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Original Research

Kolaviron, a biflavonoid of *Garcinia kola* seed mitigates ischemic/reperfusion injury by modulation of pro-survival and apoptotic signaling pathways

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ABSTRACT

Objective: The study was designed to investigate the ameliorative effect of Kolaviron (KV) on ischemic/reperfusion injury in experimental animal models. **Materials and Methods:** Male Wistar rats were randomly divided into two groups: Group 1 received corn oil as a vehicle and rats in Group 2 were administered KV at 200 mg/kg for 4 weeks. The rats were fed with rat standard chow pellet and water administered *ad libitum*. After 4 weeks of KV administration, hearts were excised and mounted on the working heart perfusion system. Western blot analysis for protein expression was carried out on frozen heart samples. **Results:** There was significant ($P < 0.05$) reduction in the activity of catalase, superoxide dismutase, and glutathione peroxidase with concomitant reduction in oxygen radical absorbance capacity in ischemic rat heart of control compared to group pre-treated with KV, respectively. Similarly, intracellular reactive oxygen species and malondialdehyde were significantly elevated in control compared to KV pre-treated rats. KV significantly increased total Akt/protein kinase B (PKB), phosphorylated Akt/PKB at serine 473 and also caused a significant reduction in p38 mitogen-activated protein kinase, Caspase 3, and cleaved poly adenosine diphosphate ribose polymerase. **Conclusion:** Taken together, KV offered significant cardioprotection via free radical scavenging activity and upregulation of pro-survival pathway.

KEY WORDS: Antioxidant, chemoprevention, Kolaviron, ischemic/reperfusion injury, oxidative stress

INTRODUCTION

Mitochondrial generation of reactive oxygen species (ROS) has been reported as one of the sources of important mechanisms of disease and redox signaling in the cardiovascular system [1]. Ischemic heart disease, myocardial infarction, and other pathologies associated with heart diseases continue to be leading causes of cardiovascular morbidity and mortality. ROS are known to induce the oxidation of membrane lipids with concomitant production of malondialdehyde (MDA), a specific biomarker of lipoperoxidation [2]. Furthermore, the restoration of coronary flow (CF) (reperfusion) after a prolonged period of ischemia therefore precipitates and aggravates oxidative stress, which is a major cause of myocardial injury. In the myocardium, nicotinamide adenine dinucleotide phosphate oxidase and xanthine oxidase have been described as the two major enzymatic sources of ROS [3,4].

The survivor activating factor enhancement (SAFE) pathway has been shown to require the activation of the signal transducer

and activator of transcription 3 and it can successfully lessen cardiomyocyte death at the time of reperfusion [5]. Tamareille *et al.* and Lecour defined SAFE pathway as all kinases which are specifically activated at reperfusion and that can improve cardiomyocyte survival [5,6]. Previously, SAFE pathway was originally described solely in terms of the extracellular-signal regulated kinase (ERK1/2), mitogen-activated protein kinase (MAPK) and Akt/PI3K kinase pathways, but Tamareille *et al.* described the JAK-STAT signaling pathway as a critical "third arm" of the RISK pathway (sometimes referred to as the SAFE pathway) [6]. Recently, antioxidant therapy has become a promising pharmacological approach for the prevention of myocardial ischemia/reperfusion (MI/R) injury [7-9].

Our laboratory has reported the beneficial effects of *Garcinia kola* (GK) and Kolaviron (KV) ranging from hepatoprotective, nephroprotective, and chemopreventive [10-18]. Hence, this study was designed to elucidate the possible mechanism of action of KV a bioflavonoid of GK seed extract on ischemic reperfusion injury in isolated rat hearts.

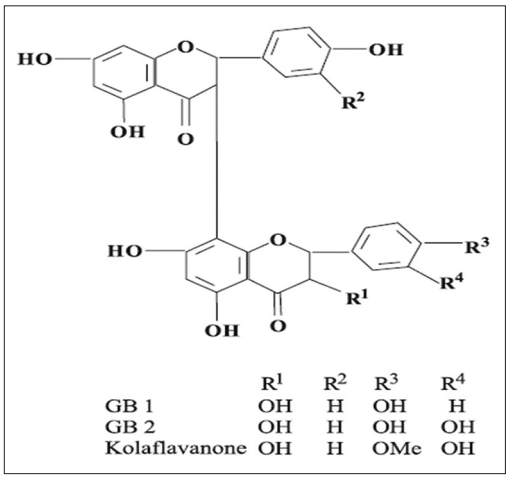
MATERIALS AND METHODS

Extraction of GK and Isolation of KV

KV was extracted from the seeds of GK according to the method of Iwu *et al.* with slight modification [19]. 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 h. The defatted dried marc was repacked and extracted with methanol. KV was fractionated from concentrated methanolic extract using chloroform to give a golden yellow solid which consists of Garcinia biflavanones – GB1, GB2, and kolaflavanone [Figure 1] [19].

Experimental Animals

A total of 40 rats were purchased from the primate colony of Tygerberg Hospital, Cape Town, South Africa. The rats were divided into 20 rats per group of two. They were housed in standard rat cages with 12 h light and 12 h dark cycle with temperature of 14 ± 4°C. They were fed with standard rat chow and given free access to water. The rats were acclimatized for 2 weeks before the commencement of the study. The rats in Group 1 received corn oil and those in Group 2 were administered KV at 200 mg/kg body weight for 4 weeks, respectively. The pharmacologic dose of KV (200 mg/kg) was used based on the previously established studies in our laboratory.



	R^1	R^2	R^3	R^4
GB 1	OH	H	OH	H
GB 2	OH	H	OH	OH
Kolaflavanone	OH	H	OMe	OH

Figure 1: Structure of kolaviron

Induction of Anesthesia and Perfusion Protocol

The rats were intraperitoneally anesthetized with sodium pentobarbital (50 mg/kg). About 10 min after anesthesia, blood was collected from the abdominal aorta into heparinized sample bottles for antioxidant capacity, and the hearts were immediately removed and mounted on a Langendorff apparatus. Krebs-Henseleit buffer (KHB) composed of NaCl 118.5 mM, KCl 4.7 mM, $MgSO_4$ 1.2 mM, KH_2PO_4 1.2 mM, $CaCl_2$ 2.5 mM, $NaHCO_3$ 25 mM, and glucose 11 mM (pH 7.4, 37°C) and saturated with 95% O_2, and 5% CO_2 as previously described [20]. The left ventricular pressure, the heart rate (HR), aortic output (AO), CF, systolic pressure (SP), and diastolic pressure (DP) were monitored via a transducer connected to a water-filled latex balloon inset into the left ventricle through the atrium and coupled to a pressure transducer (PowerLab, Australia). All the data were recorded and stored with the LG Computers, South Africa.

Animal Ethics

All animals used in this study received humane care in accordance with the Principle of Laboratory Animal Care of the National Society of Medical Research and the Guide for the Care and Use of Laboratory Animals of the National Academy of Sciences (National Institutes of Health Publications No. 80-23, revised 1978). The rats had free access to water and food. They were housed in an animal house at a constant temperature of 27°C, and they were exposed to a 12 h artificial day-night cycle. The ethical clearance for this study was approved and granted by the Health and Applied Sciences Research Ethics Committee of the Cape Peninsula University of Technology (Ethical number CPUT/HW-REC 2012/A03).

Isolated Heart Perfusion

Perfusion was maintained at a constant pressure of 75 mmHg, and the isolated hearts were stabilized for 20 min. The isolated heart was subjected to 5 min perfusion on Langendorff and 10 min perfusion on working heart with KHB. This was followed with 15 min global ischemia. The isolated rat hearts were for 10 min on Langendorff and 15 min on working heart [Chart 1].
Buffer: KHB.
Drugs: KV, GK and dimethyl sulfoxide as the control.

Blood Collection and Post-mitochondrial Fraction Preparation

About 3 ml of blood was collected from the abdominal aorta of the animals into heparinized tubes. The blood was centrifuged at 4000 g for 15 min to obtain the plasma.

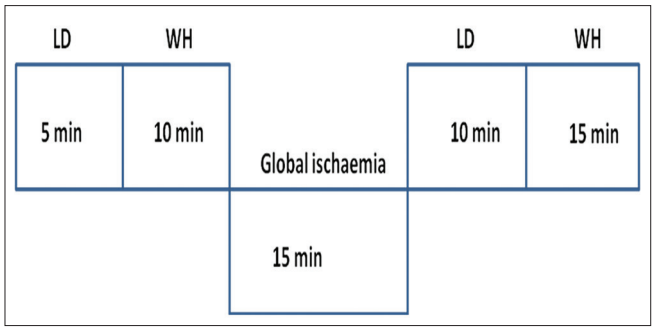


Chart 1: Heart perfusion protocol

The heart was harvested, rinsed and homogenized in aqueous potassium buffer (0.1 M, pH 7.4), and the resulting homogenate was centrifuged at 10,000 g (4°C) for 10 min to obtain the post-mitochondrial fraction.

Biochemical Assays

Protein determination was carried out bicinchoninic acid assay according to the method of Olsen and Markwell assay kit [21]. The oxygen radical absorbance capacity assay (ORAC) was determined as described by Cao and Prior [22]. The 2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical scavenging activity of the hydrophilic extract was determined according to the method described by Seeram *et al.* [23]. The ferric reducing ability of the hydrophilic fraction was determined using the method described by Benzie and Strain [24]. The phenolic content of plasma was measured by the Folin-Ciocalteu method modified to remove protein interference [25]. Glutathione peroxidase (GPx) activity was measured according to the method of Flohé and Günzler [26]. The superoxide dismutase (SOD) activity was determined according to the method of Ellerby and Bredesen [27]. Catalase activity was determined by the method of Aebi [28]. The oxidized glutathione (GSSG) was determined as described by Griffith [29]. Total reduced glutathione (GSH) was determined according to the method of Tietze [30].

The intracellular ROS were determined described by Bartosz [31]. The fluorescence intensity is proportional to the ROS levels within the cell cytosol. The assay employs the cell-permeable fluorogenic probe 2', 7'-dichlorodihydrofluorescein diacetate (DCFH-DA). In brief, DCFH-DA is diffused into cells and is deacetylated by cellular esterases to non-fluorescent DCFH, which is rapidly oxidized to highly fluorescent 2', 7'-dichlorodihydrofluorescein by ROS.

Sodium Dodecyl Sulfate (SDS)-Polyacrylamide Gel Electrophoresis (PAGE) and Western Blot Analysis

Loading gel was prepared as follows 30% acrylamide, 0.5 mM Tris (pH 6.8), double distilled water (dd.H_2O), 10% SDS, ammonium persulfate, and tetramethylethylenediamine while the separating gel was also prepared in the same manner with 1.5 mM Tris at pH 8.8 (BIO-RAD; CA, USA). Gel electrophoresis was performed in a running buffer (×10, Tris/Glycine/SDS Buffer; BIO-RAD, CA, USA). Proteins (20 μg/lane) were separated by 10% SDS-PAGE and transferred in transfer buffer Tris 25 mM, glycine 192 mM, 0.01% SDS and 10% methanol and dd.H_2O (Millipore) onto polyvinylidene difluoride membranes. The separated proteins were pre-incubated with 5% nonfat in Tris buffered saline (Tris, sodium chloride, dd.H_2O and Tween-20) and incubated overnight at room temperature. Membranes were then probed with the following primary antibodies against protein kinase B (PKB)/Akt (1:1000), p-PKB/Akt at Ser473 (1:2000), p38 MAPK (1:1000), poly adenosine diphosphate ribose polymerase (PARP) (1:1000), cleaved PARP (1:1000), Caspase 3 (1:1000), and cleaved Caspase 3 (Asp 175) (1:1000). All primary antibody probes were purchased from Cell Signaling Technology, Danvers, MA, USA. And then, the membranes were incubated with horseradish peroxidase-conjugated secondary anti-rabbit immunoglobulin G (1:500; Santa Cruz Biotechnology, CA, USA).

Protein Quantification and Analysis

Protein bands were visualized using western blot detection reagent 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium phosphatase substrate (KPL, Gaithersburg, MD, USA) according to manufacturer's instruction. The membranes were then allowed to dry, scanned and the relative intensities of the membranes were thereafter quantified with UN SCAN-IT (Skill Scientific Incorporation, Orem, Utah, USA). UN SCAN-IT programme allows the protein bands on the membrane to be marked and the pixel value of each band determined. The higher the pixel value, the higher the protein concentration.

RESULTS

Effect of KV on Markers of Oxidative Stress, Enzymatic and Non-enzymatic Antioxidant System in Reperfusion Injury of Isolated Rat Hearts

In this study, there was significant ($P < 0.05$) increase in FRAP, Trolox equivalent antioxidant capacity (TEAC)/ABTS and ORAC levels in the plasma of rats pre-treated with KV at 200 mg/kg body weight compared to the control [Table 1]. As shown in Table 2, the antioxidant enzyme (SOD, catalase [CAT] and GPx) activities were significantly increased in KV pre-treated rats at the 20 min reperfusion time point compared to the control. The markers of oxidative stress (MDA and intracellular ROS) values were significantly ($P < 0.05$) reduced in the isolated rat hearts of the KV-treated group [Table 2]. Similarly, a significant ($P < 0.05$) increase in ORAC levels was found in the perfused ischemic cardiac tissues of rats pre-treated with KV for 4 weeks at 25 min reperfusion time points [Table 2].

Effect of KV on Functional Parameters in Reperfusion Injury of Isolated Rat Hearts

Pre-treatment with 200 mg/kg body weight of KV for 4 weeks was able to significantly improve AO compared to the control [Table 3]. Similarly, there was a significant increase in CF compared to the control as shown in Table 3. The improvement

Table 1: Effect of KV on antioxidant capacity in plasma in ischemic reperfused rat hearts

Groups	Total polyphenols (mg/GAE/L)	FRAP (μmol/AAE/L)	TEAC/ABTS (μmol TE/L)	ORAC (μmol TE/L)
KV (200 mg/kg)	321.51±15.49	680.44±34.69**	444.67±17.99***	817.93±34.99***
Corn oil (2 ml/kg)	304.60±40.04	542.44±21.61	354.80±30.06	731.25±42.83

The results are expressed as the mean±SEM (n=7). **and***Indicate significant difference at $P<0.01$ and $P<0.001$, respectively, in each column. FRAP: Ferric reducing antioxidant power, TEAC: Trolox equivalent antioxidant capacity, ORAC: Oxygen radical absorbance capacity, GAE: Gallic acid equivalent, TE: Trolox equivalent, AAE: Ascorbic acid equivalent, KV: Kolaviron (200 mg/kg bodyweight), corn oil (2 mL/kg), SEM: Standard error of mean

Table 2: Effect of KV on antioxidant enzymes and markers of oxidative stress in ischemic rat hearts (20 min reperfusion time point)

Groups	SOD[a]	CAT[b]	GPx[c]	MDA[d]	ORAC[e]	ROS[f]
KV (200 mg/kg)	2.34±0.97	0.29±0.063	0.057±0.0008	4.67±0.76	16.01±2.147	415.70±36.41
Corn oil (2 ml/kg)	0.97±0.26**	0.21±0.024**	0.032±0.0006*	8.81±1.84**	12.17±1.84*	548.40±15.57**

The results are expressed as the mean±SEM of seven rat hearts per group. *,**and***Indicate significant difference at $P<0.05$, $P<0.01$ and $P<0.001$, respectively, in each column. [a]Units/μmol/mg protein, [b]μmol/min/mg protein, [c]nmol/min/mg protein, [d]fluorescence detection unit, [e]nmol of MDA formed/g tissue, [f]μmol/TE/g tissue. SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase, MDA: Malondialdehyde, ORAC: Oxygen radical absorbance capacity, ROS: Intracellular reactive oxygen species, KV: Kolaviron (200 mg/kg bodyweight), corn oil (2 mL/kg), SEM: Standard error of mean

Table 3: The effect of KV on functional parameters for 25 min reperfusion

Functional parameters	Groups	Pre-ischemia	25 min reperfusion
AO (mL/min)	KV	38.76±0.92	24.01±1.81
	Control	35.62±0.96*	18.22±2.75
CF (mL/min)	KV	20.53±1.03	8.91±1.18
	Control	16.74±1.07*	15.52±0.73*
SP (mm Hg)	KV	163.21±3.05	145.80±2.55
	Control	158.01±3.16	141.36±4.08
DP (mm Hg)	KV	71.40±2.80	70.62±2.45
	Control	68.71±1.00	71.37±1.66
HR (bpm)	KV	288.45±4.89	286.26±6.18
	Control	309.13±8.94	298.74±7.74

The results are expressed as the mean±SEM of 10 rat hearts per group. *Indicate significant difference at $P<0.05$ in each column. HR: Heart rate, LVEDP: Left ventricular developed pressure, SP: Systolic pressure, CF: Coronary flow, AO: Aortic output, bpm: Beat per minute, KV: Kolaviron (200 mg/kg bodyweight), corn oil (2 mL/kg)

observed in the hemodynamic parameters (SP, DP and HR) in KV pre-treated rats were not significantly different from the control animals at the 20 min reperfusion time point [Table 3].

Our results showed an apparent increase in plasma reduced GSH in rats pre-treated with 200 mg/kg body weight of KV but with no significant difference when compared to the control [Figure 2]. Similarly, there was a significant ($P < 0.05$) reduction in plasma GSSG in rats pre-treated with KV (200 mg/kg) when compared with the control [Figure 2]. However, the increase in GSH/GSSG ratio obtained in rats administered with 200 mg/kg KV was not significantly ($P > 0.05$) different from the control [Figure 2].

Western Blots Results

Figure 3 showed that KV pre-treatment significantly reduced the expression of Akt and phosphorylated Akt (p-Akt), respectively, at 25 min reperfusion time point in isolated rat hearts. KV pre-treatment significantly reduced the expression of pro-apoptotic protein (p38 MAPK) compared to the control at 25 min reperfusion time point [Figure 4]. Furthermore, Caspase 3 and cleaved Caspase 3 expressions were significantly reduced in isolated hearts of animals pre-treated with KV for 4 weeks at 25 min reperfusion time points [Figure 5], respectively. Results obtained in this study showed that KV significantly increased and reduced PARP and cleaved PARP expressions, respectively, after 25 min reperfusion in hearts of animals pre-treated with KV compared to the control group [Figure 6].

DISCUSSION

This study provides evidence about the antioxidant property and cardioprotective effect of KV isolated from GK seed on isolated rat heart. The antioxidant capacity of KV was demonstrated with significant elevated plasma levels of FRAP, TEAC and ORAC in the rats pre-treated with KV compared to the untreated rats. The bioflavonoids present in KV might be responsible for this antioxidant capacity and the cardioprotective potentials. In addition, our data also show that KV pre-treated significantly increased AO and CF. The improvement in these hemodynamic parameters signifies the essential components of KV that offer cardioprotection via antioxidant property.

Moreover, we observed a significant increase in MDA levels, intracellular ROS and followed by a depletion of cardiac antioxidant enzymes; CAT, SOD, and GPx activities, respectively, after 20 min ischemic reperfusion injury in untreated rat hearts compared to the KV pre-treated rats. However, it has been documented that brief episodes of MI/R are associated with the generation of ROS [32,33].

The administration of antioxidants or free radicals scavengers is able to limit the evolution of myocardial damage reducing ROS-induced lipid peroxidation [34-36]. The medicinal use of KV as antioxidant, anti-inflammatory, and chemoprevention has been extensively documented elsewhere [14-16]. Moreover, the GB - GB1, GB2, and kolaflavanone present in KV could be used as a functional food to prevent or mitigate pathologies associated with oxidative stress and myocardial damage.

Western blot analysis revealed a significant increase in the expressions of Akt and p-Akt as pro-survival proteins in the

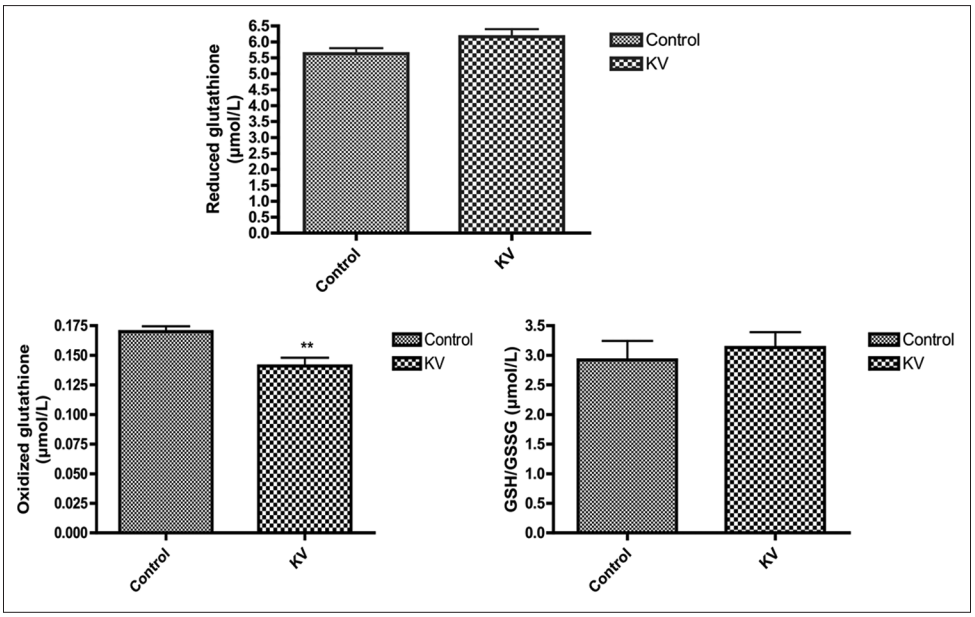


Figure 2: The effect Kolaviron (KV) on plasma reduced glutathione (GSH), oxidize GSH (GSSG) and GSH/GSSG ratio in 20 min reperfusion time point. Values are mean ± standard deviation, $n = 10$. Kolaviron; KV (200 mg/kg bodyweight), corn oil (2 mL/kg)

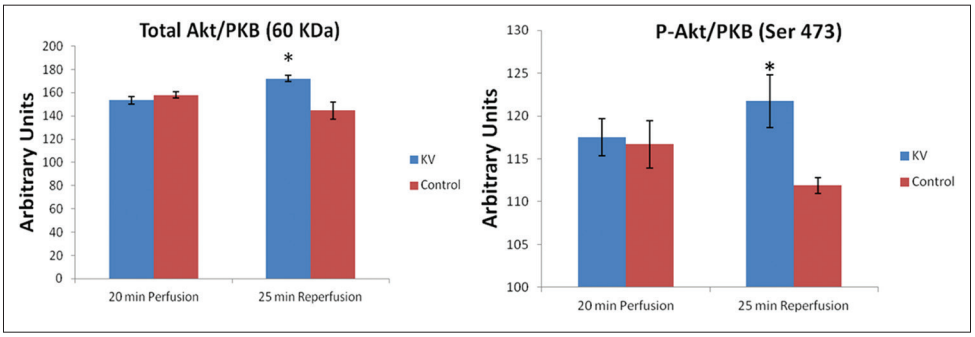


Figure 3: The effect of Kolaviron (KV) on total Akt/PKB and phosphorylated Akt/PKB at 20 min perfusion and 25 min reperfusion time point. Values are mean ± standard deviation, $n = 10$, $^*P < 0.05$ compared with control. KV (200 mg/kg bodyweight), corn oil (2 mL/kg)

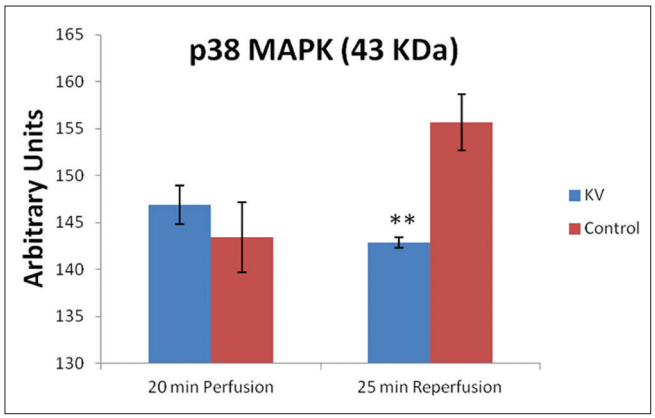


Figure 4: The effect of Kolaviron (KV) on p38 MAPK at 20 min perfusion and 25 min reperfusion time points. Values are mean ± standard deviation, $n = 10$, $^{**}P < 0.05$ compared with control. KV (200 mg/kg bodyweight), corn oil (2 mL/kg)

heart of rats pre-treated with KV compared to untreated group. This therefore also pointed to the fact that KV increased Akt phosphorylation in the pre-treated animals. On the other hand, pre-treatment with KV significantly suppressed Caspase 3, cleaved Caspase 3, p38 MAPK and cleaved PARP in the isolated rat hearts subjected to 20 reperfusion injury after global ischemia. The cytoprotecive and anti-apoptotic activity of KV might be associated with antioxidant and free radical scavenging activity of KV. This study, therefore, corroborates the previous study by Farombi *et al.* on the cytoprotecive of KV against DNA damage and oxidative stress [37]. Together, KV suppressed pro-apoptotic pathway and up-regulated pro-survival pathway in isolated rat heart of animals pre-treated with KV for 4 weeks. The antioxidant and free radical scavenging activity of KV might be responsible for the abrogation of death pathway and improvement in the survival pathway.

A great number of studies have suggested that MAPK signaling cascades were regarded as an important pathway in oxidative-stress-induced apoptotic cell death [38,39], and three MAPK subfamily members - ERK1/2 (beneficial), p38 MAPK and JNK (deleterious) - are activated following MI/R [40,41]. Furthermore, activation of ERK contributes to

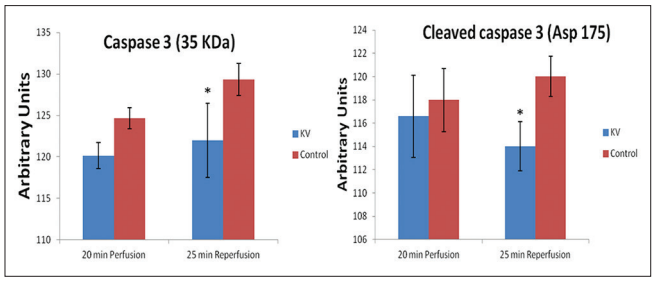


Figure 5: The effect Kolaviron on Caspase 3 and cleaved Caspase 3 at 20 min perfusion and 25 min reperfusion time points. Values are mean ± standard deviation, $n = 10$, $^*P < 0.05$ compared with control

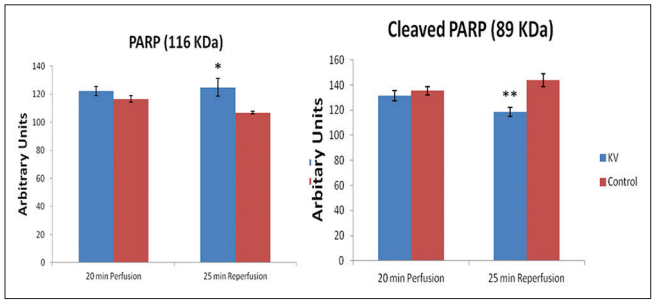


Figure 6: The effect of Kolaviron (KV) on apoptotic marker (cleaved Caspase 3) at 20 min perfusion and 25 min reperfusion time points. Values are mean ± standard deviation, $n = 10$, $^*P < 0.05$ compared with control. KV (200 mg/kg bodyweight), corn oil (2 mL/kg)

cell differentiation, proliferation, and survival; whereas JNK and p38 are activated by environmental stresses, promote apoptosis, and pro-inflammatory cytokines [42]. To clarify the possible singling pathway of KV on I/R-induced heart damage, we next tested the potential effects of KV on different pro-apoptotic cascade activation induced by I/R. The results indicated that activations of p38, Caspase 3, cleaved Caspase 3 and cleaved PARP protein expressions were observed in untreated rat hearts subjected to I/R.

In this study, we chose to study the impact of KV on p38 MAPK, another member of the MAPK family. However, pre-treatment with KV downregulated the expression of p38 MAPK in our model of MI/R. In the same vein, rats pre-treated with KV for 4 weeks significantly reduce Caspase 3, cleaved Caspase 3 and cleaved PARP protein expressions compared to the untreated group.

PI3K activates Akt by phosphorylation at Thr308, which is necessary for Akt activation, and by phosphorylation at Ser473, which is required for its maximal activity [43]. PI3K/Akt has been documented to be protective in heart I/R injury together with the inhibitory effect of KV on Sertoli cell line [44,45]. Furthermore, we, therefore, proposed that the cardioprotection produced by KV is mediated, at least partially, by PI3K/Akt activation and abrogation of apoptosis via inhibition of p38 MAPK and caspase expressions. In this study, compared with the control group, the KV group showed a significant increase in both total Akt and phosphorylated Akt, indicating its activation.

The generation of ROS from oxidative stress caused by reperfusion injury is known activated MAPKs signaling transduction with subsequent Caspase 3 and cleaved Caspase 3 activation [46]. MI/R injury has been reported to compromise myocardial function and contribute greatly to morbidity and mortality [32]. Necrosis and apoptosis are the two morphologically distinct pathways that contribute to MI/R injury. The induction of apoptosis has been shown to occur by at least two pathways, which are the extrinsic and intrinsic pathways [46]. The mitochondrion has been implicated as a major regulator of the intrinsic pathway [47]. The translocation of pro-apoptotic protein bax causes mitochondrial dysfunction and swelling and induces the efflux of cytochrome C to the cytosol. The release of cytochrome C to the cytosol has been documented to activate caspase apoptotic capacity [48].

Pre-treatment with KV inhibited the activation of p38 MAPK and promoted the activation of Akt in rats subjected to reperfusion injury. At the same time, cardioprotection by KV might be related to the inhibition of cardiomyocytes apoptosis signal via a reduction in the expressions of Caspase 3, cleaved Caspase 3 and cleaved PARP. Hence, KV offered cardioprotection via inhibition of pro-apoptotic pathway and up-regulation of survival pathway in isolated rat heart. Furthermore, the activation of the effector caspase, Caspase-3, is followed by the initiation of the multiple different stimuli that induce apoptosis. In addition, Caspase-3 is primarily responsible for the cleavage of PARP, a nuclear enzyme that is catalytically activated by DNA strand interruptions [46].

CONCLUSION

Taken together, KV abrogated pro-apoptotic pathways by inhibiting p38 MAPK, Caspase 3 and cleaved Caspase 3 and cleaved PARP. Pre-treated with KV significantly increased the expressions of survival proteins thereby attenuating apoptosis and improving recovery from reperfusion injury. Furthermore, the functional parameters also improved in isolated rat hearts of animals pre-treated with KV. Hence, the mechanism of cardioprotection of KV might be associated with antioxidant, anti-inflammatory and free radical scavenging activity of KV via up-regulation of survival pathway and abrogation death pathway. This study clearly demonstrates for the first time the cardioprotective effect of KV against reperfusion injury in isolated rat heart. These cardioprotective properties may be linked to the ability of KV to abrogate death pathway via phosphorylation of Akt and to scavenge ROS. From these results, we propose that KV could be a future therapeutic approach against myocardial dysfunction.

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