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Cardioprotective Effects of *Kalanchoe pinnata* Aqueous and Ethanolic Extracts in *Wistar* Rat

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Abstract

Introduction: *Kalanchoe pinnata* (Crassulaceae) aqueous extract potential cardioprotective property was revealed recently. This study aimed to confirm cardioprotective effects of aqueous and ethanolic extracts against Isoproterenol (ISO) -induced Myocardial Infarction (MI) in rat.

Methods: Forty adult *wistar* rats were randomly distributed into 8 groups, and then treated for 28 days. Control groups 1-3 received water (10 mL/kg, per os, neutral), ISO (150 mg/kg, sc, negative) and ISO+ propranolol (10 mg/kg, per os, positive). Test groups (4-6) received *Kalanchoe pinnata* ethanolic extract (50 mg/kg, 100 mg/kg and 200 mg/kg, per os). Cardioprotective test groups 7 and 8 received *Kalanchoe pinnata* aqueous extract (100 mg/kg, per os) +ISO and ethanolic extract (100 mg/kg, per os) +ISO, respectively. Rats were sacrificed on day 29 and blood collected for determination of serum creatine kinase-MB and Troponin I levels. Hearts were histopathologically analyzed.

Results: There was no significant change caused by the ethanolic extract alone on neither the biomarkers level nor the heart architecture. Compared to neutral control, creatine kinase-MB level (16.16 ± 6.07) U/L rose to (96.09 ± 14.07) U/L and (19.43 ± 6.24) U/L in the negative and positive controls, respectively. This biomarker rose from 16.16 U/L to (26.09 ± 4.853) U/L and (63.73 ± 10.97) U/L in aqueous *Kalanchoe pinnata* (100 mg/kg) +ISO and ethanolic *Kalanchoe pinnata* (100 mg/kg) +ISO groups, respectively. The increase in Troponin was from (0.0348 ± 0.0157) µg/L (neutral control) to (1.886 ± 0.0002) µg/L in the negative control, (0.9694 ± 0.1303) µg/L in the aqueous *Kalanchoe pinnata* (100 mg/kg) +ISO and (1.493 ± 0.177) µg/L in the ethanolic *Kalanchoe pinnata* (100 mg/kg) +ISO. Compared to the negative control, there was a significant decrease of Troponin, to (0.2312 ± 0.1314) µg/L in the positive control. The aqueous extract was more potent than ethanolic extract on infracted heart.

Conclusion: We demonstrated that both extracts (100 mg/kg/day, per os) are cardioprotective against ISO-induced MI, but the aqueous extract is more potent.

Keywords: *Kalanchoe pinnata*; Myocardial infarction; Cardioprotective effects; Rat

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Introduction

Cardiovascular Diseases (CVDs) accounted for almost a third of all deaths globally in 2013 [1]. In Cameroon, CVDs represent a public health problem, although still there is a significant gap in population awareness about this burden [2-4]. CVDs include: coronary heart disease, peripheral arterial disease, rheumatic heart disease, congenital heart, strokes, myocardial ischemia and myocardial infarction (heart attack) [5]. Myocardial Ischemia (MI) remains a major pathological cause of death worldwide despite rapid advancements made in the treatment of coronary diseases [6,7]. As an alternative medicine, active natural products from medicinal plants constitute an asset for the protection against various human diseases including CVDs [8,9]. The use of such alternative medicine has been recommended actually by WHO, especially in developing countries [10]. *Bryophyllum pinnatum* (Lamarck) Oken or *Kalanchoe pinnata* (Lamarck) Persoon (Crassulaceae) aqueous extract has been taken by populations for the management of CVDs, precisely hypertension [11-13]. Hypertension increases the risk of acute myocardial infarction [14]. Previously, we demonstrated that the above extract could induce cardioprotection of the heart against MI [15]. In

the current research, we have investigated the effects of the ethanolic extract, in comparison with the aqueous extract. The activity of the *Kalanchoe pinnata* extracts was investigated on serum creatine kinase-MB and troponin 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 3-4 months and weighting 155 g to 190 g were used. They were carefully handled according to International Guidelines (CIOMS) [16]. 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 local day/night natural cycle and temperature (18°C to 25°C). Access to feed and water was *ad-libitum*. A total of 40 adult *Wistar* rats were randomly and evenly distributed into eight groups (numbered 1-8) of five animals each. The dose of 100 mg/kg/day *Kalanchoe pinnata* has been considered, from our previous findings on the effects of aqueous extract, as the "therapeutic dose" [13]. Table 1 shows the grouping and treatment of animals.

Plant material and extraction

Fresh leaves of *Kalanchoe pinnata* were harvested in Buea (Cameroon), in April 2018 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 (3 kg) was pounded by means of porcelain laboratory pounding cup. For the preparation of aqueous extract, one portion (1 kg) was macerated with water (1.5 L) for 48 h. After filtration (Watman No 1 paper) and drying (oven, 45°C), 29.6 g (2.96% yields) of powder was obtained. For the preparation of ethanolic extract, *Kalanchoe pinnata* fresh leaves (2 kg) were air dried for 3 weeks and pounded with a porcelain laboratory pounding cup. A 69.8 g (3.49% yield) of dry powder was obtained after grinding, and was immersed into a liter of ethanol (95%), then allowed for 48 h-maceration. Filtration was done using filter paper Watman No 1. The ethanol was evaporated in a rotary evaporator and collected in a round bottom flask while the *Kalanchoe pinnata* was extracted in another one during rotation in a water bath containing heated water (60°C). The stock solution (1 g/mL) was prepared by dissolving the above extracts in distilled water.

Study of the effects of *Kalanchoe pinnata* aqueous extract on the cardiac biomarkers' levels and the architecture of the cardiac tissues

Biochemical analysis of cardiovascular biomarkers levels: Blood was collected by cardiac puncture and allowed to clot for 40 min to 60 min at room temperature, the supernatant was collected separate in labeled tubes, using a pipette, centrifuged (3000 revolutions per min, -5°C) for 10 min. 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 level of Creatine-Kinase (CK-MB) and troponin was assayed.

Determination of creatine kinase- MB level: 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 340 nm, at room temperature 24°C to 25°C. A 1 cm light path curette was used. The equipment was adjusted to zero with distilled water. Two reagents were used, R1 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). R2 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 (R1 and R2) were then mixed and incubated for 10 min. The initial absorbance (A1) of the sample was recorded and the stopwatch was started. The value was recorded after 5 min again (A2). The difference in the absorbance was then calculated as follows:

Change in Absorbance (ΔA) = Final Absorbance (A2) - Initial Absorbance (A1).

ΔA × 1651=U/L CK-MB

Determination of troponin level: Troponin was assayed using ELISA method. The troponin kit (EIA-2952-DRG Diagnostics, Germany) contained 5 standards of different concentrations (1=0 ng/ml, 2=2 ng/ml, 3=7.5 ng/ml, 4=30 ng/ml, 5=75 ng/ml) and 3 other troponin reagents (troponin I conjugate reagent, TMB reagent and Troponin I stop solution (1N HCl)) and a micro plate. The standards and samples were put into different wells and troponin I conjugate reagent added to all the wells containing both the standards and the samples. This was allowed to stand for 90 min (reaction time), after which it was discarded and the plate washed with a micro plate washer. TMB reagent (100 µl) was added to the wells and left to stand for 20 min. Troponin I stop solution (1N HCl) was then added. This stops the reaction. After 5 sec the results were obtained and read using a micro plate reader.

After plotting the standard calibration curve of absorbance against troponin concentrations, the troponin level in samples was determined by extrapolation.

Histopathological analyses: The preserved (4% Formalin) hearts were kept for two weeks and then carried to the laboratory of Animal Physiology of the University of Yaounde I for histopathological examinations. Tissues of the heart were processed with a microtome (Ernst Leitz Wetzlar 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) [17]. 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 analyzed 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

Effects of *Kalanchoe pinnata* aqueous extract on cardiac biomarkers' levels and the architecture of the cardiac tissue

Effects of *Kalanchoe pinnata* on cardiac biomarkers (creatine-

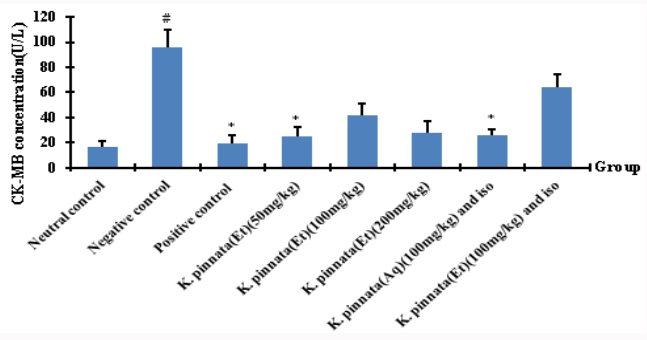


Figure 1: Effects of *Kalanchoe pinnata* extract on CK-MB of the rats per group.

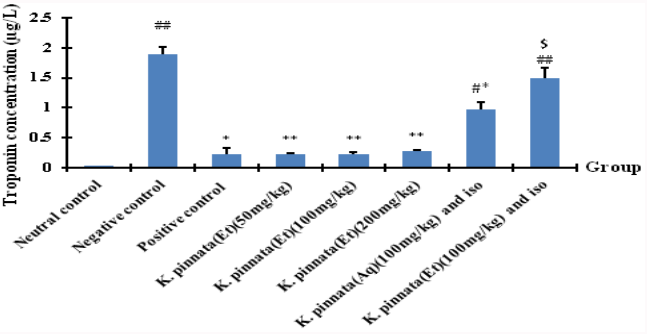


Figure 2: Effects of *Kalanchoe pinnata* extracts on troponin level in various groups.

kinase and troponin) levels

Effects of *Kalanchoe pinnata* on creatine kinase-MB (CK-MB) level in blood serum: There was no significant difference comparing the neutral control with the other groups excluding the negative control. Compared to the neutral control, the level of creatine kinase-MB (16.16 ± 6.07) u/L rose to (96.09 ± 14.07) u/L and (19.43 ± 6.24) u/L in the negative control and positive control, respectively. In the ethanolic *Kalanchoe pinnata* administered rats, the increases were up to (25.1 ± 7.07) u/L, (41.61 ± 9.55) u/L and (27.57 ± 9.554) u/L at the doses of 50 mg/kg, 100 mg/kg and 200 mg/kg respectively. The level of CK-MB increased from 16.16 u/L to (26.09 ± 4.853) u/L for the aqueous *Kalanchoe pinnata* (100 mg/kg) +isoproterenol, and to (63.73 ± 10.97) u/L for ethanolic *Kalanchoe pinnata* (100 mg/kg) +isoproterenol groups. The increase from 16.16 u/L to 96.09 u/L was obtained precisely in group 2, the negative control, which received only ISO subcutaneously. Compared to the latter, Creatine-Kinase (CK-MB) level significantly decreased in the positive control, ethanolic *Kalanchoe pinnata* (50 mg/kg), and aqueous *Kalanchoe pinnata* (100 mg/kg) +isoproterenol at ($P<0.05$). There was no significant difference in the positive control compared to the aqueous *Kalanchoe pinnata* (100 mg/kg) +isoproterenol and ethanolic *Kalanchoe pinnata* (100 mg/kg) +isoproterenol. Figure 1 below represents CK-MB concentration in all the groups observed.

Each bar represents the mean ± SEM, n=5, *$p<0.05$ significantly different from the negative control, $^{\#}p<0.05$ significantly different from the neutral control. The neutral control received only tap water, negative control received isoproterenol, positive control received propranolol+isoproterenol, and the other groups received ethanolic *Kalanchoe pinnata* (50 mg/kg, 100 mg/kg and 200 mg/kg), aqueous *Kalanchoe pinnata* (100 mg/kg) +isoproterenol and ethanolic *Kalanchoe pinnata* (100 mg/kg) +isoproterenol.

Effects of *Kalanchoe pinnata* on troponin level in blood serum

Compared to the neutral control (0.0348 ± 0.0157) ug/L, there was an increase in the level of troponin to (1.886 ± 0.0002) ug/L in the negative control, which received only ISO. Compared to the negative control, there was a significant decrease of troponin level, to (0.2312 ± 0.1314) ug/L in the positive control. In the groups treated solely with the ethanolic *Kalanchoe pinnata* the troponin level rose to (0.2286 ± 0.1023) u/L, (0.364 ± 0.0187) ug/L and (0.2778 ± 0.0115) ug/L at 50 mg/kg, 100 mg/kg, 200 mg/kg, respectively, but this increase was not significant. However, with comparison to negative control, there was a significant decrease ($p<0.05$) in the ethanolic *Kalanchoe pinnata* (50 mg/kg, 100 mg/kg, and 200 mg/kg). In the aqueous *Kalanchoe pinnata* (100 mg/kg) +isoproterenol, troponin level rose to (0.9694 ± 0.1303) ug/L, while in the ethanolic *Kalanchoe pinnata* (100 mg/kg) +isoproterenol, it rose to (1.493 ± 0.177) ug/L. There was a significant difference (increase, $p<0.05$) between the neutral control and the groups administered with ethanolic *Kalanchoe pinnata* (100 mg/kg) +isoproterenol and aqueous *Kalanchoe pinnata* (100 mg/kg) +isoproterenol. However, compared to negative control, both extracts reduced non-significantly the rise of troponin observed. There was no significant difference in the negative control compared to the aqueous *Kalanchoe pinnata* (100 mg/kg) +isoproterenol and ethanolic *Kalanchoe pinnata* (100 mg/kg) +isoproterenol. There was a significant increase ($p<0.05$) comparing the positive control and the ethanolic *Kalanchoe pinnata* (100 mg/kg) +isoproterenol while there was no significant difference between positive control and the aqueous *Kalanchoe pinnata* (100 mg/kg) +isoproterenol. Figure 2 below represents troponin concentration in all the groups observed.

Each bar represents the mean ± SEM, n=5, $^{\#}P<0.05$ and $^{\#\#}P<0.01$ significantly different from neutral control, *$p<0.05$ and **$p<0.01$ significantly different from the negative control, $^{\$}P<0.05$ significantly different from the positive control. The neutral control received only tap water, negative control received isoproterenol, positive control received propranolol+isoproterenol, the other groups received ethanolic *Kalanchoe pinnata* (50 mg/kg, 100 mg/kg, and 200 mg/kg), aqueous *Kalanchoe pinnata* (100 mg/kg) +isoproterenol and ethanolic *Kalanchoe pinnata* (100 mg/kg) +isoproterenol.

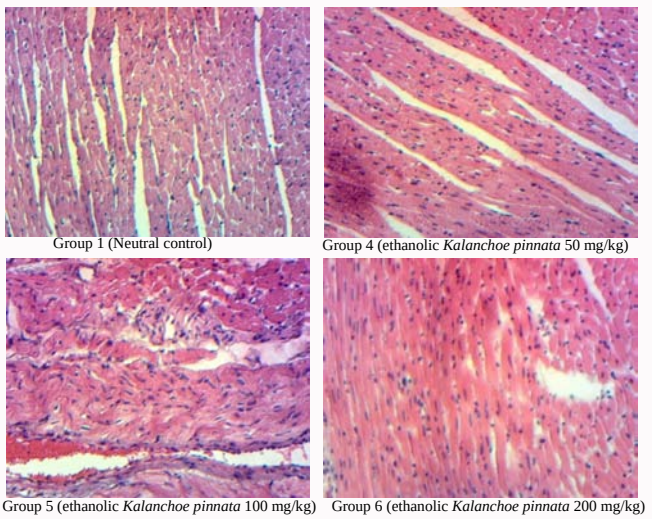
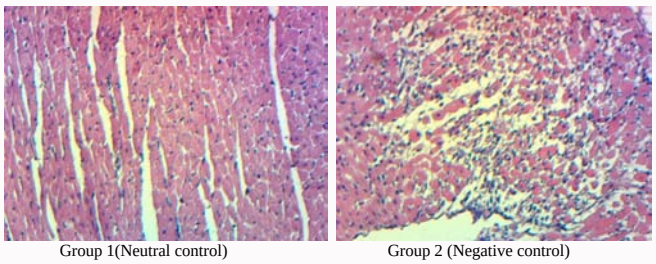
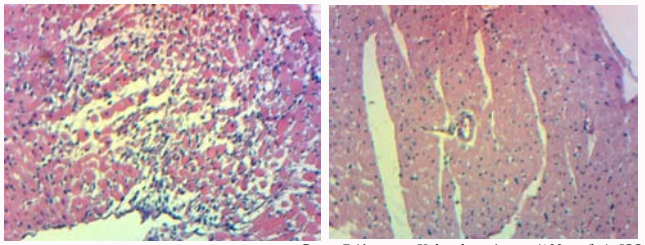


Figure 3: Micrographs of heart tissues (Groups 1,4,5 and 6). Groups 1,4,5 and 6 all present a normal architecture.



Group 1(Neutral control) Group 2 (Negative control)

Figure 4: Micrographs of heart tissues (Group 1 and Group 2).



Group 2 (Negative control) Group 7 (Aqueous *Kalanchoe pinnata* (100 mg/kg)+ISO

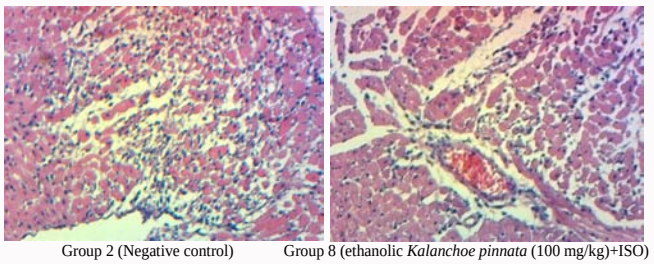
Figure 5: Micrographs of heart tissues (Group 2 and Group 7).

Effects of *Kalanchoe pinnata* on the cardiac tissue architecture: Animals treated during this experiment in the 8 groups receiving different concentrations of extracts displayed many differences in the level of damage and accent of pathological changes in the cardiac tissue. Generally, treatment with isoprenaline caused the cells to become edematous, lose the normal striations pattern and possess inhomogeneous content; some fibres and cells lysed. Myocytes showed damage caused by isoprenaline. In the negative control, the damage was very intense as compared with the other groups receiving isoprenaline after extracts administration. There was massive necrosis, cellular arrangement disappeared, nuclei disappeared or massive vacuoles formed (infarction). In the negative control, positive control, aqueous *Kalanchoe pinnata* (100 mg/kg) +isoproterenol, and ethanolic *Kalanchoe pinnata* (100 mg/kg) +isoproterenol, there were damages thus leading to the presence of infarction in the cardiac tissues. These architectural changes were absent in the other four groups which did not receive isoprenaline. Figure 3 below shows the micrographs of heart tissues in groups 1,4,5 and 6.

In the neutral control which received distilled water, there was no damage in the cardiac tissue in the contrary of the negative control treated with isoproterenol only (with many cells lysed and leucocytic infiltration). Figure 4 below represents the heart micrographs of the neutral and negative control.

In the negative control, heart tissues were damaged intensively as compared to the aqueous *Kalanchoe pinnata* (100 mg/kg) +isoproterenol. Figure 5 below represents the heart micrographs in the negative control and aqueous *Kalanchoe pinnata* (100 mg/kg).

In the negative control, heart tissues were damaged intensively as compared to the ethanolic *Kalanchoe pinnata* (100 mg/kg) +isoproterenol (group 8). However, it was observed that the repairs of the damage were less pronounced than what obtained in group 7 (administered with the aqueous extract). Figure 6 represents the heart tissues of the negative control and ethanolic.



Group 2 (Negative control) Group 8 (ethanolic *Kalanchoe pinnata* (100 mg/kg)+ISO)

Figure 6: Micrographs of heart tissues (Group 2 and Group 8).

Discussion

Natural products, such as medicinal plants have been used empirically for the treatment of various ailments. This study was carried out to determine the effects of *Kalanchoe pinnata* on the serum level of CK-MB and troponin (cardiac biomarkers) and architectural changes as an indicator of myocardial infarction in ISO treated rats.

In this study, the choice of the animal model of Acute Myocardial Infarction (AMI) by subcutaneous administration of isoprenaline (150 mg/kg/day) in rats was in line with many other authors [18-20]. There was a significant rise in serum biomarkers of ISO-treated rats. Pre treatment with *Kalanchoe pinnata* showed a significant reduction in the levels of all serum diagnostic biomarkers compared to ISO group.

As concerns Creatine Kinase-MB (CK-MB) levels, there was a significant increase in the negative control compared to neutral control. The level of CK-MB was highest in the negative and lowest in the positive control. CK-MB level was significantly higher in the negative control compared to aqueous *Kalanchoe pinnata* (50 mg/kg) and aqueous *Kalanchoe pinnata* (100 mg/kg) +isoproterenol. This marked increase in CK-MB in the negative control shows that there was a net damage in the myocardial tissues caused by isoprenaline. There was a non-significant decrease in CK-MB in *Kalanchoe pinnata* (Et) (100 mg/kg), compared to the negative control, indicating *Kalanchoe pinnata* (Et) (100 mg/kg) reduced non-significantly the level of damage caused by isoprenaline, and this was further confirmed by histopathological analysis. More fascinatingly, there was a significant decrease in the level of CK-MB in the *Kalanchoe pinnata* (Aq) (100 mg/kg) +ISO group, compared to ISO group; indicating *Kalanchoe pinnata* (Aq) (100 mg/kg) significantly prevented the rise in CK-MB in blood serum by preventing the damage of cardiomyocytes. As compared to neutral control, there were non-significant increases of the levels of CK-MB in blood in the test groups, treated with *Kalanchoe pinnata* (Et) (50 mg/kg, 100 mg/kg and 200 mg/kg). Results in the positive control demonstrated that propranolol prevented the ISO-induced rise of CK-MB in blood and hence, the damage to myocardial tissues. This corroborates the results of Acikel et al. [21], Ahmed et al. [22] and Upaganlawar et al. [23].

With regards to troponin levels, there was a significant increase in the negative control group, treated with isoprenaline, compared to the neutral control group. The significant increase in the negative control was caused by the administration of isoprenaline (150 mg/kg), consecutively to cell lyses during course myocardial infarction. Myocardial tissues damaged by isoproterenol did not only release CK-MB, but also troponin and this is clearly observed in the group with the highest quantity of troponin released (negative control). In the *Kalanchoe pinnata* (Et) (100 mg/kg) +isoprenaline(150 mg/kg), there was an increase in troponin level which also indicated a damage caused by isoprenaline (150 mg/kg), although it was slightly reduced by *Kalanchoe pinnata* ethanolic extract (100 mg/kg). In

Table 1: Grouping and treatment of animals.

Group	Treatment Administered	Duration
Group 1, neutral control	Distilled water only (10 ml/kg/day, per os)	28 days
Group 2, negative control	Isoproterenol (150 mg/kg/day, sc)	2 days before sacrifice
Group 3, positive control	Isoproterenol (150 mg/kg/day) and propranolol (10 mg/kg/day, per os)	Isoproterenol-2 days before sacrifice and propranolol-10 days to the sacrifice
Group 4-6, test groups*	50 mg, 100 mg, 200 mg of ethanolic extract of *Kalanchoe pinnata* (per os)	28days
Group 7	Aqueous *Kalanchoe pinnata* extracts (100 mg/kg/day, per os)+isoproterenol (150 mg/kg/day, sc)	Aqueous *Kalanchoe pinnata* extracts-28 days isoproterenol-2 days)
Group 8	Ethanolic *Kalanchoe pinnata* extracts (100 mg/kg/day, per os)+isoproterenol (150 mg/kg/day, sc)	Ethanolic *Kalanchoe pinnata* extracts for 28 days+isoproterenol-2 days)

*Test groups were only for the ethanolic extract, because the aqueous extract has been tested previously [15]

the aqueous *Kalanchoe pinnata* (100 mg/kg) +isoproterenol, the damage caused by administration of isoprenaline, was significantly reduced by the aqueous extract as revealed by the 49% reduction of the rise in troponin level observed in the negative control (from 1.886 ± 0.0002) ug/L to (0.9694 ± 0.1303) ug/L. Aqueous *Kalanchoe pinnata* from the above results shows more potency than *Kalanchoe pinnata* (Et) at same dose. These activities of *Kalanchoe pinnata* extracts were qualitatively comparable to that of propranolol, a known beta 1-antagonist [24]. In groups administered with ethanolic extract there was a slight dose non-dependant increase in the level of troponin, however not significant. This finding indicates that, when administered alone, *Kalanchoe pinnata* (Et) at doses 50 mg/kg, 100 mg/kg and 200 mg/kg induced non-significant increase levels of troponin in blood, comparatively to neutral control. In the contrary of the aqueous extract group, no significant difference was observed between troponin levels in the group treated with this extract and the negative control. Furthermore, we observed a significant difference between the positive control and this extract, but not same with the aqueous extract. This suggests that the ethanolic extract was not as potent as the aqueous one.

Troponin I is a unique cardiac marker, which is released from infracting 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 [25]. Cardiac Troponin I (cTnI) has consistently been shown for identifying myocardial necrosis [26]. Several evidences suggest that measurement of cTnI is at least as sensitive and specific as CK-MB [27,28]. The observed elevation in the levels of serum cTnI predicts the risk of both cardiac cell death and subsequent lysis in ISO-induced infracted rats. Treatment with aqueous and ethanolic *Kalanchoe pinnata* resulted in reduced level of troponin-I which is recognized as a better myocardial injury marker.

Findings from biochemical analyses were confirmed by histopathological analyses. Architectural changes were observed in the negative, because of the administration of isoprenaline. Histopathological examinations of the heart showed leucocytic infiltration in the negative control group which are signs of a disease or inflammation and cell necrosis as clear signs of infarction [29-31]. Infarction was pronounced in the negative control because of the administration of isoprenaline only, which intensely damaged heart tissues especially. In Group 7 (aqueous *Kalanchoe pinnata* (100 mg/kg) +isoproterenol) there was damage of heart, although the level of damage was reduced by *Kalanchoe pinnata* (100 mg/kg). This indicates that there is a preventive effect of *Kalanchoe pinnata* (100 mg/kg) against infarction caused by isoprenaline. Also, there were damages observed in Group 8 (ethanolic *Kalanchoe pinnata* (100 mg/kg) +isoproterenol). These damages were also reduced as compared to the negative control but to a lesser extent. The positive control (micrograph not shown!) has also proven the efficacy of propranolol in preventing the ISO-induced damage of the cardiomyocytes. These findings once more corroborate previous ones by Bopda et al. [15], confirming the cardioprotective effects of the leaf aqueous extract of *Kalanchoe pinnata*.

Conclusion

Our findings revealed that *Kalanchoe pinnata* extracts (aqueous and ethanolic) have the potential to prevent myocardial infarction as indicated by reduction of the rise of CK-MB and troponin, caused by isoprenaline, and prevention of the histological damage of the heart. The aqueous extract of *Kalanchoe pinnata* had a higher potency in preventing heart damage than the ethanolic *Kalanchoe pinnata* at the same dose of 100 mg/kg. Results confirm that the aqueous extract has good cardioprotective potency against isoproterenol-induced myocardial infarction in rat.

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