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Research article

Kolaviron Attenuates Elevation in Blood Pressure and Ameliorates Dyslipidemia in Salt-Induced Hypertensive Sprague-Dawley Rats

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ABSTRACT

Antilipidemic and smooth muscle inhibition are known properties of kolaviron (KV), a biflavanoid of *Garcinia kola*-fraction. This study investigated the hypothesis that KV may prevent elevation in blood pressure and dyslipidemia in hypertension. Thirty-two (32) Sprague-Dawley rats (inbred) weighing 110-130g were randomly separated into four groups of 8 rats each. Control rats (CR) received normal rat chow and water ad libitum. Group 2 received rat chow containing 8% NaCl (high salt diet-HSD); while the remaining experimental groups were concomitantly given HSD + 200 mg/kg bw/day of KV and/or 2.3 mg/kg bw/day of lisinopril (LP) respectively by oral gavage for 6 weeks. Measurements of blood pressure [(Bp) (mmHg)] were by the cuff-tail artery blood pressure and the blood pressure transducer via cannulation of the left common carotid artery following anaesthesia with urethane. The heart rate [(HR)(bpm)] was determined by counting the number of arterial pulses over a period of 60 seconds. 5 ml of blood was collected from the carotid artery for lipid profile including total cholesterol (TC), Triglycerides (TGC), low density lipoprotein (LDL) and high density lipoprotein (HDL). Results showed that systolic, diastolic, mean arterial and pulse pressures (mmHg) were significantly ($p<0.05$) higher in the HSD-fed rats (HSDFR) compared to the CR. KV attenuated elevation in blood pressure and prevented dyslipidemia in HSDFR. Also, serum levels of TC, TGC, LDL were higher while HDL levels were lower in the salt-induced hypertensive than the CR. TC, TGC, LDL, Total/HDL and LDL/HDL ratios decreased significantly ($p<0.05$) in HSDFR-co treated with KV compared to HSDFR. Heart rate showed no significant change. The results from the present study demonstrated that, kolaviron attenuates elevation in blood pressure, ameliorates dyslipidemia in salt-induced hypertension and may be effective in the improvement of atherogenic indices in the cardiovascular system.

Keywords: *Salt-induced hypertension. Blood pressure, kolaviron, and dyslipidemia.*

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INTRODUCTION

High sodium salt dietary intake has long been implicated in the aetiology and progression of hypertension (Ambard and Beaujard, 1904; Kempner,1948; Jin *et al*., 2013). Hypertension and dyslipidemia are associated with oxidative stress and are major factors in the pathogenesis of several diseases including chronic kidney disease, stroke, and increase incidence of cardiovascular disease (CVD) which is also the leading cause of death globally and presents a major public health challenge especially in light of the report that more than 25% of the adult world population has hypertension and the projection that about 29% of the adult world population will become hypertensive by the year 2025 (Morris *et al*., 1999, Drenjancevic-peric *et al* .,2011, Lewington *et al*., 2012, O'Shaughnessy and Kalam *et al*., 2016). Atherogenic indices are known predictive cardiovascular risk factors in clinical practice. Low density lipoprotein (LDL) cholesterol concentration has been the prime index of cardiovascular disease risk and the main target for therapy. In recent times however, Total/high-density lipoprotein (HDL) cholesterol and LDL/HDL cholesterol ratios are risk indicators with greater predictive value than isolated parameters used independently, particularly LDL (Millian *et al*., 2009).

Sodium is an essential element in the body for excitation of nerve and muscle function. It is also relevant in auto-regulation of water and fluid balance of the body (Paula *et al*., 2017). It has been suggested that a reduction in dietary salt from the current intake of 9-12 g/day to the recommended

level of less than 5-6 g/day will have major beneficial effects on cardiovascular health (Sung, 2014; Paula *et al*., 2017). Experimental animal models have shown that Sprague-Dawley rats [(SDR, (inbred)] rapidly develop elevated blood pressure (>170 mm Hg) when fed 8% NaCl diet (Sofola *et al*., 2002, Gu *et al*., 2008, Adejare and Sofola, 2017). In recent times however, changes in lifestyle and dietary supplementation have been shown to be effective in the management and /or prevention of hypertension in combination with routine clinical therapies (Sung, 2014; Vilskerst *et al*., 2014, Feyh *et al*.,2016).

The pharmacological properties and medicinal values of *Garcinia kola* Heckel seed (gKola)(Family: Guttiferae, sub-family: Clusoideae) are widely reported and known to contain high content of biflavonoid compounds (Iwu *et al*., 1987, Farombi, 2000; Adaramoye and Adeyemi, 2006). Kolaviron, a biflavonoid rich complex and a major active ingredient of gkola is also linked with various bioactive compounds including garcinia biflavonoid (GB)-1, GB-2 and kolaflavanone which have direct impact on organs-health (Eisner, 1990, Olayinka *et al*., 2014); including hypolipidemic, hypoglycaemic, hepatoprotective, high antioxidant and safety profile and smooth muscle relaxant effects on experimental animal models (Adaramoye and Adeyemi, 2006; Udia *et al*., 2009; Nwangwa, 2012; Adaramoye *et al*., 2014). Recently, a considerable evidence linking kolaviron, to vascular smooth muscle relaxation in isolated rabbit aortic and carotid arterial rings following agonists precontractions through receptor operated Ca2+ channels (ROCCs) and voltage-operated Ca2+ channels (VOCCs) has been reported (Uche *et al*., 2015, Uche and Ofeimu, 2017).

We hypothesized that hypertension, atherogenesis and /or dyslipidemia may be inhibited by dietary supplementation of kolaviron. Therefore the present study was designed to determine if prolonged dietary kolaviron supplementation could prevent blood pressure elevation, and ameliorate dyslipidemia or improve lipid profile in salt-induced hypertensive Sprague-Dawley rats.

MATERIALS AND METHODS

Extraction of Kolaviron (KV): Extraction of KV was essentially done by the methods of Iwu *et al*., 1990, and Adaramoye and Medeiros (2009). Briefly, 500g of the powder seeds of *Garcinia kola* were extracted with light petroleum ether (bp 40-60°C) in a soxhlet extractor. The defatted, dried Marc was repacked and then extracted with methanol. The extract was concentrated and diluted to twice its volume with distilled water and extracted with ethyl acetate (6 x 250ml). The concentrated ethyl acetate fraction gave a yellowish solid known as kolaviron (49.79g). It was stored in a wide mouth air tight glass container to prevent absorption of moisture. Kolaviron (200mg/kg) was dissolved in 5% tween 80 and diluted to desired concentration with distilled water to give a water-soluble fraction.

Animals: Experiments were carried out on 32 male Sprague-Dawley rats weighing 110-130g and randomly separated into 4 groups consisting of 8 rats each. The experiments were done in line with standard guidelines on use of animals. Animals were maintained according to standard laboratory procedure and given free access to normal rat chow and tap water ad libitum. Basal data of blood pressure (mmHg), weight (grams) and pulse rate (per min) of all the animals were measured and recorded before the commencement of treatment. Group one, control rats (CR) received normal rat chow and water ad libitum. Group 2 received high salt diet (HSD) containing 8% NaCl as described by Sofola *et al*., 2002. Groups 3 and 4 were concomitantly administered with HSD + 200 mg/kg bw/day of KV, and HSD + 2.3 mg/kg bw/day of lisinopril (LP) respectively by oral 6 weeks. Lisinopril (Lp), a known inhibitor served as a standard drug.

At the end of the feeding period, blood pressure (mmHg) and heart rate (bpm) were measured by invasive (common carotid arterial catheter via pressure transducer) and non-invasive (cuff-tail artery) methods following anaesthesia with urethane.

Determination of Lipid profile: 5 ml of blood was collected from the left carotid artery and fasting blood samples were analyzed for electrolytes, total cholesterol, HDL cholesterol (HDL-C), VLDL cholesterol, and triglyceride concentrations with an Hitachi 747 auto analyzer, LDL cholesterol (LDL-C) was calculated using the Friedwald formula (Millian *et al*., 2009). All experimental procedures complied with the standard protocols for the use of laboratory animals (National Institute of Health USA, 2002).

Statistical analysis: Graphs and statistical analyses were by means of OriginPro 8.0 software and Students t-test; followed by one-way analysis of variance (ANOVA). Data are presented as means ± SEM. P-values less than 0.05 ($p<0.05$) was considered statistically significant; while n-values denote number of animals in each experimental group.

RESULTS

As shown in Fig. 1, Both systolic and diastolic blood pressure were significantly elevated (>170 mmHg and > 110 mmHg) respectively in the salt- induced hypertensive rats (SHRs) compared to the control whereas in the high-salt diet cotreated kolaviron (KV) and lisinopril (LP), KV and LP attenuated and decreased blood pressure rise significantly ($p<0.05$). N = 8; means ±SEM.

The changes in pulse and mean arterial pressures during treatment are shown in Table 1. Mean arterial and pulse pressures were significantly ($p<0.05$) higher in SR compared to CR whereas pulse pressure decreased and mean arterial pressure attenuated in KV-treated rats. Mean and pulse pressures were significantly decreased in Lp-treated rats. N = 8; means ±SEM

The changes in lipid profile in the control and treared rats are shown in Fig. 2. Total Cholesterol level [(TCL)(A)], Triglyceride[(TGC)(B)], and LDL(A) levels were significantly ($p<0.05$) elevated in Salt loaded rats (SR) compared to the CR whereas in SR co-treated with KV serum levels rise of TCL, TGC and LDL was prevented and significantly ($p<0.05$) decreased compared to the CR. HDL levels were significantly ($p<0.05$) decreased in SR and SR-co treated with KV compared to the CR. The KV effects were comparable with lisinopril.

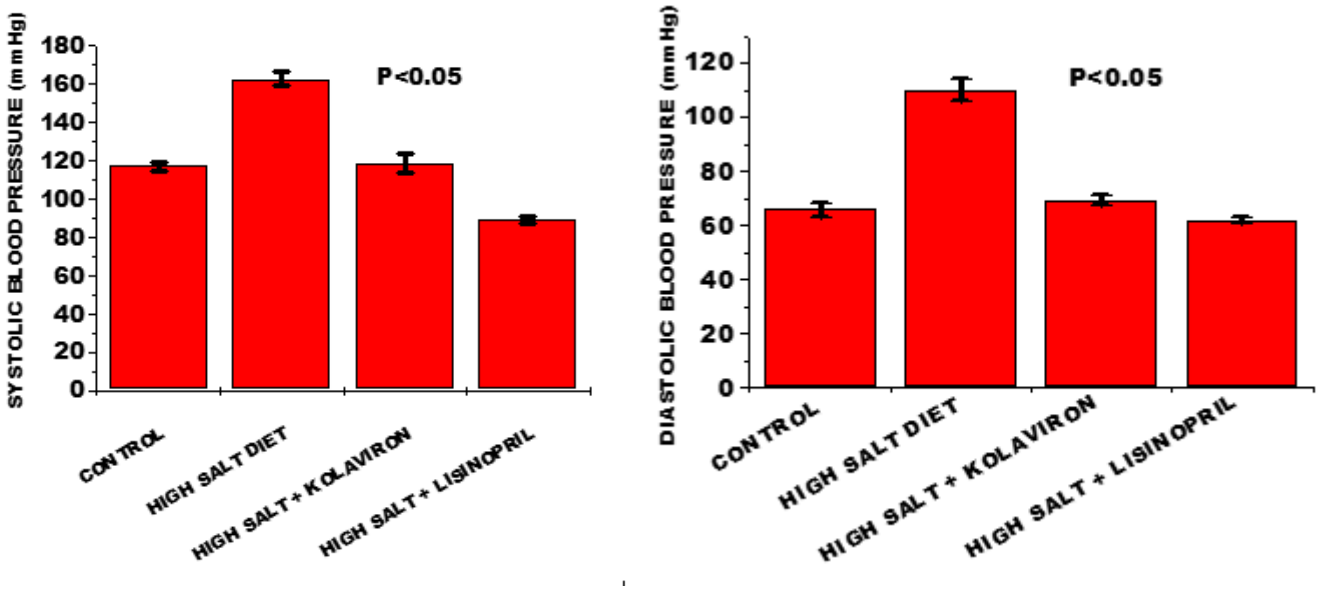

Figure 1
Changes in blood pressure following dietary supplementations

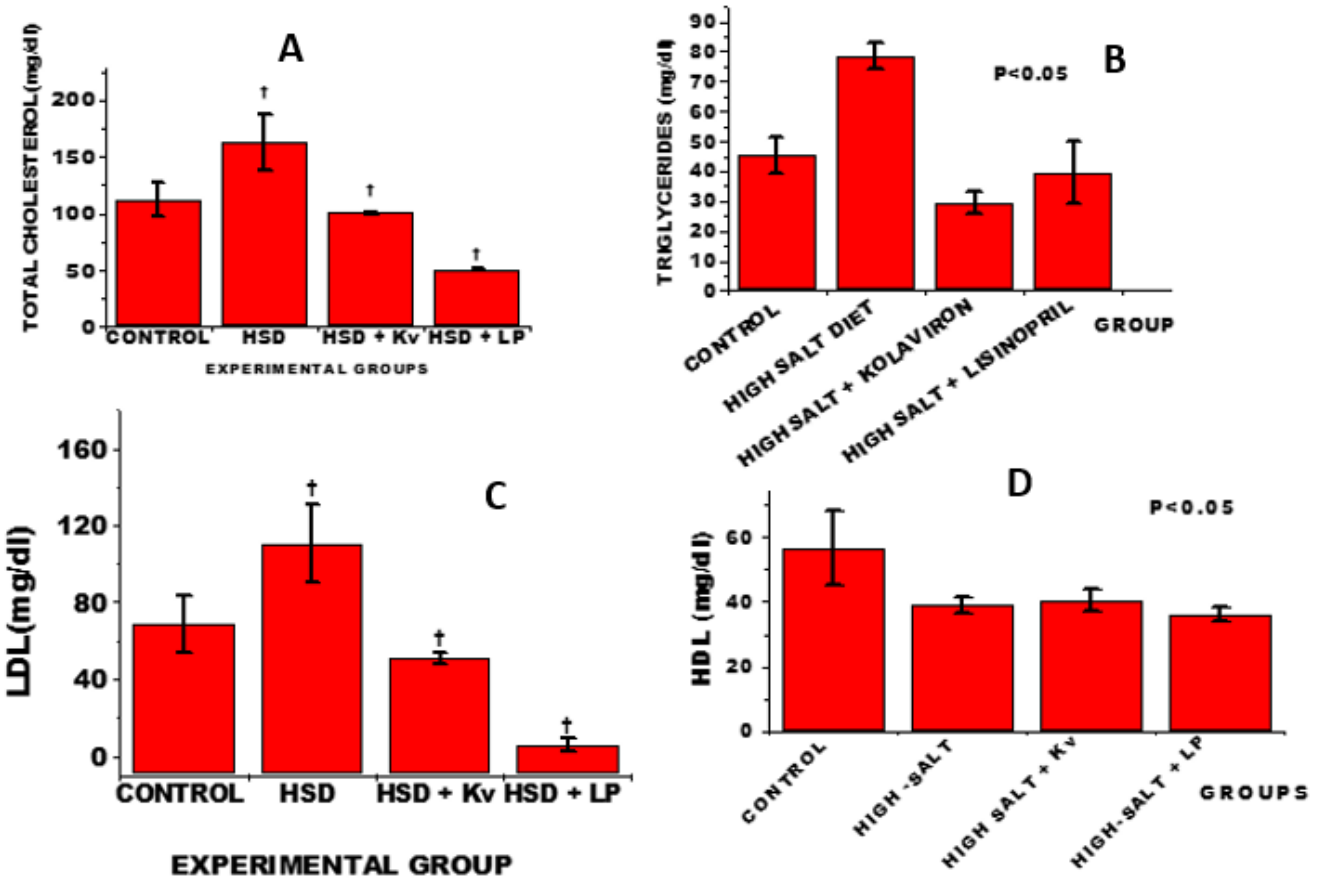

Figure 2
Changes in lipid profile

Table 2 shows high Total/HDL- cholesterol, LDL/HDL-cholesterol and TGC/HDL cholesterol ratios in SR compared to the CR whereas total/HDL-cholesterol and LDL/HDL-cholesterol ratios decreased significantly ($p<0.05$) in HSDFR -co treated with KV compared to SR

Table 1
Changes in pulse and mean arterial pressures during treatment. (N= 8)

Groups	Pulse Pressure mmHg	MAP mmHg
Control	51.10 ±0.36	83.05 ±0.34
High salt diet (HSD)	62.05 ±0.35	130.93 ±0.53
HSD + KV	49.25 ±0.37	89.92± 0.37
HSD + Lp	27.10± 0.05	71.02± 0.05

Table 2. Lipid profile ratio

PARAMETERS	GROUPS	RATIOS
TCL/HDL	**CR:HSD:HSD+KV: HSD+LP**	**2.27 : 4.63 : 1.25 : 1.17**
LDL/HDL	**CR:HSD:HSD+KV: HSD+LP**	**1.18 : 2.75 : 1.25 : 0.17**
TGC/HDL	**CR:HSD:HSD+KV: HSD+LP**	**0.85 : 2.00 : 0.75 : 0.95**

DISCUSSION

From the results, the rats used in this present study developed (>180/120 mmHg) following 6 weeks of dietary salt supplementation and therefore provides additional support to the notion that weanling Sprague-Dawley rats (inbred) fed on 8% NaCl diet for 4-6 weeks develop hypertension (Bp>180mmHg)(Sofola *et al*., 2002). Of equally interesting finding is the higher serum concentrations of total cholesterol, triglyceride, low density lipoprotein-cholesterol (LDL-C) as well as the significant reduction in HDL-C concentrations observed in the salt-induced hypertensive rats compared to the control. In contrast, results of additional experiments demonstrate that salt loaded rats co-treated with kolaviron, a biflavanoid-complex, at a dose of 200 mg/kg for six weeks significantly attenuated the elevation in blood pressure and at the same time prevented the rise in serum levels of TCL, TGC, LDL and significantly ($p<0.05$) decreased their levels compared to the CR (Figure 2). The total/high-density lipoprotein (HDL) cholesterol and LDL/HDL cholesterol ratios were significantly reduced in HSD-fed rats co-treated with kolaviron compared to the salt loaded rats (Table-2). The observations from this study of the increased levels of TCL, TGC, LDL and decreased HDL levels in hypertension are similar to the findings of some other studies (Choudhury *et al*., 2014, Kalam *et al*.,2016),

High sodium diets are usually employed to study diet-induced hypertension since increasing levels of circulating sodium causes cells to release water due to osmotic pressure which exerts pressure on vascular walls especially in resistant blood vessels (Andreas *et al*., 2016). However, salt-loading hypertension syndrome is not only limited to elevation in pressure, but also characterised by an increase in vascular resistance to blood flow, oxidative stress, cardiac hypertrophy, increase sympathetic stimulation, changes in vascular wall matrix and renal dysfunction (De Wardener and MacGregor, 2002; Meneton *et al*., 2005, O'Brien *et al*., 2007, Versari, 2009; Esler *et al*., 2010, Hulin *et al*., 2010). Hypertension and dyslipidemia are major risk factors for cardiovascular diseases (CVD) and account for more than 80% of death and disability (Reddy, 2004). It is widely accepted that CVD is associated with hypertension and increased blood level of low density lipoprotein (LDL), total cholesterol (TC), and triglycerides (TG) (Kalam *et al*., 2016). In contrast, a low level of high density lipoprotein is a risk factor for mortality from CVD (Mora *et al*., 2013). In recent times the total/HDL cholesterol ratio, known as the atherogenic index and the LDL /HDL cholesterol ratio are two important components and indicators of vascular risk, the predictive value of which is greater than isolated parameters of TCL, TGC, LDL and HDL. Other authors have demonstrated in humans the importance of the total/HDL cholesterol atherogenic index in predicting atherosclerosis while individuals with high total /HDL cholesterol or LDL/HDL cholesterol ratio have greater cardiovascular risk owing to the imbalance between the cholesterol carried by atherogenic and protective lipoproteins (Millian *et al*., 2009).The results of the present study clearly show that kolaviron was effective in decreasing the high total /HDL cholesterol and LDL/HDL cholesterol ratios in the hypertensive rats suggesting the antiatherogenic beneficiary effect of kolaviron.

Kolaviron, a biflavonoids-rich complex is a major constituent of *Garcinia kola* (Iwu *et al*., 1987, Farombi, 2000; Adaramoye and Adeyemi, 2006b). The pharmacological properties and medicinal values of *Garcinia kola* have been linked with various bioactive compounds including Garcinia biflavonoids (GB)-1, GB-2 and kola flavanone (Iwu *et al*., 1987, Adegboye *et al*.,2008) which have been shown to be effective smooth muscle relaxants (Adaramoye and Medeiros, 2009; Udia *et al*., 2009), potent antioxidants (James *et al*., 2014, Olayinka *et al*., 2014), anti-inflammatory and antigenotoxic agents believed to result from inhibition of cyclo-oxygenase enzyme (Liang,1999).

Modifications in diet and exercise are effective in managing hypertension and maintaining cardiovascular health (Morris *et al*., 1999; Feyh *et al*., 2016). Also medicinal plants have been widely reported to contain varieties of health beneficiary bioactive compounds including: polyphenols, tocopherols, alkaloids, biflavonoids etc. (Gordana *et al*., 2004, Lee *et al*., 2007). Furthermore, plants containing biflavonoids/flavonoids posses inhibitory effects on smooth muscle activities which leads to decrease in blood pressure (Kabangu *et al*., 1987). Other health benefits of flavonoids have been attributed to their actions as antioxidants, free radical scavengers, quencher of singlet and triplet oxygen and inhibitors of peroxidation reactions (Li-chem *et al*., 2006). This may have also contributed to the potency of kolaviron in attenuating the observed elevation in blood pressure in this study, which probably may have been induced by oxidative stress commonly associated with high-salt diet and hypertension (Touyz and Briones, 2011; Kalam *et al*., 2016). Previous studies have suggested the use of kolaviron as a

prophylactic agent in the protection against atherosclerosis due to its antioxidative effects on serum lipoprotein oxidation both in vitro and ex vivo (Farombi *et al*., 2002). The possible mechanisms of protection were suggested to involve metal chelating, anti-oxidant and scavenging of radical species (Farombi and Nwaokeafor, 2005). Recently, the cardioprotective and vasorelaxant effects of kolaviron on mesenteric artery of Wistar rats and rabbit aortic atrial rings via receptor and non-receptor agonist inhibition have been reported (Adaramoye and Medeiros, 2009; Uche and Ofeimu, 2017). Therefore, the observation of an improvement in lipid profile and attenuation in blood pressure elevation in salt-induced hypertensive Sprague-Dawley rats may not be unconnected with kolaviron-induced vascular smooth muscle relaxation and high antioxidant profile potency.

In this study, the improvement in lipid profile and attenuation in blood pressure elevation activities of kolaviron have been benchmarked with Lisinopril (Lp) a known ACE-antihypertensive drug. Comparatively, kolaviron and lisinopril decreased pulse pressure, total cholesterol, HDL and triglyceride levels in salt-induced hypertensive rats lower than the normal values in the control rats; and demonstrates relatively comparable blood pressure attenuation effect suggesting that kolaviron could be an effective agent in cardiovascular protection.

In conclusion, the present study demonstrates that, kolaviron attenuates elevation in blood pressure and ameliorates dyslipidemia in salt-induced hypertension which suggest that it may be an effective anti-atherogenic agent thus offer protection to the cardiovascular system. This may not be unconnected with the reported effect of kolaviron to be vascular smooth muscle relaxant with high antioxidant profile potency..

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