



Effect of *Garcinia kola* Consumption on the Blood Pressure of Normotensive Individuals

Ayomide Afolabi Coker¹ , Adebunsi Akande Ademisoye² , Julius Olugbenga Soyinka^{1*} 

¹ Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria

² Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Lead City University, Ibadan, Nigeria

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*Corresponding author:

E-mail: juliussoyinka@gmail.com

ABSTRACT

Introduction: *Garcinia kola*, commonly known as bitter kola, has been widely used in traditional African medicine for its therapeutic properties. It is particularly recognized for its potential antihypertensive effects, as demonstrated in animal studies, notably in Wistar rats. This study aimed to evaluate the effects of oral *G. kola* consumption on the blood pressure of normotensive individuals.

Methods: A single-arm, non-randomized repeated-measures study was conducted with 22 normotensive participants aged 18–50 years. Each participant consumed 15 g of *G. kola* daily for two consecutive days. Blood pressure measurements were recorded at baseline and at 30, 60, 120, and 180 minutes post-ingestion. Statistical analysis was performed using SPSS, and a paired t-test was applied to identify significant differences in blood pressure readings following *G. kola* consumption.

Results: There were no significant changes ($p > 0.05$) in mean diastolic blood pressure or mean heart rate at any time point across the two days of evaluation. However, a significant reduction ($p < 0.05$) in mean systolic blood pressure was observed at the 60-minute mark post-ingestion.

Conclusion: While *G. kola* consumption resulted in a transient significant decrease in systolic blood pressure at 60 minutes post-ingestion, this effect was not sustained beyond the first hour. These findings highlight the need for further research to elucidate the mechanisms underlying the antihypertensive properties of *G. kola*.

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Introduction

Blood pressure is the pressure exerted by the blood pushing against the walls of the arteries as it is pumped throughout the circulatory system by the heart (Whelton *et al.*, 2018). Elevated blood pressure, or hypertension, can lead to severe health issues, including an increased risk of heart disease, stroke, and other cardiovascular disorders. Conversely, abnormally low blood pressure, or hypotension, may result in inadequate blood flow to vital organs, leading to dizziness, fainting, and, in extreme cases, shock (Sharma *et al.*, 2023). Thus, maintaining a balanced blood pressure range is crucial for overall well-being and the prevention of associated health complications. According to the World Health Organization (2023), hypertension, or high blood pressure, is when blood exerts excessive force on artery walls, risking severe health issues if unmanaged (typically 140/90 mmHg or higher). Hypertension poses a significant risk to global public health, with its prevalence steadily increasing across diverse populations. While modern medicine offers various pharmaceutical interventions for managing hypertension, traditional and alternative remedies have also been historically employed to mitigate blood pressure fluctuations. Herbal supplements such as *Garcinia kola* (bitter kola), *Crataegus spp.* (hawthorn), *Allium sativum* (garlic), and the leaf extract of *Olea europaea* (olive), along with lifestyle modifications encompassing dietary adjustments and physical exercise, have been explored for their potential in regulating blood pressure (Sharma *et al.*, 2023). A normotensive individual is defined by blood pressure levels within the normal range, typically with systolic blood pressure below 120 mmHg and diastolic blood pressure below 80 mmHg (Whelton *et al.*, 2018; American College of Cardiology (ACC), 2018). Normotension signifies a healthy and balanced state of blood pressure (ACC, 2018). The pharmacological properties of *G. kola* (bitter kola) have attracted significant attention due to the plant's ethnomedicinal uses, particularly in African traditional medicine. Notably, bitter kola is known for its bioactive compounds, including flavonoids, xanthenes, and benzophenones, which have been linked to various health benefits (Emmanuel *et al.*, 2022). These compounds exhibit antioxidant, anti-inflammatory, and antimicrobial properties,

further supporting the plant's use in managing ailments like diabetes, inflammation, and digestive disorders (Adebisi, 2004).

Bitter kola's antihypertensive effects, which are the focus of this study, may be attributed to its ability to induce vasodilation and improve lipid profiles in animal studies. In rats, the bioflavonoid kolaviron was shown to lower blood pressure and enhance the production of vasodilators, such as nitric oxide, which plays a critical role in regulating vascular health (Olatoye *et al.*, 2023). These pharmacological properties provide a strong foundation for further investigation into its potential effects on human blood pressure, particularly in normotensive individuals.

Through this study, the hope is to build upon the ethnobotanical knowledge surrounding *G. kola* by providing empirical data on its cardiovascular effects, potentially paving the way for new herbal-based approaches to hypertension management.

A cultural practice involves chewing *G. kola* seeds as a masticatory substance that promotes stimulation of salivary secretions and the seeds are also widely savoured as snacks (Ajibade *et al.*, 2015). Notably, *G. kola* distinguishes itself from other kola nuts, such as *Cola nitida* and *Cola acuminata*, by its purported ability to cleanse the digestive system without inducing abdominal discomfort, even with substantial consumption (Adebisi, 2004).

Culturally, *G. kola* holds immense significance for various sub-Saharan African communities, especially the Yoruba and Igbo tribes in Nigeria. *G. kola* nuts play a pivotal role in traditional ceremonies, symbolizing significant life events like births, marriages, and the conferring of chieftaincy titles. Interestingly, the customary planting of a *G. kola* nut tree accompanies the birth of a baby, with the child becoming its lifelong owner. In marriage proposals, the presentation of *G. kola* nuts to the bride's father underscores their cultural importance in various aspects of life and relationships (Ofusori *et al.*, 2008).

G. kola has been an integral component of traditional African medicine, utilized for various therapeutic purposes like anti-inflammatory properties (Daramola *et al.*, 2011), aphrodisiac effects (Yakubu and Quadri 2012), blood sugar reduction (Etim *et al.*, 2020), and digestive aid (Icheku *et al.*, 2018). Due to this, it is commonly eaten by various people for these medicinal

properties. It has also been reported for its antihypertensive activity in Wistar rats (Naiho & Ugwu, 2009), which is the basis of this study.

G. kola, commonly known as bitter kola, contains a rich array of bioactive compounds. Phytochemical analysis of bitter kola revealed the isolation of several chemical compounds, including various *Garcinia* bioflavonoids and most importantly Kolaviron (Rao *et al.*, 2013). Notable constituents include garcinol, isogarcinol, bioflavonoids such as *Garcinia* biflavonoid-1 (GB-1) and *Garcinia* biflavonoid-2 (GB-2), tannins, saponins, and kolaviron, a complex mixture of bioflavonoids (GB-1, GB-2 and Kolaflavinone), extracted from *G. kola* seeds (Rao *et al.*, 2013; Iwu, 2013).

Kolaviron, in particular, has garnered significant attention due to its diverse array of bioactive compounds and potential physiological significance. Chemical analyses have revealed the structural complexity of kolaviron, which contributes to its many interactions *in vivo* and sets the stage for exploring its effects on blood pressure (Rao *et al.*, 2013).

Studies by Ogunlana *et al.* (2016) have explored the vasodilatory effects of kolaviron, indicating its influence on blood vessel dilation. Understanding these effects sheds light on the mechanisms through which kolaviron may contribute to maintaining healthy blood pressure levels.

The vasodilatory potential of kolaviron adds a critical dimension to its role in cardiovascular health, offering insights into its potential as a vasodilator (Ajiboye *et al.*, 2014).

Examining the connection between the consumption of bitter kola and its influence on blood pressure is of paramount importance. Given the escalating global prevalence of hypertension, it is imperative to investigate the ethnomedicinal claims to provide more information on the potential health implications, risks or benefits associated with the consumption of bitter kola. Thus, this study aimed to investigate the effects of oral consumption of *G. kola* on the blood pressure of normotensive individuals through a single-arm, non-randomized, repeated measure study over a three-day period. The study focused on understanding how *G. kola* influenced systolic and diastolic blood pressure, heart rate, and its potential relationship to antihypertensive activity. Specifically, it aimed to assess changes in both heart rate, systolic and diastolic blood pressure in response to *G. kola* consumption over the three

days and to establish a relationship between the plant's consumption and its proposed antihypertensive effects.

Materials and Methods

Materials and Equipment

The materials used include Mettler Balance (Mettler Toledo, Switzerland SNR: 1117220734), Automatic Blood Pressure Monitor OMRON M2 Basic (HEM-7121J-E), and a weighing scale OMRON HN289 (HN-289-ESL).

Plant Collection and Authentication

G. kola Heckel (Clusiaceae) seeds were obtained from herb sellers at Ikoyi quarter, Ile-Ife. The longitude of this market is 7.512278 N, and the latitude is 4.575672 E. The identification of *G. kola* was conducted through a detailed examination of its morphological characteristics, such as the seed size, shape, and texture, in comparison with standard botanical descriptions from reference materials. An experienced botanist from the Medicinal Plant Herbarium further authenticated the species, ensuring its accuracy by cross-referencing herbarium records and taxonomic keys. The verified specimen was assigned a voucher number (FPI 2492) and deposited in the Faculty of Pharmacy's herbarium for future reference.

Preparation of *G. kola*

The collected *G. kola* seeds' dark brown shafts were peeled off. The collected yellow seeds were weighed to determine the average weight measurement of the seeds using a Mettler Balance (Mettler Toledo, Switzerland SNR: 1117220734). The weighing process involved measuring ten randomly selected *G. kola* seeds to determine their average weight. After peeling the outer shaft of each seed, they were weighed individually using a calibrated Mettler Balance (Mettler Toledo, Switzerland SNR: 1117220734). The recorded weights of the ten seeds were: 16.1578, 15.9154, 14.8120, 16.2717, 14.5070, 14.4362, 15.3107, 15.0452, 13.5089, and 16.9582 g. The average weight was calculated by summing these individual weights and dividing by the total number of seeds, resulting in an average of 15.2918 g. Selecting ten seeds provided a representative sample size for the study while minimizing variability in seed size, ensuring consistent dosing across participants.

Procedures

The study design was a single-arm, non-randomized repeated measure study to investigate the effect of oral consumption of *G. kola* seed on the blood pressure of normotensive Individuals. The Participants were volunteers from the Obafemi Awolowo University students and staff population. This study enrolled a total of 22 participants between the ages of 18 and 50 years. The ethical review process for this study adhered to the Declaration of Helsinki and received approval from the Health Research Ethics Committee (HREC) of the Institute of Public Health, Obafemi Awolowo University, Nigeria (HREC Number IPH/OAU/12/2487). Informed consent was obtained through a signed questionnaire and consent form, ensuring participants understood the study's purpose, procedures, risks, and benefits. To protect confidentiality, participants were assigned unique identification numbers, and all data were anonymized. Testing was conducted in a secure and isolated room within the Clinical Pharmacy Skills Laboratory, limiting access to authorized personnel only, thus preventing any potential disclosure of participants' identities.

Twenty 22 participants were administered an average weight of 15 g of bitter kola (Ozolua & Ao, 2016; Emmanuel *et al.*, 2022) on the first day.

Before the study began, both the Mettler scale and the blood pressure monitor were calibrated to ensure accuracy in measurements. The Mettler scale was tested using standard weights to confirm its precision, and any necessary adjustments were made to align with manufacturer standards. Similarly, the blood pressure monitor was calibrated by comparing its readings with those from a mercury sphygmomanometer, ensuring they fell within an acceptable range. This ensured all weight and blood pressure measurements during the study were reliable and accurate.

The selected participants were allowed to sit down for 10 minutes in order to be in a state of rest before the process began. They were asked to take a deep breath and the OMRON blood pressure machine was fixed into their right upper arm at chest length. The participants' blood pressure (BP) was examined. The first reading (baseline reading) was taken after 15 minutes (0 mins) before consumption of *G. kola* seeds. Other readings were recorded at 30 minutes, 60

minutes, 120 minutes and 180 minutes after consumption of *G. kola* seeds. The readings were taken twice to determine the average in order to reduce variations. These procedures were repeated consecutively for the next two days (Days 2 and 3). A flow diagram illustrating the entire process of recruitment, administration of *G. kola* seeds, and measurement of blood pressure is shown in Figure 1.

For quality assurance, all measurements were taken by the researcher to ensure uniformity in findings and were taken between 8 am and 11 am each day to avoid diurnal variation of blood pressure. Participants were advised to stay away from alcohol, caffeine products, caffeinated drinks, and energy drinks starting five days before the experiment and during the period of the study as they have been shown to raise blood pressure (Emmanuel *et al.*, 2022).

Inclusion Criteria

Healthy individuals, both male and female, aged between 18 and 50 years, with normal blood pressure, systolic blood pressure below 120 mmHg and diastolic blood pressure below 80 mmHg (American College of Cardiology [ACC] *et al.*, 2018; Whelton *et al.*, 2018) were eligible to participate in the study.

Exclusion Criteria

Participants were excluded if they fell outside the normal body weight range or had a history of hypertension (blood pressure $\geq 140/90$ mmHg). Participants were also excluded if they had diabetes mellitus, cardiovascular disease, contraindication to *G. kola* (hypotension, palpitation, and dizziness), gastrointestinal ulcer, and Glucose-6-phosphate dehydrogenase (G-6-P) deficiency. Smokers, alcoholics, pregnant women and breastfeeding mothers were also excluded. These criteria are implemented to minimize the influence of confounding variables on blood pressure.

Data Analysis

The analysis was done using SPSS (Statistical Package for Social Sciences), version 21.0 for Windows. Descriptive statistics using percentage frequency distribution mean and the standard deviation were employed, while a paired t-test was used to analyze the data for this study. The level of significance was set at $p < 0.05$.

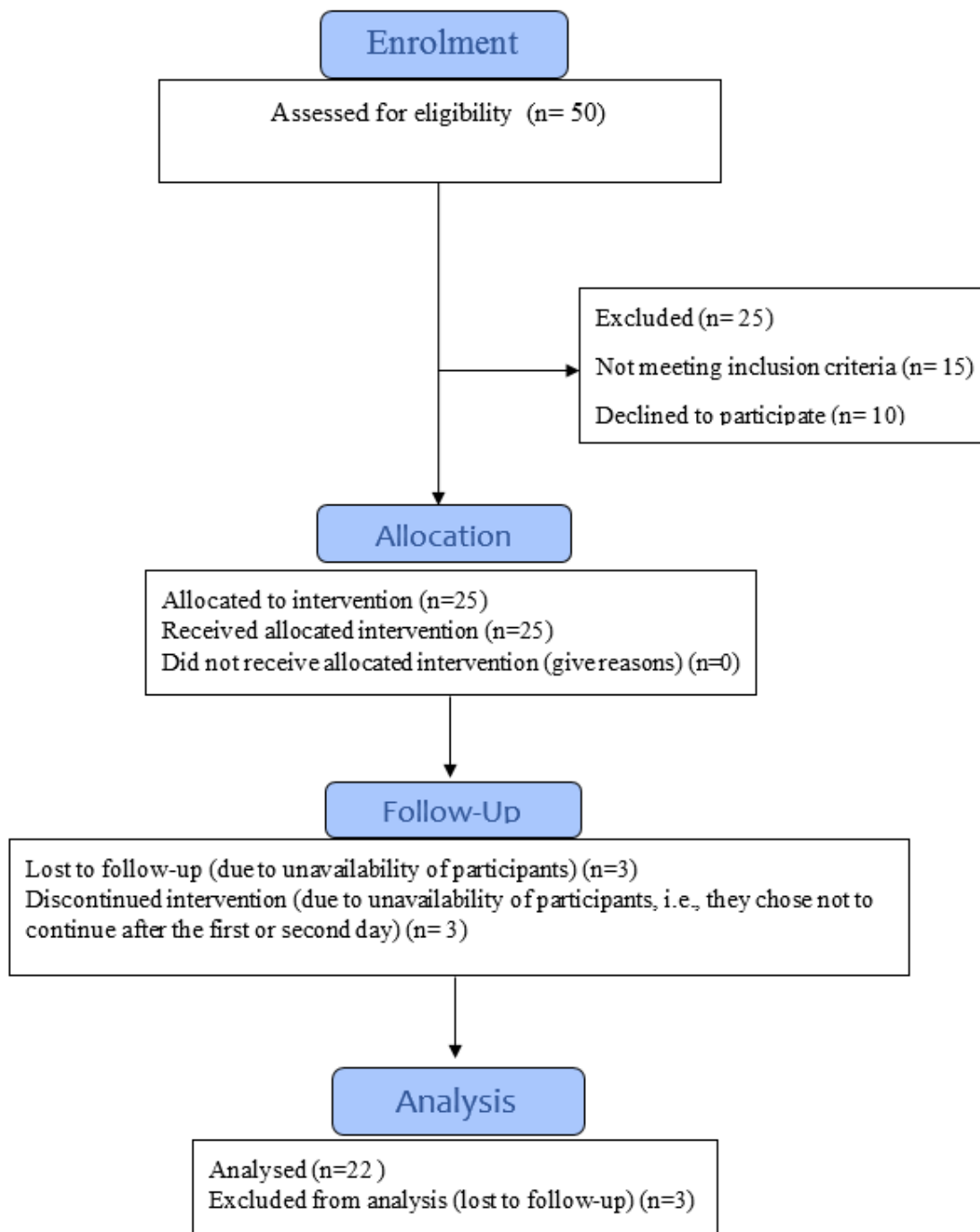


Figure 1: A flow diagram illustrating the recruitment, seed administration and blood pressure measurement.

Results

Descriptive Analysis of Demographic Variables

A total of twenty-two (n=22) participants took part in this study. The majority of the participants were females (54.5%) while 45.5% were males.

Tables 1 shows the sociodemographic information of the respondents. Participants' weight ranged from 45.0 to 81.9 kg with a mean of 65.0 ± 9.2 kg. Also, their age ranged from 18 to 28 years, with a mean of 24.1 ± 2.5 years. The height of the participants ranged from 152 to 185 cm with a mean of 169.0 ± 8.3 cm.

Table 1: Statistical data on the weight, age and height of the participants

Statistical data	Weight (Kg)	Age (years)	Height (cm)
Mean	66.2	24.1	169.5
Median	65.0	24	169
Standard Deviation	9.2	2.5	8.3
Maximum	81.9	28	185
Minimum	45.0	18	152

Mean Systolic, Diastolic Blood Pressure and Heart Rate of Participants after Consumption of *Garcinia kola* after Different Time Intervals

The mean change in systolic blood pressure at all times of assessment after ingestion of *G. kola* was statistically significant only at 60 minutes to the baseline systolic blood pressure (At 30 minutes, $p = 0.8561$, $t = 0.4016$; at 60 minutes, $p = 0.0052$, $t = 3.115$; at 120 minutes $p = 0.1809$, $t = 1.384$; at 180 minutes $p = 0.1037$, $t = 1.701$). The mean change in diastolic blood pressure at all times of assessment after ingestion of *G. kola* was not

statistically significant to the baseline diastolic blood pressure (At 30 minutes, $p = 0.8672$, $t = 0.1693$; at 60 minutes, $p = 0.3384$, $t = 0.9796$; at 120 minutes $p = 0.1195$, $t = 1.623$; at 180 minutes $p = 0.1534$, $t = 1.481$). The mean change in heart rate was not statistically significant at all the time of assessment (At 30 minutes, $p = 0.3582$, $t = 0.9393$; at 60 minutes, $p = 0.8657$, $t = 0.1712$; at 120 minutes $p = 0.9866$, $t = 0.0170$; at 180 minutes $p = 0.5192$, $t = 0.6555$).

The mean systolic, and diastolic blood pressure and heart rate of participants after consumption of *G. kola* after different time intervals are presented in Table 2, and in Figures 2, 3 and 4 respectively.

Paired T-test

The p-value and t-statistic of the Systolic Blood Pressure, Diastolic Blood Pressure and Heart Rate at 30, 60, 120 and 180 minutes are shown in Tables 3.

Table 2: Mean systolic, diastolic blood pressure and heart rate of participants

Time (min)	Systolic blood pressure (mmhg)			Diastolic blood pressure (mmhg)			Heart rate (mmhg)		
	Mean \pm S.D	Diff from 0 MIN	Diff (%)	Mean \pm S.D	Diff from 0 MIN	Diff (%)	Mean \pm S.D	Diff from 0 MIN	Diff (%)
0	109.1 \pm 7.3			71.6 \pm 7.2			75.8 \pm 8.2		
30	108.7 \pm 8.0	-0.44	0.40	71.4 \pm 6.9	-0.11	0.15	75.0 \pm 8.9	-0.86	1.13
60	107.0 \pm 7.6	-2.16	1.99	70.7 \pm 7.2	-0.87	1.22	75.6 \pm 8.7	-0.17	0.23
120	107.8 \pm 9.4	-1.32	1.23	70.0 \pm 7.4	-1.55	2.19	75.8 \pm 9.3	-0.02	0.03
180	107.7 \pm 8.7	-1.42	1.32	70.4 \pm 7.3	-1.14	1.63	76.7 \pm 9.3	0.91	1.20

Table 3: Systolic blood pressure, diastolic blood pressure and heart rate at 30, 60, 120 and 180 minutes

Time	Systolic blood pressure (mmhg)		Diastolic blood pressure (mmhg)		Heart rate	
	p-value	t-statistic	p-value	t-statistic	p-value	t-statistic
30 MIN	0.8561	0.4016	0.8672	0.1693	0.3582	0.9393
60 MIN	0.0052	3.115	0.3384	0.9796	0.8657	0.1712
120 MIN	0.1809	1.384	0.1195	1.623	0.9866	0.0170
180 MIN	0.1037	1.701	0.1534	1.481	0.5192	0.6555

Null hypothesis: There is no significant difference between blood pressure at 0 min and at each subsequent time point (30, 60, 120, 180 min); Alternative hypothesis: There is a significant difference between blood pressure at 0 min and at each subsequent time point (30, 60, 120, 180 min); *Significant level: 0.05.*

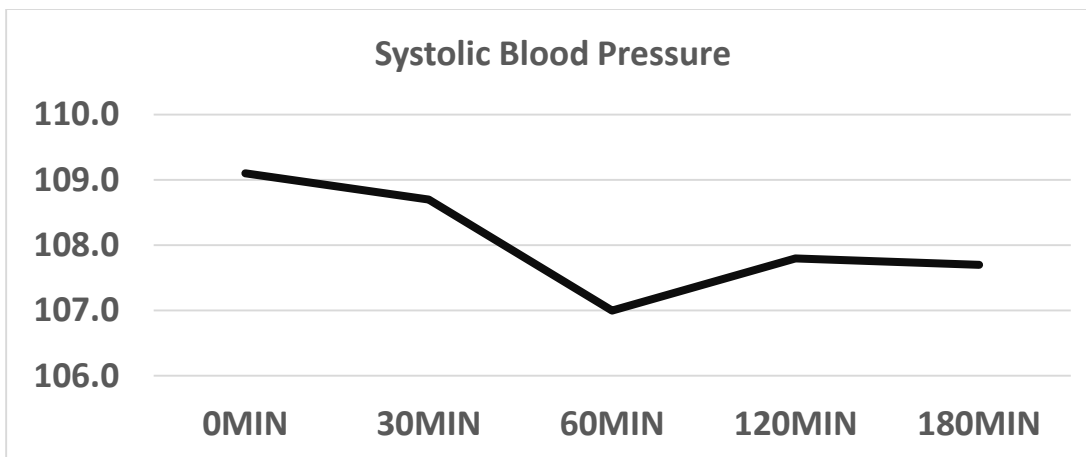


Figure 2: Line graph showing the mean systolic blood pressure of the participants

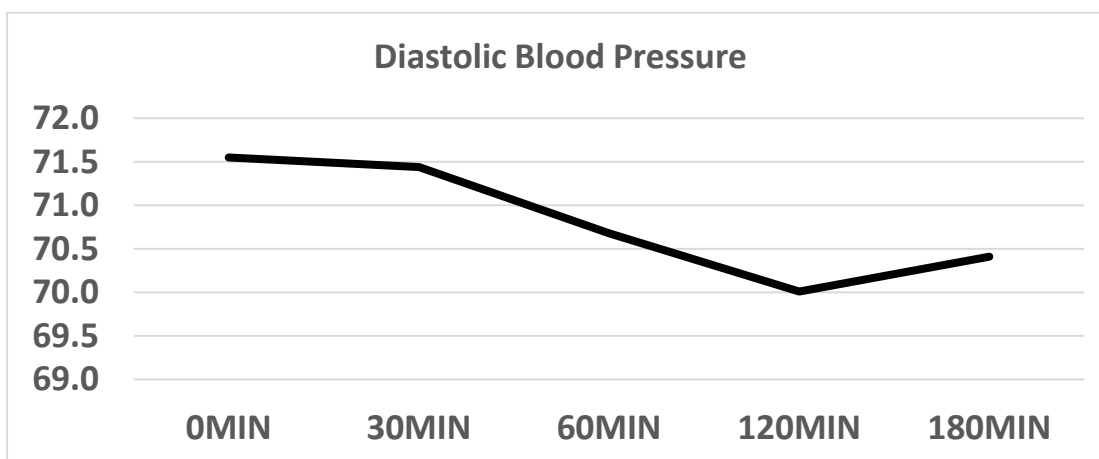


Figure 3: Line graph showing the mean diastolic blood pressure of the participants

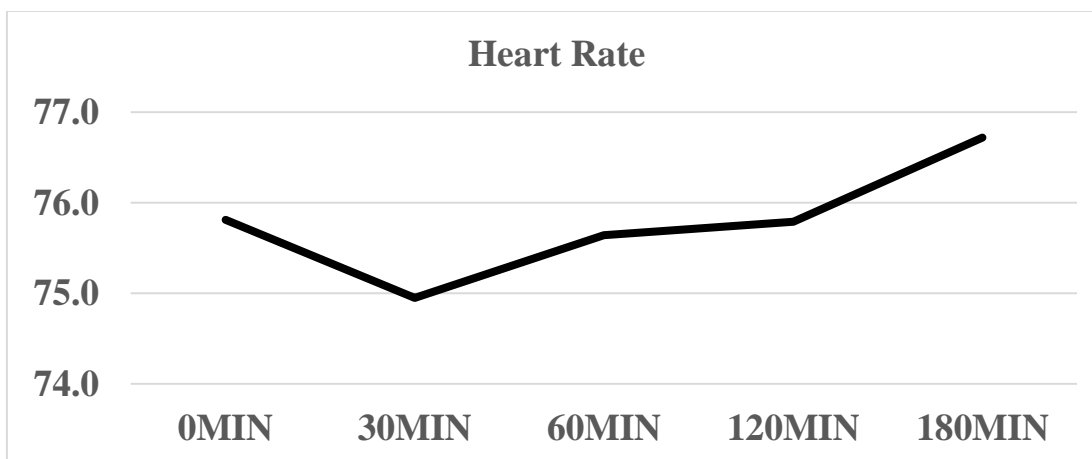


Figure 4: Line graph showing the mean heart rate of the participants

Discussion

Hypertensive disorders are among the leading causes of adult morbidity and mortality in Africa (Rao et al., 2006). Numerous plant materials have been studied and documented for their blood pressure-lowering properties, including the seeds of pawpaw, *Hibiscus sabdariffa*, and garlic (Naiho & Ugwu, 2009; Jalalyazdi et al., 2019; Ried, 2020). The study design was a single-arm, non-randomized repeated measure study to determine whether the average daily oral consumption of *G. kola* seed significantly reduces blood pressure in normotensive individuals. Twenty-two participants, aged 18 to 28 years, were recruited for this study. To clarify the study conditions, participants were not required to fast prior to the blood pressure measurements, allowing them to consume their regular meals. During data collection, environmental factors were carefully controlled to ensure accurate results. All measurements were taken in a quiet, comfortable setting within the Clinical Pharmacy Skills laboratory, minimizing distractions and stress. Participants were seated with their backs supported, feet flat on the floor, and arms at heart level to standardize the measurement conditions. Additionally, all readings were conducted during the morning hours (between 8 am and 11 am) to reduce variability caused by diurnal fluctuations in blood pressure. Participants were encouraged to maintain their regular lifestyle and advised to refrain from alcohol, smoking, and caffeinated beverages for 3-5 days before and throughout the study. These precautions aimed to minimize external factors that could influence blood pressure readings, enhancing the objectivity and reliability of the data collected.

The mean age was 24.1 ± 2.5 years. The age indicates that all the participants were youth in their productive years, which falls within the estimated average blood pressure age. The normal blood pressure within this age group is 119/70 mmHg for males and 110/68 mmHg for females. It has been reported that ageing increases systolic blood pressure (Singh et al., 2023). The average weight of the participants is 66.2 kg. Mertens and van Gaal (2012) revealed that an increase in body weight results in an increase in blood pressure. The results indicated that most participants were female (54.5%) while 45.5% were males. Studies have shown that there is a relationship between gender and blood pressure. It revealed that men typically have higher blood pressure and develop cardiovascular diseases earlier than women (Maranon & Reckelhoff, 2013). This study demonstrated that oral consumption of *G. kola* led to a reduction in systolic blood pressure over time. Specifically, there was a 0.40% (0.44 mmHg) decrease in mean systolic blood pressure after 30 minutes, followed by a 1.99% (2.16 mmHg) reduction after 60 minutes, a 1.23% (1.32 mmHg) reduction after 120 minutes, and a 1.32% (1.42 mmHg) reduction after 180 minutes (Table 3).

Similarly, *G. kola* induced a 0.15% (0.11 mmHg) decrease in mean diastolic blood pressure after 30 minutes of ingestion, with a 1.22% (0.87 mmHg) reduction after 60 minutes, a 2.19% (1.55 mmHg) reduction after 120 minutes, and a 1.63% (1.14 mmHg) reduction after 180 minutes (Table 3).

This study showed that the effect of *G. kola* on the blood pressure of normotensive individuals was significant ($p < 0.05$) on the first and second days of the assessment. The significant reduction ($p < 0.05$) observed in the systolic blood pressure by intake of *G. kola* is transient since the reduction could not be consistently sustained beyond 60 minutes after ingestion. It can be observed from this study that oral consumption of *G. kola* poses no adverse effect on the blood pressure of the participants. It was also observed that *G. kola* reduced the mean blood pressure of the participants significantly ($p < 0.05$) after 60 minutes of ingestion. This result confirmed the earlier reports that *G. kola* seed supplementation significantly ($p < 0.05$) reduced the blood pressure in 24 adult patients with high normal blood pressure (Kuate et al., 2021). Heart rate plays a crucial role in the comprehensive assessment of hypertensive patients (Reule & Drawz, 2012). Elevated heart rate has been reported to be associated with increased blood pressure and the potential risk for hypertension (Reule & Drawz, 2012). Assessing heart rate in hypertensive patients can be a useful indicator for evaluating treatment adherence (Fodor et al., 2005). The relationship between antihypertensive medications and heart rate reduction has not been validated. This study revealed that *G. kola* reduced the heart rate by 1.13% (0.86 mmHg) after 30 minutes of ingestion, 0.23% (0.17 mmHg) after 60 minutes of ingestion, and 0.03% (0.02 mmHg) after 120 minutes of ingestion. The heart rate increased by 1.20% (0.91 mmHg) after 180 minutes of ingestion (Table 3). Naiho and Ugwu (2009) conducted a study investigating the effects of *G. kola* on blood pressure in Wistar rats. Their findings indicated that *G. kola* reduced mean arterial pressure while simultaneously increasing basal heart rate. This rise in heart rate was likely a compensatory response to decreased total peripheral resistance, which resulted from the extract's action on vascular smooth muscle. The study concluded that *G. kola* contains a vasoactive compound capable of lowering blood pressure. The paired t-test result showed that at 60 minutes, the p-value is less than 0.05 for systolic blood pressure. The result means that there was a significant decrease in systolic blood pressure at the baseline (0 min) and at 60 minutes after consuming *G. kola*. Normality testing was conducted to confirm the assumptions for paired t-tests. To enhance the study's validity, alternative statistical tests, such as Wilcoxon signed-rank tests for non-parametric data, could have been employed, providing additional insights into the effects of *G. kola* on blood pressure.

The study showed that *G. kola* may contain active compounds with good antihypertensive activity. Olatoye *et al.* (2023) reported that Kolaviron significantly affected ethanol- and sucrose-induced hypertension. The blood pressure-lowering effect of the seed of *G. kola* may be due to the presence of this compound or in combination with other compounds due to synergistic actions. The study utilized a single-arm, non-randomized, repeated measure design to evaluate the effects of *G. kola* on blood pressure in normotensive individuals, with participants serving as their own controls. A placebo group was not included due to the exploratory nature of the study, which aimed to gather preliminary data on the acute effects of *G. kola*. However, this design presents limitations, as the absence of a placebo makes it difficult to distinguish the substance's effects from placebo responses, and potential confounding variables may not be evenly distributed among participants. Future research should consider incorporating a controlled design with randomization and a placebo group to validate these findings. While this study provides valuable insights into the short-term effects of *G. kola* on systolic blood pressure in normotensive individuals, it highlights the necessity for further exploration of its underlying mechanisms. Future research could embark on longer-term trials to assess the sustained effects of *G. kola* on blood pressure and explore different dosages to identify optimal levels for therapeutic benefit. Additionally, investigating the potential interactions of *G. kola* with common antihypertensive medications could provide crucial insights into its role in comprehensive hypertension management. By expanding the scope of research, we can unlock a deeper understanding of how *G. kola* functions and its potential as a viable option in blood pressure regulation.

Conclusion

Consumption of *G. kola* can lower systolic blood pressure in normotensive individuals. However, this effect was found to be transient as the reduction observed was not consistently sustained after 1 hour of ingestion. The study revealed that *G. kola* may contain constituents (like kolaviron) with potential as active antihypertensive drugs. However, the mechanism of action should be subjected to further study.

Declaration

Conflict of interest

The authors report that there is no conflict of interest.

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Consent to participate

All participants agreed to consent to participate anonymously.

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Author's contributions

JOS designed the research study, AAC carried out the study procedures, JOS, AAC and AAA analyzed the results, wrote and reviewed the manuscript.

Availability of data and material

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Ethical considerations

This study was performed in line with the principles of the Declaration of Helsinki.

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