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Kolaviron and *Garcinia kola* attenuate homocysteine-induced arteriosclerosis and cardiotoxicity in Wistar rats

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Abstract

Sixty male Wistar rats were divided into six groups of 10 rats each. Group I was the control. Group II received homocysteine (Hcy) (200 mg/kg body weight) alone daily for 14 days. Rats in groups III and IV received Kolaviron orally at 100 and 200 mg/kg respectively, in addition to Hcy. Rats in groups V and VI received *Garcinia kola* (GK) orally (100 and 200 mg/kg respectively) in addition to Hcy (200 mg/kg) for a period of 14 days. The results showed that Hcy caused a reduction in heart rate, shortened QT and QTc intervals together with low voltage QRS which were reversed by Kolaviron (100 & 200 mg/kg body weight) and *Garcinia kola* (100 & 200 mg/kg body weight). Hcy alone also caused a significant ($p<0.05$) increase in MDA, H_2O_2 generation, Xanthine oxidase, Myeloperoxidase, Lactate dehydrogenase (LDH), Creatinine kinase cardiac specific (CK-MB) and nitrite with a significant ($p<0.05$) reduction in non-enzymic and enzymic antioxidants such as Reduced glutathione (GSH), Glutathione peroxidase (GPx), Glutathione-s-transferase (GST), Superoxide dismutase (SOD) and Catalase (CAT) coupled with a significantly high serum values of total cholesterol, triglycerides and low density lipoproteins (LDL) and a significant reduction in ($p<0.05$) high density lipoprotein (HDL) cholesterol. Overall, co-administration of Hcy with *Garcinia kola* at 200 mg/kg body weight showed a better cardioprotective and anti-atherosclerotic effect on (Hcy)-induced cardiotoxicity and atherosclerosis than Kolaviron in this study.

Keywords: Homocysteine (Hcy); *Garcinia kola*; Kolaviron; anti-atherosclerosis; ECG changes

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1. Introduction

Elevated plasma homocysteine (Hcy) is one of the risk factors for cardiovascular disease. Recent finding showed that Hcy is associated with an increased risk for cardiovascular disease, possibly leading to myocardial damage.[1] Epidemiological studies have associated hyperhomocysteinemia (HHcy) with an increased risk of coronary artery disease, peripheral vascular disease, venous thrombosis, myocardial infarction, and stroke.[2] In the same vein, HHcy has been shown to promote endothelial dysfunction by decreasing the bioavailability of nitric oxide (NO) and increasing oxidative stress in the vasculature.[3] The generation of peroxynitrite is one of the mechanisms recently shown for Hcy-induced cell death in H9c2 cells.[4] Kolling *et al.*[5] reported that chronic Hcy administration increased lipid peroxidation and reactive oxygen species (ROS) production with decrease in the antioxidant defence system of rats. However, significant high levels of Hcy and its derivatives have been shown to contribute to the modification of LDL and HDL particles, inflammation and disorders in coagulation and fibrinolysis.[6] The specific inhibition of Hcy on cellular antioxidant enzymes, like superoxide dismutase and glutathione peroxidase has also been reported.[7] Furthermore, increased oxidative stress in the vasculature in HHcy has been shown to activate the nuclear transcription factor (NF-kappaB) as one of the

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key elements of pro-inflammatory signaling pathways in endothelial cells. The activation of NF-κB promotes increased endothelial expression of chemokines and adhesion molecules that facilitate the recruitment, adhesion and transmigration of circulating leukocytes to the vessel wall.[7]

Kolaviron (KV), a biflavonoid from *Garcinia kola* (GK) seed extract is reportedly known as the most active phytochemical present in GK. The antioxidant, free radical scavenging activity, anti-inflammatory, cytoprotective and other chemopreventive activities of *Garcinia kola* and KV have been reported.[8] This present study was undertaken to investigate the possible ameliorative effect of KV and *Garcinia kola* on Hcy-induced arteriosclerosis in Wistar rats.

2. Materials and methods

Chemicals: Glutathione, 1, 2-dichloro-4-nitrobenzene (CDNB), Doxorubicin, thiobarbituric acid (TBA), Trichloroacetic acid, sodium hydroxide, xylenol orange, potassium hydroxide and hydrogen peroxide were purchased from Sigma (St Louis, MO, USA). All other chemicals were of analytical grade and were obtained from British Drug Houses (Poole, Dorset, UK).

Extraction of *Garcinia kola* and Isolation of Kolaviron (KV): Kolaviron was extracted from the seeds of Garcinia Kola according to the method of Iwu *et al.* with slight modification. [9] The seeds were sliced, air-dried and powdered. The powdered seeds were defatted by extraction using n-hexane in a Soxhlet extractor apparatus for 24 hours. The defatted dried marc was repacked and extracted with methanol. Kolaviron was fractionated from concentrated methanolic extract using chloroform to give a golden tallow solid which consists of Garcinia biflavanones – GB1, GB2 and kolaflavanone.[9]

Animal treatment: Sixty adult male rats weighing approximately 101-122 g obtained from the Experimental Animal Unit of faculty of Veterinary Medicine, University of Ibadan, Nigeria were randomly divided into 4 groups of 10 animals per group. The animals were kept in wire mesh cages under controlled light cycle (12 h light/12 h dark) and fed with commercial rat chow *ad libitum* and liberally supplied with water. Sixty male Wistar rats were divided into six groups of 10 rats. Group I was the control. Group II received Hcy (200 mg/kg body weight) alone daily for 14 days. Rats in groups III and IV were co-administered with Hcy (200 mg/kg body weight) with KV (100 & 200 mg/kg body weight) while rats in groups V & VI were also co-administered with Hcy (200 mg/kg body weight) with *Garcinia kola* (GK) (100 & 200 mg/kg body weight) respectively, for 14 days.

Animal ethics: All of the animals received humane care according to the criteria outline in the Guide for the Care and the Use of Laboratory Animals prepared by the National Academy of Science and published by the National Institute of Health. The ethics regulations were followed in accordance with national and institutional guidelines for the protection of the animals' welfare during experiments.

Preparation of microsomal fraction: Rats were starved overnight and sacrificed by cervical dislocation. Hearts were removed, rinsed in 1.15% KCl and homogenized in aqueous potassium phosphate buffer (0.1 M, pH 7.4) and homogenates were centrifuged at 10,000 g for 20 minutes to obtain the supernatant fraction.

Determination of myocardial tissue antioxidant status, inflammation, markers of oxidative stress and myocardial damage: The supernatant was collected for the estimation of Catalase (CAT) activity according to the method of Shinha.[10] Superoxide dismutase (SOD) was determined by measuring the inhibition of auto-oxidation of epinephrine at pH 7.2 at 30^{O}C as described by Misra and Fridovich.[11] Glutathione-S-transferase (GST) was estimated by the method of Habig *et al.*[12] using 1-chloro-2, 4-dinitrobenzene (CDNB) as substrate. Protein concentration was determined by the method of Lowry *et al.*[13] Reduced GSH was determined at 412 nm using the method described by Jollow *et al.*[14] Hydrogen peroxide generation was determined as described by Wolff.[15] The MDA level was extrapolated according to Farombi *et al.*[16] Glutathione peroxidase activity was measured according to Buetler *et al.*[17] The activity of xanthine oxidase (XO) was determined according to the method of Akaike *et al.*[18] The serum CK-MB and lactate dehydrogenase (LDH) was determined using Randox kit according to manufacturer's instruction. Nitrite was determined according to Olaleye *et al.*[19] while myeloperoxidase activity was determined by Xia and Zweier.[20]

Electrocardiogram: Standard lead II electrocardiogram was recorded in conscious rats using a 7-lead ECG machine (EDAN VE-1010, Shanghai, China). The machine was calibrated at 20 mm/mV paper speed and 50 mm/s paper speed. From the electrocardiogram, parameters such as heart rate, PR interval, QRS wave duration, R-wave amplitude and QT segment were determined.

Histopathology: Small slices of hearts tissues were collected in 10% buffered formalin for proper fixation. These tissues were processed and embedded in paraffin wax. Sections about 5-6 µm thick were made and stained with haematoxylin and eosin for histopathology.

3. Statistical analysis

All values are expressed as mean±S.D. The test of significance between two groups was estimated by Student's t test. "One-way ANOVA with Dunnett's posttest was also performed using GraphPad Prism version 4.00.

4. Results

The effect of high Hcy on body weight, heart weight and relative body weights of rats

Our data show that administration of Hcy (200 mg/kg body weight) significantly increased body, heart and relative heart weights compared to the control and co-administration with KV or GK at 100 and 200 mg/kg body respectively (Table 1).

The effect of high Hcy on cardiac markers of oxidative and antioxidant defence system

Treatment with Hcy alone caused a significant ($p<0.05$) increase in cardiac malondialdehyde (MDA), H_2O_2 generation and xanthine oxidase activity relative to the control (Table 2). The significant ($p<0.05$) increase in markers of oxidative stress was accompanied with a significant ($p<0.05$) depletion in cardiac GSH content in Hcy treated rats compared to the control and other treatment groups. The activities of GPX, SOD, GST and CAT were significantly reduced in rats treated with Hcy alone and that co-administration of Hcy with KV (100 & 200 mg/kg body weight) and GK (100 & 200 mg/kg body weight) caused a significant increase in the activities of these antioxidant enzymes. Hence, co-administration with KV and GK reversed the cardiotoxic effects of Hcy-induced arteriosclerosis via a significant ($p<0.05$) reduction in markers of oxidative stress accompanied by significant ($p<0.05$) increase in in enzymic and non-enzymic antioxidants (Table 2).

Table 1. The effect of high Hcy on body weight, heart weight and relative heart weights of rats

Groups	Control	Hcy	Hcy + KV1	Hcy + KV2	Hcy + GK1	Hcy + GK2
Final Body weight (g)	153.75±26.69	172.00±4.47[a]	154.17±17.13[b]	154.29±12.72[b]	155.56±15.09[b]	153.00±20.58[b]
Heart weight (g)	0.57±0.08	0.64±0.06[a]	0.53±0.04[b]	0.54±0.07[b]	0.52±0.06[b]	0.52±0.04[b]
Relative heart weight	0.40±0.04	0.43±0.02[a]	0.35±0.02[b]	0.32±0.06[a,b]	0.32±0.07[a,b]	0.36±0.03[a,b]

Values are mean ± SD, n = 10, [a]P < 0.05 compared with control, [b]P < 0.05 compared with Homocysteine (Hcy) alone.
Control = (2 mL/kg), Hcy = (200 mg/kg), KV1 = (100 mg/kg), KV2 = (200 mg/kg), GK1 (100 mg/kg) = GK2 (200 mg/kg)

Table 2. The effects of *Garcinia kola* and Kolaviron on oxidative stress markers in high Hcy-treated rats

Groups	Control	Hcy	Hcy+ KV1	Hcy + KV2	Hcy + GK1	Hcy + GK2
MDA	10.93±0.14	15.43±2.06[a]	11.55±1.06[b]	12.00±1.05[b]	13.04±0.26[b]	12.46±1.63[b]
H_2O_2 generation	15.29±1.74	19.15±1.77[a]	15.81±3.92[b]	15.24±2.46[b]	15.64±1.79[b]	12.21±1.63[b]
GSH	36.29±0.91	32.83±2.56[a]	36.64±6.28	37.96±3.17[b]	36.98±2.03	37.85±0.82[b]
GPx	81.62±5.12	66.46±7.41[a]	82.84±6.08[b]	81.97±7.28[b]	78.67±4.06[b]	76.85±3.54[b]
GST	7.27±0.61	4.60±0.60[a]	8.00±0.48[b]	8.03±0.48[b]	7.39±0.80[b]	8.21±1.32[b]
SOD	18.72±0.91	14.51±1.49[a]	18.17±1.82[b]	17.81±1.68[b]	16.16±1.64[b]	16.80±2.05[b]
CAT	294.22±11.29	238.63±14.17[a]	296.23±21.29[b]	299.71±14.22[b]	281.68±28.03[b]	277.73±6.13[b]
XO	0.085±0.009	0.110±0.007[a]	0.102±0.010[b]	0.084±0.004[b]	0.078±0.008[b]	0.021±0.004[a,b]

Values are mean ± SD, n = 10, [a]P < 0.05 compared with control, [b]P < 0.05 compared with Homocysteine (Hcy) alone. Control = (2 mL/kg), Hcy = (200 mg/kg), KV1 = (100 mg/kg), KV2 = (200 mg/kg), GK1 (100 mg/kg) = GK2 (200 mg/kg)

The effect of high Hcy-treated on electrocardiography (ECG) changes

Table 3 and figure 1 show that Hcy treatment caused a significant ($p<0.05$) decrease in heart rate (HR), R-amplitude, QRS duration and QT interval compared to the control and rats co-administered with KV or GK. Similarly, Hcy treatment caused a significant ($p<0.05$) reduction in P-duration, QRS complex and QT interval compared to the control and co-administration of Hcy with KV (100 & 200 mg/kg body weight) and GK (100 & 200 mg/kg body weight) respectively (Table 3).

The effect of high Hcy on markers of inflammation and cardiac damage

Table 4 shows that markers of inflammation (MPO) and serum markers of cardiac damage (nitrite, LDH and CK-MB) were significantly ($p<0.05$) elevated in Hcy alone treated rats. Also, co-administration with KV and GK ameliorated the cardiotoxic and arteriosclerotic effect of Hcy by a significant ($p<0.05$) reduction in serum MPO, cardiac nitrite content, LDH and CK-MB to near normal values (Table 4).

Table 3. The effect of *Garcinia kola* and Kolaviron on the electrocardiograph (ECG) in Hcy-induced arteriosclerosis in rats

Groups	Heart rate (Rate /min)	P-Duration (ms)	PR (ms)	R-Amp (mV)	QRS (ms)	QTc (ms)	QT (ms)
Control	257.33±23.46	20.25±2.22	41.00±4.00	0.44±0.01	20.30±3.74	109.50±10.47	56.60±4.50
Hcy	225.00 ±21.21[a]	24.67 ±2.89[a]	45.00±2.00[a]	0.14±0.03[a]	11.00±2.83[a]	100.75±11.30	46.33±9.02[a]
Hcy + KV1	244.50 ±36.06	20.25±2.06	43.75±.96	0.39±0.01[a,b]	15.20±1.30[a,b]	118.56±14.84	59.60±2.41[b]
Hcy + KV2	230.00 ±22.63	20.50±5.51	44.00±1.41	0.57±0.01[a,b]	15.00±2.22[a,b]	103.75±13.89	66.00±8.49[b]
Hcy + GK2	266.50 ±45.38	20.67±0.8	43.00±1.41	0.37±0.01[a,b]	18.00±1.15[b]	114.75±13.45	57.00±6.52
Hcy + GK2	242.00 ±26.23	21.75±1.71	43.67±2.52	0.27±0.06[a,b]	16.00±1.41[b]	114.60±9.29	59.50±2.89[b]

Values are mean ± SD, n = 10, [a]P < 0.05 compared with control, [b]P < 0.05 compared with Homocysteine (Hcy) alone.
Control = (2 mL/kg), Hcy = (200 mg/kg), KV1 = (100 mg/kg), KV2 = (200 mg/kg), GK1 (100 mg/kg) = GK2 (200 mg/kg)

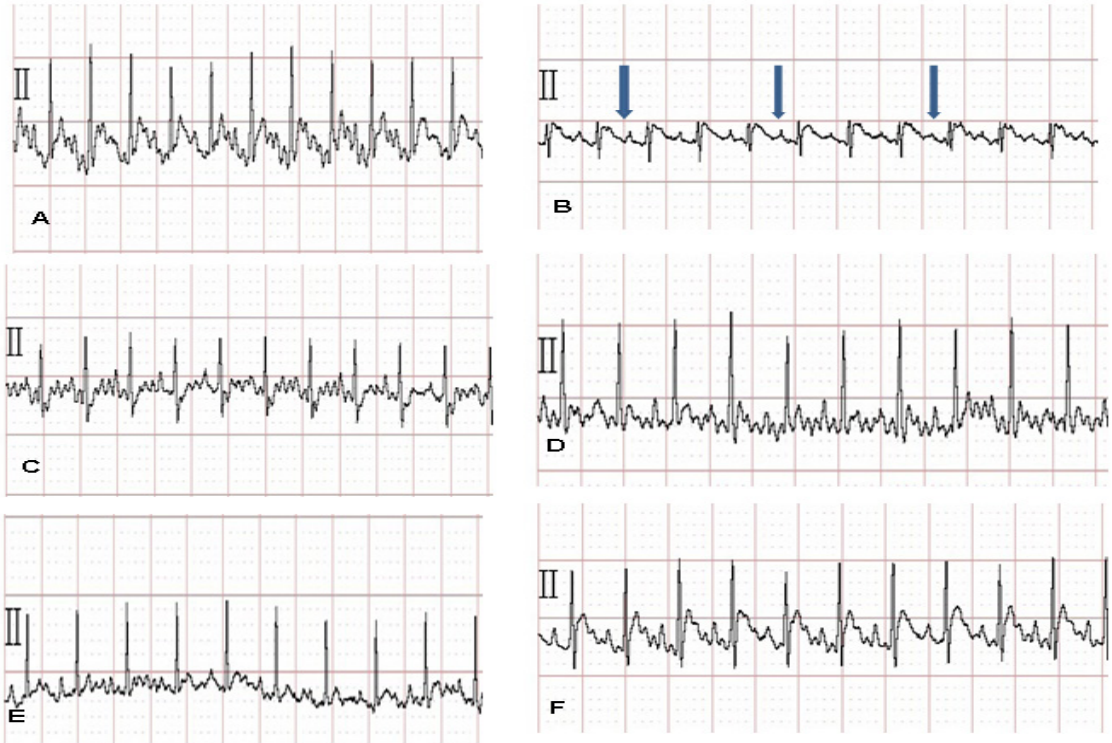


Figure 1. The effects of methanol extract of *Garcinia kola* and Kolaviron on the electrocardiogram (ECG) of rats administered homocysteine (Hcy). Control = (2 mL/kg), Hcy = (200 mg/kg), KV1 = (100 mg/kg), KV2 = (200 mg/kg), GK1 (100 mg/kg) = GK2 (200 mg/kg)

Table 4. The effect of *Garcinia kola* and Kolaviron on markers of inflammation and cardiac damage in Hcy-treated rats

Groups	Control	Hcy	Hcy+ KV1	Hcy + KV2	Hcy + GK1	Hcy + GK2
MPO (U/mg protein)	4.28±0.26	7.38±0.16[a]	4.14±0.46[b]	3.74±0.64[b]	3.02±0.51b	4.24±0.35[b]
Nitrite (μmole/L)	0.98±0.02	1.03±0.04[a]	0.90±0.06[a,b]	0.90±0.05[a,b]	0.90±0.07[a,b]	0.89±0.03[a,b]
LDH (U/L)	965.72±95.25	1060.64±17.99[a]	1037.94±37.94	986.94±61.84[b]	967.27±6.56	883.69±45.96[a,b]
CK-MB (U/L)	137.03±1.42	92.63±9.58[a]	98.73±16.69[a]	99.84±1.17[a]	104.84±1.17[a,b]	74.29±4.67[a,b]

Values are mean ± SD, n = 10, [a]P < 0.05 compared with control, [b]P < 0.05 compared with Homocysteine (Hcy) alone.
Control = (2 mL/kg), Hcy = (200 mg/kg), KV1 = (100 mg/kg), KV2 = (200 mg/kg), GK1 (100 mg/kg) = GK2 (200 mg/kg)

The effect of high Hcy on lipid profiles of high Hcy-induced arteriosclerosis

In this study, administration of Hcy alone significantly ($p<0.05$) elevated serum triglycerides (TAG), total cholesterol (TC) and low density lipoprotein (LDL-cholesterol). Co-administration of Hcy with KV (100 & 200 mg/kg body weight) and GK (100 & 200 mg/kg body weight) reversed the aberration in the lipid profiles to near normal values indicating the cholesterol lowing property of KV and GK. Co-administration of Hcy with GK at 200 mg/kg body weight showed better results in terms of lipid lowering capacity (Table 5). Similarly, Co-administration of Hcy with KV (100 & 200 mg/kg body weight) and GK (100 & 200 mg/kg body weight) significantly ($p<0.05$) increased serum HDL-cholesterol level which declined significantly by administration of Hcy alone (Table 5).

Histopathological examination

The histology of the heart revealed loss of striation and apoptotic bodies in Hcy-treated rats compared to the control and other treatment groups (Fig. 1). Also, necrosis and degeneration in the cardiac tissues was observed in Hcy-treated group compared with the control and groups co-administered with KV or GK (Fig. 2). However, histology of the heart of rats that received co-administration of Hcy with KV (100 & 200 mg/kg body weight) and GK (100 & 200 mg/kg body weight) was near normal in architecture. Also, the histology of the aorta of the rats administered with Hcy alone showed the presence of fibfrin thrombi in the endothelium of the aorta (Fig. 3).

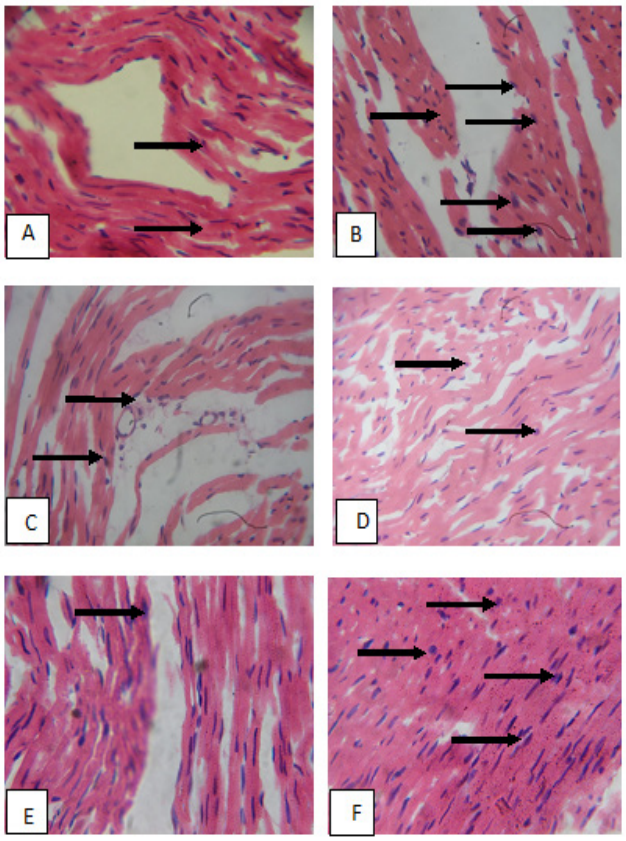


Figure 2. The histology of the cardiac tissues in homocysteine-induced arteriosclerosis in rats. Group A = (Control), Groups B = (Hcy treated), C = (Hcy + 100 mg/kg KV), D = (Hcy + 200 mg/kg KV), E = (Hcy + 100 mg/kg GK), = F (Hcy + 200 mg/kg GK). Heart tissues were stained with haematoxylin and eosin (H & E) stains (Magnification: X100).

Table 5. The effect of *Garcinia kola* and Kolaviron on lipid profiles in Hcy-induced arteriosclerosis

Groups	Control	Hcy	Hcy +KV1	Hcy + KV2	Hcy + GK1	Hcy + GK2
Triglycerides (mg/dL)	70.56±1.17	77.36±3.79[a]	71.73±1.29[b]	72.96±2.41b	71.59±0.20[b]	71.41±3.55[b]
Total cholesterol (mg/dL)	50.77±1.27	54.26±0.63[a]	52.66±1.37[b]	50.66±2.43[b]	50.87±0.29[b]	52.17±0.59[b]
HDL-cholesterol (mg/dL)	75.38±5.43	34.23±2.72[a]	44.42±4.74[a,b]	41.15±8.16[a,b]	35.38±5.98[a]	62.43±4.59[a,b]
LDL-cholesterol (mg/dL)	11.78±2.08	69.83±6.70[a]	64.98±1.76[a,b]	49.07±6.36[a,b]	44.24±4.55[a,b]	37.18±4.34[a,b]

Values are mean ± SD, n = 10, [a]P < 0.05 compared with control, [b]P < 0.05 compared with Homocysteine (Hcy) alone.
Control = (2 mL/kg), Hcy = (200 mg/kg), KV1 = (100 mg/kg), KV2 = (200 mg/kg), GK1 (100 mg/kg) = GK2 (200 mg/kg)

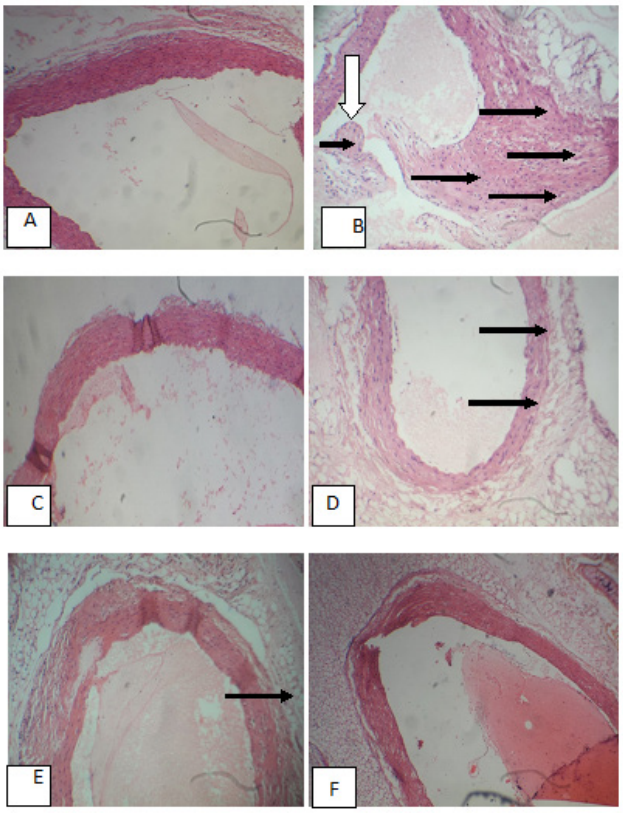


Figure 3. The histology of the aota in homocysteine-induced arteriosclerosis in rats. Group A = (Control), Groups B = (Hcy treated), C = (Hcy + 100 mg/kg KV), D = (Hcy + 200 mg/kg KV), E = (Hcy + 100 mg/kg GK), = F (Hcy + 200 mg/kg GK). Aorta were stained with haematoxylin and eosin (H & E) stains (Magnification: X100).

5. Discussion and conclusion

Cardiovascular disease (CVD) has been reported as the leading cause of death in the Western world with high mortality and morbidity.[36] The major risk factors associated with CVD include hyperlipidaemia, hypertension, and smoking. Endothelial dysfunction, atherosclerotic plaque formation, and rupture are a sequence of event leading to CVD.

The major non-enzymic intracellular defence known as reduced glutathione (GSH) was depleted while malondialdehyde (MDA), hydrogen peroxide (H_2O_2) generation and xanthine oxidase activity as markers of oxidative stress were elevated in Hcy alone treated rats. The reduction in GSH and increase in markers of oxidative stress also contributed significantly to Hcy-induced arteriosclerosis in this study. The aforementioned elevated markers of oxidative stress induced by Hcy administration was ameliorated with KV (100 & 200 mg/kg body weight) and GK (100 & 200 mg/kg body weight), respectively. It is interesting to note that GK at 200 mg/kg body weight gave better improvement in attenuating oxidative stress by significantly reducing markers of oxidative stress and increasing antioxidant enzymes. The LPO is the process of oxidative degradation of polyunsaturated fatty acid (PUFA) and its incidence in biological membranes could be linked to impaired membrane function, structural integrity, decreased membrane fluidity and the inactivation of several membrane-bound enzymes.

The CAT is a major primary antioxidant defence component which along with GPx catalyses the decomposition of H_2O_2 to H_2O. However, the decrease in the activities of CAT, GPx and GR could be attributed to their utilization in converting H_2O_2 to H_2O with concomitant increase in the activity of SOD. According to the present study, the enzymic antioxidant activities of SOD, CAT, GPx and GST were significantly reduced in Hcy alone treated rats. We therefore proposed that the superoxide anion radical generated from the coronary arteries might be responsible for the reduced activities of these vital antioxidant enzymes. This therefore contributed immensely to the oxidative stress and ultimately cardiovascular dysfunction as evidenced in the aberration observed in the lipid profiles. Results from the present study showed that co-administration of Hcy with KV (100 & 200 mg/kg body weight) and GK (100 & 200 mg/kg body weight) led to significant increase in CAT, SOD, GPx, GST and significant reduction in MPO and xanthine oxidase activities.

Wang *et al.*[22] reported that the activity of serum SOD and GPx significantly decreased, whereas the MDA content and hydroxyl radical level noticeably increased with decrease in the mRNA and protein expression of SOD2 and GPX1 in the aortic wall tissue of Hcy treated rats. However, Ungvari *et al.*[23] reported increased production of superoxide anion radical from the coronary arteries by HHcy. The increase in SOD activity indicates elevation in the formation of oxy-free radicals thereby enhancing SOD activity to thwart the toxicity of free radicals particularly during oxidative stress as reported by Garg *et al.*[22] Moreover, this increase can be contributed to the physiological response of the animal in mitigating the toxic stress on the body as SOD plays a vital role in the detoxification of ROS. The increase in the activity of the

antioxidant enzymes in rats that received both Hcy and KV or GK pointed to the antioxidant capacity of both KV and GK. Similarly, the anti-inflammatory effect of KV and GK was demonstrated with a significant reduction in the activity of serum MPO.

The MPO is a heme protein released by leukocytes and it plays a crucial role in inflammation and oxidative stress as reported by Anatoliotakis *et al.*[23] MPO is abundantly expressed in the azurophilic granules of most leukocyte including neutrophils and monocytes. Co-administration of Hcy with KV or GK significantly reduced the activity of MPO. This is an indication that KV and GK have anti-artherosclerotic and cardioprotective properties via antioxidant and free radical scavenging activities. The phytochemicals present in GK might also be responsible for its cholesterol lowering effect and cardioprotective property. Hence, MPO assay in the blood can be used as a diagnostic and prognostic tool for a number of cardiovascular diseases.

The data from the present study showed a significant increase in low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides and total cholesterol in Hcy treated rats. It was observed that co-administration of Hcy with KV (100 & 200 mg/kg body weight) and GK (100 & 200 mg/kg body weight) improved the lipid profiles with a concomitant attenuation of arteriosclerosis. The increase in LDL-C, triglycerides and total cholesterol and decrease in serum HDL-C might have contributed to the vascular thickness and arterial blood pressure, increase body weight and heart weight together with the presence of fibrin thrombi into the wall of the aorta. The serum levels of LDL-C, triglycerides and total cholesterol were significantly reduced in rats that received Hcy and then co-administered with KV or GK. The electrocardiograph (ECG) results showed a significant reduction in heart rate (HR), R-amplitude and shortened QT interval. In dogs, low voltages R-waves have been associated with clinical complications such as obesity, effusions, hyperkalaemia, pneumothorax and respiratory diseases.[24] We therefore speculate that the aberration in the lipid profile might have caused reduction in the HR and altered the ECG in Hcy treated rats compared to the Hcy treated rats co-administered with KV (100 & 200 mg/kg body weight) and GK (100 & 200 mg/kg body weight) respectively. In fact, there were no significant differences in the ECG results of control group and rats that received KV or GK with Hcy.

According to our results, significant increase in LDH and CK-MB activity was observed. The use of CK-MB and LDH as diagnostic cardiac marker enzymes have been extensively studied and reported.[25] Hcy-induced myocardial damage via atherosclerosis was indicated by increased activities of CK-MB and LDH in the serum of Hcy-treated rats compared to the control and other treatment groups that were co-administered with KV or GK. The cardioprotective and anti-atherosclerotic effect of KV and GK was demonstrated by significant reduction LDL-cholesterol, triglycerides and total cholesterol, LDH and CK-MB respectively.

In conclusion, co-administration of KV or GK caused attenuation of (Hcy)-induced atherosclerosis and oxidative stress by increasing the activity of the antioxidant enzymes and reducing the markers of oxidative stress and inflammation. Overall, co-administration of Hcy with *Garcinia kola* at 200 mg/kg body weight showed a better cardioprotective and anti-atherosclerotic effect on (Hcy)-induced cardiotoxicity and atherosclerosis than KV in this study.

6. Conflict of Interest

None Declared

7. References

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