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Orelien Sylvain Mtopi Bopda
Department of Zoology and Animal Physiology, Faculty of Science, University of Buea, Buea, Cameroon

Nathalie Nadège Chendjou Koumtouzi
Department of Zoology and Animal Physiology, Faculty of Science, University of Buea, Buea, Cameroon

Danielle Bilanda
Department of Animal Biology and Physiology, Faculty of Science, University of Yaoundé I, Yaoundé, Cameroon

Désiré Paul Djomeni Dzeufiet
Department of Animal Biology and Physiology, Faculty of Science, University of Yaoundé I, Yaoundé, Cameroon

Christian Tiambo Keambou
Department of Zoology and Animal Physiology, Faculty of Science, University of Buea, Buea, Cameroon

Théophile Dimo
Department of Animal Biology and Physiology, Faculty of Science, University of Yaoundé I, Yaoundé, Cameroon

Correspondence:
Orelien Sylvain Mtopi Bopda
Department of Zoology and Animal Physiology, Faculty of Science, University of Buea, Buea, Cameroon
Email: bopda.mtopi@ubuea.cm, boresyl@yahoo.fr

Kalanchoe pinnata aqueous extract safety and potential cardioprotective effects in isoprenaline treated rats

Orelien Sylvain Mtopi Bopda, Nathalie Nadège Chendjou Koumtouzi, Danielle Bilanda, Désiré Paul Djomeni Dzeufiet, Christian Tiambo Keambou, Théophile Dimo

ABSTRACT

Introduction: *Kalanchoe pinnata* (Crassulaceae) aqueous extract antihypertensive property has been proven. This study aimed to evaluate the safety and potential cardioprotective effects of the extract in isoproterenol treated rat. **Methods:** A total of 35 adult wistar rats were randomly and evenly distributed into groups 1-7 then treated for 28days. Control groups 1-3 received 10mL/kg water (per os, neutral), 150mg/kg isoproterenol (ISO, sc, 48 and 24hours prior to sacrifice, negative) and ISO plus 10mg/kg propranolol (per os, ten days prior to sacrifice, positive). Safety test groups (4-6) received *K. pinnata* extract (50, 100 and 200mg/kg per os). Cardioprotective test group 7 received *K. pinnata* (100mg/kg per os) and ISO. The sacrifice of rats was carried out on day 29 and blood collected for haematological and biochemical analyses. Liver, kidney and heart were collected weighed and preserved for histopathological analysis. **Results:** There was no observable sign of toxicity. No significant difference was observed between the relative weight of the heart from groups 1-7. The platelet value dropped from 794.7x109/L (group 2) to 284x109/L (group 7, $p<0.05$). An elevated value of creatine Kinase-MB (20.64U/L) was obtained in group 2, which decreased in other groups ($P<0.05$). In group 7, however, the extract (100mg/kg/day) reduced ($P>0.05$) the level of CK-MB compared to group 2. The level of triglycerides was elevated in group 2, 4 and 6 compared to group 1. Histopathological analysis of the heart showed the safety of the extract. **Conclusion:** We demonstrated that *K. pinnata* extract (100-200mg/kg/day, per os) might be cardioprotective.

Keywords: K. pinnata; safety; myocardial infarction; cardioprotective effects; rat

INTRODUCTION

Cardiovascular diseases (CVD) accounted for almost a third of all deaths globally in 2013 [1]. In Cameroon, CVD represent a public health problem [2-3], although still there is a significant gap in population awareness about this burden [4]. Myocardial Ischemia (M.I.), one of those CVD, remains a major pathological cause of death worldwide despite rapid advancements made in the treatment of coronary diseases [5-6]. Beside conventional medicine, medicinal plants constitute an important source of active natural products useful for the protection against various human diseases including CVD [7-8]. WHO has recommended the use of herbal medicines as an alternative medicine, especially in developing countries [9]. *Bryophyllum pinnatum* (Lamarck) Oken or *Kalanchoe pinnata* (Lamarck) Persoon (Crassulaceae) aqueous extract has been taken by populations for the management of CVD, precisely hypertension [10-11]. However, less attention has been paid to the toxicity of this extract. Hypertension increases the risk of acute myocardial infarction [12]. Previously, we demonstrated the antihypertensive properties of the *K. pinnata* leaf aqueous extract in salt-loaded hypertensive rats [13]. In line with this previous research [13] and as pioneer investigators, we carried out the present work aiming to evaluate the safety and activity of the *K. pinnata* extract on some cardiovascular biomarker levels, as well as myocardial architecture, in untreated and isoprenaline (a beta-agonist)-treated rats.

MATERIALS AND METHODS

Animals and distribution of groups

Wistar rats aged three months and weighting 150-180g were used. They were carefully handled according to International Guidelines (CIOMS) [14]. 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 Zoology and Animal Physiology, University of Buea, in plastic cages; under 12hour-day/night natural cycle and 25ºC-temperature. Access

to feed and water was *ad-libitum*. A total of 35 adult *wistar* rats were randomly and evenly distributed into seven groups (numbered 1-7) of five animals each, and treated for 28days. Control groups 1-3 were treated as follows: Group 1 served as neutral control and received distilled water (10mL/kg/day *per os*) only, for 28 days. Group 2, negative control, received only 150 mg/kg isoproterenol, an inducer of ischemia and myocardial infarction twice (ISO, *sc*, 48 and 24hours prior to sacrifice). Group 3, positive control group, received 150 mg/kg isoproterenol (similarly to group 2) plus 10mg/kg propranolol (*per os*, ten days prior to sacrifice) [15]. Groups 4, 5, 6 served as test groups for the safety and were gavaged with *K. pinnata* extract (50, 100 and 200mg/kg respectively). Group 7, test group for the cardioprotective activity, received *K. pinnata* extract (100mg/kg/day, 28 days *per os*) plus ISO (150 mg/kg, *sc*). The dose of 100mg/kg/day *K. pinnata* administered above because we considered it, from our previous findings [13], as the "therapeutic dose".

Plant material and extraction

Fresh leaves of *Kalanchoe pinnata* were harvested in Buea (Cameroon), in April 2017 and only matured leaves without signs of lesion were used. The fresh leaves 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 (2kg) was pounded by means of porcelain laboratory pounding cup, and then macerated with water (3L) for 24hours. After filtration and lyophilisation, 59.2 g (2.96% yields) of powder was obtained. The stock solution (1g/mL) was prepared by dissolving the above extract in distilled water.

Toxicity/safety study of *Kalanchoe pinnata* aqueous extract

This toxicity study being carried out essentially to check the safety of the plant extract, we deliberately decided not to go above the dose of 200mg/kg/day, which is double of the "therapeutic dose" of 100mg/kg/day considered from our previous study [13]. The abovementioned groups 1, 4, 5 and 6 were the groups of interest. During the experiment, body weights and food intake were assessed weekly. The rats were also observed daily (two hours following gavage) to detect any abnormality in the rat's behaviour. All animals were starved overnight previous to the sacrifice in the morning of day 29. For the sacrifice, the rats were anaesthetised using chloroform 99%. By cardiac puncture on each animal, blood (about 4mL) was collected in a 5ML syringe; 2mL of blood was collected in EDTA tubes for haematological studies and the rest in a non-heparinised tube for biochemical analysis of a key biomarker of the liver toxicity (ALT). Rats were then dissected and the organs of interest (liver, kidney and heart) removed and weighed. The curve of the body weight variation was plotted. The absolute organ weights for each animal were measured and then the relative organ weights calculated using the following formula:

Relative organ weight =[Absolute organ weight (g)/Body weight of rat on sacrifice day (g)]x100

The organs of each animal were then preserved separately in 10% neutral buffered formalin for histopathological analysis.

Study of the effects of *Kalanchoe pinnata* aqueous extract on the haematological, hepatic and cardiovascular biomarkers levels and the architecture of the cardiac tissues

For this study, all groups (1-7) were taken into consideration i.e. including those considered above for safety evaluation. Therefore, the 28 day-treatment was carried out as described above (see 2.1). At the end of the treatment, haematological, biochemical and histopathological analyses were carried out.

Haematological analyses

Blood collected from each animal in properly labelled 5 mL EDTA tubes was shaken gently to allow it homogenize. A blood count was then ran using the Full Blood Count machine, URIT 3300 following the manufacturer's instructions, to automatically get the following indices: mean corpuscular haemoglobin concentration (MCHC), white blood cells (WBC), haematocrit (HCT), red blood cell (RBC), platelet (PLT), plateletcrit (PCT), red blood cell distribution widthcoefficient of variation (RDWcv), red blood cell distribution-standard deviation (RDW-SD), haemoglobin (Hb), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV).

Biochemical analyses: hepatic and cardiovascular biomarkers levels

The remaining blood after collection for haematological analysis was all used for biochemical studies. It was allowed to clot for 40-60mins at room temperature, the supernatant was collected separate in labelled tubes, using a pipette, centrifuged (3000 revolutions per minute, -5°C) for 10mins. 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 two-week deadline following the collection of serum.

Determination of the hepatic biomarker, Alanine Aminotransferase (ALT)

Following the manufacturer (Chronolab, Diputación 260, Spain, 2014 version) instructions, the assay was done at wavelength 340nm, at 37°C. The cuvette was 1cm light path. The instrument was adjusted to zero with distilled water. The content of R_2 substrate was dissolved in the corresponding volume of R_1, capped and mixed gently to dissolve the contents. The contents were pipetted into a cuvette. The cuvette with the blank contained 1000µL of distilled water and 10µL of the sample. The second cuvette (the standard) contained 1000µL of distilled water and 10µL of the standard. The third cuvette (sample) contained 1000µL of distilled water and 10µL of the sample. They were mixed together and incubated for one minute after which the stopwatch was started and the initial absorbance (A_1) recorded. Absorbance was read at 1 minute intervals for three minutes. The absorbance difference per minute (ΔA/min) was calculated.

ΔA/min x 1750 = Concentration (U/L) of ALT, Where (U/L) = units per litre of sample and ΔA is the absorbance difference.

Determination of Creatine Kinase – MB

The experimental conditions and procedure were respected, as instructed by the manufacturer (Chronolab, Travessia Prat de la Riba, Spain and 2017 Version). The spectrophotometer was set at 340nm, at room temperature 24-25°C. A 1cm light path cuvette was used. The equipment was adjusted to zero with distilled water. Two reagents were used, R_1 being the buffer containing imidazole at pH 6.7 (100 mmol/L), glucose (20 mmol/L), magnesium acetate (10 mmol/L) and EDTA (2 mmol/L). R_2 was the anti CK-M which contained an anti CK-M (2000 U/L), ADP (2 mmol/L), AMP (5 mmol/L), di-Adenosine-5-pentaphosphate (10 mmol/L), $NADP^+$ (2 mmol/L), hexoquinase (HK, 2500 U/L), glucose-6-phosphate dehydrogenase (1500 U/L), N-acetyl cysteine (20 mmol/L) and creatinine phosphate (30 mmol/L). The two reagents (R_1 and R_2) were then mixed and incubated for 10 minutes. The initial absorbance (A_1) of the sample was recorded and the stopwatch was started. The value was recorded after 5 minutes again (A_2). The difference in the absorbance was then calculated as follows:

Change in Absorbance (ΔA) = Final Absorbance (A_2) – Initial Absorbance (A_1).

$$\Delta A \times 1651 = \text{U/L CK-MB.}$$

Determination of Triglyceride and Cholesterol

The analysis was carried out following the CHRONOLAB SYSTEMS manual (CHRONOLAB SYSTEMS, Diputación 260, Spain, 2014 version). Triglycerides and cholesterol have similar methods of assessment. The kits were used following the manual procedure. There were two containers of solutions (R2 and R1) in the kit of enzymes. The content of container R2 was dissolved in container R1. It was used immediately after being removed from the fridge and was recapped immediately. The instrument was adjusted to zero with distilled water. Three cuvettes were used for the working reagent. One was containing the blank, the other contained the standard and the last contained the test sample. The cuvette with the blank contained 1000µL of distilled water and 10µL of the sample. The second cuvette (the standard) contained 1000µL of distilled water and 10µL of the standard. The third cuvette (sample) contained 1000µL of distilled water and 10µL of the sample. The three were then mixed and incubated for 5 minutes at 37°C. The absorbance (A) of the samples was read and calibrator, against the Blank. The calculation was done as follows:

$$\frac{(A)\text{Sample} \times \text{Standard concentration}}{(A)\text{Standard}} = \text{mg/dL triglycerides in the sample.}$$

Conversion factor: mg/dL x 0.0113= mmol/L

Histopathological analyses

The preserved tissues 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, kidney, liver and aorta 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 stain (HE) [16]. Micrographs were snapped with the aid of a digital camera attached to the eyepiece of the light microscope.

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 $P < 0.05$ was considered significant.

Results

Evaluation of the Safety of *Kalanchoe pinnata* extract

In the course of our experiment no death or considerable change in behaviour was observed in the animals that were treated with *K. pinnata* (50, 100 and 200mg/kg/day) as compared to the neutral control group.

Effects of *Kalanchoe pinnata* Extract on Physical Parameters (body weight, relative organ weight)

There was an increase in body weights of the rats in all groups from day 0 to day 28. However, the mean body weight gain did not vary among various groups (Figure 1).

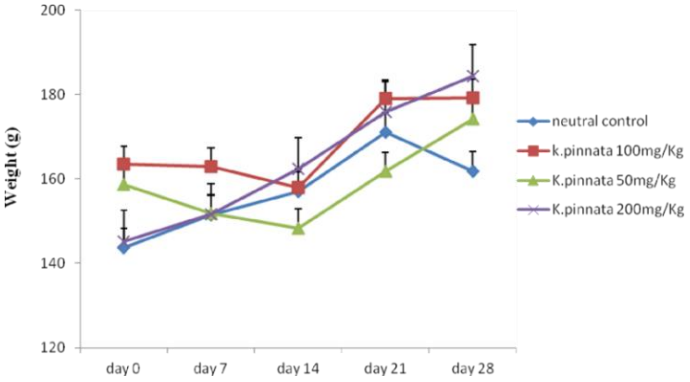


Figure 1: Variation of body weight during sub-chronic treatment with *K. pinnata*.

Each point on the figure represents the mean ± SEM with n=5. No significant difference was observed between groups (P>0.05).

Moreover, there was a significant difference in the relative weight of the liver between the various groups but not in the relative weights of the kidney and heart. The values obtained for the relative weight of the liver were 1.4%, 1.01%, 0.98%, 1.48%, 1.4%, 1.16%, and 0.99% of the body weight of animals from group 1-7 respectively. For the kidney the relative weights were 0.37%, 0.37%, 0.36%, 0.34%, 0.3%, 0.31%, 0.32% and for the heart 0.37%, 0.41%, 0.42%, 0.38%, 0.38%, 0.33%, 0.39% respectively from group 1-7.

Effect of *K. pinnata* aqueous extract on a Key Biomarker of the liver toxicity (Alanine Aminotransferase).

A significant decrease in the level of alanine aminotransferase ALT was observed in all groups as compared to the neutral control group (47.4 ± 3.67 U/L). The greatest decrease was in group 5 (with a value of 22.27 ± 0.07 U/L) followed by group 7 (22.42 ± 2.48 U/L), group 2 (26.78 ± 1.61 U/L), group 6 (28.37 ± 0.88 U/L), group 4 (38.13 ± 1.93 U/L) and finally group 3 (33.84 ± 1.37 U/L). A significant difference was observed between the neutral control and the other groups (P<0.05). Also, a significant difference was observed between the negative control and group 4 (P<0.05) and between the positive control and groups 5 and 7 (P<0.05). Lastly, a significant difference was observed between group 4 and groups 5 and 7 (P<0.01) (Figure 2.

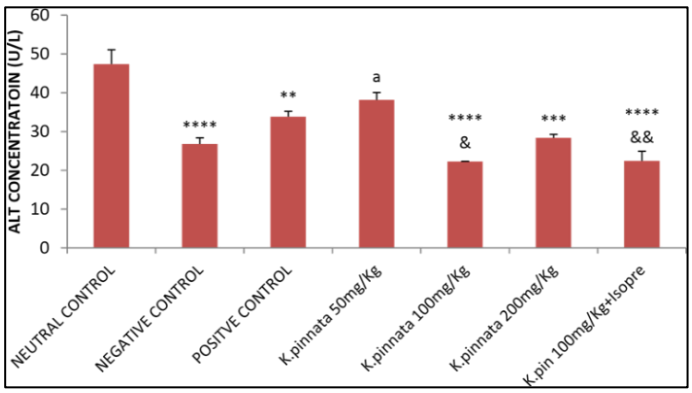


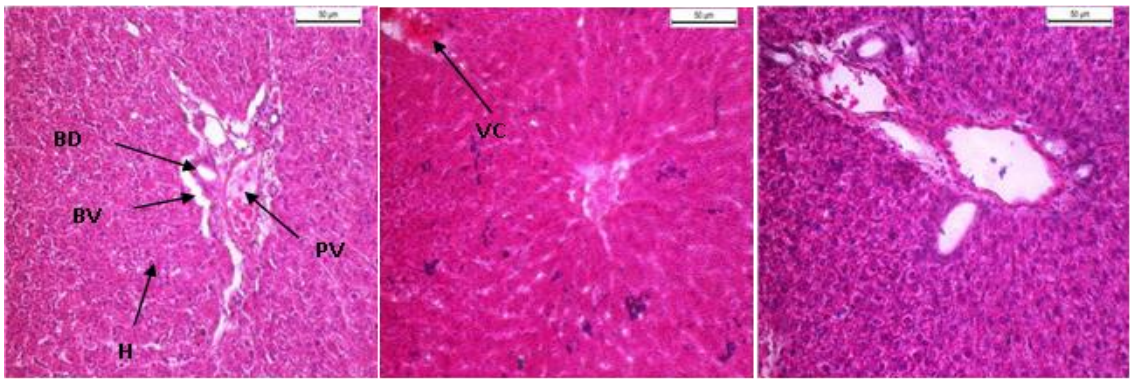
Figure 2: Effects of aqueous extract of *K. pinnata* leaf on the levels of ALT after 4 weeks of treatment.

Each bar represents the mean ± SEM, n=5. *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001 (significant differences between the neutral control and the other groups). ^{a} P < 0.05 (significant difference between the negative control and group 4). $^{\&}$ P < 0.05, $^{\&\&}$ P < 0.01 (significant differences between the positive control and groups 5 and 7).

Histopathological examinations of detoxifying organs (liver, kidney)

The plant extract provoked no damage of the liver and kidney architecture in treated groups. Figure 3 shows histological sections of sample livers of group 1 (neutral control, 3A), group 2 (negative control, 3B) and group 6 (*K. pinnata* 200mg/kg, 3C). In the pictures, we can observe the hepatocytes (H), the portal space (PS), the portal vein (PV) and the bile duct (BD) as indicated with the arrows. Only on Fig. 3B can be observed a vascular congestion (VC). This is the primary sign for inflammation in an organ.

Likewise, the architecture of kidneys from rat treated with plant extract was similar to that of the neutral group.



A B C

Figure 3: Micrographs of liver histological sections.

Neutral control (A) and group 6 (*K. pinnata* 200mg/kg) (C) with no noticeable change, vascular congestion (VC) observed in the negative control (B).

Effects of *Kalanchoe pinnata* aqueous extract on the haematological, cardiovascular biomarkers levels and the architecture of the cardiac tissue

Effects on Haematological Parameters

There was globally no significant difference between the mean values of haematological parameters while comparing the different groups, except for platelets. The platelets value dropped from

794.7±142.7 x10^9/L in group 2 (negative control) to 284±138 x10^9/L in group 7 (*K. pinnata*

100mg/kg+ISO) ($p<0.05$).

Effects of the aqueous extract of *K. pinnata* on the CK-MB levels

The values obtained while measuring the level of CK-MB were as follows: 20.64 ± 3.26, 8.59 ± 3.44, 2.75 ± 1.46, 5.37 ± 1.56, 4.13 ± 0.48 and 9.49 ± 2.18 U/L respectively from group 2 to 7. The elevated value of 20.64 ± 3.26U/L was obtained precisely in group 2, the negative control, which received ISO subcutaneously. A significant decrease of the level of CK-MB was observed while comparing the negative control with other groups ($P<0.05$). In group 7, the plant extract (100mg/kg/day) reduced non significantly ($p>0.05$) the level of CK-MB compared to the negative control group administered solely with ISO (Figure 4).

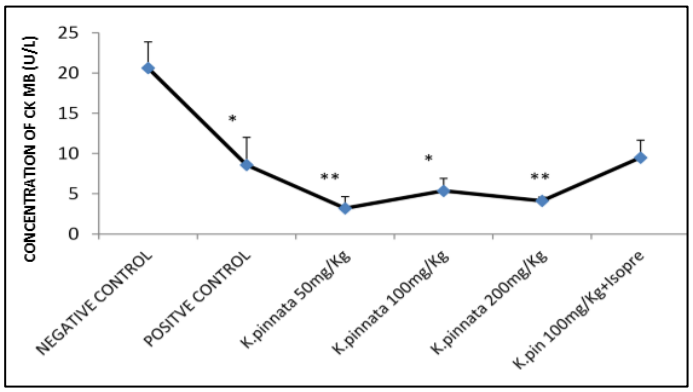


Figure 4: Effects of ISO and aqueous extract of *K. pinnata* on the levels of Creatine KinaseMB in serum.

Each point on the figure represents the mean ± SEM, n =5. *$P<0.05$ and **$P<0.01$ (Significant difference between negative control and other groups).

Effects on Cholesterol Levels

Sub chronic administration of the aqueous extract of *K. pinnata* to the different groups of rats for four weeks caused an increase of the cholesterol levels in rats. The highest increase was from the negative control with a mean value of 94.89 ± 3.98 followed by group 7 (ISO+100mg/kg/day) with 78.81 ± 3.59, group 5 (100mg/kg/day of *K. pinnata*) with a value of 72.93 ± 8.95, group 3 (positive control) with a value of 66.4 ± 0.19, group 6 with 65.45 ± 11.68 and lastly group 4 (50mg/kg/day *K. pinnata*) with 52.8 ± 11.24. These values were compared to that of group 1 (neutral control) with value 49.31 ± 8.13. A significant difference was observed between the different groups ($P<0.05$). Furthermore, the turkey test showed that there was a significant difference between the neutral control and the negative control ($P<0.01$) and between the negative control and group 4 ($P<0.05$) (Figure 5).

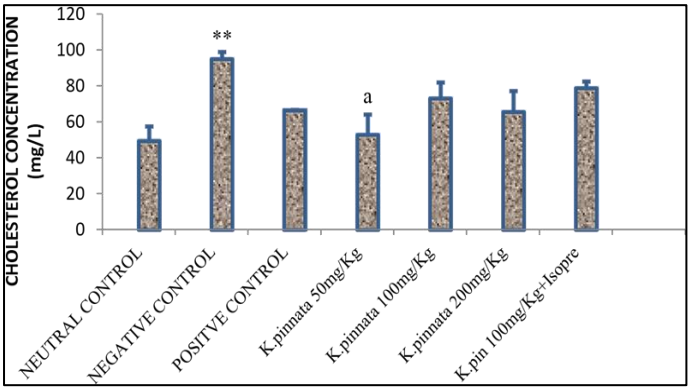


Figure 5: Effects of aqueous extract of *K. pinnata* leaf on the levels of cholesterol in blood after 4 weeks of treatment.

Each bar represents the mean ± SEM, n =5. **P<0.01 and [a] P<0.05 (significant difference between the neutral control and the negative control and between the negative control and group 4, respectively).

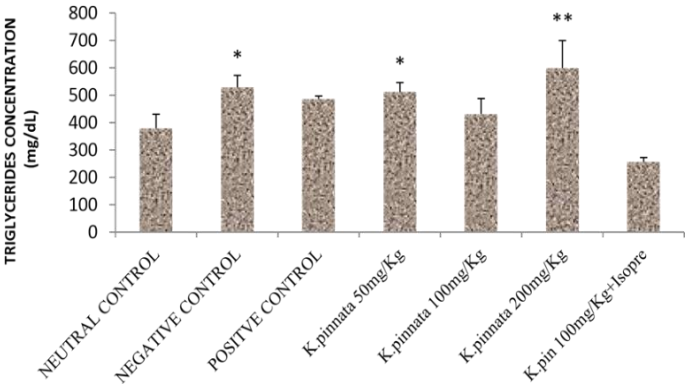


Figure 6: Effects of aqueous extract of *K. pinnata* leaf on the levels of triglycerides in blood after 4 weeks of treatment.

Each bar represents the mean ± SEM, n =5. *P<0.05 and **P<0.01 (significant difference between group 7 and other groups).

Effects on Triglycerides Levels

Figure 6 shows that in group 7 (ISO+100mg/kg/day of *K. pinnata*) there was a significant decrease in the level of triglycerides as compared to the negative control group (P<0.05), group 4 (P<0.05) and group 6 (P<0.01). Generally, there was an increase of the triglyceride levels in all the other groups as compared to the neutral group after 28 days of treatment. The values obtained were 379.01 ± 51.39, 528.1 ± 44.27, 486.5 ± 10.27, 511.8 ± 34.39, 431.2 ± 56.58, 599 ± 100.6 and 256 ± 16.51 mg/dL.

Histopathological examinations of the heart

Figure 7 displays sample pictures of the heart's histological sections in the neutral control (A), the negative control (B), group 5 (100mg/kg/day of *K. pinnata* (C)) and group 7 (ISO+100mg/kg/day *K. pinnata* (D)). Leucocytic infiltration (LIF) and vascular congestion were observed in the negative control group, but not in group 7.

Discussion

For centuries, natural products, such as medicinal plants have been the basis for the treatment of various ailments [17]. In screening natural products for the pharmacological activity, the evaluation of toxic characteristics of a natural product extract, fraction, or compound are usually an initial step [18]. This study investigated the toxicity and effects of *K. pinnata* on cardiovascular biomarker levels and architecture in isoprenaline administered rats. The plant extract did not elicit any death in rats which proves that the plant has a relatively high safety margin at the doses of 50, 100 and 200mg/kg. Moreover, in our study we adopted the model of induction of acute myocardial infarction (AMI) by isoprenaline (150mg/kg/day) subcutaneous administration in rats since it is effective, reliable and cause a low morbidity and mortality in animals [19]. It has been widely used in physiological studies [19, 20, 21]. It has been reported that isoprenaline administration in high doses to animals produces 'infarct like' lesions in the heart similar to those present in AMI in humans [22, 23, 24]. The resulting data from our study gave no mortality in the negative control group (ISO treated) as compared to 33.3% reported by Acikel *et al.* [25] and 25% by Heraldo *et al.* [19]. This difference may be due to the administration of the drug 24 hours and then 5 hours prior to the sacrifice as oppose to 48 hours and 24 hours prior to the sacrifice in the other studies. The drug may not have affected its complete action in the rats before they were sacrificed.

Increases in the body weights of the rats were observed in all the groups from day 0 to 28. No significant difference was observed between the neutral control (gavaged with water) and the test groups (gavage with *K. pinnata*). Thus, the increase in body weight cannot be attributed directly to the components of the plant. The observed increases in body weight could be due to the nutritive components in their chow since they were well fed and therefore surely assimilated their food normally [26]. The experimental animals that received ISO showed a significant decrease in both the absolute and relative weights of their livers as compared to the neutral control while the groups that received the plant extract rather showed a significant increase in both their absolute and relative weights as compared to the negative control with the exception of group 6 (receiving 200mg/kg/day of *K. pinnata*). On the other hand, the mean relative kidney and hearts weights of the rats did not vary significantly among the groups; however, an increase in heart's relative weight of the animals that received ISO was noticed. This increase in the heart's relative weight could be due to increase water, oedematous intramuscular spaces, increased protein content and infiltration of inflammatory cells to damaged areas of the heart tissue [15, 27].

Histopathological examinations of the heart showed leucocytic infiltration in the negative control group which are signs of a disease or inflammation. This result is in accordance with Mai *et al.* [28] who reported that inflammation is a key process involved in mediating myocardial tissue damage after an ischemic event. Pre-treatment with *K. pinnata* prevented the occurrence of such a leucocytic infiltration and reduced both the relative and the absolute heart weight (group 7). This is indicative of the protective effect of *K. pinnata* on the myocardium against inflammation or oedema. It has been proven that when myocardial cells are damaged or destroyed due to a deficiency in the oxygen supply or glucose, the cardiac membrane becomes permeable or may rupture entirely, resulting in the leakage of enzymes [29]. The activity assay for CK-MB in serum is an important diagnosis because of the marked abundance of this enzyme in myocardial tissue and its virtual absence from most other tissues and its consequent sensitivity [27]. CK-MB isoenzyme activity is useful as an index for the early diagnosis of not only myocardial infarction, but also any type of myocardial injury. Leakage of cytosolic enzymes including CK-MB, AST, and ALT into the blood stream may occur when cell membranes become more permeable or rupture [27]. The levels of these markers in serum reflect the alterations in plasma membrane integrity and/or permeability [30]. In this study, rats that received ISO (negative control) showed elevated levels of CK-MB in serum as compared to all the other groups and this is in line with the literature [25, 31, 32]. There was a considerable reduction in the level of CK-MB in the group that received both the extract and ISO proving that the aqueous extract of *K. pinnata* could help in maintaining membrane integrity and permeability and therefore might have cardio protective properties. However, the preventive effect of *K. pinnata* (100mg/kg/day) being non-significant, more investigation should be carried out to confirm this biochemical activity of the plant extract.

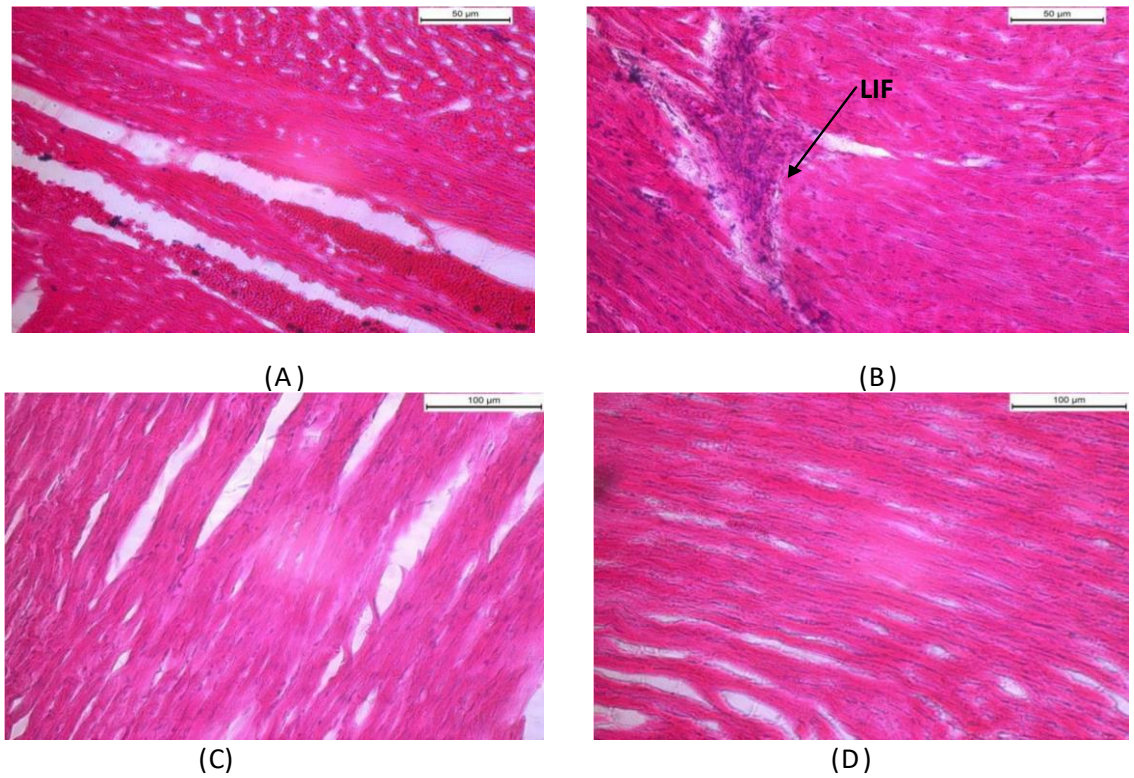


Figure 7: Light micrographs of the heart's histological sections (HEx50 & x100).

Lipids play an important role in CVD, not only by contributing to the development of atherosclerosis but also by modifying the composition, structure, and stability of the cellular membrane [27]. Elevated cholesterol levels and its accumulation in heart tissue have been associated with cardiovascular damage [33]. In our study, the rats that received isoprenaline subcutaneously showed a significant ($P<0.05$) increase in serum levels of cholesterol as compared to the neutral group. This is in line with previous studies [34, 35]. This change in cholesterol levels might be due to enhanced lipid biosynthesis by cardiac cyclic adenosine monophosphate. Generally, the mechanism of actions of lipolytic hormones, including ISO, on fat cells are believed to be mediated by the cAMP cascade, in which lipolytic hormones activate adenylate cyclase, thereby increasing cAMP formation. Subsequently, cAMP promotes lipolytic activity by activating cAMP-dependent protein kinase, which phosphorylates hormone-sensitive lipase. This results in the hydrolysis of stored triacylglycerol, which may contribute to hyperlipidaemia [27]. The cholesterol was reduced in the positive control group which in addition to ISO received propranolol (10mg/kg/day). Pre-treatment with the *K. pinnata* extract (100mg/kg) also decreased the level of cholesterol in the serum of ISO treated rats thereby maintaining the normal fluidity and function of the myocardium. Phytochemical analysis of *Kalanchoe pinnata* leaf extracts showed the presence of phenols [36]. Polyphenols have been reported to inhibit cholesterol esterase [37]. In general, pancreatic cholesterol esterase has an important role to play in the hydrolysis of dietary cholesterol esters, which liberates free cholesterol in the lumen of the small intestine [38]. Therefore, the inhibition of cholesterol esterase is expected to limit the absorbance of dietary cholesterol, resulting in reduced cholesterol absorption. Hypertriglyceridemia is one of the major pathological conditions related to AMI [34]. A significant increase in serum levels of triglycerides (TG) was observed in the negative control group. It might be due to decreased activity of lipoprotein lipase, and decreased uptake of TG from the circulation [34]. The TG level was considerably reduced in group 7 that was pre-treated with *K. pinnata* before ISO administration and slidely in the positive control group. A significant increase in the TG level was also observed in the test groups as compared to the neutral control group with the greatest increase being in group 6 (receiving 200mg/kg of *K. pinnata*). The increase in this case is difficult to explain. Thus, more studies should be carried out to better understand the relationship between the plant extract and TG levels in rats. ALT and AST are the two most important transaminases and are present in high concentrations in the liver and muscles [26]. An increase in ALT level is a strong indicator of liver disease [39] but it can be used as a cardiovascular damage biomarker in some cases. Treatment with the aqueous extract of *K. pinnata* revealed highly significant decreases in the levels of ALT in all test groups. This can be confirmed using histopathological findings since no noticeable change was observed in the architecture of the liver in the groups treated with the extract. This result reinforces the idea that *K. pinnata* might have hepatoprotective activities more precisely at the dose of 100mg/kg where the rats had the least ALT levels. In our previous study [13] concomitant administration of NaCl 18% and the extract (100 mg/kg/day) significantly reduced the AST level as compared to that in the hypertensive rats suggesting that the plant extract might protect various tissues, including hepatic tissues. This gives more credit to the results obtained in this study. The significant decrease in the ALT level observed in the negative control group as compared to the neutral group was not expected and is therefore difficult to explain since it opposes the results obtained in previous literature [19, 31, 25].

For histopathological examinations of the liver, vascular congestion was noticed in the negative control which is known to be a sign of inflammation in an organ. This may be indicating that there is a damage of blood vessels and/or liver inflammation. The group pre-treated with *K. pinnata* and then ISO did not show any noticeable modification in the architecture of the liver. This might be another proof that the aqueous extract of *K. pinnata* has a hepatoprotective effect and is not noticeably toxic at any of the doses used in this study. The presence of compounds such as flavonoids and polyphenols in *Kalanchoe pinnata* [40, 41, 13] argues in favour of its displayed hepatoprotective, antioxidant and antihypertensive properties. Histopathological examinations of the kidney revealed leucocytic invasion in the negative control group but in all the other groups, there was no clear difference viewed as compared to the neutral control kidney. From this finding, we can say that the extract did not affect renal functioning since there was no change in the architecture of the kidney after treatment with the extract at various doses.

No significant difference was observed between the means of the groups as concerns haematological parameters with the exception of the platelets count. There was a non-significant increase in the mean

platelets count (P<0.05) of the negative control as compared to the neutral control group. This result is in line with Ren *et al*. [42] who reported that MI is associated with an inflammatory response, ultimately leading to healing and scar formation. A significant decrease in the mean platelets count was noticed in the group pre-treated with *K. pinnata* before subcutaneous administration of ISO. Thrombocytosis is a disorder in which the body produces too many platelets (thrombocytes), which play an important role in blood clotting [43]. Platelets are blood cells in plasma that stop bleeding by sticking together to form a clot. Excessive levels of platelets in the blood can lead to certain conditions, including stroke, heart attack, or a clot in the blood vessels [44]. Pathologies that are known to cause an increase in the platelet counts include anaemia, inflammation or infection and surgery, especially splenectomy (removal of the spleen) [43-44]. This result is once again confirmed by the histopathological examinations of the heart in which inflammation and leucocytic invasion was observed in the negative control group and no modification was observed in group 7 (that received ISO+ 100mg/kg of *K. pinnata* extract). It once more reinforces the potential cardioprotective activities of the *K. pinnata* aqueous extract. Giving that the antihypertensive activity was earlier established with, 100mg/kg/day as the most potent, and that at doses as high as 200mg/kg/day no sign or symptom of sub-chronic toxicity was recorded, we suggest a 100-200mg/kg/day dosage, of this aqueous extract; *per os*.

Conclusion

The leaf aqueous extract of *K. pinnata* did not have a noticeable toxic effect in rats showing its relatively good safety margin at 50-200mg/kg b.w. of rats. The extract did not cause any significant variation in the growth parameters of the rats. *K. pinnata* extract had a hepatoprotective effect in rats which was best observed at the dose of 100mg/kg. Also, the leucocytic invasion was prevented in the kidney of ISO-treated rat by the same extract. The extract decreased cardiovascular biomarkers (cholesterol, CK-MB) levels in ISO administered rats, while protecting the heart from injury. The increase in the PLT level by ISO was significantly prevented by *K. pinnata* at the dose of 100mg/kg/day. Therefore, the extract might have cardioprotective properties, if administered at 100-200mg/kg/day.

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Conflict of Interest

None

Authors' Contributions

Conceived and designed the experiments: OSMB. Carried out the major part of experiments: NNCK. Performed histopathological examinations: DPDD, DB. Read and contributed ideas in the collection of blood and haematological analysis: CTK, Analyzed the data: OSMB, NNCK. Wrote the paper: OSMB. Made provision of reagents and proof-read the paper: TD, OSMB. All authors read and approved the final manuscript.

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