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Positive Inotropic And Negative Chronotrophic Effect Of *Aloe Vera* Extract On Cardiomyocytes In Frogs: An *in-vitro* Study.

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ABSTRACT

Aloe vera belongs to the class of *Xanthorrhoeaceae* native to Arabian Peninsula. The *Aloe vera* extract was evaluated for its cardio-protective activity using isolated frog's heart perfusion technique. The positive inotropic and negative chronotropic activities were recorded using power lab setup. Besides that, digoxin was used as a standard drug when compare to *Aloe vera* extract. *Aloe vera* extract produced positive inotropic and negative chronotropic activities on isolated frog's heart. The pharmacological action was specifically triggers by *Aloe vera* extract indicating that these might have been mediated through Na^+, K^+ and ATPase on cardio myocytes.

Keywords: *Aloe vera*, Inotropic, frog, cardiomyocytes

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INTRODUCTION

Aloe vera potentially shown to have cardio protective effects and property.*Aloe vera* is one of the most important herbal plants that maintain its popularity for decades. Several medicinal properties had been attributed to this plant. *Aloe vera* promoted for different kind of human disease such as diabetes, constipation, cough, wound, ulcer, cancer, headache, arthritis, immune system deficiencies and many other conditions [1]. *Aloe vera* has been proved to have antioxidant effect, antimicrobial effect and much other effect [2] [3]. Apart from this, *Aloe vera* has been promoted to various kinds of cardiovascular condition but the real mechanism and pathway is unknown.

Aloe vera is cultivated all over the world and is native to Arabian Peninsula. It has become neutralized North Africa, Mediterranean, Caribbean, South America and the Indian subcontinent [4]. This plant is a rich source of many natural health-promoting substances including many phytochemicals vitamins such as Vitamin C, A, E, β-carotene, Zinc, Calcium, Copper, Magnesium, Manganese, and Phosphorous, plant sterols such ascampestrol, cholesterol, and β-sitosterol, polysaccharides including B1-3 and B1-4 Glucomannans known for their immune stimulating effects. Based on its constituent make up, *Aloe vera* has a wide array of applications [5].

Researchers able to investigate the physiology and pharmacology of various tissue sample using isolated organ and tissue preparation in controlled environment without the complication of intact animal model. These is an *in vitro* experiment, which can done on various tissue and organs include smooth muscle, skeletal muscle, cardiac muscle, gastrointestinal and urogenital tissue samples and arterial rings. Typically, these studies done on controlled temperature by tissue perfused in oxygenated physiological buffer. Pharmacological agents and electrical stimulations can be used to carry out evoked-response protocol. The isolated heart preparation will be most commonly used model in pharmacological study [6]. Some of the rodents such as guinea pig, rats, rabbits and frogs are common source used in this study.

This study was undertaken to enhance scientifically the positive inotropic and negative chronotropic effect of *Aloe vera* extract on isolated frog heart preparation by using power lab setup.

MATERIALS AND METHODS

Collection of plant

The whole plant of *Aloe vera* was collected from Cheras, Selangor, Malaysia, in the month of July, 2014. The plant material was identified and authenticated at University Putra Malaysia (UPM), Kajang, Selangor, Malaysia. The leaves of *Aloe vera* were washed to remove dirt and impurities. Leaves were dried for two weeks under low sun intensity [7]. The procedure for extraction was maceration extraction method using ethanolic extract.

Chemicals

Digoxin was purchased from Sigma Aldrich, USA and other chemicals such as sodium chloride (NaCl), potassium chloride (KCl), calcium chloride ($CaCl_2$), sodium bicarbonate ($NaHCO_3$), sodium dihydrogen phosphate (NaH_2PO_4) and glucose were obtained from Pharmaniaga Manufacturing Bhd.

Preparation of digoxin

Digoxin was purchased with the concentration of 500 µg in 2 ml. The working concentration of 50 µg in 0.2 ml was of digoxin was added on heart to produce effect.

Preparation of *Aloe vera* extract

Aloe vera with 100 mg and 1 mg of Carboxyl Methyl Cellulose (CMC) was mix together in mortar and dilute with 100 ml of distilled water. A stock solution of 100 mg / 100 ml was prepared. The following working concentrations were used from the stock: 500µg/0.1 ml and 1000µg/0.1 ml were added.

Isolated frog's heart preparation

The frog was pithed and destroyed by passing a stiletto through the occipito-atlantic junction. The anterior chest wall was opened and a pericardiectomy was performed to expose the heart.The one end of the aorta, inferior vena cava was identified. A small cut inferior vena cava and a Syme's cannula was inserted towards the heart. A steady flow of the perfusion Frog-Ringer solution containing oxygenated, fluid of the following composition: NaCl 6.5, KCl 0.14, $CaCl_2$ 0.12, and $NaHCO_3$ 0.2, NaH_2PO_4 0.01, Glucose 2.0 in g / l.A steady flow of the perfusion Frog-Ringer solution was perfused through this cannula and there was an opening in the cannula through which drugs could be injected by pushing a capillary tube attached to a syringe through an injection needle [8]. A very thin hook was attached to the apex of beating heart and connects it to force transducer on power lab to find the cardiac parameter such as force of contraction, heart rate and cardiac output.

Statistical analysis

Values were represented as Mean ± SE. Significant difference was calculated using one way Analysis of Variance (ANOVA) using Duncan multiple range test. Values not sharing common signs were differ significantly at $p<0.05$.

RESULTS AND DISCUSSION

The inotropic and chronotropic effects of isolated heart were recorded from apex of the heart connected to a power lab setup 26T (T26-0750). The normal rate of contraction of isolated heart was recorded after stabilization for 30 minutes.The doses of 500 µg and 1000 µg of *Aloe vera* extract were injected directly to the isolated heart. The lower doses of *Aloe vera* extract increased the cardiac output (12.75±0.95 ml / min) and force of contraction (0.282±0.000 N) but decreased in heart rate (42.00±0.810 beats / min) when compare to normal cardiac output (12.50±0.547 ml / min), force of contraction (-0.284±0.000 N) and heart rate (39.60±0.540 beats / min) (Table 1). However, the maximum response was produced by 500 µg of *Aloe vera* extract. Besides that, digoxin was administered at the dose of 50 µg and the cardiac output (11.60±0.547 ml / min), force of contraction (0.300±0.000 N) and heart rate (32.00±0.700 beats / min).

Table 1: Effect of *Aloe vera* extracton cardiac output, heart rate and force of contraction in frog heart.

Group	Drugs/Extract & dose	Cardiac Output (ml / min)	Heart rate (Beats / min)	Force (N)
1	Physiological Salt solution	$12.50±0.547^{d}$	$39.60±0.540^{d}$	$-0.284±0.000^{a}$
2	*Aloe vera* extract 500 µg	$12.75±0.950^{b}$	$42.00±0.810^{a}$	$-0.282±0.000^{b}$
3	*Aloe vera* extract 1000 µg	$10.50±0.577^{a}$	$55.25±4.030^{b}$	$-0.282±0.005^{b}$
4	Digoxin 50 µg	$11.60±0.547^{c}$	$32.00±0.700^{a}$	$-0.300±0.000^{d}$

Values are mean ± SE (n = 5). Significant difference is calculated using one way ANOVA using Duncan multiple range test. Values not sharing common signs are differ significantly at $p < 0.05$.

This study was on isolated frog heart by using physiological salt solution. Ethanol extract of *Aloe vera*produced positive inotropic and negative chronotropic on myocardium. The similar effect was also shown by digoxin at different concentration (Table 1). Cardiac enhance the action indicates that the ethanol extract revealed potent cardiac tonic activity similar as a result of digoxin.

In the isolated heart preparation, increase in force of contractionis due to inhibition of Na^+, K^+ and ATPaseand the effect is mediated via cardiac glycoside action in the myocardium. In our study, the effect will beinhibition of Na^+, K^+ and ATPase, which suggests that the active component present in *Aloe vera* may be acting either on the cardiac glycoside or any other receptors that are inhibited by Na^+, K^+, ATPase [9].In our study it seems thatNa^+, K^+, ATPase inhibition by cardiac glycosides indicates rise in intracellular Ca^{2+}concentrations through Na^+/ Ca^{2+}exchange and an associated rise in slow internal Ca^{2+}current as well as in transient Ca^{2+}current [8].

An enhanced intracellular sodium transient was caused by the positive ionotropic effects of digitalis and its derivatives improved intracellular calcium, therefore, increases myocardial contractility [10]. Membrane bound Na^+ and K^+ activated adenosine triphosphatase inhibited by cardiac glycoside.This enzyme provides the energy for sodium to force the system in the sarcolemma of cardiac fibres by hydrolysis of adenosine triphosphate that actively extrudes sodium and passage of potassium into the fibres. It attached to the Na^+, K^+ and ATPase, inhibits its enzymatic activity and impairs the sodium and potassium's active transport. Na^+, K^+ and ATPase inhibition stimulated by*Aloe vera* extractthat produce positive ionotropic effects similar to cardiac glycosides.

The active ingredients in Aloe vera gel are vitamins, minerals, saponins (glycoside), amino acids and enzymes [9].We areunable to identify the exact ingredients which are accountable for the positive inotropic and negative chronotropic effect on isolated heart with this initial study.Moreover, *Aloe vera* juice and gel consists of cardiac glycoside properties through the phytochemical analysis by the presents of brown ring interface which indicates deoxysugarcharacteristics of cardenolides. Besides that, component of *Aloe vera* proved to have antioxidant activity which is used to prevent heart disease and protect the cardiovascular system [1].

The result obtained reveals that therapeutic efficacy of *Aloe vera* extract is similar to digoxin. The *Aloe vera* extract, which have been found to poses exact cardiac glycoside activity similar as digoxin, having wide margin of safety compare to digoxin.

CONCLUSION

The ethanol extraction of *Aloe vera*exhibits positive inotropic and negative chronotropic activity. Furthermore, the mechanism of action of the extract is evaluated as the inhibition of Na^+, K^+ and ATPase is similar to the action of cardiac glycosides and these might be due to the presence of cardiac glycoside properties in the *Aloe vera* plant. In future, the major compound of the extract may be isolated for further studies.

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