{abc}	104.78 ± 0.38^{abcd}	101.96 ± 1.45^{abcde}
PP (mmHg)	19.73 ± 1.51	37.00 ± 0.21	35.55 ± 2.15^{b}	37.44 ± 0.66^{b}	38.98 ± 0.05	37.16 ± 2.57^{b}	41.56 ± 1.93^{b}
MABP (mmHg)	91.76 ± 2.90	113.26 ± 5.11^{a}	113.86 ± 5.27^{a}	115.35 ± 4.26^{a}	110.94 ± 2.09^{a}	123.03 ± 0.92^{a}	117.46 ± 0.85^{a}
HR (beat/min)	453.50 ± 3.50	452.85 ± 5.85^{a}	453.85 ± 6.25^{a}	454.35 ± 6.15^{a}	456.00 ± 6.00	456.66 ± 6.06^{a}	453.30 ± 12.30^{a}
RPP (mmHg.beat/min)	46721.30 ± 213.89	58233.15 ± 978.66	59265.40 ± 653.45	58578.43 ± 1567.28	60948.23 ± 1151.51	63288.15 ± 130.52	62351.49 ± 328.00

SBP – Systolic Blood Pressure (mmHg); MABP – Mean Arterial Blood Pressure (mmHg); DBP – Diastolic Blood Pressure (mmHg); HR – Heart Rate (b/min); PP – Pulse Pressure (mmHg); RPP – Rate Pressure Product (dimensionless).
a – significant when compared to baseline; b – significant when compared to 0.008mg/kg; c – significant when compared to 0.016mg/kg; d – significant when compared to 0.032mg/kg; e – significant when compared to 0.064mg/kg; f – significant when compared to 0.128mg/kg

Table 2. Blood pressure responses to intravenous administration of graded concentration of genistein after administration of indomethacin (cyclooxygenase (COX) inhibitor

Parameters	Genistein concentration (mg/kg body wt.) administered after indomethacin (n=4)						
	0.000	0.008	0.016	0.032	0.064	0.128	0.256
SBP (mmHg)	145.90 ± 6.08	171.39 ± 1.66^{a}	112.58 ±0.39^{ab}	114.67 ± 1.15^{ab}	110.38 ± 0.46^{ab}	108.90 ± 3.17^{ab}	100.96 ± 0.73^{abcde}
DBP (mmHg)	90.27 ± 3.71	102.88 ± 1.40^{a}	87.88 ± 0.34^{b}	86.27 ± 0.99^{b}	79.94 ± 0.94^{abc}	76.59 ± 1.93^{abcd}	63.41 ± 1.90^{abcdef}
PP (mmHg)	55.63 ± 2.37	68.52 ± 1.25^{a}	24.70 ± 0.54^{ab}	28.40 ± 1.90^{ab}	30.45 ± 1.81^{ab}	32.30 ± 1.81^{abc}	36.26 ± 0.70^{abcd}
MABP (mmHg)	108.81 ± 4.50	125.72 ± 1.37^{a}	96.12 ± 0.25^{ab}	95.73 ± 0.54^{ab}	90.08 ± 0.75^{abd}	87.36 ± 2.26^{abcd}	75.93 ± 1.45^{abcdef}
HR (beat/min)	168.00 ± 0.00	180.00 ± 0.00	228.00 ± 0.00^{ab}	282.00 ± 6.00^{ab}	267.00 ± 3.00^{abd}	276.00 ± 0.00^{ab}	264.00 ± 0.00^{abd}
RPP (mmHg.beat/min)	24510.36 ± 1020.60	31044.60 ± 214.20^{a}	25749.18 ± 119.70^{ab}	32798.40 ± 318.72^{ab}	29583.96 ± 364.44^{ab}	30958.92 ± 1656.00^{abcd}	26673.24 ± 336.60^{abcdef}

SBP – Systolic Blood Pressure (mmHg); MABP – Mean Arterial Blood Pressure (mmHg); DBP – Diastolic Blood Pressure (mmHg); HR – Heart Rate (b/min); PP – Pulse Pressure (mmHg); RPP – Rate Pressure Product (dimensionless).
a – significant when compared to baseline; b – significant when compared to 0.008mg/kg; c – significant when compared to 0.016mg/kg; d – significant when compared to 0.032mg/kg; e – significant when compared to 0.064mg/kg; f – significant when compared to 0.128mg/kg

Table 3. Blood pressure responses to intravenous administration of graded concentration of genistein after administration of methylene blue (monoamine oxidase inhibitor (MAOI)

Parameters	Genistein concentration (mg/kg body wt.) administered after methylene blue (n=4)						
	0.000	0.008	0.016	0.032	0.064	0.128	0.256
SBP (mmHg)	130.78 ± 0.93	130.91 ± 4.81[a]	132.37 ± 4.35[b]	134.76 ± 4.63[ac]	141.13 ± 6.95[a]	144.12 ± 10.81[ac]	156.05 ± 0.18[bd]
DBP (mmHg)	94.62 ± 3.14	95.10 ± 3.32	96.17 ± 2.85	97.38 ± 2.67	102.56 ± 2.40	102.29 ± 4.10	107.85 ± 0.06
PP (mmHg)	35.99 ± 2.03	35.82 ± 1.50	36.20 ± 1.50	37.39 ± 1.97	38.58 ± 4.54	41.84 ± 6.72	48.20 ± 0.24
MABP (mmHg)	106.79 ± 2.28	107.03 ± 3.81	108.22 ± 3.35	109.84 ± 3.32	115.41 ± 3.92	116.23 ± 6.33	123.92 ± 0.02
HR (beat/min)	417.00 ± 9.00	408.00 ± 0.00	408.00 ± 6.00	414.00 ± 6.00	423.00 ± 9.00	417.00 ± 9.00	408.00 ± 12.00
RPP (mmHg.beat/min)	54543.63 ± 1564.83	53411.28 ± 1962.48	54030.99 ± 2566.95	55818.42 ± 2725.38	59635.44 ± 1669.68	60000.75 ± 3210.69	63664.14 ± 1797.06

SBP – Systolic Blood Pressure (mmHg); MABP – Mean Arterial Blood Pressure (mmHg); DBP – Diastolic Blood Pressure (mmHg); HR – Heart Rate (b/min); PP – Pulse Pressure (mmHg); RPP – Rate Pressure Product (dimensionless).
a – significant when compared to baseline; b – significant when compared to 0.008mg/kg; c – significant when compared to 0.016mg/kg; d – significant when compared to 0.032mg/kg; e – significant when compared to 0.064mg/kg; f – significant when compared to 0.128mg/kg

Table 4. Blood pressure responses to intravenous administration of graded concentration of genistein after administration of Nifedipine (calcium channel blocker)

Parameters	Genistein concentration (mg/kg body wt.) administered after nifedipine (n=4)						
	Baseline	0.008	0.016	0.032	0.064	0.128	0.256
SBP (mmHg)	87.86 ± 1.30	88.85 ± 0.79	89.35 ± 1.02	95.49 ± 1.79	97.21 ± 1.48	101.16 ± 0.72	100.44 ± 0.82
DBP (mmHg)	39.55 ± 0.63	39.99 ± 0.34	40.76 ± 0.43	40.37 ± 1.31[abc]	43.99 ± 0.87[abc]	45.21 ± 1.36[abcd]	44.36 ± 0.67[abc]
PP (mmHg)	48.31 ± 0.85	48.86 ± 0.46	48.59 ± 1.37	55.12 ± 1.55	53.21 ± 1.97[a]	55.95 ± 1.07[abcd]	56.08 ± 0.68[ab]
MABP (mmHg)	55.65 ± 0.82	56.28 ± 0.49	59.95 ± 0.23	58.74 ± 1.30[abc]	61.73 ± 0.61	63.86 ± 1.08[abc]	63.06 ± 0.65[abc]
HR (beat/min)	372.00 ± 0.00	373.20 ± 1.20	370.20 ± 1.80	363.00 ± 3.00	361.20 ± 1.20[abc]	358.80 ± 1.20[abcd]	348.00 ± 0.00[abcd]
RPP (mmHg.beat/min)	32877.36 ± 1134.60	32782.48 ± 288.28	33062.08 ± 318.64	34797.30 ± 1235.10[ab]	35464.92 ± 819.48[abc]	36026.93 ± 66.67[abc]	35031.42 ± 523.74[abcdef]

SBP – Systolic Blood Pressure (mmHg); MABP – Mean Arterial Blood Pressure (mmHg); DBP – Diastolic Blood Pressure (mmHg); HR – Heart Rate (b/min); PP – Pulse Pressure (mmHg); RPP – Rate Pressure Product (dimensionless).
a – significant when compared to baseline; b – significant when compared to 0.008mg/kg; c – significant when compared to 0.016mg/kg; d – significant when compared to 0.032mg/kg; e – significant when compared to 0.064mg/kg; f – significant when compared to 0.128mg/kg

Table 5. Blood pressure responses to intravenous administration of graded concentration of genistein after administration of tetraethylammonium (potassium channel blocker)

Parameters	Genistein concentration (mg/kg body wt.) administered after Tetraethylammonium (n=4)						
	0.000	0.008	0.016	0.032	0.064	0.128	0.256
SBP (mmHg)	125.56 ± 2.44	131.03 ± 2.57	137.22 ± 1.85	145.02 ± 0.46	154.51 ± 4.11	162.29 ± 3.60	150.54 ± 13.61
DBP (mmHg)	103.25 ± 1.32	108.09 ± 0.74	110.13 ± 0.96	114.07 ± 1.39	115.65 ± 1.64[a]	115.93 ± 0.67[ab]	111.39 ± 1.25[a]
PP (mmHg)	22.31 ± 1.44	22.94 ± 3.31	27.09 ± 0.89[a]	30.95 ± 1.85[a]	38.86 ± 5.75[ab]	46.36 ± 4.27[ab]	39.16 ± 12.37[a]
MABP (mmHg)	110.68 ± 1.64	115.74 ± 0.36	119.16 ± 1.26	124.38 ± 0.77	128.60 ± 0.28	131.38 ± 0.75[a]	124.44 ± 5.37
HR (beat/min)	444.00 ± 0.00	435.00 ± 9.00	439.20 ± 7.20	436.80 ± 7.20[a]	428.40 ± 6.00[ab]	420.00 ± 12.00[abc]	414.00 ± 6.00[a]
RPP (mmHg.beat/min)	55804.14 ± 1702.74	56974.92 ± 61.32	60251.54 ± 177.62	63339.20 ± 841.00	66167.42 ± 833.66	68116.56 ± 437.52	62405.22 ± 6537.78

SBP – Systolic Blood Pressure (mmHg); MABP – Mean Arterial Blood Pressure (mmHg); DBP – Diastolic Blood Pressure (mmHg); HR – Heart Rate (b/min); PP – Pulse Pressure (mmHg); RPP – Rate Pressure Product (dimensionless).
a – significant when compared to baseline; b – significant when compared to 0.008mg/kg; c – significant when compared to 0.016mg/kg; d – significant when compared to 0.032mg/kg; e – significant when compared to 0.064mg/kg; f – significant when compared to 0.128mg/kg

0.032mg/kg, 0.128mg/kg, 0.256mg/kg significantly increased the HR when compared to baseline ($p<0.05$) (Fig. 2, plate E).

Changes to blood pressure parameter after administration of Indomethacin (cyclooxygenase (cox) inhibitor) and genistein. Changes to blood pressure after the administration of indomethacin and genistein are presented in Table 2. SBP was significantly increased ($p<0.05$) at 0.008 mg/kg compared to baseline. SBP was significantly decreased at genistein doses above 0.008 mg/kg, compared to values recorded at baseline. SBP was significantly decreased at 0.256 mg/kg compared with values recorded with 0.016mg/kg, 0.032mg/kg, 0.064mg/kg (Fig. 2, plate A) There was a significant increase in DBP when 0.008mg/kg was compared to baseline ($p<0.05$). There was also a significant decrease in DBP when graded doses 0.064mg/kg, 0.128mg/kg, and 0.256mg/kg were compared to values at baseline (Fig. 2, plate B). PP was significantly increased at 0.08mg/kg and significantly decreased in graded doses 0.016mg/kg, 0.032mg/kg, 0.064mg/kg, 0.128mg/kg, and 0.256mg/kg when compared to baseline ($p<0.05$). PP was significantly decreased at 0.016mg/kg, 0.032mg/kg, 0.064mg/kg, 0.128mg/kg and 0.256mg/kg compared to values at baseline (Fig. 2, plate C). MABP was significantly increased with all other doses of genistein compared to the

derived values at baseline (p<0.05) (Fig. 2, plate D). HR was significantly decreased with all higher doses of genistein above 0.08 mg/kg compared to values recorded at baseline (Fig. 2, plate F). RPP was significantly increased at all doses of genistein compared to the derived values at baseline (Fig. 2, plate F).

Changes in blood pressure after administration of methylene blue (monoamine oxidase and guanylate cyclase (cGMP) inhibitor) and genistein. Changesino blood pressure after the administration of methylene blue and genistein are presented in Table 3. SBP was significantly increased(p<0.05) at 0.008 mg/kg, compared to baseline. SBP was significantly increased(p<0.05) at genistein doses above 0.008 mg/kg, compared to baseline, except for 0.016 mg/kg and 0.0256 mg/kg doses. Significant increase in SBP occurred at 0.016mg/kg and 0.256mg/kg were compared to 0.008mg/kg. Similar response was recorded at 032mg/kg and 0.128mg/kg compared to 0.016mg/kg (p<0.05). 0.256mg/kg response was significantly increased when compared to 0.032mg/kg (p<0.05) (Fig. 2, plate A).

Changes in blood pressure after administration of nifedipine (calcium channel blocker) and genistein. Changes to blood pressure after the administration of nifedipine and genistein are presented in Table 4. DBP was significantly increased (p<0.05) at genistein doses above 0.016 mg/kg when compared to baseline, 0.008 mg/kg and 0.016 mg/kg doses. Similarly, 0.0128 mg/kg genistein significantly increased DBP when compared to the 0.032 mg/kg dose (Fig. 2, Plate B). The PP was significantly increased when 0.064mg/kg, 0.128mg/kg and 0.256mg/kg were compared to baseline (p<0.05). PP was significantly increased when 0.128mg/kg and 0.256mg/kg were compared to 0.008mg/kg (p<0.05); there was also a significant increase when 0.128mg/kg was compared to 0.016mg/kg and 0.032mg/kg (p<0.05) (Fig.2, Plate C). MABP was significantly increased at doses above 0.016 mg/kg, excluding 0.064 mg/kg, compared to baseline, 0.008mg/kg, and 0.016mg/kg (p<0.05) (Fig. 2, Plate D). HR was significantly increased when 0.064mg/kg, 0.128mg/kg, and 0.256mg/kg were compared to baseline, 0.008mg/kg, and 0.016mg/kg (p<0.05); there was a significantly increase when 0.128mg/kg and 0.256mg/kg were compared to 0.032mg/kg (p<0.05) (Fig. 2, Plate F). RPP was significantly increased at doses above 0.016 mg/kg compared to baseline,0.008mg/kg and 0.016 mg/kg doses (p<0.05). RPP was significantly increased when 0.064mg/kg, 0.125mg/kg, and 0.256mg/kg were compared to 0.016mg/kg (p<0.05). RPP was also significantly increased at doses above 0.016 mg/kg compared to baseline, 0.008 mg/kg and 0.016 mg/kg. A 0.256 mg/kg dose recorded a significant rise in RPP when compared to doses above 0.016 mg/kg. These RPP changes are illustrated in Figure 2, Plate F.

Changes in blood pressure after administration of tetraethylammonium (potassium channel blocker) and genistein. Changes to blood pressure after the administration of tetraethylammonium and genistein are presented in Table 5.There was a significant increase in DBP when genistein doses above 0.016 mg/kg were compared to baseline, and when 0.128mg/kg was compared to 0.008mg/kg (p<0.05) (Fig. 2, Plate B). PP was significantly increased when doses above 0.08mg/kg were compared to baseline (p<0.05). PP was also significantly increased when 0.064mg/kg and 0.128mg/kg were compared to 0.008mg/kg (p<0.05) (Fig. 2, Plate C). MABP was significantly increased when 0.128mg/kg is compared to baseline (p<0.05) (Fig. 2, Plate D). HR was significantly decreased when doses above 0.016 mg/kg is compared to baseline (p<0.05). 0.064mg/kg and 0.128mg/kg significantly decreased. HR compared to 0.008mg/kg and 0.128mg/kg when compared to 0.016mg/kg (Fig. 2, Plate E).

DISCUSSION

While high blood pressure is an important risk factor for heart-related diseases [1], studies have shown that combining pharmacological therapy with dietary adjustment and regular exercise are more effective in the management of hypertension than pharmacotherapy alone [15,16]. In the present study, intravenous administration of genistein increased blood pressure at doses above 0.016 mg/kg when compared to baseline contrary to many of the previous reports [17]. In previous reports, either from human or animal experiments in which genistein lowered blood pressure, the route of genistein administration was the oral route. It thus appears from this study, and with recourse to previous studies by other authors, that the route of administration of genistein significantly affects the effect produced on blood pressure. It has been reported that the route of administration of genistein determines its bioavailability in the blood [18–20]. The report showed that about 80% of genistein was converted into glucoronides and sulphates, rendering the absolute bioavailability as being very low (<15% – 23.4%) when administered orally, while the bioavailability of the total genistein remain very high (>55 – 90 %) [18, 19]. Genistein was also reported to express a longer half-life when administered orally [19]. The bioavailability of genistein therefore may have contributed to the disparity recorded in the effect of genistein on blood pressure between the intravenous route, as recorded in this study, and that of the oral route as reported by other authors [17, 21].

A reduction in blood pressure was recorded, however, in animals pre-treated with indomethacin before the administration of genistein, corroborating the findings of Antoni *et al*., (2017) [22] that genistein relaxation of indomethacin constricted rat aorta and main pulmonary artery was via the endothelium dependent mechanism. On the other hand, genistein significantly raised the blood pressure in animals pre-treated with methylene blue (a monoamine oxidase and cGMP inhibitor), Tetraethylammonium (a potassium channel blocker) and Nifedipine (a calcium channel inhibitor). Although Tetraethylammonium, methylene blue and nifedipine are known blood pressure lowering drugs that produce a reduction in blood pressure via blockade of K^+, cGMP induced pressure natriuresis and calcium channels respectively [11, 13, 14], intravenous genistein administration attenuated these blood pressure lowering effects of the drugs. Richardson et al., (2016) [23] reported that methylene blue had no effect on relaxation induced by genistein. This may be a result of possible decrease in the total bioavailability of genistein when administered intravenously [22], or as a result of the well- established dual effects of phytoestrogen, depending of *in-vivo* concentrations and the greater affinity for the β-estrogen receptor [6].

The Rate pressure product was significantly increased in all genistein groups compared to the values obtained at baseline, and this increase is equally as a result of the increase in blood pressure recorded after intravenous infusion of genistein. There will be a need to evaluate the bioavailability of genistein when administered through routes apart from the usual oral route, and to further determine the reasons behind the change in pressure response when administered intravenously compared with the oral route.

CONCLUSION

The results of this study indicated that intravenous administration of genestein precipitated an increase in blood pressure in experimental normotensive male rats, and also attenuated the blood pressure lowering effects of indomethacin, Tetraethyylammonium, methyline blue and nifedipine in normotensive rats.

REFERENCES

1. Al Ghorani H, Götzinger F, Böhm M, Mahfoud F. Arterial hypertension-Clinical trials update 2021. Nutrition, Metabolism, and cardiovascular diseases NMCD. 2021;32(1):21–31.
2. Adejare AA, Sofola OA. Amlodipine corrects changes in blood pressure and baroreceptor reflex sensitivity in Sprague Dawley rats fed a high salt diet. Niger J Physiol Sci. 2017;32:63–67.
3. UJ J, Myung-Sook C. Obesity and Its Metabolic Complications: The Role of Adipokines and the Relationship between Obesity, Inflammation, Insulin Resistance, Dyslipidemia and Nonalcoholic Fatty Liver Disease. Int J Mol Sci. 2014;15(4):6184–6223.
4. Jia-Liu W, Xin-Yan W, Fang-Chao L, Ji-Chun C, Jie C, Jian-Xin L, et all. Associations of soybean products intake with blood pressure changes and hypertension incidence: the China-PAR project. J Geriatr Cardiol. 2020;17(7):384–392.
5. Toktay E, Selli J, Gurbuz MA, Tastan TB, Ugan RA, Un H, Halici Z. Effects of soy isoflavonoids (genistein and daidzein) on endometrial receptivity. Iran J Basic Med Sci. 2020;23(12):1603–1609. doi:10.22038/ijbms.2020.48294.11089
6. Xueyan Chen, Ugur Uzuner, Man Li, Weibing Shi, Joshua S. Yuan, Susie Y. Dai Phytoestrogens and Mycoestrogens Induce Signature Structure Dynamics Changes on Estrogen Receptor α. Int J Environ Res Public Health. 2016;13(9):869.
7. Zhang T, Chi XX. The effect of genistein on lipid levels and LDLR, LXRα and ABCG1 expression in postmenopausal women with hyperlipidemia. Diabetol Metab Syndr. 2019;11:111.
8. National Research Council NRC. 2011. guide for the care and use of laboratory animals:eighth edition. The national academies press. 1–247.
9. Harikrishnan V, Hansen AK, Abelson KS, Sørensen DB. A comparison of various methods of blood sampling in mice and rats: Effects on animal welfare. Laboratory Animals. 2018;52(3):253–264.
10. O'Connor TP, Liesen DA, Mann PC, Rolando L, Banz WJ. A high isoflavone soy protein diet and intravenous genistein delay rejection of rat cardiav allografts. J Nutr. 2002;132(8):2283–7.
11. Morgan TO, Anderson A, Bertram D. Effect of indomethacin on blood pressure in elderly people with essential hypertension well controlled on amlodipine or enalapril. Am J Hypertension. 2000;13(11):1161–1167. https://doi.org/10.1016/S0895-7061(00)01204-8
12. Evora PRB, Soares ROS, Bassettol S, Martins MA, Silva FLS, Filho AB. After Thirty Years, We Still Cannot Understand Why Methylene Blue is not a Reference to Treat Vasoplegic Syndrome in Cardiac Surgery. Braz J Cardiovasc Surg. 2021;36(3):406–11.
13. Zicha J, Behuliak M, Pintérová M, Bencze M, Kuneš J, Vaněčková I. The interaction of calcium entry and calcium sensitization in the control of vascular tone and blood pressure of normotensive and hypertensive rats. Physiol Res. 2014; 63(Suppl 1).
14. Haghdoost-Yazdi H, Piri H, Faraji A, Fraidouni N, Dargahi T, Mahmudi M, Heidari MA. Pretreatment with potassium channel blockers of 4-aminopyridine and tetraethylammonium attenuates behavioural symptoms of Parkinsonism induced by intrastriatal injection of 6-hydroxydopamine; the role of lipid peroxidation. Neurol Res. 2016;38(4):294–300. doi:10.1080/01616412.2015.1114290
15. Elliot, CA, Hamlin, MJ. Combined diet and physical activity is better than diet or physical activity alone at improving health outcomes for patients in New Zealand's primary care intervention. BMC Public Health 2018;18:230. https://doi.org/10.1186/s12889-018-5152-z
16. Venkatakrishnan K, Chiu HF, Wang CK. Impact of functional foods and nutraceuticals on high blood pressure with a special +focus on meta-analysis: Review from a public health perspective. Food Funct. 2020;11:27922804.
17. Yu J, Bi X, Yu B, Chen D. Isoflavones: Anti-Inflammatory Benefit and Possible Caveats. Nutrients. 2016 Jun 10;8(6):361. doi:10.3390/nu8060361
18. Farmer C, Robertson P, Gilani GS. Effects of dose and route of administration of genistein on isoflavone concentrations in post-weaned and gestating sows. Animal. 2013 Jun;7(6):983–9. doi:10.1017/S1751731113000037
19. Fuloria S, Yusri MAA, Sekar M, Gan SH, Rani NNIM, Lum PT, et al. Genistein: A Potential Natural Lead Molecule for New Drug Design and Development for Treating Memory Impairment. Molecules. 2022 Jan 1;27(1):265. doi:10.3390/molecules27010265
20. Farmer C, Robertson P, Gilani GS. Effects of dose and route of administration of genistein on isoflavone concentrations in post-weaned and gestating sows. Animal. 2013;7(6):983–989.
21. Yang Z, Zhu W, Gao S, Xu H, Wu B, Kulkarni K, et al. Simultaneous determination of genistein and its four phase II metabolites in blood by a sensitive and robust UPLC-MS/MS method: Application to an oral bioavailability study of genistein in mice. J Pharm Biomed Anal. 2010;53(1):819.
22. Antoni S, Ana SS, Dalia IS, Jaime L, Maria D, Seyed FN, Seyed MN. Hypotensive effects of genistein: From chemistry to medicine. Chemico-Biological Interactions. 2017;268:37–46. ISSN 0009-2797
23. Richardson SI, Steffen LM, Swett K, Smith C, Burke L, Zhou X. Dietary Total Isoflavone Intake Is Associated With Lower Systolic Blood Pressure: The Coronary Artery Risk Development in Young Adults (CARDIA) Study. J Clin Hypertens (Greenwich). 2016 Aug;18(8):778–83. doi: 10.1111/jch.12760