The freeze-dried extracts of *Salvia coccinea* Juss. Ex Murray attenuate myocardial ischemia

reperfusion injury in a global ischemia Rat model

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Abstract

Background: Ischemia reperfusion injury is the leading cause of myocardial cell death in Ischemic Heart Disease. Thus intensive research efforts are geared at discovering pharmacological approaches that prevent it. Over twenty species from the genus *Salvia* are widely applied in traditional Chinese medicine in the management of heart diseases with *Salvia miltiorrhiza* (Danshen) being a canonical example. Our study aimed to investigate the cardio-protective effects of the freeze-dried extracts of *salvia coccinea* against ischemia reperfusion injury in a rodent *in-vitro* model of global ischemia.

Methods: Forty two (42) Sprague Dawley rats were randomly assigned into five groups: positive control (Glucosamine 1000mg/kg), negative control group (Krebs Henseleit buffer), low dose test (50 mg/100ml), medium dose test (100 mg/100ml), and high dose test (200 mg/100ml).

The cardio-protective effects of the different treatments were evaluated in a global ischemia model using isolated rat hearts mounted on a Langendorff system.

Naloxone 2.2 µmol/L (µ opioid receptor blocker), and theophylline 1000 µmol/L (non-specific adenosine receptor blocker) were co-administered with 50 mg of *S.coccinea* in the mechanism of action experiments.

The following indices of cardiac function were recorded pre- and post- ischemia: left ventricular developed pressure (LVDP), heart rate, and maximum rate of contraction and relaxation. All data were expressed as Mean ± Standard Error of Mean and analyzed using one-way ANOVA and Tukey post-hoc tests. Significance was set at $p < 0.05$.

Results: The freeze-dried extracts of *S. coccinea* had significant effects on post-ischemic contractile function recovery in the early [51.4 ± 9.7% (low dose test) vs. 14.9 ± 3.3% (medium

dose test) vs. 12.7 ± 2.6% (high dose test) vs. 13.7 ± 5.7% (negative control): p<0.05] and late

[38.6 ± 8.9% (low dose test) vs. 22.0± 7.1% (medium dose test) vs. 14.6 ± 5.8 (high dose test) vs.

12.5 ± 4.2% (negative control): p< 0.05]. Reperfusion phases with the highest LVDP recovery

were observed at the 50 mg dosage level.

The freeze-dried extracts of *S. coccinea* had significant negative chronotropic effects on heart rate

[234.0 ± 2.4 beats/min to 90.0 ± 7.0 beats/min, 50 mg vs. 102.0 ± 13.9 beats/min to 135.0 ± 25.9

beats/min, control P<0.05].

The cardioprotective effects of *S. coccinea* displayed an inverted U-shaped dose-response curve

with low dose stimulation and high dose inhibition.

Naloxone completely abolished the LVDP recovery afforded by the freeze-dried extracts of *S.*

coccinea at the 50 mg dosage level while adenosine only partly abolished the LVDP recovery (9.5

± 3.2% (naloxone) *vs.* 15.5 ± 5.8% (adenosine): P>0.05).

Conclusion: The freeze-dried extracts of *S. coccinea* possessed significant cardioprotective effects

which appear to be mediated by activation of the opioidergic pathway in the heart.

Key Words: Ischemia, Reperfusion Injury, Cardio-protection, Opioid Pathway, *S. coccinea*

1.0 Introduction

Cardiovascular diseases are the leading cause of death globally with a 31% annual death rate (1).

Ischemic heart disease (IHD) is responsible for more than 40% of these cardiovascular deaths and

thus poses a major global health problem(2).

The clinical manifestations of IHD result from subjection of the heart to ischemia-reperfusion

injury which leads to myocardial injury, cardiac dysfunction, arrhythmias, heart failure and

ultimately death (3).

Cardioprotection aims to prevent the death of cells during acute injury and may be achieved

through myocardial or pharmacological conditioning carried out pre- or post- ischemia (4).

Salvia species are used widely in Traditional Chinese Medicine (TCM) as Danshen to manage

cardiac conditions. *Salvia coccinea* has been used since the 17th century by the Chinese who refer

to it as "cure all" or "sage the savior'' for the management of common maladies (5). In South

America a boiled mixture is used to bathe varicosities and treat blood clots (6). The aqueous and

alcohol extracts of *Salvia coccinea* contain potent phenolic antioxidants (7). The present study

aimed to investigate the cardioprotective effects of the freeze-dried extract of *S. coccinea* against

ischemia reperfusion injury and to determine its mode of action.

2.0 Materials and methods

2.1 Plant material and freeze dried extract preparation

The specimen of *Salvia coccinea* was harvested from Naivasha County, Kenya, and its identity

confirmed by resident taxonomists of the herbarium situated at the Department of Botany, School

of Biological sciences, University of Nairobi.

The whole plant was used in the study. The plant parts were cut into small pieces and air dried for

one week. The dried plant was milled using a standard mill and the resulting powder macerated in

cold distilled water in a ratio of 1:10 (weight: volume) with vigorous shaking for an hour. The

suspension obtained was filtered consecutively using cotton wool and filter paper (Whatman's^{TM}).

The filtrates were frozen and lyophilized and the resultant freeze-dried extracts weighed and placed

in sample containers. These were stored in a standard laboratory freezer (Hotpoint™). During each

procedure, the freeze- dried extracts were reconstituted to form the required concentrations.

2.2 Experimental Animals

Forty-two (42) six month old adult male Sprague Dawley rats, weighing 300-400 g were used. The

animals were housed in standard cages containing five rats in each. The experiments were

performed in the animal house at the Department of Medical Physiology, University of Nairobi.

The following conditions were maintained in the animal house: constant room temperature (23 +/-

2°C) and 30-70% relative humidity with a 12-hr. light/day cycle. The animals were provided with

standard rat chow pellets and tap water ad libitum. The protocol was approved by the Biosafety,

Animal Use and Ethics Committee, Faculty of Veterinary Medicine, University of Nairobi. Ref.

Number: (FVM BAUEC/2016/113). All experiments were based on internationally accepted

directions for the use and care of laboratory animals as stipulated by the FELASA guidelines (8).

2.3 Drugs

Naloxone HCL (Sanofi Ltd.), Theophylline (GlaxoSmithKline Ltd.), Glucosamine (Power Health

Ltd.), and Heparin (Rotex Medica Ltd.) were obtained from Dischem Pharmacy, Nairobi. Sodium

pentobarbital (Bayer Ltd.) was obtained from Lesukut Ltd. NaCI, KCl, KH_2PO4, $MgSO_4$,

$NaHCO_3$, $CaCl_2$, and glucose (LOBA Ltd.) were obtained from Chem-labs, Nairobi. All drugs

were freshly prepared in normal saline on the day of the experiment.

2.4 Isolated heart preparation

Each rat was anaesthetized with sodium pentobarbital 6% (40 mg/kg) intraperitoneally. There-after heparin (500 u/kg) was administered intravenously immediately the rat lost its righting reflex. The hearts were excised through a trans-abdominal incision and immediately immersed in ice-cold Krebs' Henseleit (KH) buffer solution. A plastic cannula was inserted through the aorta, clamped with a blunt artery clip and retrograde perfusion with Krebs Henseleit buffer (10 ml/min) initiated.

2.5 Measurement of isovolumic cardiac performance

A latex balloon connected to a pressure transducer was inserted into the left ventricle via the mitral valve and inflated to increase the diastolic pressure by regulating the volume of saline in it. The pressure transducer was connected to a power lab (AD Instruments, Colorado Springs, CO, USA) recording system. The following cardiac indices were then measured and averaged from twelve (12) beats: Left ventricular developed pressure (LVDP), maximum rate of contraction ($+dp/dt_{max}$), maximum rate of relaxation ($-dp/dt_{min}$), and the heart rate.

2.6 Cardioprotection experiment

The hearts were equilibrated for twenty (20) minutes and subjected to thirty (30) minutes global ischemia by halting perfusion and immersing the hearts in a KH buffer filled organ bath. The hearts were divided into five (5) groups and on reperfusion treated (Table 1).

Table 1: Heart treatment during reperfusion

TREATMENT STEPS Time (minutes)	EXPERIMENTAL GROUPS				
	POSITIVE CONTROL	**CONTROL**	**50 mg**	**100 mg**	**200 mg**
15	Equilibration	Equilibration	Equilibration	Equilibration	Equilibration
30	Global Ischemia	Global Ischemia	Global Ischemia	Global Ischemia	Global Ischemia
90	Reperfusion with KH buffer + Glucosamine	Reperfusion with KH buffer containing *Salvia coccinea* extract (50 mg per liter)	Reperfusion with KH buffer containing *Salvia coccinea* extract (100 mg per liter)	Reperfusion with KH buffer containing *Salvia coccinea* extract (200 mg per liter)	Reperfusion with plain KH buffer

130 *2.7 Mechanism of action experiments*

131 Theophylline and Naloxone, which are non-selective adenosine and opioid receptor blockers

132 respectively, were used to determine the signaling pathway of cardioprotection by *Salvia coccinea*.

133 These blockers were administered for 20 minutes at the onset of reperfusion before treatment with

134 *Salvia coccinea* extracts (Table 2).

Table 2: Heart treatment during reperfusion

TREATMENT STEPS Time (minutes)	EXPERIMENTAL GROUPS				
	POSITIVE CONTROL	**CONTROL**	**50 mg**	**100 mg**	**200 mg**
15	Equilibration	Equilibration	Equilibration	Equilibration	Equilibration
30	Global Ischemia	Global Ischemia	Global Ischemia	Global Ischemia	Global Ischemia
90	Reperfusion with KH buffer + Glucosamine	Reperfusion with KH buffer containing *Salvia coccinea* extract (50 mg per liter)	Reperfusion with KH buffer containing *Salvia coccinea* extract (100 mg per liter)	Reperfusion with KH buffer containing *Salvia coccinea* extract (200 mg per liter)	Reperfusion with plain KH buffer

2.9 Data and statistical analyses

The experimental data were expressed as the mean +/-SEM. The data were analyzed using One-

way ANOVA with post- hoc statistical analysis being performed using Tukey HSD in cases of

significance which was set at p<0.05. The Kruskall Wallis non-parametric with Dunns' post- hoc

statistical test in cases of significance (p<0.05) was used to analyze the extent of the histological

damage. GraphPad Prism 6 suite of statistical software was used for carrying out the statistical

tests.

3.0 Results

3.1 Effects on cardiac function

3.1.1 Baseline cardiac function

There were no significant differences in LVDP between the groups during equilibration [43.8 ±

2.5 mmHg (positive control) vs. 40.9 ± 7.5 mmHg (negative control) vs. 51.1 ± 1.4 mmHg (low

dose test) vs. 54.8 ± 1.3 mmHg (medium dose test) vs. 40.6 ± 2.6 mmHg (high dose test); P=0.05].

The graphical representation of the equilibration LVDP values is shown in Figure 1.

Figure 1. Equilibration Left Ventricular Developed Pressure (LVDP) for each group. PC

(positive control), NC (Negative control).

3.1.2 Effects of S. coccinea extracts on the left ventricular developed pressure (LVDP)

The freeze-dried extracts of *Salvia coccinea* had significant effects on the LVDP recovery both in

the early [51.4 ± 9.7% (low dose test) vs. 14.9 ± 3.3% (medium dose test) vs. 12.7 ± 2.6% (high

dose test) vs. 13.7 ± 5.7% (negative control): p<0.05] and late [38.6 ± 8.9% (low dose test) vs.

22.0± 7.1% (medium dose test) vs. 14.6 ± 5.8 (high dose test) vs. 12.5 ± 4.2% (negative control):

p< 0.05] phases of reperfusion. Post-hoc statistical analysis using Tukey's multiple comparison

test showed that the low dose (50 mg) had a significantly higher cardioprotective activity in the

early phase compared to the medium dose (100 mg), high dose (200 mg) and negative control

(KH) groups (p<0.05). The 50 mg dose also had significant cardioprotective activity in the late

phase compared to the KH and 200 mg group (p<0.01). A graphical representation of the LVDP

percentage recovery is shown in Figure 2.

Figure 2. Post ischemic Left Ventricular Developed Pressure A) Percentage early recovery

after post ischemic post conditioning with *S. coccinea*. B) Percentage late recovery after post

ischemic post conditioning with *S. coccinea*. LVDP (Left Ventricular Developed Pressure), PC

(positive control), NC (Negative control). KEY: *= $P \leq 0.05$; **$P \leq 0.01$

3.1.3 Effects of the S. coccinea extracts on the maximum rate of contraction (+dp/dt max)

The freeze-dried extracts of *S.coccinea* had a significant effect on the recovery of the $+dp/dt_{max}$

in the early [78.2 ± 3.8% (low dose test) vs. 18.7 ± 3.9% (medium dose test) vs. 37.9 ± 8.1%

(negative control) vs. 41.4 ± 8.9% (high dose test): P<0.05] and late phases [(69.8 ± 6.9% (low

dose test) vs. 43.5 ± 6.6% (medium dose test) vs. 40.2 ± 14.6% (high dose test) vs. 44.8 ± 2.5%

(negative control): p<0.05] of reperfusion. Post-hoc statistical analysis using Tukey's multiple

comparison test showed that the extract at 50 mg dose had significant cardioprotective activity in

the early phase compared to the glucosamine group (P < 0.05) and 100 mg group (P<0.01). A

graphical representation of the $+dp/dt_{max}$ percentage recovery is shown in Figure 3.

Figure 3. Post ischemic calculated maximum rate of contraction. A) Equilibration values B)

Percentage early recovery after treatment with *s. coccinea* C) Late recovery after treatment with *s.*

coccinea. LVDP (Left Ventricular Developed Pressure), PC (positive control), NC (Negative

control).KEY: *=P $\leq$ 0.05; **=P $\leq$ 0.01.

3.1.4 Effects of the S. coccinea extracts on the maximum rate of relaxation (-dp/dtmin)

The freeze-dried extracts of *Salvia coccinea* caused a significant -dp/dt $_{min}$ recovery in the early

[131.6 $\pm$ 8.3 (low dose test) vs. 45.6 $\pm$ 5.3% (medium dose test) vs. 55.2 $\pm$ 13.1% (high dose test)

vs. 83.8 $\pm$ 11.8% (negative control): P<0.05] and late phases [149.0 $\pm$ 23.7% (low dose test) vs.

97.4 $\pm$ 10.8% (medium dose test) vs. 78.0 $\pm$ 31.0% (high dose test) vs. 113.6 $\pm$ 4.6% (negative

control): P<0.05] of reperfusion. Post-hoc statistical analysis using Tukey's multiple comparison

test showed that the low dose test (50 mg) possessed significant cardioprotective activity in the

early phase compared to the negative control (KH) group (P <0.01), the medium dose test (100

mg) group (P<0.001), the high dose test (200 mg) group (p<0.05) and the test positive control

(glucosamine) group (P<0.01). The low dose also had significant cardioprotective activity in the

late phase compared to the negative control (P <0.05), the high dose test (p<0.01), the medium

dose test (P<0.05) and the positive control (P < 0.01) A graphical representation of the   -dp/dt

$_{min}$ percentage recovery is shown in Figure 4.

Figure 4. Post ischemic calculated maximum rate of relaxation. A) Equilibration values B)

Percentage early recovery after treatment with *S. coccinea* C) percentage late recovery after

treatment with *S. coccinea*. LVDP (Left Ventricular Developed Pressure), PC (positive control),

NC (Negative control).KEY: *=P$\leq$0.05; **=P$\leq$0.01; ***=P$\leq$ 0.001**.**

3.1.5 Trends of recovery in the early and late phases of reperfusion

The trend of recovery was obtained by plotting the ratios of the percentage recovery in both phases

for each of the cardiac indices to determine the effectiveness of *Salvia coccinea* in early and late

reperfusion. The low dose test group had the highest recovery rate in both phases. The graphical

representation of these trends is shown in figure 5, 6 and 7.

Figure 5. Trends of LVDP recovery in the early and late reperfusion phases.

Figure 6. Percentage +dp/dt $_{max}$ recovery in the early and late reperfusion phases.

Figure 7. Trends of -dp/dt $_{min}$ recovery in the early and late reperfusion phases.

3.1.6 Effects of the freeze-dried extracts of S. coccinea on heart rate

The freeze-dried extracts of *S.coccinea* caused a significant decrease in heart rate [234.0 ± 2.4

beats/min (low dose test) vs. 258.0 ± 11.5 beats/min (medium dose test) vs. 174.0 ± 32.6 beats/min

(high dose test)] in the early [90.0 ± 7.0 beats/min (low dose test) vs. 98.0 ± 2.0 beats/min (medium

dose test) vs. 107.5 ± 7.5 beats/min (high dose test): $P<0.05$] and late phases [106.0 ± 6.7 beats/min

(low dose test) vs. 84.0 ± 10.7 beats/min (medium dose test) vs. 117.5 ± 14.3 beats/min (high dose

test): $P<0.05$] of reperfusion. The heart rate in the negative and the positive control groups

increased from the equilibration values [150.0±13.7 beats/min (positive control) vs. 102.0 ± 13.9

beats/min (negative control)] in the early [146.0 ± 19.3 beats/min (positive control) vs. 135.0 ±

25.9 beats/min (negative control)] and late phases [175.0 ± 30.9 beats/min (positive control) vs.

205.0 ± 41.1 beats/min (negative control)] of reperfusion. A graphical representation of the heart

rates in the equilibration, and reperfusion phases is shown in Figure 8.

Figure 8. Heart rate; A) Equilibration heart rate values B) Early reperfusion phase heart rate C)

Late reperfusion phase heart rate. LVDP (Left Ventricular Developed Pressure), PC (positive

control), NC (Negative control)

3.3 Mechanism of action experiments

Co-administration of naloxone along the low dose test (50 mg) led to decreased $+dp/dt_{max}$ recovery compared to treatment with 50 mg *S. coccinea* alone [0.09570±0.03294 (naloxone) vs.0.3868±0.1090(low dose test): P= 0.0338]. Similarly, naloxone led to a lower $-dp/dt_{min}$ recovery compared to the 50 mg dose [0.7430±0.06739 (naloxone) vs.1.686±0.2693 (low dose test): P= 0.0179].The graphical representation for the late recovery of the assessed indices of cardiac function when blockers were used is shown in Figure 9.

Figure 9. Indices of cardiac function. A) % recovery of Left ventricular developed pressure B) % recovery of maximum rate of contraction C) % recovery of maximum rate of relaxation. LVDP (Left Ventricular Developed Pressure), PC (positive control), NC (Negative control) KEY: * = P<0.05.

3.4 Dose-response analysis

The dose response curve for the cardioprotective effects of *S. coccinea* displayed a biphasic U-shaped pattern. As the dose was increased, the percentage recovery decreased up to a minimum point [x=153.2: y=1.0 (early phase) vs. x=173.97: y=12.49 (late phase)] beyond which the percentage recovery started to increase as shown in Figure 10.

Figure 10. Dose response curve showing the percentage LVDP early reperfusion phase recovery after pharmacological post conditioning with the aqueous freeze dried extracts of *S. coccinea* following global ischemia.

4.0 Discussion

Ischemic heart disease (IHD) is among the leading causes of death and disability worldwide. Myocardial infarction induced by Ischemia/reperfusion (I/R) injury is one of the most severe manifestation of IHD, which makes its attenuation critical. The treatment of choice for myocardial ischemic injury is prompt and efficient reperfusion which paradoxically induces further cardiomyocyte damage known as reperfusion injury (9). Cardioprotection through pharmacological therapy in addition to early reperfusion is needed to further reduce the infarct size and reduce the mortality (10). Despite the burden caused by I/R injury there is still no effective therapy for preventing reperfusion injury which necessitates the search for more potent therapies (11).

Several salvia species are used as Danshen to treat heart diseases (5). However, the cardioprotective effects of *S. coccinea* and its possible mechanism of action have not yet been investigated.

Treatment with *S. coccinea* extract led to a significant dose-dependent recovery of the LVDP and +dp/dt $_{max}$ after 30 minutes of global ischemia, with the highest recovery recorded at the low dose test (50 mg). Since LVDP and +dp/dt $_{max}$ are measures of systolic function, this finding indicates that *S. coccinea* has the ability to prevent the systolic dysfunction that arises during I/R injury impairing the heart's ability to build enough pressure at the required rate to pump blood commensurate to the body's demands (12). These findings are in concurrence with those of a study that investigated the cardioprotective effects of another Salvia species: *S. miltiorhiza* (5). It is noteworthy that the percentage recovery in this study was higher compared to other cardioprotective herbal remedies (13).

The *S. coccinea* extract was shown to preserve the post-ischemic diastolic function in a dose

dependent manner. The hearts treated with the herb had a higher percentage -dp/dt $_{min}$ recovery

compared to the control group hearts. Myocardial ischemia precipitates the development of

diastolic dysfunction characterized by delayed relaxation, impaired filling and left ventricular

stiffening (14). This finding is consistent with a previous study on other salvia species which

preserved diastolic function (13). The finding was in contrast with other studies in which the -dp/dt

$_{min}$ recovered to a lower level than the equilibration value (15). Accordingly, the aqueous extract

of *S. coccinea* may be more potent in abating the diastolic dysfunction that arises in I/R injury.

The *S. coccinea* extracts led to a significant decrease in heart rate at all doses in both the early and

late phases of reperfusion in contrast to the control groups in which the heart rates were increased.

Reperfusion following ischemia induces tachycardia which initiates life threatening ventricular

arrhythmias (16). Low heart rates post-ischemia have thus been shown to be protective (17). These

previous studies suggest that the heart rate lowering effects of *S. coccinea* might have been

protective to the injured myocardium. This finding was however contradictory to *S. miltiorhiza*

and its isolated components where the heart rate remained constant with no significant decrease

(18).

This study also sought to assess the degree of cardioprotection by *S. coccinea* in the early and late

phases of reperfusion. This is because most cardioprotective agents show potency only in the early

phase (19). The events occurring in the early and late reperfusion phases differ and the

cardioprotective potency of an agent in both phases depends on its ability to ameliorate the

injurious events in both phases (20). Lethal reperfusion injury in the early phase is due to necrosis

while that in the late phase is attributed to apoptosis (21). It is interesting to note that the

cardioprotective effects of the *S. coccinea* extract were highly maintained in the early and late

phases of reperfusion, a clear indication that the aqueous extracts may have the potential to prevent

both necrosis and apoptosis.

S. coccinea was most potent at the 50 mg dose with the 100 mg and 200 mg providing

cardioprotection to a lesser degree as indicated by the lower recovery of the LVDP, dp/dt $_{max}$ and

dp/dt $_{min}$ in early and late reperfusion. This finding corroborates a study that investigated the

cardioprotective effects of *S. miltiorhiza* where a lower dose, less than 100 mg was suggested as

the 100 mg dose caused stiffening of the left ventricle and consequently diastolic dysfunction (13).

The dose response analysis of *S.coccinea* generated an inverted dose response curve with

characterized by low dose inhibition and high dose stimulation of cardioprotection as opposed to

the monophasic S-shape favored in pharmacological analysis (22). The probable molecular

explanation for the inverted dose - response curve is biased agonism, a property of the ligand-

receptor complex where a ligand favors one signaling pathway over another. β-arrestins usually

associated with desensitization of the GPCRs, are involved in signaling (23) and have been shown

to stimulate signal transduction pathways including MAPK, PKC, PI3K (24). Biochemical data

shows that signaling mediated by β arrestins and G proteins have very distinct physiological

consequences (25).

Pretreatment with naloxone at the onset of reperfusion completely abolished the cardioprotective

effects of *S. coccinea*. This indicates that the cardioprotection by *S. coccinea* is mediated via the

opioid pathway. Agonist binding to the opioid receptor usually inhibits adenylyl cyclase and

activates phospholipase C signaling (26). Previous studies have shown that PLC signaling leads to

the activation of the PKC and RISK kinases which are activated by salvianolic acid A and B which

are phenolic compounds commonly found in salvia species (27). It is key to note that opioid

receptors were among the first to portray biased agonism (24), a phenomenon that was observed

in the dose response analysis of *S.coccinea*.

The synthesis and release of endogenous opioids into the circulation has been shown to increase

in response to stressful stimuli including ischemia. Opioids mediate organ responses to stress by

inducing hibernation and metabolic protection and are thus vital in enhancing the ability of the

heart to withstand ischemia reperfusion injury (Akil et al., 1984). Myocardial binding studies have

shown the presence of *kappa* and *delta* opioid receptors in ventricular myocytes. The capacity of

the heart to synthesize all the three opioid peptides has been extensively verified experimentally

(Krumins et al., 1985) (28) (29).

Pretreatment with theophylline partially abolished the cardio protection indicating that the

adenosine pathway does not work in isolation to mediate the cardioprotection by *S. coccinea*.

Adenosine receptors are known to interact with opioid receptors via receptor oligomerization (30).

Adenosine is phosphorylated to AMP by adenosine kinase which is inhibited upon opioid receptor

activation leading to a rise in adenosine levels (30). Consequently, it follows that, when

theophylline blocks the adenosine receptors, only one pathway of cardioprotection is obstructed

leaving the opioid pathway operational and hence the partially abolished cardioprotection.

Preliminary photochemistry analysis of *S.coccinea* confirmed the presence of saponins, sugars,

phenols, tannins, amino groups, triterpenes, alkaloids and flavonoids (31). The pharmacological

activities of the compounds from *S. coccinea* have not yet been fully elucidated and further studies

would reveal the actual constituents that are involved in mediating the cardioprotective effects as

observed in this present study.

Further work needs to be done to identify the active component of *S. coccinea* responsible for the

cardioprotection observed. The cardioprotection experiments should also be performed in *in-vivo*

models of ischemia.

Conclusion

The freeze-dried extracts of *S. coccinea* possessed significant activity in the prevention of ischemia

reperfusion injury. These actions were probably mediated by activation of the opioidergic system

in the heart and lowering the heart rate through activation of the opioid pathway.

Competing Interests

The authors(s) declare that they have no competing interests.

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Author Contributions.

PWM, FOB and NMN designed the study and analyzed the experimental data. PWM and NMN

wrote the paper. NMN did the experimental work. All authors read and approved the final

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Left Ventricular Developed Pressure(mmHg)

A LVDP Early Recovery

B LVDP Late Recovery

Maximum Rate of Contraction (dp/dt$_{max}$)

A

Equilibriation Values

B

Early Recovery (mmHg/s)

C

Late Recovery mmHg/s

MAXIMUM RATE OF RELAXATION (mmHg/s)

A

Equilibriation Values

B   Early Recovery

C   Late Recovery

HEART RATE

A   Equilibriation

B   Early Phase

C   Late Phase

RECOVERY AFTER BLOCKER TREATMENT

A

LVDP

B   Maximum Rate of Contraction

C   Maximum Rate of Relaxation

y=0.0047x2-1.4403x+111.64
dy/dx=0.00472x-1.4403
1.4407=0.0094x
x=153.265: y=1.0052