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Kalanchoe pinnata aqueous extract possesses vasorelaxant activities contributing to its antihypertensive effects in a model of rat-induced hypertension and myocardial infarction

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ABSTRACT

Background and aim: *Kalanchoe pinnata* is an herbal medicine used in Cameroon against cardiovascular diseases. Hypertension and myocardial infarction are among key risk factors of cardiovascular diseases. This research aimed to investigate antihypertensive effect of *Kalanchoe pinnata* in salt loaded myocardial infarcted rats and possible mechanism of action on vascular smooth muscle. **Experimental procedure**: Thirty rats were equally distributed into six groups: neutral, negative and positive controls and three test groups (administered extract 50, 100 and 150 mg/kg). Hypertension was induced by salt loading (18%) meanwhile myocardial infarction was by injection of 100mg/kg isoproterenol. The 28 day-*In vivo* treatment was followed by evaluation of systolic and diastolic arterial pressures, and troponin level. Ten untreated rats were used for the *in vitro* study, to investigate vasorelaxant mechanism. **Results and conclusion:** Treated rats showed significant increase of systolic (46.3%) and diastolic (73.5%) pressures. Troponine level increased by 400%. Extract at highest dose (150mg/kg) reduced these increases by 29.1%, 41.3% and 52% respectively. Extract (1.5mg/mL) caused a vasorelaxation (62.79%) in the presence of endothelium. Endothelium removal did not significantly modify the effect of the extract in KCl pre-contracted rings. In the same way, in rings pre-contracted with phenylephrine, the extract (1.5mg/mL) did not significantly modify the relaxation induced by sodium nitroprusside. However, methylene blue, a NO-cGMP inhibitor significantly reduced ($P<0.001$) the vasorelaxant effect of *K. pinnata* extract (1.5mg/mL). This indicated that the extract exhibits its antihypertensive activity by relaxing vascular smooth muscle, partially through endothelium mediators.

Keywords: *Kalanchoe pinnata*, Antihypertensive effects, Rat-induced hypertension, Myocardial infarction.

INTRODUCTION

Hypertension is the leading cause of cardiovascular diseases [1]. Positive associations do exist between blood pressure (BP), risk of CVD and mortality [2]. Hypertension is defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg [3]. Two-thirds of the world widely estimated 1.13 billion hypertensive people live in low- and middle-income countries [3]. Currently, 30.9% Cameroonians are hypertensive [4]. According to the World Health Organization Traditional Medicine Strategies 2014-2023, member states should be supported in strengthening the contribution of traditional and complementary medicine to health, wellness and people centred health care [5]. The perceived efficacy and availability of medicinal plants especially in Africa might account for this, since about 25% of the total number of higher plants in the world are found in the continent; with over 5,400 plants species reported to have about 16,300 medicinal uses [6]. Furthermore, in this same continent, the ratio of traditional healers to population is 1:500 whereas the ratio of medical doctors to population is only 1:40,000 [5]. Several medicinal plants have been and are in current use for multiple disease conditions [7, 8]. However, the medical use of plants and their derivatives has been undermined by inadequate scientific research, lack of standardization, and poor documentation [9, 10]. Hypertension and related illnesses feature prominently with some of these herbal preparations [11]. This medicinal system requires an extensive research into the untapped natural resources of plants in Africa, especially West and Central Africa. However, because the cost of modern drug therapy is prohibitive, many patients especially in rural and semi-urban areas rely on traditional medicine for the management of Hypertension [12, 13, 14].

Previous researches have shown beneficial activity of *Kalanchoe pinnata* in the alleviation of hypertension [15, 16]. It has been proven that Almost 40% of patients with ischaemic heart disease (IHD) who die suddenly have a history of hypertension [17]. Pandit [18] reported in a study that 72% of Myocardial Infarcted (MI) patients were hypertensive. This reflected high incidence of hypertension

in MI patients as also reported by Frazier *et al* [19]. With the increasing prevalence of hypertension and other heart diseases, it is therefore imperative to intensify research on the use of *Kalanchoe pinnata* leaf extracts on hypertension. More so, the vascular activity of these extracts has not been elucidated. In this experiment, pharmacological effects of *Kalanchoe pinnata* leaf aqueous extract was explored on the blood pressure of an animal (rat) model of concurrent NaCl-induced hypertension and Isoproterenol-induced myocardial infarction. We also investigated the underlying mechanistic contribution of vasorelaxant activity of the plant extract.

MATERIALS AND METHODS

Plant collection and extraction

Fresh mature leaves of the plant, without lesion, were harvested in Buea (Cameroon), in April 2018. They were wrapped with plastic sheets during transportation. The sample authentication was confirmed at the South western Cameroon Herbarium, Limbe (Voucher Number SCA 2770). The material (3kg) was pounded by means of porcelain laboratory pounding cup. For the preparation of aqueous extract, one portion (1kg) was macerated with water (1.5L) for 48hours. After filtration (Watman No 1 paper) and drying (oven, 45ºC), 29.6 g (2.96% yields) of powder was obtained. The stock solution (1g/mL) was prepared by dissolving the above extracts in distilled water.

Animals and distribution of groups

Wistar rats aged 3-4 months and weighting 155-200g were used. They were carefully handled according to International Guidelines (CIOMS) [20]. Furthermore, an ethical clearance was obtained from the University of Buea Institutional Animal Care and Use Committee (2018/001/UB/IACUC/BTU/FS). Animals were raised in the Animal House of Department of Animal Biology, University of Buea, in plastic cages; under local day/night natural cycle and temperature (18-25ºC). Access to feed and water was *ad-libitum*. A total of 40 adult *Wistar* rats were used. For the study of the effects of the plant extract on blood pressure and heart rate, 30 rats were randomly and evenly distributed into six groups (numbered 1-6) of five animals each. The dose of 100mg/kg/day *K. pinnata* has been considered, from our previous findings as the "therapeutic dose" [16].

Table 1: Grouping and treatment of animals

Group	Treatment received	Duration/steps
Group 1,	Water (10mL/kg)	28days
Group 2,	18% NaCl (10mL/kg) + isoproterenol 100mg/kg	28 days Isoproterenol -2 days
Group 3-5	18% NaCl +*K. pinnata* (150mg/kg) + isoproterenol 150mg/kg	(50- 18% NaCl - 28 days Isoproterenol -2days
Group 6	18%NaCl (10mL/kg) +isoproterenol(150mg/kg) +propranolol(10mg/kg) +spironolactone(150mg/kg)	18%NaCl+spironolactone-28days Isoproterenol -2 days propranolol -10 days

In vivo study

Normotensive rats (30) were randomly divided into 6 groups of 5 animals each and treated as presented in Table 1.

In Group 2, representing negative control, rats received 18% NaCl (daily for four weeks) and Isoproterenol (100mg/kg) was injected subcutaneously for two consecutive days before sacrifice. In Group 3, animals received 18%NaCl (daily for four weeks) and plant extract at 50mg/kg/day then Isoproterenol (150mg/kg) was injected subcutaneously for two consecutive days before sacrifice. Group 4 comprises animals that received 18% NaCl (daily for four weeks) and plant extract at 100mg/kg/day then Isoproterenol (150mg/kg) was injected subcutaneously for two consecutive days before sacrifice. In Group 5 they received 18%NaCl (daily for four weeks) and the plant extract at 150mg/kg/day, and then Isoproterenol (150 mg/kg) was injected subcutaneously for two consecutive days before sacrifice. Finally, Group 6, the positive control group received 18%NaCl and spironolactone (0.71mg/kg, for four weeks), then propanolol (10mg/kg/day for 10 days) while Isoproterenol (150mg/kg/day) was injected subcutaneously for two consecutive days before sacrifice.

Effects of Kalanchoe pinnata on blood pressure and heart rate

After 28 days of treatment, the effect of aqueous extract of *K. pinnata* on 18%NaCl-hypertension was evaluated. Intra-peritoneal injection of 15% urethane (1.5g/kg) led to rat anaesthesia. The trachea was exposed and cannulated to facilitate spontaneous respiration. A polyethylene catheter (PE50) was inserted into the right femoral vein and a bolus injection of 10% heparin (0.1mL/100gbw) was immediately administered.[16] Cardiovascular parameters were recorded by invasive method. A catheter connected to a pressure transducer (coupled with a Biopac Student Lab MP35 hemodynamic recorder and a computer) was inserted into the left carotid artery. The Biopac StudentLabMP35 device recorded simultaneously the systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR), following a 30–60min stabilisation period [21].

Determination of serum cardiac troponin

At the end of the blood pressure measurement, blood was collected from these animals. Blood (3mL) was collected by cardiac puncture using 5mL syringe, and kept in plain tubes for 1 h at room temperature. Serum was separated by centrifugation at 3000 rounds per minute (rpm) for 15 minutes. Cardiac troponin values were determined by spectrophotometry: 100μL of the samples and standards were dispersed into separate wells. 100μL of the enzyme conjugate reagent were added and mixed for 30s then incubated for 90 minutes. The wells were then flick into a waste container and rinsed five times with distilled water. The wells were dried with tissue paper. 100μL of TMB reagent was dispense gently into each wells and mixed for 5 seconds before being incubated for 20 minutes. The reaction was stopped by adding and mixed for 30 seconds with 100μL of stop solution until colour changed from blue to yellow, the absorbances were read at 450nm. The absorbance of the standard was plotted against the concentration and troponin concentration for the samples were extrapolated from the standard curves. Results obtained were in ng/dL.

In vitro study

A total of ten male rats weighing between 180g – 200g were used in this study. The rats were sacrificed by cervical dislocation, and dissected the thoracic aorta. A midline incision was made from the abdominal region just below the diaphragm towards the chest region in order to expose the thoracic aorta. The descending thoracic aorta was rapidly dissected out and immersed in an ice cold Petri dish containing Krebs physiological salt solution, composed of (g): NaCl,

68.96; KCl, 3.5; $MgSO_4$, 2.955; KH_2PO_4, 1.63; $CaCl_2$, 2.775; $NaHCO_3$, 2.1; glucose, 2.18; and bubbled with 100% O_2 (pH 7.4).[22] After the perivascular tissue was carefully removed, aortic rings (2-4mm) in length were cut, while taking care not to abrade the endothelial layer. Aortic rings were mounted in organ chambers (Organ Bath, 2 channels, EMKA Technologies, France) containing 20mL Krebs' solution at 37◦C, and aerated with 100% O_2. Equilibration was done under 0.5g, 1g, 1.5g and 2g tensions. During the equilibration period, the solution was renewed every 15 min. At the beginning, rings were stimulated with 3M KCl until a reproducible contraction was obtained. The presence of functional endothelium was ascertained by the ability of acetylcholine (Ach) (10^{-4}M) to induce more than 60% reduction of phenylephrine (PE) (10^{-4}M) vascular pre-contraction.

Effects of K. pinnata aqueous extract on phenylephrine or potassium chloride-induced vascular contraction

Vasodilatory effects of *K. pinnata* were evaluated on phenylephrine (10^{-4}M PE) or potassium chloride (60mM KCl) contracted rings in two separate experiments. When the vasoconstriction curves reached the plateau phase, the extract was added in the organ bath making a concentration of 0.40mg/mL for the contraction induced by PE; or cumulatively 0.25mg/mL, 0.35mg/mL and 0.40mg/mL respectively for the contraction induced by KCl. The tensions were recorded and the vasorelaxant effect of *K. pinnata* was calculated as a percentage of the relaxation in response to PE and KCl induced contractions of the aortic rings. From this preliminary screening of concentrations we decided to work, for better efficacy, with the following concentrations: 0.5, 1, 1.5mg/mL.

Effects of K. pinnata aqueous extract on endothelium intact or endothelium-denuded rings pre-contracted with phenylephrine or potassium chloride

To check if endothelium plays a role in the vasorelaxant effect of *K. pinnata* aqueous extract, the vasodilatory effect of the extract on contraction induced by PE or KCl was evaluated for both endothelium intact and endothelium denuded vessels. To confirm that the endothelial had been removed, we selected the rings with a maximum relaxation induced by acetylcholine less than 30% after phenylephrine (10^{-4}M)-induced contraction in denuded vessels [22].

Studies of endothelium-related vasorelaxant mechanisms of K. pinnata aqueous extract

To determine the endothelium-underlying mechanisms, the role of nitric oxide (NO) and guanylate cyclase inhibition was determine using methylene blue (MB). Endothelium-intact rings were incubated with MB (10^{-3}M) for 30min before addition of extract (1.5mg/mL) and the percentage of relaxation calculated. Also the vasorelaxant response in the presence of a NO donor sodium nitroprusside (SNP) was evaluated after incubation with the extract (1.5mg/mL) and PE induced pre-contraction.

Statistical analyses

Data was entered into Excel spread sheets and analysis was done with the statistical package Graph pad prism version 6. Data was presented in the form of tables and graphs and was analysed using the one-way analysis of variance (ANOVA) followed by a multiple comparison turkey test. Results were expressed as mean ± standard error of mean (SEM) and difference was considered significant at $P < 0.05$.

RESULTS

Effects of Kalanchoe pinnata on arterial pressure and heart rate

Treatment with 18% salt for 28 days and ISO for 2 days, significantly caused an elevation in systolic arterial pressure (135.34±2.38 mmHg) in the negative control compared to the normal control (92.49±0.6 mmHg) giving a 46.3% ($p<0.001$) increase in systolic arterial pressure. Administration of *K. pinnata* at dose of 100mg/kg and 150mg/kg significantly prevented an increase in systolic arterial pressure compared to the negative control, with the dose of 150 mg/kg being more efficacious (135.34±2.38 mmHg vs 95.6±1.76 mmHg), corresponding to a 29.4% ($p<0.001$) reduction than the dose of 100 mg/kg (135.34±2.38 mmHg vs 119.4±4.71 mmHg) corresponding to a 11.78% ($P<0.05$) reduction. Propranolol and spironolactone also significantly reduced systolic arterial pressure by (29.1%) ($p<0.001$) compared to the negative control (Figure 1)

On the other hand treatment with 18% salt and ISO significantly caused an increased in diastolic arterial pressure in the negative control (106.71±5.6 mmHg) compared to the normal control (61.49±0.8mmHg) corresponding to a 73.5% ($p<0.001$) increase. Both doses (100 mg/kg and 150 mg/kg) of *K. pinnata* significantly reduced diastolic arterial pressure (81.7±2.9 mmHg and 62.6±3.1 mmHg respectively) corresponding to a 23.4% and 41.3% ($P<0.05$) respectively compared to negative control (106.71±5.6 mmHg). No significant differences in the efficacy of both doses of the extract. Propranolol also significantly reduced diastolic arterial pressure by 52.58% (50.6±1.2 mmHg) compared to the negative control (106.71±5.6 mmHg) (Figure 2). In the positive control, administration of propranolol to salt loaded myocardial infarcted rats significantly lowered heart rate) by 39.1 % ($P< 0.001$) compared to the negative control (200.37±0.98bpm vs 329.02±7.59bpm). There was no significant reduction in heart rate by both doses of the extract (100 mg/kg and 150 mg/kg) (317.71±8.8bpm and 302.59±5.1bpm respectively vs 329.02±7.59bpm) compared to the negative control.

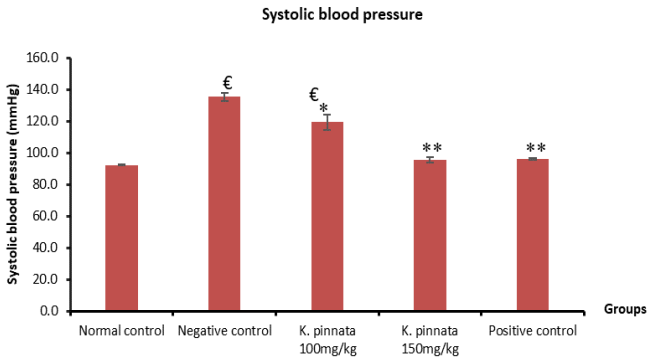


Figure 1: Effect of *K. pinnata* on systolic blood pressure in 18%NaCl-loaded, myocardial infarcted rats. Each bar represents mean ± SEM. n=5, *P<0.05 and **P< 0.001 vs negative control, €p<0.001 vs normal control.

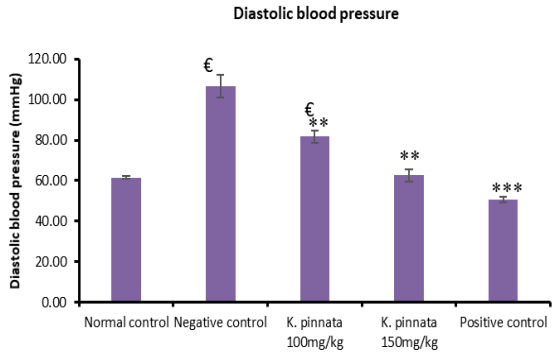


Figure 2: Effect of *K. pinnata* on diastolic blood pressure in 18%NaCl-loaded, myocardial infarcted rats. Each bar represents mean ± SEM. n=5, **P<0.05 and ***P<0.001 vs negative control, €p<0.001 vs normal control

Effects of aqueous extract of Kalanchoe pinnata on cardiac troponin

Kalanchoe pinnata has a positive effect on salt loaded isoproterenol induced myocardial infarction in rats. The level of cardiac troponin was significantly elevated in the negative control group by almost 400% (2.49±0.21 ng/dl vs 0.49±0.04 ng/dL) compared to the normal (Figure 3). The extract (100 mg/kg and 150 mg/kg) significantly reduced the level of cardiac troponin by 52% compared to the negative control meanwhile propranolol significantly reduced the level of troponin by 61% compared to the negative control.

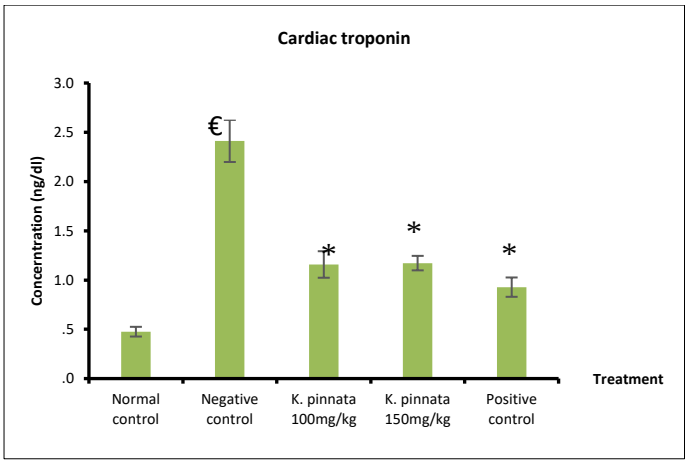


Figure 3: Effects of aqueous extract of K. pinnata leaf on cardiac troponin levels. Each bar represents the mean ± SEM, n =5. *P<0.05 vs negative control, €P<0.001 vs normal control

Effects of K. Pinnata aqueous extract on endothelium intact or endothelium-denuded aortic rings pre-contracted with potassium chloride

The effect of *Kalanchoe pinnata* on vascular smooth muscle was investigated on endothelium denuded and endothelium intact aortic ring as shown in Figure 4. *Kalanchoe pinnata* induced relaxation in a dose dependent manner in both the endothelium intact and endothelium denuded aortic rings. There was no significant difference (p>0.05) between the relaxation induced by *Kalanchoe pinnata* on both the endothelium intact versus endothelium denuded aortic ring. Ring with endothelium had a maximum percentage relaxation of 31.44±5.1% as compared to 23.64±1.63% in rings without endothelium.

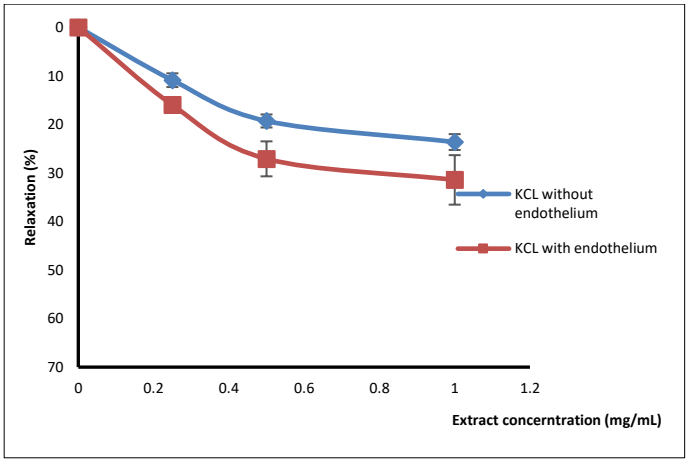


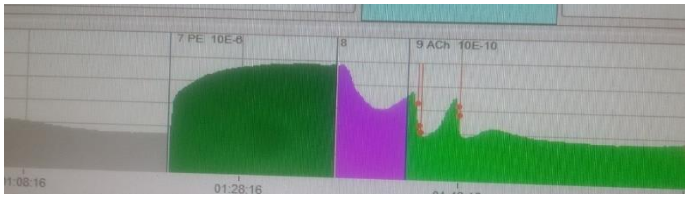
Figure 4: Concentration dependent relaxation effect of *Kalanchoe pinnata* aqueous extract on KCl (60mM) pre-contracted endothelium intact and endothelium denuded aortic rings. Values are expressed as means ±SEM (n=5). P<0.05.

Effects of K. pinnata aqueous extract on endothelium intact or endothelium-denuded aortic rings pre-contracted with phenylephrine

We found out that the rings pre-contracted with phenylephrine without endothelium caused an additional increase in tension followed by a non-significant relaxation when the maximum dose was administered (result not shown).

For rings with endothelium the increase in tension was observed only when the least concentration was administered (Figure 5A). At the concentrations of 0.5mg/mL, 1mg/mL and 1.5mg/mL of extract, a 35.24±6.02% contraction followed by relaxation of 20.12±4.01% and 64.02±4.31%, were observed respectively. For that reason, we investigated the effect of the maximum concentration of the extract alone on endothelium intact aortic ring pre-contracted with phenylephrine and a percentage relaxation of 62.79 ± 4.02 was obtained (Figure 5B).

A



B

Figure 5: Effects of *K. pinnata* aqueous extract on endothelium intact aortic rings

5A: Effect of different doses of *K. pinnata*. Dark green represent contraction with phenylephrine, purple contraction with 0.5mg/mL extract, light green relaxation with 1mg/mL, pink relaxation with maximum dose 1.5mg/mL of extract. Ach was administered following washout to check on the functionality of the endothelium (green color at the end).

5B: Effect of maximum dose of *K. pinnata*. Dark green represent contraction with phenylephrine, purple relaxation with 1.5mg/mL extract. Ach was administered following washout to check on the functionality of the endothelium (green color at the end). (n=5)

Effect of Kalanchoe pinnata in the presence of Methylene blue, a NO-cGMP pathway inhibitor

Pre-treatment of endothelium intact aortic rings with methylene blue (MB, 10^{-3}M) produced a significant reduction in the relaxation induced by *Kalanchoe pinnata*. In the presence of MB, the E*max* was 43.858 ± 6.524% *vs.* 62.792± 4.022% (P<0.001**)** in the aortic rings contracted with phenylephrine and relaxed with the maximum dose of the extract (Figure 6).

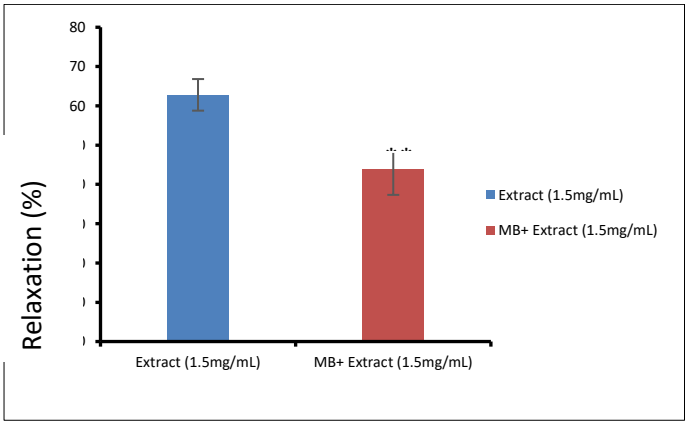


Figure 6: The effects of methylene blue (MB, 10^{-3}M) on intact aortic rings contracted with PE (10^{-4}M) incubated with the maximum dose of *Kalanchoe pinnata* aqueous extract (1.5mg/mL). Values are expressed as mean ± SEM (n=5). ***P<0.001 vs. relaxation with phenylephrine.

Under basic tone of 2g, in aortic rings pre-contracted with phenylephrine, relaxation of the vascular muscle by sodium nitropruside (SNP) was not significantly different from the relaxation of SNP in the presence of *K. pinnata* (1.5mg/mL) (Figure 7)

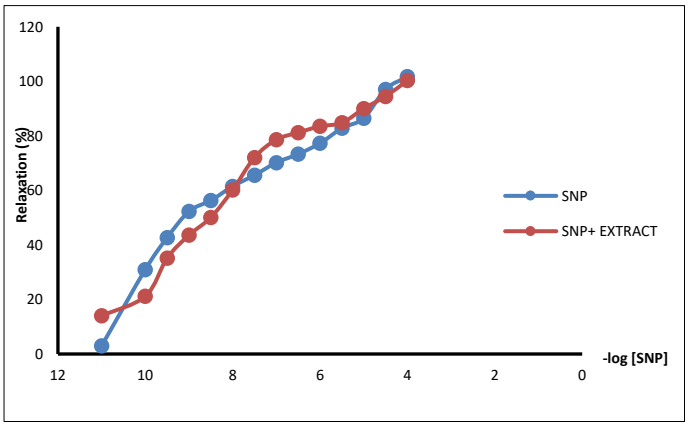


Figure 7: Effect of aqueous extract *Kalanchoe pinnata* 1.5 mg/mL on SNP induced relaxation of rat aortic rings pre-contracted with PE (10^{-4}M). Values are expressed as means (n=4)

DISCUSSION

This study was aimed at investigating the antihypertensive effects of *Kalanchoe pinnata* on concurrent salt loaded and myocardial infarcted rats and to investigate the vasorelaxant effect of the extract on rat's thoracic aorta. Rats which were treated only with salts and ISO plus those treated with the least dose of the extract died within the three hours following the administration of isoproterenol (150mg/kg), corresponding to a 33% dead. This is similar to the work of Heraldo *et al.* [23] who reported 25%. The negative control group was then repeated with a smaller dose of isoproterenol 100 mg/kg which is known to cause sub-epicardial infarction in rats [24].

The aqueous extract of *Kalanchoe pinnata* produced a decreased in systolic, diastolic arterial pressure without a significant effect on the heart rate. The results obtained from this study have demonstrated that *Kalanchoe pinnata* possessed significant antihypertensive effect against salt loaded myocardial infarcted rats. To the best of our knowledge, this is the first report to show that *Kalanchoe pinnata* can ameliorate the development of hypertension in simultaneously salt loaded and ISO-induced myocardial infarcted rat model. These findings support the traditional use of the plant leaves for the treatment of hypertension and other cardiovascular dysfunctions.

In this study, we demonstrated that salt loading (18% NaCl) by gavage significantly increased blood pressure in rats compared to the normotensive animals in agreement with Bopda *et al.* [16]. Constant high levels of NaCl in internal medium easily brings about hypertension by hardening of blood vessels [13, 25].

Administration of different doses of the extract (100mg/kg and 150mg/kg) prevented the elevation of both systolic and diastolic arterial pressure in rats. *Kalanchoe pinnata*, spironolactone and propanolol all reversed the abnormalities in salt loaded myocardial infarcted rats. Maximum decrease in blood pressure was observed at the dose of 150mg/kg body weight. Flavonoids, alkaloids, quercetin rutin and tannins have been reported to be the main component in *Kalanchoe pinnata* [26]. These compounds have been reported to play very important roles in lipid metabolism and protective functions against cardiovascular diseases [27]. The hypotensive effect of rutin previously demonstrated [28] was attributed to an increase plasmatic level of quercetin in mice [29].

With regards to troponin levels, there was a significant increase in the negative control group compared to the neutral control group. The significant increase in the negative control is caused by the administration of ISO (100mg/kg), which is an inducer of myocardial infarction. Myocardial tissues were damaged by ISO leading to the release of troponin by myocytes. In the groups treated with *Kalanchoe pinnata* extract (100mg/kg and 150mg/kg) + isoproterenol, the extract significantly reduced the damage caused by the administration of isoproterenol compared to the negative control. Administration of propranolol, a known antagonist significantly reduced the level of damage, in muscles, caused by isoproterenol; this was in line with the findings of Thiringer and Svedmyr [30].

In vitro studies revealed that aqueous extract of *Kalanchoe pinnata* possesses vasorelaxant properties in thoracic aorta with or without an intact endothelium. The antihypertensive properties of *Kalanchoe pinnata* is therefore partly exhibited by its ability to relax vascular smooth muscle as speculated by Bopda *et al.* [16], this is in conformity with the work of Salahdeen and Yemitan [31] who evaluated the muscle-relaxant activity of the aqueous extract and observed reduction

in the muscle tone of laboratory animals. The extract exhibited a slight reduction in the baseline tension of unstimulated thoracic rings. This reinforces the observation that the plant extract prevents vasoconstriction.

Cumulative concentrations of the extract (0.25–1mg/mL), caused dose-dependent relaxation of KCl (60mM) pre-contraction in both endothelium intact and denuded rings but rings with endothelium demonstrated a more pronounced relaxation than rings without endothelium. Because the difference in the relaxation of endothelium and endothelium-denuded aortas was not significant, we concluded that relaxation evoked by *K. pinnata* extract is mostly endothelium-independent in conditions where hyperpolarization mechanisms are dampened. Endothelium plays an important role in vasorelaxation. NO is one of the potent vasodilators secreted from vascular endothelium and vascular smooth muscle is relaxed via NO-cGMP pathway [32]. In the present study, the vasorelaxant effect of *K. pinnata* was reduced in endothelium intact aortic rings when incubated in methylene blue (MB) a soluble guanylate cyclase inhibitor. These results suggested that the vasorelaxant effect of *K. pinnata* is related to the induction of NO formation from l-arginine and NO-cGMP pathways. Vasodilation of aortic rings pre-contracted by phenylephrine is endothelium-dependent (at least partly).

Application of Sodium nitroprusside (SNP) induced a significant relaxation in endothelium denuded rings suggesting that the relaxation of *K. pinnata* extract is not fully endothelium-dependent. Therefore, it is more likely that *K. pinnata* may lead to either an increase in endothelium production of NO or premature activation of produced NO in the aortic rings. It is well known that SNP breaks down in circulation to release nitric oxide, which plays a significant role in relaxation.

Ring pre-contracted with phenylephrine in the absence of an endothelium developed additional tension when cumulative doses (starting from 0.5mg/mL) were added to the rings. There was a relaxation when the dose of 1.5mg/mL was added but relaxation was not significant. This is similar to results obtained by Rodrıguez *et al*. [33] when they investigated the endothelium-dependent effects of the ethanolic extract of the *mistletoe Psittacanthus calyculatus* in rat aorta.

Rings pre-contracted with phenylephrine showed similar relaxation in the presence of endothelium and without endothelium when the dose 0.5mg/mL was administered, but when higher doses (1mg/mL and 1.5mg/mL) were added cumulatively, the relaxation of endothelium intact rings was concentration-dependent. The increase in tension, induced by low concentrations of the extract suggests that it might contain also some vasoconstrictor compounds. The tension development induced by the extract occurred in rings already maximally pre-contracted by an α-adrenergic agonist. Since the extract-induced tension increment was pronounced in rings without a functional endothelium, it seems clear the extract contains some active ingredients, which could act in synergy with PE; especially at lower concentrations of extract. These findings however should not devalue the significant vasorelaxant activity recorded in rings pre-contracted with KCl (60mM) and the similar effect by high doses on PE pre-contracted rings. The importance of Ca^{2+} in smooth muscle cell excitation-contraction coupling needs not new demonstration [34]. It is known that vasoconstriction inducers act by activating both intracellular and extracellular calcium. The prior pathway for catecholamines like phenylephrine is mobilisation of intracellular calcium, while prior mechanism for KCl is stimulation of extracellular calcium influx, via voltage dependent calcium channels (VDCCs). Stimulation of extracellular Ca^{2+} influx through receptor-operated Ca^{2+} channels (ROCCs) by phenylephrine or through VDCCs by KCl and release of Ca^{2+} from sarcoplasmic reticulum result in increased intracellular Ca^{2+} [35, 36], which causes contraction. The main bioactive constituents in *K. pinnata* leaf are flavonoids, such as rutin, quercetin [37]. An inhibition of Ca^{2+} influx is a contributing mechanism of the vasorelaxation induced by rutin, quercetin [38]. The flavones and flavonols also inhibit contraction caused by the intracellular release of Ca^{2+} [38]. We suggest that the relaxation effect of *K. pinnata* is also related to the inhibition of intracellular Ca^{2+} increase.

Phenylephrine causes aortic contraction by Ca^{2+} influx through ROCCs and by release of Ca^{2+} from the sarcoplasmic reticulum [36, 39]. The intracellular pathway involves phenylephrine stimulation of phospholipase C to produce diacylglycerol (DG) and IP3, and subsequent Ca^{2+} release from the sarcoplasmic reticulum by opening IP3 receptors [36]. The aqueous extract of *Kalanchoe pinnata* relaxes aortic rings pre-contracted with phenylephrine implying that it may inhibit the IP3 and/or block ROCCs to decrease intracellular Ca^{2+} and relax the aorta.

KCl provokes the contraction of smooth myocytes mainly by extracellular Ca^{2+}influx and subsequent opening of VDCC [40]. *Kalanchoe pinnata* cumulatively relaxed aortic rings that were pre-contracted with KCl (60mM). This result suggests that *K. pinnata* could also inhibit vasoconstriction induced by extracellular Ca^{2+}entry via the VDCC.

CONCLUSION

The aqueous extract of *Kalanchoe pinnata* has the ability to prevent the development of high blood pressure in concurrent hypertensive and myocardial infarcted rat model but has no significant effect on the heart rat. One of the main mechanisms of action of the extract is by relaxing vascular smooth muscle mostly when the endothelium is present; the maximum effect of the extract can be achieved when the highest dose is administered. *Kalanchoe pinnata* might relax vascular smooth muscle via an endothelium dependent pathway such as the NO pathway. In that light more studies are required to determine precisely the intimate endothelium- dependent vasorelaxant mechanism of the extract.

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Conflicts of interest

The authors declare no conflict of interest.

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