International Journal of Medical and Health Research
ISSN: 2454-9142
Received: 16-11-2020; Accepted: 30-11-2020; Published: 16-12-2020
www.medicalsciencejournal.com
Volume 6; Issue 12; 2020; Page No. 75-79

Effect of *Zingiber officinale* roscoe on myocardial ischemic-reperfusion injury

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Abstract
Cardiovascular disease is the leading cause of death around the world. Of all the spectra of cardiovascular disease, myocardial infarction (MI) contributes the biggest proportion of mortality. MI results when perfusion of the myocardium is interrupted, thus, can only be treated by reperfusion therapy, a strategy that may cause ischemic reperfusion injury. This injury currently lacks therapy; therefore, there is need for more investigation to devise its preventive measures. Twenty five male Sprague dawley rats were divided into 4 groups (N = 5). Three of these were treated with varying doses of *Zingiber officinale* extract and one was treated with vehicle for 30 days. Ischemia and reperfusion (I &R) was performed on Langendorf perfused hearts and contractile function was measured during the pre-ischemic and post ischemic periods. These readings were then compared to assess the degree of myocardial recovery after I & R. Serum catalase activity was also determined. Groups were compared using ANOVA (α=0.05) There were statistically significant differences between groups in left ventricular developed pressure (P= 0.04), maximum rate of contraction (P=0.036), maximum rate of relaxation (P=0.04) and heart rate (p=0.041). There was also statistically significant difference between groups in serum catalase activity (p=0.032). *Zingiber officinale* may be having protective effects against ischemic reperfusion injury (IRI). This effect was demonstrated in the current study by improved recovery of left ventricular contractile function following myocardial ischemic-reperfusion injury in the treated groups. The study also depicts that *Zingiber officinale* exerts antioxidant effect by increasing catalase activity.

Keywords: *Zingiber officinale*, myocardial infarction, ischemic reperfusion injury

Introduction
Cardiovascular disease is the leading cause of death worldwide. It is estimated to cause fifteen million deaths annually translating to 31 % of global mortality [1]. Among the types of cardiovascular disease, ischemic heart disease contributes to the biggest proportion of deaths (13%) [1]. Myocardial infarction is the main spectrum of ischemic heart disease that contributes to this mortality, as it is estimated to account for 20% of global deaths [2]. In sub-Saharan Africa, cardiovascular disease accounts for 9.2% of total deaths and is the leading cause of death among the elderly [3]. Further, this disease contributes to 7-10% of all adults admitted to hospital in this region [4]. Eighty percent of global deaths attributed to cardiovascular disease occur in low and middle income countries [4]. In Kenya, ischemic heart disease account for 25% of all admissions and 13% of deaths with the biggest contributor being myocardial infarction [5].

Myocardial infarction results from an imbalance between myocardial oxygen demand and supply. When demand exceeds supply, ischemic necrosis of the myocardium may ensue [6]. Etiology of myocardial infarction is multifactorial but the most prevalent causal factor is occlusion of the coronary artery which leads to interruption of perfusion to the myocardium [7]. When such happens, reperfusion therapy is the only treatment option that can be employed. This, can be achieved either through per cutaneous coronary intervention (PCI) or use of thrombolytic agents [8]. However, reperfusion therapy can cause reperfusion injury which inflicts additional damage to the infarcted myocardium. This injury has contributed immensely to morbidity among the survivors of myocardial infarction and has significantly negated the benefits of reperfusion therapy [9]. Up to date, there are no curative measures of ischemic reperfusion injury that have been documented. Therefore, it calls for more investigation to come up with treatment options for this condition, as this will positively influence the outcome of reperfusion therapy.

Medicinal plants like *Zingiber officinale* (ginger) have been used since time immemorial for treatment or prevention of many diseases including cardiovascular ailments [10]. However, the usefulness of these herbal preparations often lack a backing from scientific evidence [11]. Though empirical data exists on the medicinal value of *Zingiber officinale* in management of many conditions, there is paucity of data on its proven therapeutic or preventive efficacy on myocardial ischemic reperfusion injury. The purpose of this study was to determine the effect of *Zingiber officinale* on ventricular contractile function in the setting of myocardial ischemic reperfusion injury

Materials and methods
Study site and design
The current study was carried out in the department of medical physiology of the University of Nairobi. A laboratory based true experimental study design was employed.

Preparation of *Zingiber officinale* extract
Zingiber officinale rhizomes were purchased from a local

farmer and wrapped in a paper foil in order to maintain freshness during the pre-extraction period. Botanical description and methanol extraction was done at the institution's departments of botany and pharmacognosy respectively. To carry out the extraction, fresh *Zingiber officinale* rhizomes were washed with water, peeled and cut into small pieces. Extraction of *Zingiber officinale* was done by soaking the rhizome in 70% methanol over 48 hours. The mixture was then filtered twice; first using a filter cloth and finally using a filter paper and the methanol in the filtrate was evaporated using rotary evaporator. The remaining brown extract was then weighed and dissolved in 5% Dimethyl sulfoxide (DMSO). This solution was stored at 4^0C, ready for administration to the experimental animals [12].

Experimental animals

Sprague dawley rats were obtained from the institution's breeding facility. They were kept in appropriate rodent cages and had free access to approved food pellets and water *ad libitum*. Ethical conduct was observed at all times during the course of this study. FELASA guidelines were adhered to as the principles of Replacement, Reduction and Refinement were observed [12]. Ethical approval was sought from the institution's animal use ethics committee. Twenty male Sprague dawley rats weighing between 250-300 grams were randomized into 4 groups (N=5). They were then treated through oral gavage daily at 9.00 AM for 30 days as follows:

1. **Negative control:** This received 2ml of 5% DMSO (vehicle)
2. **Low dose ginger:** This received 50mg/kg of *Zingiber officinale* extract
3. **Medium dose ginger:** This received 250mg/kg of *Zingiber officinale* extract
4. **High dose ginger:** This received 500mg/kg of *Zingiber officinale* extract

Experimental protocol

On the 31th day, animals were administered with 7.5mg/kg of unfractionated heparin and szzacrificed by using intraperitoneal injection of ketamine (100mg/kg) [13]. Hearts were then harvested by making an incision on tshe anterior abdominal wall and then advancing to the thoracic cavity by excising the diaphragm. The sternum was then dissected from the xiphoid process upwards while care was observed not to damage the aorta. The aorta was then identified, held at the root and cut at a point just proximal the aortic arch. With the secured aorta, the entire heart was then excised out of the thoracic cavity and mounted by the aorta on the Langedorff isolated heart perfusion apparatus, to provide retrograde perfusion using Krebs Henseleit buffer solution. The perfusate flow rate was set at 8ml/min. A barometer was inserted into the left ventricle through the mitral valve to measure changes in the left ventricular pressure. After 15 minutes, perfusion was stopped for 30 minutes to induce global myocardial ischemia and thereafter resumed for another 30 minutes. Left ventricular developed pressure (LVDP), maximum rate of contraction (dP/dt_{max}), maximum rate of relaxation(dP/dt_{min}) and heart rate (HR) were measured five minutes before induction of ischemia and 20 minutes after reinstatement of perfusion and recorded using Lab chart pro software [14] These parameters were used to assess the degree of myocardial recovery following ischemia and reperfusion by comparing the pre-ischemic and post-ischemic readings as percentage recovery as shown in the following formula:

$$\frac{\text{Post} - \text{ischemic value}}{\text{Pre} - \text{ischemic value}} \times 100$$

Catalase activity was determined using the method described by Aebi, 1984. Blood was collected from the retro-orbital plexus into plain vacutainers and centrifuged at 2000r/m for 10 minutes to obtain serum. 0.1 ml of serum was mixed with 2ml of potassium phosphate buffer (50mM; PH7.4) in a cuvette. The cuvette was placed inside a spectrophotometer and 1ml of hydrogen peroxide (20mM) was added. Absorbance was then read using a spectrophotometer at wavelength of 240nM for 1 minute at 15 minutes interval to assess the rate of hydrogen peroxide decomposition. Values were recorded as units of H_2O_2 decomposed /µL of serum /min.

Statistical analysis

Data was collected using customized data acquisition tables and entered into SPSS version 21(SPSS Inc. Chicago, IL) software. Shapiro Wilk's test and Levine's test were used to check for normality and homoscedasticity of data respectively. Groups were compared using one way ANOVA where $P < 0.05$ was considered to be statistically significant. Post hoc statistical analysis by Tukey test was performed to identify groups with significant differences. Results are presented in bar charts as Means ± SEM presented.

Results

Left ventricular developed pressure (% recovery)

There were statistically significant differences between groups in left ventricular developed pressure (LVDP) (P = 0.04). Post hoc statistical analysis with Tukey revealed that there were statistically significant differences between high dose group and negative control (P = 0.044). Results for Left ventricular developed pressure % recovery are shown in figure 1.

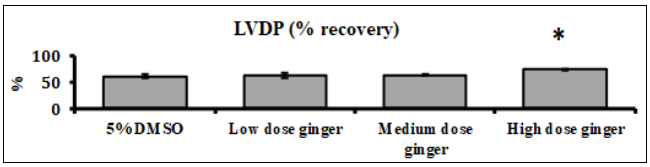

Fig 1: LVDP % recovery; P=0.04

Heart rate

There were statistically significant differences between groups in heart rate (P= 0.041). Post hoc statistical analysis with Tukey showed that there were statistically significant differences between high dose group and negative control (P = 0.028). Results and dose response curve for heart rate recovery are shown in figure 2.

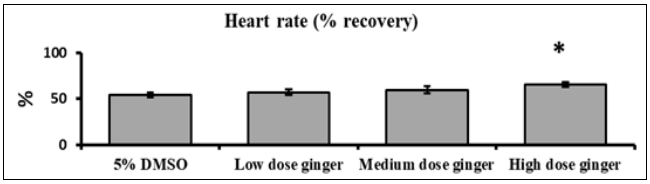

Fig 2: Heart rate (% recovery); P= 0.041

Maximum rate of contraction (dP/dt_{max})

There were statistically significant differences between groups in dP/dt_{max} (P=0.036). Post hoc statistical analysis with Tukey showed that there were statistically significant differences between high dose group and negative control. Results for dP/dt_{max} are shown in figure 3.

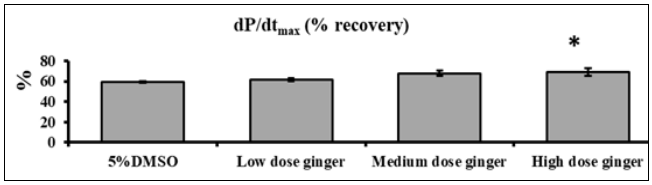

Fig 3: Maximum rate of contraction (% recovery); P= 0.036

Maximum rate of relaxation (dP/dt_{min})

There were statistically significant differences between groups in dP/dt_{min}. Post hoc statistical analysis with Tukey showed that there were statistically significant differences between high dose group and negative control. Results and the dose response curve for dP/dt_{min} recovery are shown in figure 4.

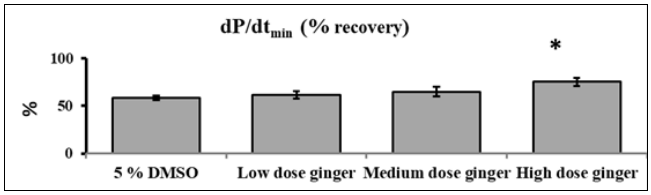

Fig 4: Maximum rate of relaxation (dP/dt_{min}) (% recovery): P = 0.04

Serum catalase activity

There were statistically significant differences between the groups in serum catalase activity (P =0.032). Post hoc statistical analysis with Tukey revealed that there were differences between high dose and negative control groups. Results for serum catalase activity are presented in figure 13. The catalase activity increased in a dose dependent manner as shown in the dose response curve is depicted in figure 14

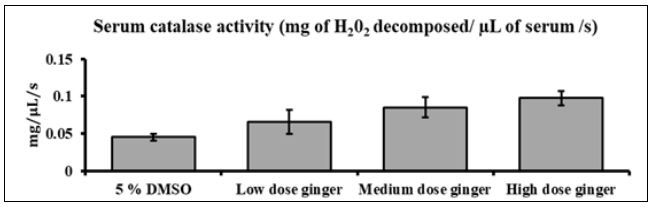

Fig 5: Serum catalase activity; P = 0.032

Discussion

In the current study, methanol extract of *Zingiber officinale* possessed a protective effect against myocardial IRI as demonstrated by a statistically significant recovery of the left ventricular function in the treated groups. There were statistically significant differences between different groups in percentage recovery of Left Ventricular Developed Pressure (LVDP) (P= 0.040), heart rate (HR) (P=0.041), maximum rate of contraction (dP/dt_{max}) (P= 0.036) and maximum rate of relaxation (dP/dt_{min}) (P=0.047). Preservation of LVDP, and dP/dt_{max} in the high dose *Zingiber officinale* treated group could mean that this group had more functional myocytes suggesting the potential prophylaxis against myocardial apoptosis and necrosis during ischemic reperfusion injury [15]. In addition, high dose of *Zingiber officinale* may have prevented the occurrence of myocardial stunning, a pathology which would have impacted negatively on the myocardial contractile function [16]. It is probable that the extract exerted these prophylactic actions by curbing accumulation of reactive oxygen species (ROS). This is because ROS are thought to cause apoptosis, necrosis and myocardial stunning during myocardial IRI [17]. Better recovery of the dP/dt_{min} could imply that the cellular elements needed for myocardial relaxation were enhanced. For instance, increasing the action of Sarcoplasmic Reticulum-Calcium ATPase (SERCA) facilitates faster and more efficient re-uptake of calcium into the sarcoplasmic reticulum. This optimizes the ventricular diastolic function [18]. Phospholamban, in its phosphorylated state increases the activity of SERCA. This protein is a potential target of destruction by oxidative stress during ischemic reperfusion injury. Therefore, treatment with high dose of *Zingiber officinale* could have prevented this oxidative damage of phospholamban, leading to better SERCA activity and consequently improved dP/dt_{min} in this group [19] [20] [21]. Preservation of heart rate in the high dose *Zingiber officinale* treated group meant that the integrity of sinus node was maintained [22]. ROS generated during the ischemic reperfusion injury may inappropriately inactivate ion channels [23]. If slow sodium channels in the pacemaker cells are inactivated, rate of phase 4 depolarization of the pacemaker potential will be reduced, leading to a small number of action potential generated per unit time, and subsequently a reduction in heart rate [24]. High dose of *Zingiber officinale* may have dampened the oxidative inactivation of these sodium channels in the pacemaker cells, a factor that could explain the higher heart rate in the group that was treated with this dose.

These effects of *Zingiber officinale* on left ventricular contractile function in the setting of ischemic reperfusion injury, to the best of our knowledge, have never been described in past studies. However, *Zingiber officinale* has been documented to cause significant reduction of cardiac biomarkers when cardiotoxicity is induced in animal models [25]. Troponins and creatinine kinase are myocardial biomarkers that are inappropriately released into the circulation following myocardial injury and their plasma concentration is directly proportional to the magnitude of cellular damage [26]. The utility of elevated plasma cardiac markers as a surrogate measure of myocardial injury cannot be disputed. Indeed its usefulness in clinical practice is immense owing to the non-invasive nature of its measurement [27]. However, it does not entirely eliminate the need to study the ventricular contractile function as that is a true measure of heart function [28]. The current study focused on the assessment of ventricular contractile activity and hence sheds additional light in that *Zingiber officinale* may also preserve ventricular contractility after myocardial ischemic reperfusion injury just as it prevents post ischemic elevation of cardiac biomarkers as shown by past investigations.

The role of oxidative stress in the pathogenesis of ischemic reperfusion injury has been emphasized in past studies [29]. Therefore, it can be deduced that the possible mechanism at which *Zingiber officinale* exerted its cardio-protective effect in the current study is by reducing the levels of reactive

oxygen species that are responsible for aggravating this injury. As in the current study, a past study reported the antioxidant effect of *Zingiber officinale* where it significantly increased the activities of superoxide dismutase and catalase in rats [25]. The role of these two enzymatic antioxidants in pathogenesis of ischemic reperfusion injury has been clearly described. They are highly responsible for the protection from the oxidative damage of the myocardium during this injury possibly due to their ability to scavenge for reactive oxygen species [30]. Furthermore, flavonoids have been isolated from *Zingiber officinale* in some studies in the past [31]. Flavonoids are polyphenols with potent non enzymatic anti-oxidant activity [32]. Therefore, the observed cardio-protective effect of *Zingiber officinale* could be partly ascribed to the antioxidant activity of the contained flavonoids.

Conclusion and recommendations

The current study has demonstrated that methanol extract of *Zingiber officinale* may be having protective effects against IRI in rats. This effect was demonstrated by preservation of left ventricular function following myocardial ischemic-reperfusion injury since the left ventricular pressure dynamics swere improved in treatment group than negative control. The study also depicts that *Zingiber officinale* exerts antioxidant effect by increasing catalase activity. The findings of the current hence give insight on the way forward in terms of development of treatment or preventive options for myocardial ischemic reperfusion injury. The authors recommend further investigations to; identify the exact phytochemical components of *Zingiber officinale* that confers these effects, and to determine the mechanisms by which this plant increases catalase activity.

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