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Original Research Article

High levels of cardiac and oxidative stress biomarkers ameliorated following treatment with aspirin, vitamin C, and *Justicia secunda* hydromethanol leaf extract on epinephrine induced-myocardial infarction in male Wistar rats

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ABSTRACT

Background: Myocardial infarction is an increasing cardiac disorder globally. This study investigated the ameliorative potentials of *Justicia secunda* hydromethanol leaf extract on epinephrine-induced myocardial infarction in male Wistar rats.
Methods: Twenty-four rats weighing (70-170 gm) were assigned into 6 groups (n=4). Group A was control, group B received epinephrine (0.08 mg/kg) intraperitoneally for 2 days; groups C-F received epinephrine followed by oral treatments: Aspirin (2 mg/kg), *Justicia secunda* (1 mg/kg), *Justicia secunda* (2 mg/kg) and vitamin C (2 mg/kg) respectively for 14 days.
Results: There was a significant elevation of lactate dehydrogenase and troponin T and disruption in electrolyte balance, indicative of myocardial injury. Treatment with *Justicia secunda* (2 mg/kg), aspirin, and vitamin C significantly reduced LDH and troponin T levels ($p<0.01$), improved antioxidant status (increased SOD, CAT, GSH; decreased MDA), and slightly normalized electrolytes. *Justicia secunda* (2 mg/kg), exhibited improvement in high-density-lipoproteins and reduction in low-density-lipoprotein levels.
Conclusions: Treatment with *Justicia secunda* (particularly at 2 mg/kg), aspirin, and vitamin C ameliorated the epinephrine-induced myocardial infarction. Hence, if these results apply to humans, using *Justicia secunda* in management of myocardial infarction and oxidative stress should be encouraged as a potential natural adjunct.

Keywords: Epinephrine, *Justicia secunda*, Lactate dehydrogenase, Myocardial infarction, Troponin T

INTRODUCTION

In recent years, the world population has increased and human lifestyle has undergone significant changes, leading to an increase in the incidence of several diseases such as cancer, diabetes, and cardiovascular disorders.[1] Therefore, this study aimed to determine the effect of hydromethanol extract of *Justicia secunda* leaves on epinephrine-induced myocardial infarction in male Wistar rats. Cardiovascular disease is the most common cause of

morbidity and mortality worldwide, with approximately 32.4 million cases of myocardial infarction and stroke reported worldwide each year and its incidence is constantly increasing in the southern region of Nigeria.[1]

Myocardial infarction, also known as heart attack, is a serious and life-threatening condition that damages the heart muscle due to lack of oxygen due to obstruction of blood flow to the heart.[2] According to the third universal definition of myocardial infarction, it is myocardial necrosis in a clinical setting compatible with myocardial ischemia.[3] MI occurs when blood flow is impaired and myocardial cells are damaged due to a lack of oxygen supply.[4] Early and rapid diagnosis is crucial to deciding on treatment and improving survival. Essential cardiac biomarkers such as cardiac troponin T, lactate dehydrogenase, myoglobin, ischemic-modified albumin, etc., can be used to determine the presence of myocardial infarction. Serum biomarkers of myocardial necrosis, such as cardiac troponin (I or T), can specifically reflect myocardial injury, have high clinical sensitivity, and may improve diagnostic accuracy.[5] It has been well-described that oxidative stress and inflammation are the main pathophysiological processes involved in myocardial infarction.[6,7] Therefore, it is essential to control stress and prevent chronic exposure to epinephrine to reduce the risk of myocardial infarction and other cardiovascular diseases. Nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin are effective agents against various diseases ranging from musculoskeletal to cardiovascular diseases and as an analgesic and anti-inflammatory agent.[8]

Despite improvements in healthcare delivery systems, medicinal plants still play an important role in human and animal health systems and approximately 60% of the world's population depends on herbs for primary health care.[9,10] French medicinal plants are the most common source of drugs used in traditional medicine.[11] In various parts of Africa, several *Justicia* species are used in traditional medicine for the treatment of anemia, inflammation, fever, diarrhea, liver disease, arthritis, and respiratory and gastrointestinal disorders.[12] The leaves of *Justicia secunda* have anti-inflammatory, hepatoprotective, nephroprotective, immunomodulatory, hemostatic, anti-sickling, antihypertensive and antibacterial properties.[12-14] Therefore, this study investigated the potential of hydromethanolic extract of *Justicia secunda* leaves to ameliorate epinephrine-induced myocardial infarction in male Wistar rats.

METHODS

Study design, location and duration

The study was experimental animal research (in vivo) using male Wistar rats as models in a controlled laboratory setting with the aim being to study and make assessment of biochemical and histological outcomes. The experiment was conducted in the animal house of the department of physiology, University of Calabar, Cross River State, Nigeria. Ethical clearance for the study was obtained from the University of Calabar animal ethics committee (approval number 040PHY3719) and all animal handling procedures complied with the guidelines of the Helsinki Declaration (1964).

The total duration of the experiment was approximately 23 days (June 2023), comprising: a 7-day acclimatization period for all animals. 2 days of intraperitoneal epinephrine administration (0.8 mg/kg body weight) to induce myocardial infarction.

Followed by 14 days of oral treatment with aspirin (2 mg/kg), *Justicia secunda* (1 mg/kg and 2 mg/kg) and vitamin C (2 mg/kg) via oral gavage. At the end of the administration, the rats were anesthetized with chloroform, blood samples were collected via ocular puncture, and the hearts of the control and treated rats were harvested and preserved with normal saline for tissue homogenization analysis. The samples were stored in an ice pack and immediately utilized for analysis.

Selection criteria

A total of 24 healthy adult Wistar rats weighing between 70-170 mg were selected by random sampling method within its male population. Animals showing signs of illness or injury were excluded from the study.

Procedure

The animals were arbitrarily allotted into 6 separate groups (n=4) and housed in standard laboratory cages (425×290×150 mm) with wooden shavings as bedding and maintained under standard environmental conditions (12-hour light/dark cycle, temperature of 22±2˚C).

Preparation of Justicia secunda

Fresh leaves of *Justicia secunda* were obtained and sent to a Botanist in the faculty of pharmacy, Madonna University for identification. It was dried at room temperature and ground to powder. 590 gm of pulverized *Justicia secunda* was steeped in 1450 ml of methanol and 750 ml of distilled water for 48 hours. The obtained filtrate was concentrated at 60°C and residue at 4°C until use. The LD50 of *Justicia secunda used* for this study was 3800 mg/kg body weight as reported by.[15] The choice of hydromethanol extraction was to extract both hydrophilic and hydrophobic compounds of *Justicia secunda*.

Cardiac biomarkers assessment

Lactate dehydrogenase concentration was determined using the method described by Freyer et al.[16]

Table 1: Study design and drugs administration.

Groups	No. of rats	Treatment
Group A (control)	4	Received feed and 0.5 ml of normal saline as vehicle throughout the experiment
Group B (epinephrine control)	4	Received epinephrine 0.8 mg/kg (no treatment)
Group C	4	Received epinephrine + 2 mg/kg of aspirin
Group D	4	Received epinephrine + 1 mg/kg of *J. secunda* extract
Group E	4	Received epinephrine + 2 mg/kg of *J. secunda* extract
Group F	4	Received epinephrine + 2 mg/kg of vitamin C

Principle

By measuring the per-time absorbance reduction at 340 nm, the activating reaction of pyruvate + NADH + H LDA> L-lactate + NAD + LDH in the sample is determined.

Procedure

Test tubes were carefully categorized and 1ml of the prepared reagent was introduced into the tubes and mixed with 0.02 ml of the sample. Incubate for 60 minutes at 37°C after transferring to the culture. The sample extinction decrease was read at 340 nm at time 0, 60, 120, and 180 calE/minute.

Troponin T concentration was determined using the method of Jaffe et al.[17]

Principle

A specific antibody to troponin T is immobilized on a microplate. The sample was added to the wells and incubated, allowing troponin T to bind to the capture antibody. After washing to remove unbound materials, a detected antibody branded with an enzyme, such as horseradish peroxidase (HRP) was added. The detection antibody recognizes a different epitope on the troponin T molecule. After another incubation and washing step, a substrate solution was added that reacted with the enzyme, creating a measurable indication directly proportional to the concentration of troponin T in the sample.

Procedure

The supernatant of the homogenized heart was used. The sample was collected into an EDTA tube and centrifuged to obtain serum used for troponin T measurement. The sample was diluted using an assay buffer after calibration and was added to appropriate wells of the coated microplates and incubated for 60 minutes at 37°C to allow troponin T present in the sample to bind to the capture antibody. Then the detection antibody was added to each well and incubated to allow the formation of sandwich complexes. The microplate was thoroughly washed to discard unbound detection antibody-enzyme conjugate. A substrate solution [tetramethylbenzidine (TMB)] was added to the wells and incubated on the plate to allow the enzyme-conjugated detection antibody to react with the substrate to produce a colored product. Thereafter, sulfuric acid was added to stop the enzyme reaction and stabilize the color change and the sample absorbance values were read at 450 nm.

Cardiac antioxidant assessment

The hearts of each mouse were harvested and homogenized using a Potter-Elvehjem homogenizer. The 20% (1/5 w/v) tissue homogenate was placed in 50 mm Tris-HCl buffer (pH 7.4) with 1.15% potassium chloride and centrifuged at 10,000 rpm at 4°C for 10 minutes. The supernatant was obtained for catalase assay with hydrogen peroxide as substrate. Reduced glutathione was measured at 412 nm using the method of Lucchese et al.[18] Glutathione peroxidase was measured using hydrogen peroxide as substrate.[18] Superoxide dismutase was measured using the method described by Misra et al.[19] Malondialdehyde was evaluated in thiobarbituric acid reactive substances (TBARS) as explained by Meenakshi et al and Ohkawa et al.[20,21] Then, the reaction mixture yielding 0.2 ml of 8.1% sodium dodecyl sulfate solution, 1.5 ml of 20-ethyl acid solution adapted to pH 3.5 with sodium hydroxide and 1.5 ml of 0.8% thiobarbituric acid solution was added to 0.2 ml of 10% (w/v) solution. The mixture was made up to 4.0 ml with distilled water and heated to 95°C for 60 min. Approximately 1.0 ml of distilled water and 5.0 ml of a mixture of n-butanol and pyridine (15: 1 v/v) were added and centrifuged while cooling on ice at 4000 rpm. The coarse layer was removed and the absorbance was summed at 532 nm and added to the result obtained from the MDA standards. Concentrations were calculated from the absorbance values as normal absorbance. This method was recently used by Mobisson et al.[22]

Measurement of serum electrolytes

Serum calcium ion concentration was determined by the o-cresol phthalein complex method.[23] This method is based on the reaction of calcium ions with the o-cresol phthalein complex in an alkaline solution to form a complex with a deep purple color.

Procedure

Test tubes (Becton, Dickinson, and Company, USA) were labeled as test tubes, standard tubes, and blank tubes. Then, 1.0 ml of reagent was added to all test tubes. Then, 0.025 ml of the sample was added to the appropriate tubes and mixed vigorously. The mixture was allowed to stand for 5 minutes, after which the absorbance is read and recorded at 590 nm. The increase in absorbance of the mixture was proportional to the calcium ion concentration in the sample. The serum calcium ion concentration is calculated by dividing the absorbance of the test by the absorbance of the standard, multiplied by the concentration of the standard, which was 2.5 mmol/l.

Serum sodium ion concentration is measured by the method of Maruna et al.[24]

Principle

The present method is based on the interaction of sodium with a specific chromophore, resulting in the formation of a chromophore whose absorbance fluctuates proportionally to the sodium concentration in the sample being tested.

Procedure

Test tubes were labeled as standard, blank, and test tubes, and 1.0 ml of reagent was added to each test tube. Samples from the tubes were added in 0.01 ml increments, mixed well, and incubated at 25°C for 5 minutes. At 630 nm, the absorbance value was recorded. This method was used by Mobisson et al.[25] Serum potassium ion concentration was determined by the method of Chow et al.[26]

Principle

Determination of potassium involves measuring the concentration of potassium ions in a sample using an ion-selective electrode. The ISE consists of a membrane that selectively allows potassium ions to pass through, generating an electrical potential proportional to the concentration of potassium ions in the sample.

Procedure

Calibrate the ion-selective electrode and place it in the sample, then allow sufficient time for equilibration, during which the potassium ions in the sample will bind to the sensing elements of the electrode. The electromotive force produced by the electrode was measured using a potentiometer and converted to potassium concentration using calibration parameters. The measured potassium concentration in the sample was then reported.

Lipid profile assay

Serum total cholesterol and other lipid components were measured by the Randox assay.[27,28]

Principle

Cholesterol esterase catalyzes the hydrolysis of cholesterol esters to free cholesterol and fatty acids. Cholesterol oxidase then catalyzes the oxidation of free cholesterol to cholesterin-3-one and hydrogen peroxide. Phenol and 4-amino-antipyrine then combine with hydrogen peroxide in the presence of peroxidase to produce red-colored quinonemine and then read colorimetrically at 540 nm. The color intensity obtained was equal to the amount of total cholesterol. The serum triglyceride level in the samples was determined according to the method described by Negele et al.[29]

Principle

Lipoprotein lipase A hydrolyses triglycerides to glycerol and fatty acids. The resulting glycerol is phosphorylated to glycerol-3-phosphate by glycerol kinase. Glycerol phosphate oxidase then oxidizes glycerol-3-phosphate to produce dihydroxyacetone phosphate and hydrogen peroxide. The chromogen consists of n-ethyl-n-sulfohydroxypropyl-n-foludine which is then oxidized. The quinonenine dye (purple) produced from the reactions was analyzed colorimetrically at 540 nm. LDL cholesterol was measured using the difference between serum total cholesterol, total HDL cholesterol, and triglycerides as follows: LDL-c = TC - (VLDL-c + HDL-c).

Statistical analysis

Data obtained were presented as mean±SEM, n=4. One-way analysis of variance (ANOVA) was utilized in comparing the differences within groups, followed by post hoc multiple comparisons. Statistical software SPSS version 17.0 and Microsoft Excel were used for the analysis. The p value of <0.05 was considered statistically significant.

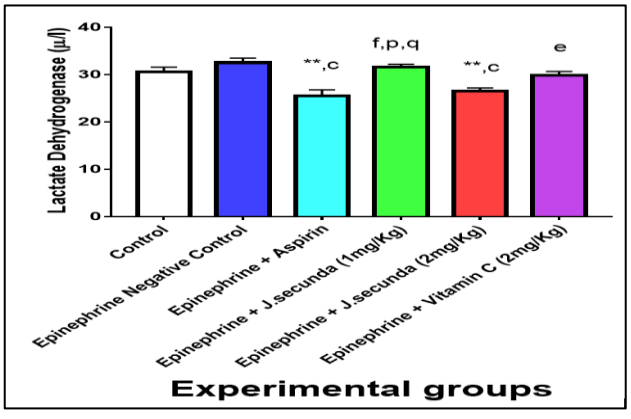

Figure 1: Comparison of cardiac lactate dehydrogenase of the different experimental groups.

Values are expressed as mean±SEM, n=4. **p<0.01 versus control, c- p<0.001 versus epinephrine negative control, e- p<0.01 versus epinephrine + aspirin, f- p<0.001 versus epinephrine + aspirin, p- p<0.05 versus epinephrine + *J. secunda* (2 mg/kg), q- p<0.01 versus epinephrine + *J. secunda* (2 mg/kg).

RESULTS

Comparison of mean cardiac biomarkers in control and treated groups

Figure 1 below showed epinephrine administration significantly increased serum lactate dehydrogenase (LDH) and troponin T levels compared to the control group, confirming myocardial damage. Treatment with *Justicia secunda* (2 mg/kg), aspirin and vitamin C significantly ($p<0.01$) reduced LDH and troponin T levels, with *Justicia secunda* (2 mg/kg) showing the most reduction.

Comparison of mean serum electrolyte concentration in control and treated groups

In Table 2, the serum concentration of calcium, potassium and sodium levels in negative control groups were significantly altered by epinephrine administration. Calcium increased markedly ($p<0.001$), while potassium and sodium were also elevated ($p<0.001$).

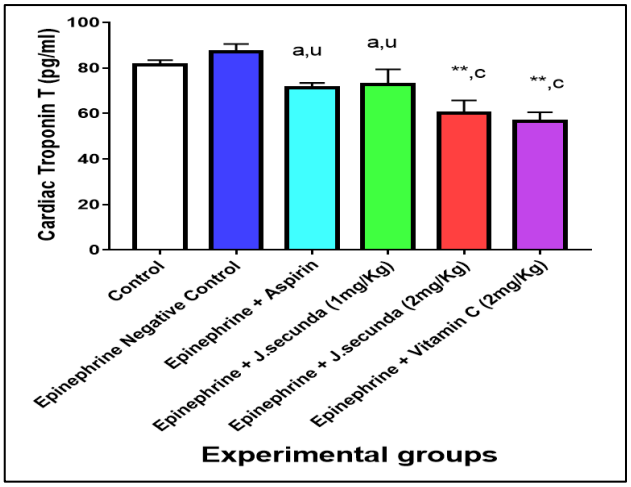

Figure 2: Comparison of cardiac troponin of the different experimental groups.

Values are expressed as mean±SEM, n=4. **p<0.01 versus control, a- p<0.05 versus epinephrine negative control, c- p<0.001 versus epinephrine negative control, u- p<0.05 versusepinephrine +vitamin C (2 mg/kg).

Table 2: Comparison of serum electrolyte concentration of control and experimental groups.

Parameters	Group A control	Group B epinephrine control	Group C Epi + aspirin	Group D Epi + *J. secunda* (1 mg)	Group E Epi + *J. secunda* (2 mg)	Group F Epi + vitamin C (2 mg)
Calcium (mmol/l)	2.19±0.01	3.07±0.02***	2.62±0.01***,c	2.65±0.01***,c	3.11±0.01***,f,o	3.20±0.01***,c,f,o,q
Potassium (mmol/l)	3.72±0.08	4.45±0.12***	3.55±0.10 c,p,v	3.30±0.10*,c	3.15±0.10**,c	3.02±0.12***,c
Sodium (mmol/l)	142.00±1.22	152.25±1.18*	135.00±3.87 c,n	123.50±3.59***,c,v	117.00±2.79***,c,f	112.00±1.87***,c,f

Values are expressed in mean±SEM, n=4. *- represents values with significant difference. *- p<0.05 versus control; **- p<0.01 versus control; ***- p<0.001 versus control; c- p<0.001 versus epinephrine negative control; f- p<0.001 versus epinephrine + aspirin; n- p<0.0 versus epinephrine + *J. secunda* (2 mg/kg); o- p<0.001 versus epinephrine + *J. secunda* (1 mg/kg); p- p<0.05 versus epinephrine + *J. secunda* (2 mg/kg); q- p<0.01 versus epinephrine + *J. secunda* (2 mg/kg); v- p<0.01 versus epinephrine + vitamin C (2 mg/kg).

Table 3: Comparison of serum lipid profile concentration of control and experimental groups.

Parameters	Group A control	Group B epinephrine control	Group C Epi + Aspirin	Group D Epi + *J. secunda* (1 mg)	Group E Epi + *J. secunda* (2 mg)	Group F Epi + vitamin C(2mg)
TC (mmol/l)	3.62±0.10	2.57±0.14***	3.50±0.09c	3.47±0.13c	4.27±0.06**,c,f,o	4.42±0.11***,c,f,o
LDL (mmol/l)	3.10±0.11	2.03±0.14***	3.13±0.07c,n	2.62±0.08**,b,w	2.86±0.05 c,w	3.48±0.09*,c,d
TG (mmol/l)	1.93±0.06	1.42±0.02	1.59±0.08	1.69±0.03	1.89±0.02	161.74±53.25***,c,f,o,r
HDL (mmol/l)	1.56±0.03	1.29±0.02***	1.12±0.00***,c	1.58±0.01 c,f	2.13±0.01***,c,f,o,w	1.78±0.01***,c,f,o

Values are expressed in mean±SEM, n=4. *=represents values with significant difference. *=p<0.05 versus control; **=p<0.01 versus control; ***=p<0.001 versus control; b=p<0.01 versus epinephrine negative control; c=p<0.001 versus epinephrine negative control; d= p<0.05 versus epinephrine + aspirin; e=p<0.01 versus epinephrine + aspirin, f= p<0.001 versus epinephrine + aspirin; n= p<0.01 versus epinephrine + *J. secunda* (1 mg/kg); o= p<0.001 ersuvs Epinephrine + *J. secunda* (1 mg/kg); w=p<0.001 versus epinephrine + vitamin C (2mg/kg).

However, calcium groups treated with aspirin and *J. secunda* (1 mg/kg) showed the most improvement.

Treatment groups showed normalization of electrolyte levels, with *J. secunda* (2 mg/kg) and vitamin C restoring potassium and sodium balance more effectively than aspirin.

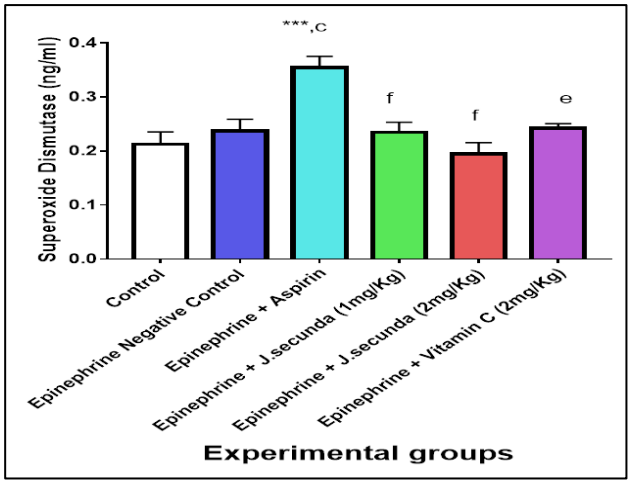

Figure 3: Comparison of cardiac superoxide dismutase of the different experimental groups.

Values are expressed as mean±SEM, n=4. ***=p<0.001 versus control, c= p<0.001 versus Epinephrine negative control, e= p<0.01 versus epinephrine + aspirin, f= p<0.001 versus epinephrine + aspirin.

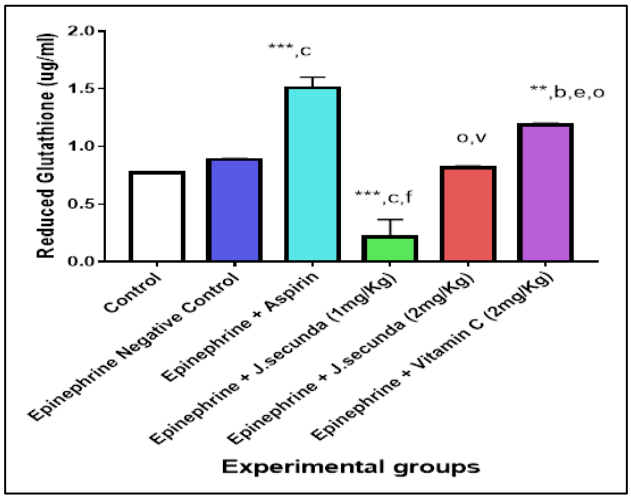

Figure 4: Comparison of cardiac reduced glutathione of the different experimental groups.

Values are expressed as mean±SEM, n=4. **=p<0.01 versus control, ***=p<0.001 versus control, b= p<0.01 versus epinephrine negative control, c= p<0.001 versus epinephrine negative control, e= p<0.01 versus epinephrine + aspirin, f= p<0.001 versus epinephrine + aspirin, o = p<0.001 versus epinephrine + *J. secunda* (1 mg/kg), v= p<0.01 versus epinephrine + vitamin C (2 mg/kg).

Comparison of mean cardiac oxidative stress markers in control and treated groups

In Figure 3 below, epinephrine significantly elevated malondialdehyde (MDA) levels and decreased superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) concentrations, indicating oxidative stress. Treatment with *J. secunda* (2 mg/kg), aspirin, and vitamin C significantly improved antioxidant enzyme levels (p<0.01) and reduced MDA. Notably, *J. secunda* (2 mg/kg) improved GSH and CAT comparably to vitamin C.

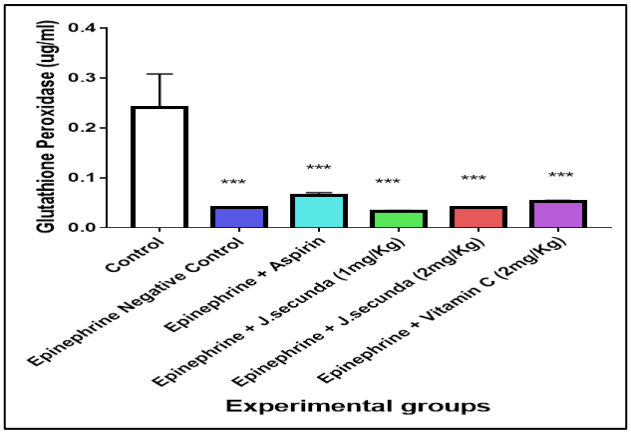

Figure 5: Comparison of cardiac glutathione peroxidase of the different experimental groups.

Values are expressed as mean ± SEM, n=4. ***=p<0.001 versus control.

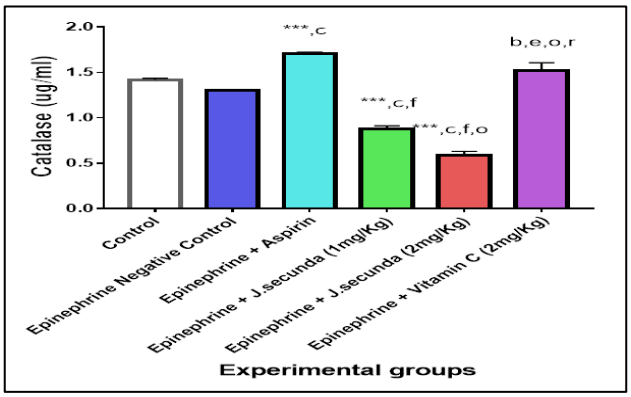

Figure 6: Comparison of cardiac catalase of the different experimental groups.

Values are expressed as mean ± SEM, n=4. ***=p<0.001 versus control, b=p<0.01 versus epinephrine negative control, c=p<0.001 versus epinephrine negative control, e=p<0.01 versus epinephrine + aspirin, f= p<0.001 versus epinephrine + aspirin, o =p<0.001 versus epinephrine + *J. Secunda* (1 mg/kg), r=p<0.01 versus Epinephrine + *J. Secunda* (2 mg/kg).

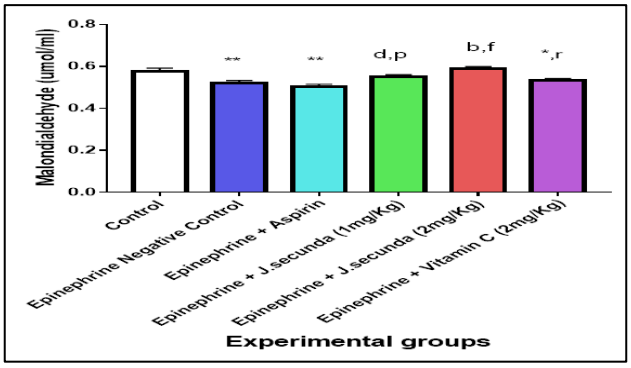

Figure 7: Comparison of cardiac malondialdehyde of the different experimental groups.

Values are expressed as mean±SEM, n=4. *=p<0.05 versus control, **=p<0.01 versus control, b=p<0.01 versus epinephrine negative control, d=p<0.05 versus epinephrine + aspirin, f= p<0.001 versus epinephrine + aspirin, p=p<0.05 versus epinephrine + *J. secunda* (2 mg/kg), r=p<0.001 versus epinephrine + *J. secunda* (2 mg/kg).

Comparison of mean serum lipid profile in control and different treated groups

In Table 3, epinephrine reduced serum total cholesterol, LDL, and HDL concentration. Treatment with *J. secunda* (2 mg/kg) and vitamin C significantly increased HDL ($p<0.001$) and total cholesterol while normalizing LDL. Rats treated with vitamin C had the highest triglyceride levels.

DISCUSSION

This study evaluated the ameliorative effect of *Justicia secunda* hydromethanolic leaf extract on epinephrine-induced myocardial infarction on male Wistar rats. The following parameters were assayed: LACTATE dehydrogenase, cardiac troponin T, calcium ion, potassium ion, sodium ion, superoxide dismutase (SOD), malondialdehyde (MDA), catalase, reduced glutathione (GSH), glutathione peroxidase (Gpx), total cholesterol (TC), low density lipoprotein (LDL), triglyceride (TG) and high-density lipoprotein (HDL).

Impact of cardiac biomarkers in control and treated groups

Epinephrine administration led to a marked increase in lactate dehydrogenase and troponin T concentration confirming myocardial cell injury. These biomarkers are widely accepted indicators of cardiomyocyte membrane disruption and necrosis.[30,31] The significant reduction in their levels following treatment with *Justicia secunda*, aspirin and vitamin C suggest a positive protective role in preserving myocardial integrity. This aligns with previous studies highlighting the therapeutic effects of particularly aspirin and *J. secunda* in cardiovascular models.[32,33]

Impact of serum electrolyte concentration in control and treated groups

The electrolyte imbalance of calcium, potassium, and sodium is a hallmark of MI as observed in the epinephrine group. These disruptions, likely driven by β-adrenergic overstimulation and RASS activation, were effectively normalized by the action of *J. secunda* and vitamin C properties.[34,35] Electrolyte imbalance or elevation can lead abnormal heart rhythms (arrhythmias) and other serious health complications. The correction of calcium and sodium homeostasis may reflect the influence of *J. secunda* extract on ionic transport or its antihypertensive properties.[13,36] The decrease in sodium concentration in vitamin C might be due to its potential to act as a diuretic, causing the kidney to remove more sodium and water from the body.

Evaluation of cardiac oxidative stress markers in control and experimental groups

Cardiac oxidative stress markers are specific molecules or compounds that indicate pathophysiology in cardiac tissue.[37] The observed increase in malondialdehyde (MDA) and concurrent decrease in key antioxidant enzymes- superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), and glutathione peroxidase (GPx) indicate significant oxidative damage induced by epinephrine. Excessive production of reactive oxygen species can lead to disruption of the redox balance and cause oxidative stress.[22,38,39]

Treatment with *J. secunda*, particularly at 2 mg/kg, restored antioxidant enzyme levels and suppressed lipid peroxidation, demonstrating a strong antioxidative capacity. These results are consistent with related reports of *J. secunda's* phytochemical constituents exerting free radical-scavenging effects.[12,32]

Impact of lipid profile parameters in control and treated groups

Dyslipidemia is a known cardiovascular risk factor, and although epinephrine caused a reduction in total cholesterol and HDL levels, treatment with *J. secunda* led to significant increase in HDL and mild normalization of LDL. These effects imply that the extract may also confer anti-atherogenic benefits, supporting its broad effect on cardiovascular integrity.[40] The significant increase in LDL in the vitamin C-treated group may be due to increased lipid peroxidation.[41]

CONCLUSION

Treatment of epinephrine induced myocardial infarction with *Justicia secunda*, aspirin, and vitamin C caused a significant decrease in lactate dehydrogenase and troponin T, altered serum calcium ion, sodium ion, and potassium ion, cardiac oxidative stress markers, lipid profile, and decreased cardiac malondialdehyde concentration. Hence, treatment of myocardial infarction particularly using *Justicia secunda* (2 mg/kg) demonstrated significant cardioprotective effects.

Therefore, if this result is to be applied to humans the combined use of *Justicia secunda*, aspirin, and vitamin C could be beneficial in the management of myocardial infarction. Further studies are recommended to elucidate physiologic mechanism and potential clinical applications through which *J. secunda* mediated its effects in the heart.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee of University of Calabar with approval number 040PHY3719

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