Research Paper

Fresh and decaying stem juice of *Musa acuminata x balbisiana (Musa paradisiaca*) reduce the force and rate of contractility of an isolated perfused rabbit heart

ABSTRACT

Background

Decaying stem juice of *Musa acuminata* × *balbisiana* is commonly used by local communities and traditional herbalist in Central Uganda in the management of cardiovascular conditions like hypertension.

Aims: The study investigated the ionotropic and chronotropic effect of fresh and decaying stem juice of *Musa acuminata* × *balbisiana* on the isolated perfused rabbit heart.

Meterials and methods

Study design: An experimental study.

Place and Duration of Study: Study was done at the Dept of Pharmacology & Therapeutics Pharmacology Lab between December 2012 to March 2013.

Experimental procedure: An experimental study determined the effects of fresh and decayed stem juices of *Musa acuminate X balbisiana* on the rate and force of contraction of an isolated rabbit heart using Langendorff's heart perfusion experiment and methods. The heart rate (beats/minute) was determined. The force of contraction of the heart was determined by measuring the height of each peak on the kymogram.

Results: Both the fresh and decayed stem juices reduced the force and rate of contraction of an isolated perfused rabbit heart just like acetylcholine. The effect of the fresh stem juice was short lived and at very high concentrations, it caused a cardiac arrest while the effect of the decayed stem juice was prolonged.

Conclusion: Fresh and decayed stem juice of *Musa acuminata* × *balbisiana* have compounds that cause a negative ionotropic and chronotropic effect on an isolated perfused rabbit heart.

Keywords: Musa acuminata X balbisiana, heart contractility, stem juices, Banana

1. INTRODUCTION

Medicinal herbs have long been used by various communities and traditional herbalist in the management and treatment of various disease conditions that affects heart contractility worldwide [1-4]. Heart contractility abnormalities leads to heart failure (HF), which is the

inability of the heart to pump blood to the different parts of the body in order to meet the bodies nutritional and oxygen demands [5-9]. Approximately 5 million people worldwide are currently diagnosed with HF and about 500,000 new cases are reported annually (NCCD, 1998). HF is a non- communicable chronic disease and the leading contributor to hospitalization of patients in many countries [10]. It is commonly secondary to a variety of primary cardiovascular diseases that include coronary artery disease, hypertension, valvular heart disease, and ischemic heart disease. In Uganda, according to the Uganda Heart Institute records, there has been a 500% increase in outpatient attendance due to heart related conditions over the past 7 years [11, 12]. The increase in heart diseases has been associated with changes in life style and poor nutrition in many of the developing countries like Uganda [8, 12-15]. The lack of exercise and poor feeding habits greatly affect the normal rhythm of heart contraction by interfering with the mechanisms of cardiac regulation like sympathetic and parasympathetic nervous systems. They also affect molecular mechanism of the cardiac muscle contraction [16, 17]. However, lack of access to drugs and the high cost of management of cardiovascular diseases have forced the poor communities in developing countries to seek alternative sources from medicinal herbs that are thought to be cheaper and safe in managing hypertension [13, 18]. One of the traditional herbs commonly used in the management of heart diseases especially hypertension in central Uganda is the decayed stem juices of *Musa acuminata x balbisiana (AAB*) or *M. paradisiaca* locally known as plantain or banana *[19-23]*. It belongs to the family Musaceae and has been reported to have various medicinal properties and used in the treatment of various disease conditions[24, 25]. The banana flower extracts have been reported to contain various phytochemical compounds including alkaloids, glycosides, steroids, saponins, tannins, phenols, flavanoids and terpenoids [26, 27]. Its also reported that frequent consumption of banana fruits has been associated with a lowered risk of cancer, heart disease, hypertension and stroke [25, 27]. Though the herb is commonly used in the management of heart diseases like hypertension, its efficacy has not been scientifically evaluated for its effects on the force and rate of heart contractility. The study investigated the ionotropic and chronotropic effects of fresh and decayed stem juice of *Musa acuminata* × *balbisiana* on the isolated perfused rabbit heart using the Langendorff's heart perfusion experimental methods.

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

2.1 Study design

It was an experimental study design that investigated the effect of the fresh and decaying stem juice of *Musa acuminata* × *balbisiana* on the rate and force of contractility of an isolated rabbit heart using the Langendorff's heart perfusion methods of experiment [28-32].

2.2 Plant material selection and processing

The plant was selected because it is commonly used by local communities and traditional herbalist in the management of heart diseases. The plant was collected from Kasangati, Wakiso district in central Uganda and was authenticated at the Makerere University Herbarium by a taxonomist with a reference and a voucher number as NO-01-PHARM. The stem of Musa acuminata × balbisiana was cut half way leaving part of it rooted in the ground to allow decay of the stem core to occur similar to what is done in the local communities. After two weeks, the decaying stem juice that had collected in the middle was removed using a spoon and collected in a flask. The juice was filtered using Whatman No. 1 filter paper in a Buchner funnel. To the 500mls of the filtrate of the juice, 300mls of absolute ethanol was added for preservation purposes. Also fresh stem juice from a freshly cut stem was got and treated as above. The stem juice were dried in the an oven at constant temperature of 25°C to allow slow evaporation without destroying the active compounds in order to obtain solid extracts.

2.3 Selection and treatment of experimental animals

Healthy hybrid male rabbits, weighing 2.5kg were purchased from local vendors. The animals were treated humanely according to international guidelines of laboratory animal use according to OECD (2001) guideline [33]. They were provided with food and water. They were allowed to acclimatize for a period of two weeks before the experiment.

2.4 Preparation of stock solutions and different doses of the fresh and decayed stem juice, adrenaline and acetylcholine solutions

The 0.5g of each of the dry fresh and decayed stem juice of *Musa acuminata* × *balbisiana* were dissolved in 5ml Locke's solution to obtain stock solution of 100mg/ml of each stem juice. Serial dilutions were made to obtain concentrations of 10mg/ml, 5mg/ml, 2.5mg/ml,

0.625mg/ml and 0.15625mg/ml of each of the stem juices that were used in the experimental study. 0.1g of acetylcholine was dissolved in 10ml of Locke's solution to obtain a concentration of 10mg/ml which was serially diluted to obtain concentrations of 5mg/ml, 2.5mg/ml, 0.625mg/ml, and 0.15625mg/ml. To 1ml of 1mg/ml of epinephrine, 9ml of Locke's solution was added to obtain a concentration of 100µg/ml that was also diluted serially to obtain concentrations of 1.625µg/ml, 3.125µg/ml, 6.25µg/ml and 12.5µg/ml.

2.5 Isolation and preparation of rabbit heart

The rabbits that were used in the experiment were anaesthetized by injecting them with sodium pentobarbitone 30mg/kg bwt via the intraperitoneal route. The chest of the rabbit was then opened immediately and the heart dissected out with about 1 cm of aorta attached. The heart was washed as quickly as possible with warm oxygenated Locke solution. It was then mounted on the Langendorff's apparatus in preparation for heart contractility activity study [28, 29, 32].

2.6 Procedures for the heart contractility activity study

The heart was then transferred to the Langendorff's heart perfusion apparatus, tied to a stainless steel cannula through the aorta. Warm perfusion fluid, Locke solution was continuously bubbled with a mixture of 95% oxygen and 5% carbon dioxide at a constant perfusion pressure of 70mmHg. The temperatures were maintained between 36.5°C and 37.5°C and continuous monitoring was done using a thermometer inserted into the perfusion fluid chamber. The heart was allowed to stabilize for 5 seconds before addition of any drug. Recording on the kymograph was done to obtain normal contractility of the heart which was considered as baseline for the different concentrations of the drugs. Adrenaline was used first in increasing concentrations; each concentration was added after return of the contractility to the baseline. Adrenaline was followed by decayed stem extract, then fresh and lastly acetylcholine, all of them added in increasing concentrations and addition of a particular concentration done after return of the contractility to baseline. Each drug concentration was added using a 1ml syringe through the perfusion line above the aortic line and the changes in the cardiac contraction were parameters recorded using the kymograph using a tracing paper. Each experiment was run for three minutes with a contact time of 5 seconds. The baseline recording before perfusion of a particular drug was considered the baseline reading for each dose. The parameters that were measured were the heart rate and mean force of contraction that was measured using the height of the peak of heart contraction on the kymogram. The peak was measured using a calibrated ruler in

millimeters. The heart rate for each dose of each drug was measured by counting the number of heart beats for 15 seconds and the heart beats per minute were then calculated.

2.7 Data collection and analysis

Data was recorded for each of the experiments that were carried out on the heart muscle. For each concentration of each drug, the cyclic height was measured at five different points on the kymogram. The percentage change in height using the baseline was calculated for each dose used for fresh and decayed stem juice, acetylcholine and adrenaline and this measured the force of contraction of the heart. The rate of contraction of the heart (heart beats/ minute) were counted for each experiment and this was done by counting the number of heart beats for 15minutes and this was later calculated for the number of heart beats/ minute. Data was entered in to the Excel spread sheet and simple statistics for each test was calculated to obtain the mean standard deviation values. For the percentage response of the cardiac muscle contractility (force of contraction), the following formula was used.

% Response of tissue = (experimental value – baseline value) x 100/baseline value

2.8 Ethical consideration

Permission was obtained from the Pharmacology and Therapeutics department and Department of Pharmacy Institution review ethics committee to carry out the experiment and the animals were treated according to the International guidelines on the laboratory animal use and care protocols of OECD (2001) guideline [33]. The animals were handled with utmost care before the experiments and at the time of the experiments. The animals were put to rest in a humane way by injecting them intraperitoneally with 30mg/kg bwt of sodium pentobarbitone.

3. RESULTS AND DISCUSSION

The results showed that there was a reduction trend in the percentage change of the force of heart contractility (ionotropy) with increasing doses from 0.156mg/mL to 100.000mg/mL of the fresh and decayed stem juice of *Musa acuminata X balbisiana* as compared to the baseline. The results were similar to that of the acetylcholine that was used as a negative control and opposite to that observed with adrenaline which was used as a positive control. However, the doses used in the experiment as controls (pure drugs) were slightly lower than those of the stem juices that were in crude form (Table 1 and figure 1). For the rate of heart contraction (heart beats/minute) showed a reduction trend with increasing doses from 0.156mg/mL to 100.000mg/mL of the fresh and decayed stem juice of *Musa acuminata X*

balbisiana. The results were similar to that observed with acetylcholine and opposite to that of adrenaline. However, the ionotropic and chronotropic effect of the stem juices of *Musa acuminata X balbisiana* were observed to be stronger for decayed stem juice as compared to the fresh stem juice (table 2 and figure 2). In figure 3, it shows the Kymograms of adrenaline, acetylcholine and different doses of fresh and decayed stem juices of *Musa acuminata X balbisiana* on contractility of the isolated rabbit heart. The arrows on the Kymogram, shows the point of contact of the drug with the heart muscle and the effect caused by the drug on the heart at that point. They show the ionotropic and chronotropic effect observed at the baseline and that of adrenaline, acetylcholine and the fresh and decayed stem juices of *Musa acuminata X balbisiana.* At a dose of 100.0mg/mL of the fresh stem juices of *Musa acuminata X balbisiana* and at the doses of 2.5 mg/mL and 5.0mg/mL of acetylcholine, the heart was observed to go into cardiac arrest. In all the cases of cardiac arrest for the stem juices and acetylcholine, adrenaline at 0.005mg/ml was used to resuscitate the heart while for adrenaline cardiac arrest, 0.01mg/ml of acetylcholine was used to overcome the cardiac arrest due to high concentration of adrenaline that was used in the experiment. The force of contractility of the heart (ionotropic effect) was observed to be sustained for a longer period of time at the dose of 100.0mg/mL for the decayed stem juice as compared to the fresh stem juices of *Musa acuminata X balbisiana.*

The observed decrease in the force and rate of heart contractility of the isolated rabbit heart using the Langendorff's heart perfusion experiment with the fresh and decayed stem juices could be due to the agonistic effect of the compounds in the juices that mimicked the physiological effect of acetylcholine, a neurotransmittere released by the parasympathetic nervous system nerve terminals in the heart [16, 17]. Similar effects observed, can occur with the vagal stimulation to the heart [16, 17]. The compounds that have been reported in *Musa acuminata* × *paradisiaca* that could contribute to the reduced chronotropic and ionotropic effects of the heart include starch and fructosans, phenolic acids, anthocyanins, terpenoids and sterols, tannins, eugenol, and tyramine [4, 34]. Other compounds reported in ripe fruit include serotonin, levarterenol and dopamine [4, 20, 34]. The decayed stem juice has also been reported to contain increased concentrations of the potassium ions, molybdenum and phosphorus [16, 17, 23]. Whereas the serotonin, dopamine and levarterenol are catecholamines that would cause an increase in chronotropic and ionotropic effects, there effects may be counteracted with the presence of the high potassium ions present in the stem juices [16, 17, 23]. A high concentration of potassium ions outside the cardiac cells' membrane lead to hyperpolarization of the cells of the myocardium thus preventing depolarization of the cells. This reduces electrical impulses generation and

passage in the myocardium and hence reduction in heart rate and force of contractility [16] as observed in the experiment. So the high concentration of potassium ions in both the fresh and decayed stem juices could have contributed to decreased force and rate of heart contraction [16, 17, 23]. On the other hand, adrenaline released by the sympathetic nerve terminals in the heart increases the cardiac muscle fiber membrane to sodium and calcium ions. An increase of sodium and calcium ion permeability causes a more positive resting potential hence bringing it nearer to the threshold level for self-excitation [16, 17, 35]. In the A-V node and A-V bundles, increased sodium- calcium permeability increases excitability of each succeeding portion of the conducting fibres by the action potential hence decreasing conduction time from the atria to the ventricles [16, 17, 35]. The increase in permeability to calcium ions is partially responsible for the increase in the force of contraction of the cardiac muscle because calcium ions play a major role in the contractile process of myofibrils [16, 17, 35]. Increasing concentrations of adrenaline leads to an increase in the contractility of the heart but overstimulation of heart overworks the heart muscle leading to cardiac arrest and even the death of tissue due to insufficient oxygen and nutrient supply and this could have been caused the heart to go in to cardiac arrest observed in the experiment with the high dose of adrenaline used as a control drug. Acetylcholine decreases the rate of rhythm of the sinus node and decreases the excitability of the A-V node junctional fibers between the A-V node and the atria hence slowing passage of impulses [16, 17, 35]. Acetylcholine increases permeability of the fiber membranes to potassium ions leading to rapid leakage of potassium out of the conductive fibers. This makes the fibers hyperpolarized making them much less excitable [16, 17, 35]. This leads to decrease in the force and rate of heart contractility. In the sinus node, hyperpolarization decreases the resting membrane potential requiring more time to reach the threshold for excitation [16, 17, 35]. At high concentrations of acetylcholine, it is possible to stop entirely the rhythmical self-excitation of the sinus node. In the A-V node, hyperpolarization makes it difficult for atrial fibers entering the node to excite the nodal fibers while the low concentration of acetylcholine simply delays conduction of the impulse and at high concentration blocks conduction entirely. The results therefore show that both the fresh and decayed stem juices of *Musa acuminata X balbisiana* decrease the ionotropic and chronotropic effects of the heart and hence its increased use by the local communities and traditional herbalist in Uganda in management of heart diseases especially hypertension.

Table 1: Effect of different doses of fresh and decayed stem juices of *Musa acuminata X balbisiana* on the force of contraction (ionotropic effect) of the isolated rabbit heart

Dose (mg/mL)	% ±SD change in the heart contractility for different test substances (%±SD)			
	Adrenaline	Acetylcholine	Fresh Stem Juice	Decaying Stem Juices
0.00 (Baseline)	0.00±00	0.00±00	0.00±00	0.00±00
0.002	41.86±5.93	-	-	-
0.003	129.07±12.06	-	-	-
0.006	134.88±6.63	-	-	-
0.010	202.33±12.33	-	-	-
0.156	-	-11.63±-2.12	-25.58±-4.87	-22.09±-7.80
0.625	-	-46.51±-4.87	-27.91±-3.18	-35.05±-8.22
2.500	-	-54.65±-7.58	-22.09±-5.20	-37.21±-4.87
5.000	-	-53.49±4.11	-19.76±-4.88	-55.81±-3.18
10.000	-	-56.12±3.14	-19.36±-2.96	-56.98±-7.80
100.000	-	-61.45±4.34	-88.37±-4.11	-83.73±-4.87

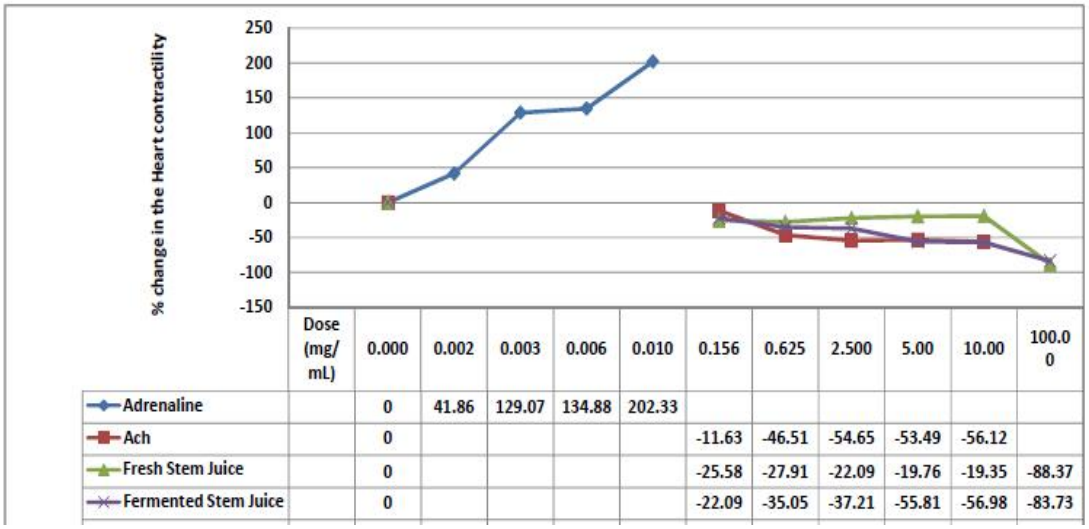

Figure 1: Effect of different doses of fresh and decayed stem juices of *Musa acuminata X balbisiana* on the force of contraction (ionotropic effect) of the isolated rabbit heart

Table 2: Effect of different doses of fresh and decayed stem juices of *Musa acuminata X balbisiana* on the heart rate (chronotropic effect) of an isolated perfused rabbit heart

Dose (mg/mL)	Mean heart beats ±SD per minute (Heart rate/chronotropic effect)			
	Adrenaline	Acetylcholine	Fresh Stem Juice	Decaying Stem Juice
0.00 (Baseline)	120.00±2.94	120.00±2.94	120.00±2.94	120.00±2.94
0.002	124.00±2.75	-	-	-
0.003	128.00±2.22	-	-	-
0.006	148.00±1.71	-	-	-
0.010	164.00±3.30	-	-	-
0.156	-	72.00±1.71	120.00±1.63	116.00±1.71
0.625	-	44.00±1.69	116.00±1.29	112.00±2.38
2.500	-	36.00±0.96	100.00±2.22	100.00±1.73
5.000	-	24.00±1.70	84.00±0.95	72.00±1.69
10.000	-	12.00±1.80	68.00±1.71	48.00±1.26
100.000	-	3.00±0.94	48.00±1.27	36.00±1.70

Figure 2: Effect of different doses of fresh and decayed stem juices of *Musa acuminata X balbisiana* on the heart rate (chronotropic effect) of an isolated perfused rabbit heart

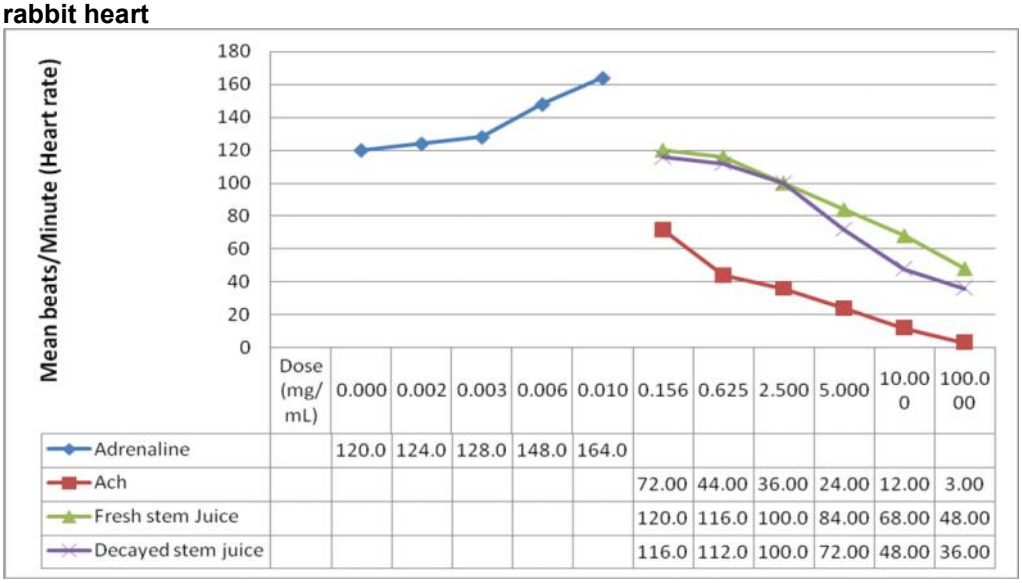

Figure 3: Kymograms of adrenaline, acetylcholine and different doses of fresh and decayed stem juices of *Musa acuminata X balbisiana* on contractility of the isolated rabbit heart

4. CONCLUSION

The fresh and decayed stem juice of *Musa acuminata* × *balbisiana* contains compounds that decreased the force and rate of contraction of an isolated perfused rabbit heart. The effects of the decayed stem juice were more prolonged than the fresh stem juices. The fresh stem juices were observed to cause a short- lived cardiac arrest at high concentrations. The herb is generally used in management of hypertension by local communities but they have to take precautions during its use especially at high concentrations since it can cause cardiac arrest and possibly the death.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

First author and second designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Third author and last author were involved in the execution of the experiments and writing of the protocol also. All authors read and approved the final manuscript."

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