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(RESEARCH ARTICLE)

Kalanchoe pinnata aqueous extract efficacy against myocardial infarction and effects on three cardio-metabolic biomarkers in NaCl-overloaded rat

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Abstract

Cardiovascular diseases are among the causes of global mortality in the world with hypertension and myocardial infarction being one of the key risk factors. *Kalanchoe pinnata* is a plant that has shown antihypertensive and cardioprotective effects in rat model. This research aimed at investigating the cardioprotective effect of *Kalanchoe pinnata* on concurrent NaCl overloading and myocardial infarction condition in rats. Thirty animals both males and females were divided into 5 groups of 6 animals each, a normal/neutral control, negative control group, positive control group and two groups that received two doses of the leaf aqueous extract (100 mg/kg and 150 mg/kg). Hypertension was induced by excess salt loading (18%NaCl) meanwhile myocardial infarction was induced by subcutaneous injection of isoproterenol (100 mg/kg). Levels of total cholesterol, triglyceride, low density lipoprotein and cardiac troponin were determined using commercial kits. Administration of excess NaCl concurrently with isoproterenol caused a significant 82% elevation in triglyceride levels. Both doses of the extract reduced non-significantly the level of triglycerides, total cholesterol and low density lipoprotein. Meanwhile the level of troponin was also significantly elevated ($P<0.001$) in the negative control group (2.49±0.21 *n*g/dL) compared to the normal (0.49±0.04 *n*g/dL). Cardiac troponin level was significantly reduced ($P<0.05$) by 52% in the extract treated groups compared to the negative control. Examination of heart tissues revealed damages in the negative control group meanwhile positive control and groups that received the extract showed no damage. Therefore, *Kalanchoe pinnata* is efficacious in preventing myocardial infarction even in salt loaded rats.

Keywords: Cardioprotective effects; Excess NaCl; Infarction; Isoproterenol; *Kalanchoe pinnata*.

1. Introduction

Cardiovascular diseases (CVDs, which include coronary heart disease and stroke among others) are the most common non-communicable diseases globally, responsible for an estimated 17.8 million deaths in 2017, of which more than three quarters were in low-income and middle-income countries. In Cameroon, CVDs represent a public health problem [1, 2], although still there is a significant gap in population awareness about this burden [3].

Hypertension appears to be the most prevalent risk factor for heart failure, coronary artery disease and stroke in economically emerging countries [4, 5]. Hypertension increases the risk of acute myocardial infarction [6]. In fact, almost 40% of patients with ischaemic heart disease (IHD) who die suddenly have a history of hypertension [7].

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Myocardial Ischaemia (MI) remains a major pathological cause of death worldwide despite rapid advancements made in the treatment of coronary diseases [8, 9]. As an alternative medicine, active natural products from medicinal plants constitute an asset for the protection against various human diseases including CVDs [10, 11]. The use of such alternative medicine has been recommended actually by WHO, especially in developing countries [12]. *Bryophyllum pinnatum* (Lamarck) Oken or *Kalanchoe pinnata* (Lamarck) Persoon (Crassulaceae) aqueous extract has been taken by populations for the management of CVDs, precisely hypertension [13-15]. Previously, we demonstrated that in addition to being antihypertensive [13], the above extract could induce cardioprotection of the heart against MI in Isoproterenol (a beta-agonist)-treated normotensive rat [16]. We have recently demonstrated that the plant extract, when administered to concurrent salt overloaded and myocardial infarcted animal model, is still able to lower blood pressure, through vasodilation [17]. In the current research, we have investigated the effects of the aqueous extract in NaCl-overloaded rat with concomitant Isoproterenol infarcted heart. The activity of the *K. pinnata* extracts was investigated on serum cardiometabolic biomarkers (cholesterols, triglyceride and troponin) levels, and myocardial structure, in NaCl-overloaded-isoproterenol-treated rats.

2. Materials and methods

2.1. Animals and experimental design

Wistar rats were raised in the Animal House of the Department of Animal Biology and Conservation, University of Buea, under the same conditions previously described by Tom *et al.* [17]. A total of 30 adult rats aged 3-4 months, weighting 155-190g, were randomly distributed, as previously [17], into five groups of six animals each (Table 1).

Table 1 Grouping and treatment of animals

Group	Treatment administered	Duration
Group 1, neutral control	Water (10mL/kg)	28 days
Group 2, negative control	18% NaCl (10mL/kg) + isoproterenol subcutaneous injection (100mg/kg)	18% NaCl, 28 days before sacrifice Isoproterenol, 2 days before sacrifice
Groups 3 and 4, *Kalanchoe pinnata.*	18% NaCl (10mL/kg) + Aqueous *Kalanchoe pinnata* extracts (100 mg/kg or 150 mg/kg) + isoproterenol (100mg/kg)	18% NaCl, 28 days Plant extract, 28 days Isoproterenol, 2 days before sacrifice
Group 5, positive control	18% NaCl (10mL/kg) + isoproterenol (100mg/kg) + propranolol (10mg/kg) + spironolactone (150mg/kg)	18% NaCl and spironolactone, 28 days Isoproterenol, 2 days before sacrifice Propranolol, 10 days to the sacrifice

The dose of 100mg/kg/day of *K. pinnata* aqueous extract has been considered, from our previous findings [14], as the "therapeutic dose" [17].

2.2. Ethics

Animals were carefully handled according to International Guidelines [18]. Experimental procedures and protocols used in this study were approved by Institutional Animal Care and Use Committee, University of Buea (reference number: 2019/008/UB/IACUC/BTU/FS of 02/04/2019).

2.3. Plant material and extraction

Fresh leaves of *Kalanchoe pinnata* were harvested in Buea (Cameroon), in April 2018 and authenticated (at South-western Cameroon Herbarium, Limbe, Voucher Number SCA 2770). From 3kg of plant material, extraction was carried out as previously described [17], and 0.99% yields powder was obtained. The stock solution (1g/mL) was prepared [17] for subsequent experiments.

2.4. Biochemical analysis of cardiometabolic biomarkers level

Blood was collected by cardiac puncture and allowed to clot for 40-60 mins at room temperature, the supernatant was collected separate in labelled tubes, using a pipette, and centrifuged (3000 revolutions per minute, -5°C) for 10 mins. The collected serum was stored at -28°C in the freezer, since all analyses could not be carried out on the same day. All

biochemical analyses were carried out within a ten-day deadline following the collection of serum. Serum levels of total cholesterol, low density lipoprotein cholesterol, triglyceride and troponin were assayed.

2.4.1. Determination of serum total cholesterol

Total cholesterol was quantified after enzymatic hydrolysis and oxidation using a commercial kit (AGAPE, Switzerland). The level of cholesterol was estimated by spectrophotometric methods, and the absorbance (A) was read at wavelength of 505nm. The values were expressed in mg/dL.

$$\text{Total cholesterol in sample (mg/dL)} = \frac{\text{A(SAMPLE)}}{\text{A(STANDARD)}} \times 200 \text{ (STANDARD CONC)}$$

2.4.2. Determination of serum triglyceride levels

Triglycerides were quantified after enzymatic hydrolysis of the sample with lipases. A volume of 1000 µL of reagent was added to each of the standard and sample. This was incubated for 5 minutes at room temperature after mixing. The absorbance of both the standard and the sample were measured against the blank within 30 minutes. The concentration of triglycerides was expressed in mg/dL.

$$\text{Triglyceride (mg/dL)} = \frac{\text{A(SAMPLE)}}{\text{A(STANDARD)}} \times 200\text{(STANDARD CONC)}$$

2.4.3. Determination of serum low density lipoprotein.

The level of low density lipoprotein was quantified after enzymatic hydrolysis of the sample. A volume of 1000 µL of the reagent was added to each of the standard and sample. This was incubated for 5 minutes at room temperature after mixing. The absorbance of the standard and the sample were measured against the blank within 30 minutes. The concentration of low density lipoprotein was expressed in mg/dL.

$$\text{LDL (mg/dL)} = = \frac{\text{A(SAMPLE)} - \text{A(BLANK)}}{\text{A(STANDARD)} - \text{A(BLANK)}} \times 79.8 \text{ (STANDARD CONC)}$$

2.4.4. Determination of serum cardiac troponin

Cardiac troponin values were determined by spectrophotometry, following the protocol previously described by Tom *et al.* [17], the volume of the samples and standards dispersed into separate wells was 100 µL. The absorbance was read at 450nm and the troponin concentrations in the samples were determined by extrapolation from the standard curves.

2.5. Histopathological assessment of cardiac damage

Hearts obtained from all groups were washed immediately with saline and then fixed in 10% buffered neutral formalin solution. The preserved (10%Formalin) hearts were kept for two weeks and then carried to the laboratory of Animal Physiology of the University of Yaoundé I for histopathological examinations. Tissues of the heart were processed with a microtome (ErnstLeitzWetzlar GMBH 530497 No. 537, Germany) and an automated tissue processor (USA). It was afterward embedded, placed on a slide and stained with Haematoxylin and Eosin solution (HE) [19]. Micrographs were snapped with the aid of a digital camera attached to the eyepiece of the light microscope.

2.6. Statistical analyses

Data analysis was done with the statistical package Graph pad prism version 6. Data 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). Differences between means were considered significant at $P < 0.05$.

3. Results

3.1. Effects of *Kalanchoe Pinnata* Aqueous Extract on Cardiometabolic Biomarkers' Levels

3.1.1. Effects of Kalanchoe pinnata on triglycerides, total cholesterol and low density lipoprotein cholesterol levels in serum

Figures 1-3 show serum lipid profiles in normotensive, salt loaded myocardial infarcted rats administered with aqueous extract of *Kalanchoe pinnata.* There was an 82.76% significant ($p<0.05$) elevation in triglycerides level (42.4±5.1 mg/mL) in the salt loaded myocardial infarcted rats compared to the normal group (23.2±2.7 mg/mL). There was a reduction of triglycerides level in groups that received the extract compared with the negative control, but the difference

was not significant (Figure 1). Additionally, total cholesterol and low density lipoproteins did not significantly vary (p>0.05) (Figures 2 and 3).

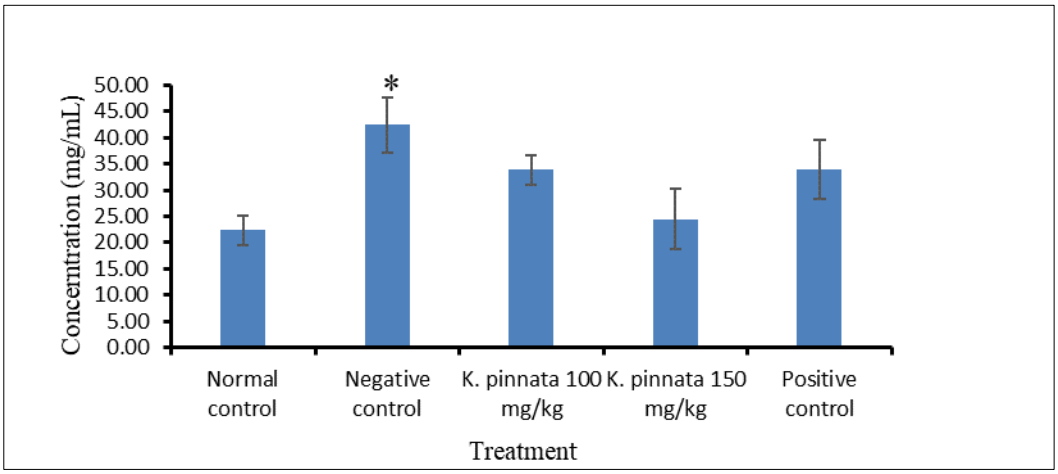


Figure 1 Serum Levels of triglycerides in normotensive rats, salt loaded myocardial infarcted rats, rats treated with extract (100 mg/kg, 150 mg/kg*)* and spironolactone (0.71 mg/kg). Each bar represent mean ± SEM, n= 6, *p<0.05 vs normal control.

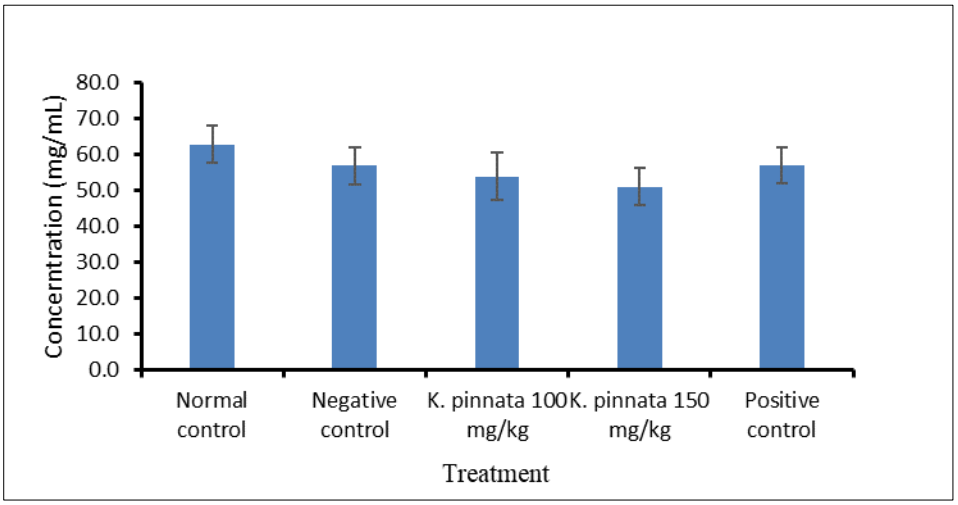


Figure 2 Levels of total cholesterol in normotensive rats, salt loaded myocardial infarcted rats, and rats treated with extract (100 mg/kg, 150 mg/kg) and spironolactone (0.71 mg/kg). Each bar represent mean ± SEM, n= 6.

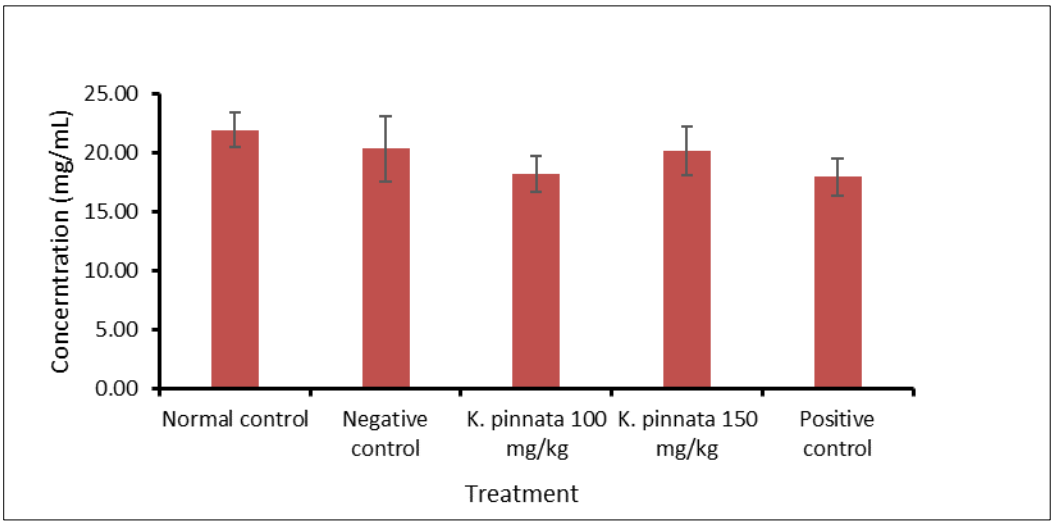


Figure 3 Concentrations of low density lipoprotein in normotensive rats, salt loaded myocardial infarcted rats, rats treated with extract (100 mg/kg, 150 mg/kg) and spironolactone (0.71 mg/kg). Each bar represent mean ± SEM, n= 6.

3.1.2. Effects of Kalanchoe pinnata on troponin level in serum

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 (2.49±1.21 ng/dL) compared to the normal (0.49±0.04 ng/dL). The extract (100 mg/kg and 150 mg/kg) significantly reduced the level of cardiac troponin by 52% compared to the negative control. The reference antagonist, propranolol, significantly reduced the level of troponin by 61% compared to the negative control. Both doses of the extract had similar efficacy (Figure 4).

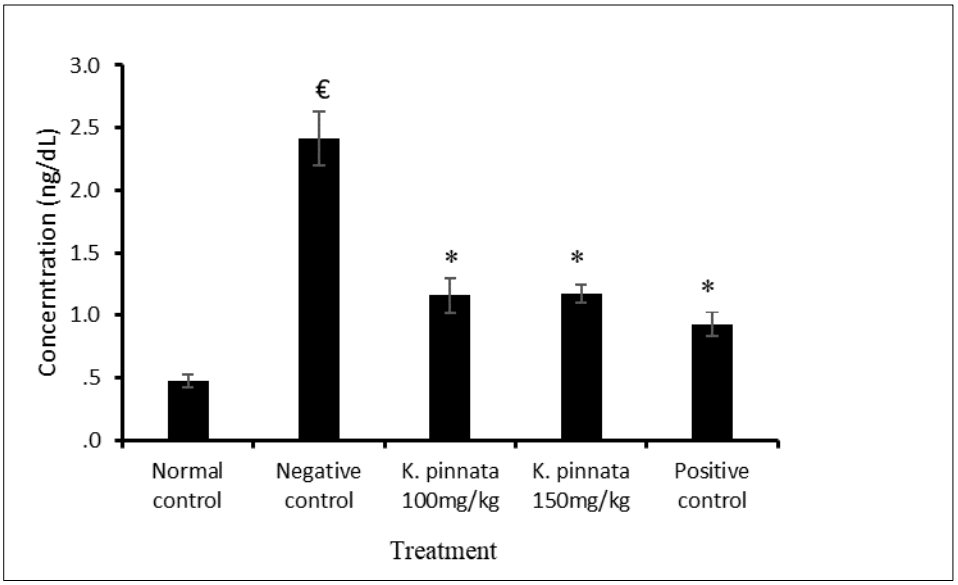
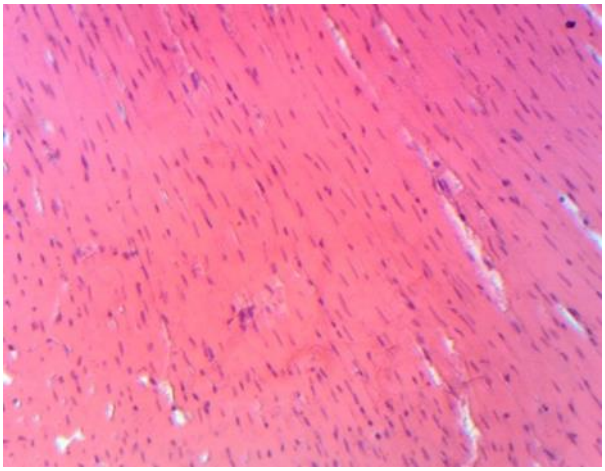


Figure 4 Effects of *K. pinnata* leaf aqueous extract on cardiac troponin levels.

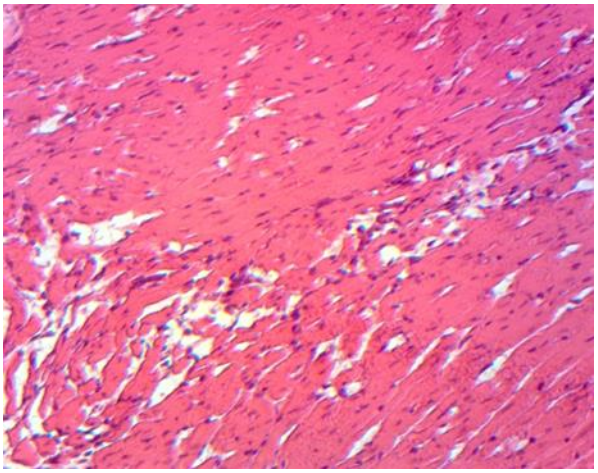
Each bar represents the mean ± SEM, n =6. *P<0.05 vs negative control, €P<0.001 vs normal control

3.2. Histology of the myocardial tissue

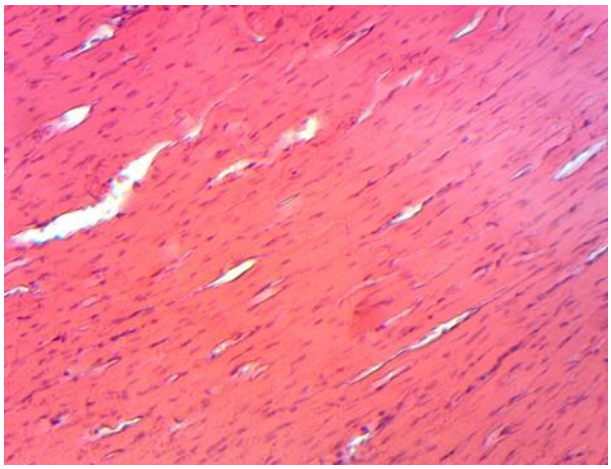
The following micrographs display structural changes in the heart. Myocytes in the negative control (micrograph B) showed damage caused by isoproterenol at 100 mg/kg. The white patches commonly seen in the negative control group are lysed cells. In the negative control, the damage was very intense as compared with the other groups receiving isoproterenol after extracts administration (micrograph C and D), normal control (micrograph A) and the positive control (micrograph E). There was necrosis, and cellular arrangement disappeared only in the negative control group.



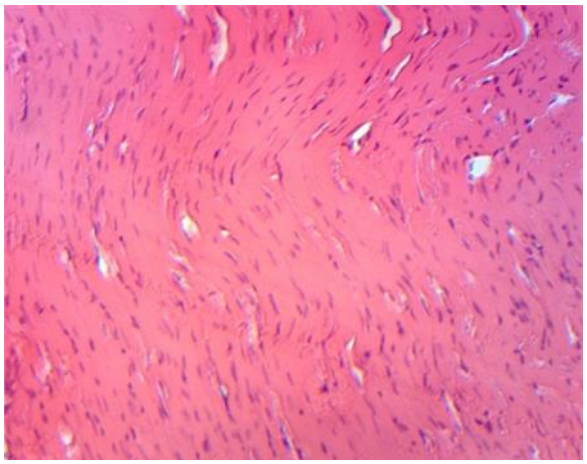
(A) Neutral control



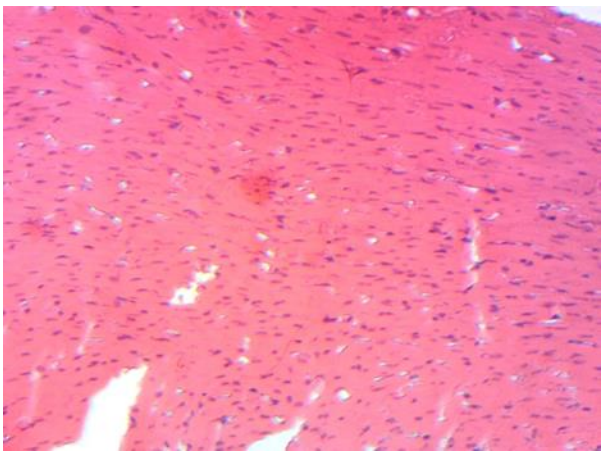
(B) Negative control



(C)*K. pinnata* 100 mg/kg



(D) *K. pinnata* 150 mg/kg



(E) Positive control

Figure 5 Photomicrographs of rat myocardium (H & E x 100).

Myocardial micrographs (A) normal control received tap water showing normal architecture of myocardium; (B) negative control group received subcutaneous injection of isoproterenol (100 mg/kg) showing necrosis of myocytes and change in the cellular arrangement of the tissue; (C) *K. pinnata* 100 mg/kg showing lesser or no change in heart architecture following ISO (100 mg/kg) administration; (D) *K. pinnata* 150 mg/kg showing lesser or no change in heart architecture following ISO (100 mg/kg) administration; (E) propranolol (10 mg/kg) showing no change in heart architecture following ISO (100 mg/kg) administration.

4. Discussion

Herbal medications, especially plant-derived substances, have been used to treat illnesses within local or regional healing practices. This form of medication is gaining steam in Africa where almost 80% of people use some form of herbal medication.

We have recently demonstrated that the plant extract, when administered to concurrent salt overloaded and myocardial infarcted animal model, was able to lower blood pressure, through vasodilation [17]. As a moving step in our investigation, this study was carried out to determine the cardioprotective effects of *Kalanchoe pinnata* aqueous extract in the same model of NaCl-overloaded-Isoproterenol-treated rat. In this light we evaluated the effects of *Kalanchoe pinnata* on triglycerides, total cholesterol, low density lipoprotein cholesterol and cardiac troponin. We also examined the architectural changes in the cardiac tissue. The model for the induction of myocardial infarction via subcutaneous route in rat was in line with other authors [20-22], while hypertension was induced in the same manner as our previous work [13].

Hyperlipidemia is one among other risk factors that contribute and predispose people to cardiovascular diseases [23]. Excessive levels of total cholesterol and low density lipoprotein cholesterol (LDL-C) promote atherosclerosis and other heart related disorders. Elevated levels of triglycerides and LDL-C are subject to oxidative modification and transformation into foam cells, which accumulate in the form of fatty streaks and fibro fatty plaques that lead to the development of atherosclerosis [24]. It is therefore important that approaches to lower these lipid concentrations are used.

The results indicate a slight reduction in total cholesterol and LDL-C levels in groups administered with the extract, however not significant. These results are similar to those obtained by Menon *et al.* [25] when they investigated the effect of *Kalanchoe pinnata* on streptozotocin-induced diabetes in mice. ISO causes oxidative stress via production of reactive oxygen species (ROS), which cause lipid peroxidation and irreversible membrane damage [26]. Rather, there was a significant elevation of triglycerides levels in the group overloaded with salts and isoproterenol. The extract was able to bring down the level of triglycerides although the reduction was not significant. Thus, *Kalanchoe pinnata* aqueous extract has no significantly potent effect on the lipid profile of NaCl-loaded-ISO-treated rats. In fact, we highly expected that the aqueous extract of *Kalanchoe pinnata* would have significantly lowered the levels of triglyceride and low density lipoprotein cholesterol because of its chemical constituent. The phytochemicals present in medicinal plants are basically responsible for the definite pharmacological effects they exert in rats. Flavonoids, alkaloids, cardiac glycosides, and tannins have been reported to play very important roles in lipid metabolism and protective functions against the incidence of lipid peroxidation and cardiovascular diseases. *Kalanchoe pinnata* has been reported to possess such compounds [27]. Also, flavone, a class of flavonoids, has been reported to induce lipid lowering action in hyperlipidaemic rats [28]. Gwon *et al*. [29] suggested that flavones may be one of the candidates for an active component in *Zanthoxylum piperitum* extract and in *Kalanchoe pinnata* aqueous preparation. In perspective, we should investigate the effects of *Kalanchoe pinnata* extract in rat models of diet-induced obesity and hyperlipidaemia, to actually confirm the non-significant potency of the extract on lipid profile.

With regards to troponin levels, there was a significant increase in the negative control group, loaded with salt and ISO, compared to the neutral control group. The significant increase in the negative control is caused by the administration of ISO (100 mg/kg) only, which is an inducer of myocardial infarction. Myocardial tissues were damaged by ISO leading to the release of troponin by myocytes in the negative control group, representing the group with the highest quantity of troponin released (2.49±1.21 ng/dL). Administration of *Kalanchoe pinnata* at the doses of 100 mg/kg and 150 mg/kg significantly reduced the damages caused by ISO on the heart. This can be seen by the reduced levels of cardiac troponin in these groups (1.15±0.78 ng/dL and 1.17±0.96 ng/dL compared to 2.49±1.21 ng/dL in the negative control group). Administration of propranolol, a known antagonist significantly reduced the level of damage in the muscles caused by isoproterenol; this was in line with the findings of Patel *et al.* [30].

Troponin I is a unique cardiac marker, which is released from infarcted myocardium. Troponin C, I and T are proteins that form thin filaments of muscle fibers and regulate the movement of contractile proteins in muscle tissue [31]. Cardiac Troponin I (cTnI) has consistently been shown for identifying myocardial necrosis [32]. The observed elevation in the levels of serum cTnI predicts the risk of both cardiac cell death and subsequent lysis in ISO-induced infarcted rats. Treatment with aqueous extract of *Kalanchoe pinnata* resulted in reduced level of troponin-I.

Examination of the heart for architectural changes illustrates that there was an observable change in the tissue distribution of cardiomyocytes only in the negative control group. The architectural change in the heart was due to the administration of ISO. The latter has deleterious effects on the heart, inducing hypertrophy, fibrosis, necrosis, and

inflammatory cell infiltration [33] and also depletes the energy reserve of cardiomyocytes. It causes myocardial stress resulting in the development of infarct-like necrosis. Administration of isoprenaline by subcutaneous injection causes imbalance between oxygen supply and demand by the cardiomyocytes through increasing the chronotropism and inotropism, important to overt myocardial function and increase the calcium overload in the myocardium [22]. Both doses (100 mg/kg and 150 mg/kg) of the extract proved to be very efficient in protecting against myocardial injury. This implies that aqueous extract of *K. pinnata* has cardioprotective effect on isoproterenol induced myocardial infarction in rats. Isoproterenol (100 mg/kg) is known to induce sub-epicardial myocardial infarction [26, 34]. This can be likening to the work of Nayak *et al.* [35] who showed that, the extract of *K. pinnata* possesses significant wound-healing activity by decreasing the size of the affected area as well as oedema at the wounded site. The phyto-constituents, sterols, phenolic acids, flavonoids and flavanols, which are present in aqueous extract of *Kalanchoe pinnata* are believed to be potential cardio-protective agents.

5. Conclusion

Our findings revealed that *Kalanchoe pinnata* aqueous extracts has cardioprotective effects on Isoproterenol-treated-NaCl-hypertensive rat by reducing the level of cardiac troponin and preventing histological damage of the heart. Both doses of the extract exhibited similar efficacy.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that they have no conflicts of interest.

Statement of ethical approval

Animals were carefully handled according to International Guidelines [18]. Experimental procedures and protocols used in this study were approved by Institutional Animal Care and Use Committee, University of Buea (reference number: 2019/008/UB/IACUC/BTU/FS of 02/04/2019).

Data availability

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

Funding

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Authors' Contributions

Conceived and designed the experiments: OSMB. Harvested the plant and prepared the extract: BK, PMM. Carried out the major part of experiments: PMM, ALA. Performed histopathological examinations: OSMB, ENLT, TD. Analyzed the data and wrote the paper: PMM, OSMB. Made provision of reagents and proof-read the paper: TD, ENLT, OSMB. All authors read and approved the final manuscript.

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