

**MYOCARDIAL POTENCY OF AQUEOUS EXTRACT OF *HARUNGANA MADAGASCARIENSIS* STEM BARK AGAINST ISOPROTERENOL-INDUCED MYOCARDIAL DAMAGE IN RATS**

**ABSTRACT**

The present study was undertaken to evaluate the effects of *Harungana madagascariensis* on electrocardiographical, biochemical and histopathological changes in isoproterenol (ISO)-induced myocardial infarction. Male *Wistar* albino rats were randomly divided and treated with aqueous extract of *Harungana madagascariensis* stem bark (AEHM, 200 and 400 mg/kg per os), or normal saline or vitamin E for 7 days with concomitant administration of ISO (85 mg/kg, subcutaneously) on 8th and 9th days, at 24 h interval. The ISO injections to the rats caused cardiac dysfunction evidenced by a marked ( $P < 0.01$ ) elevation in ST-segment, a reduction in R wave amplitude ( $P < 0.01$ ), decrease in endogenous antioxidant reduced glutathione (GSH), increase in malondialdehyde (MDA), a lipid peroxidation marker, increase of cardiac marker enzymes lactate dehydrogenase (LDH), aspartate amino transferase (AST) and alanine amino transferase (ALT). All these changes in cardiac function as well as GSH, MDA and the enzymes (LDH, AST and ALT) were ameliorated when the rats were pretreated with AEHM. Additionally, the protective effects were strengthened by improved histopathological changes, which specifies the protection of cardiomyocytes from the deleterious effects of ISO. This study demonstrates the cardioprotective effect of *Harungana madagascariensis* on isoproterenol-induced myocardial infarction in rats. The mechanism might be associated with the enhancement of antioxidant defense, reduction of lipid peroxidation and is confirmed by amending electrocardiographic pattern, improvement of cardiac markers and less histopathological damages following ISO-induced myocardial infarction. It could provide experimental evidence to support the use of *Harungana madagascariensis* used in traditional medicine to treat cardiovascular disorders.

**Keywords:** Antioxydants, electrocardiography, *Harungana madagascariensis*, isoproterenol, myocardial infarction.

**INTRODUCTION**

According to the recent world health organization survey, an estimated 17.7 million people died from cardiovascular disease (CVDs) in 2015, representing 31% of all global deaths. Of these deaths, an estimated 80% were due to myocardial infarctions (MI) and strokes<sup>1</sup>. According to the same survey, over three quarters of CVDs deaths occurred in low- and middle-income countries. MI is followed by several biochemical alterations, such as lipid peroxidation, free radical damage, hyperglycemia, hyperlipidemia, elevation in cardiac markers and pro-inflammatory cytokines leading to qualitative and quantitative alterations of myocardium<sup>2</sup>. Catecholamines at low concentrations are beneficial in regulating heart functions by exerting a positive inotropic action on the myocardium<sup>3</sup>, whereas high concentrations of catecholamines or chronic exposure to catecholamines over a prolonged period produces deleterious effects on the cardiovascular system<sup>4</sup>. Isoproterenol (ISO) is a synthetic catecholamine, a nonselective  $\beta$ -adrenoreceptor agonist, which causes severe stress in the myocardium and produces infarct like lesions, when injected in rats<sup>5</sup>. The ISO model is a well standardized and most reliable model for assessing the cardioprotective activity of several drugs, since its pathophysiological and morphological changes following ISO administration are comparable to those taking place in human MI<sup>6</sup>. Now a days, a number of pharmacological interventions such as beta-blockers, ACE inhibitors, antiplatelet agents, thrombolytics, calcium antagonist, nitrates, antioxidants have been shown to counteract the ill effect of myocardial ischemic injury, and to reduce morbidity and mortality in patients with

ischemic heart disease<sup>7, 8</sup>. However, their chronic usage is often associated with adverse effects<sup>9</sup>. Therefore, the development of new and safer drugs for the treatment and prevention of ischemic heart disease is still a major concern. There is increasing trend towards the application of herbal medicines to treat the cardiovascular diseases<sup>10, 11</sup>. *Harungana madagascariensis* is one of the most popular trees in the African traditional medicine system. It is used as an abortifacient and antiseptic, in the treatment of cardiovascular disorders, anemia, asthma, tuberculosis, fever, angina, , diarrhea, dysentery, syphilis, gonorrhoea, malaria, parasitic skin diseases, and wounds, as a natural source of dermatological agents and cosmetics<sup>12-16</sup>, asthma, liver diseases, diabetes, pancreatic and biliary problems<sup>17,18</sup>. Studies on the barks or leaves of this plant revealed antihelminthiase properties<sup>19</sup>, anti-plasmodial<sup>20</sup>, antidiabetic<sup>21</sup>, antimicrobial activities<sup>22</sup>, analgesic and anti-inflammatory activities<sup>23</sup>. A prenylated 1, 4-anthraquinone isolated from the hexane extract of the stem-bark of *H. madagascariensis* possess alpha-glucosidase inhibition and antioxidant activities<sup>24</sup>. In this context, an attempt has been made to investigate the effect of anaqueous extract of *H. madagascariensis* on maintaining the myocardial integrity in animals employing electrocardiographical, biochemical and histopathological parameters in ISO-induced myocardial infarction.

## **MATERIALS AND METHODS**

### **Plant material collection and extraction**

Fresh *H. madagascariensis* stem barks were collected at Essezok, Mbalmayo (Center Region, Cameroon) in June 2016. The identification of the plant was done at the Cameroon National Herbarium where voucher sample were deposited under the registration number N° 4224 HNC. Bark pieces were dried under room temperature and powdered with the help of electrical grinder. 500 g of powder was introduced into 3.5 L of distilled water and boiled for 20 minutes. The resulting decoction was filtered through Whatman paper N°3 and further lyophilized. A crude brown extract powder (HM extract, 31.73g) was obtained, giving a yield of 6.35%.

### **Experimental animals**

Male albino *Wistar* rats (150-200g) were obtained from the Animal House of the Faculty of Science at the University of Yaoundé I (Cameroon). They were kept at standard laboratory conditions under natural light and dark cycles, at constant room temperature (20±5°C) and were allowed to have standard food and tap water freely. This study was approved by the Cameroonian National Ethical Committee (Ref n°FW-IRB00001954).

### **Drugs and chemicals**

Isoproterenol hydrochloride was purchased from Sigma Aldrich, USA. Lactate dehydrogenase (LDH) kit for enzyme estimation was purchased from Hospitex Diagnostics. Aspartate amino transferase (AST) and alanine amino transferase (ALT) kits were from Fortress Diagnostics Biosystems. All chemicals used in the present study were of analytical grade.

### **Induction of experimental myocardial infarction**

Isoproterenol was freshly dissolved in 0.9% saline and injected (85 mg/kg) subcutaneously to the rats for two successive days (on days 8th and 9th respectively) at an interval of 24h. Animals were sacrificed 48h after the first injection of isoproterenol.

### **Experimental design**

The animals were randomized into 7 groups consisting of 7 rats each. HM extract was dissolved in distilled water. Vitamin E was used as standard drug. Rats in group 1 (normal control) received distilled water (10 mL/kg) orally, for 9 days. Rats in group 2 (ISO control) received distilled water for 9 days and were injected isoproterenol (85 mg/kg, SC) on the 8th and 9th days at an interval of 24 hour. Animals of groups 3 to 5 were pretreated with the

aqueous extract of HM (200 and 400 mg/kg/day) or vitamin E (100 mg/kg/day) orally for 9 days and on the 8th and 9th days they received isoproterenol SC at an interval of 24 hour. Rats in groups 6 and 7 were treated with the aqueous extract of HM (400 mg/kg/day) and vitamin E (100 mg/kg/day) orally for 9 days and on the 8th and 9th days they were injected saline (0.1mL/100g SC) at an interval of 24 hour. Changes in body weight in all groups were noted every 2 days during the experimental period.

#### **Electrocardiogram measurement**

Twenty four hours (24h) after the last administration of the drugs, the animals were anesthetized by intraperitoneal injection of urethane (15 mg/kg). Needle electrodes were inserted under the skin of the animals in lead II position. Electrocardiogram recordings were made using Biopac Student Lab Experiment system (BSL 3.7, USA).

#### **Blood collection and assessment of cardiac hypertrophy**

After recording the ECG, blood was collected from the abdominal aorta and allowed to clot for 1 h at room temperature. It serum was subsequently separated by centrifugation at 3000g for 15 min at 4°C and stored at -20°C for biochemical assays. After the blood collection, the animals were euthanized. Their hearts were removed, rinsed in ice-cold normal saline and weighed. The wet heart weight to body weight ratio was calculated to assess the degree of myocardial weight gain.

#### **Assay of cardiac marker enzymes**

Activities of lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the serum were measured using commercial kits (from Hospitex Diagnostics for LDH and FortressDiagnostics for AST and ALT).

#### **Estimation of lipid peroxidation product and reduced glutathione in myocardium**

After weighing, the heart tissue was divided into two transversal parts. One part was homogenized in Mc Ewen physiological ice-cold solution (pH 7.4, 1:5 w/v). The homogenate was centrifuged at 3000g for 30 min at 4°C and the supernatant was stored at -20°C for biochemical assays. Malondialdehyde (MDA), a thiobarbiturate reactive substance, was measured as a marker for oxidative stress in myocardial homogenates using trichloroacetic acid (TCA, 20%) and thiobarbituric acid (TBA, 0.67%)<sup>25</sup>. The level of reduced glutathione (GSH) was estimated as previously described<sup>26</sup>.

#### **Histopathological examination**

After weighing, the second part of the heart was fixed in 10% buffered formalin. The fixed tissues were embedded in paraffin, sectioned at 5 mm and stained with hematoxylin and eosin (H&E). The sections were examined under a light microscope (Scientico STM-50) and photomicrographs were taken by a photomicroscope (Minisee 1.0) at x200 magnification.

#### **Statistical analysis**

Results are shown as mean  $\pm$  SEM. The statistical comparisons among the groups were performed with t-students test using SigmaStat3.5 statistical package program. Mann Whitney post-test was employed to compare the mean values between the treated groups and the control. P-values less than 0.05 were considered as statistically significant.

## **RESULTS**

### **Effect of *Harungana madagascariensis* on electrocardiogram**

The Lead II electrocardiograms obtained from the animals are shown in Figure 1. The rats receiving distilled water (control group) showed normal patterns of ECG, while those treated with isoproterenol alone (ISO group) demonstrated significant changes in ECG pattern. The changes included a marked elevation of ST segment from  $0.11 \pm 0.01$  mv in control group to  $0.18 \pm 0.01$  mv in ISO-treated group ( $P < 0.01$ ) and a reduction in R wave amplitude from  $0.63 \pm 0.03$  mv in control group to  $0.37 \pm 0.02$  mv in ISO-treated group ( $P < 0.01$ ) which both are indicative of myocardial infarction (Figure 2). Oral treatment with *H. madagascariensis* at a dose of 200 mg/kg or vitamin C (100 mg/kg) resulted in a significant

increase in the R-amplitude from  $0.37 \pm 0.02$  mv in ISO-treated group to  $0.52 \pm 0.01$  and  $0.51 \pm 0.02$  mv in extract-treated group and vitamin C, respectively ( $P < 0.01$ ), Figure 2A). However, treatment with all doses of *H. madagascariensis* resulted in a non-significant decrease in the ST-elevation as compared to the rats treated with isoproterenol alone (Figure 2B).

### **Effects of *Harungana madagascariensis* on the heart weight to body weight ratio and body weight**

The mean body weight of the rats at the end of the experiment in all experimental groups had no significant change (Table 1). The heart weight and the ratio of heart weight to the body weight were increased significantly ( $P < 0.05$  and  $P < 0.001$  respectively) in ISO-administered groups when compared with control group. The extract of *H. madagascariensis* when given alone, significantly reduce the heart weight and the ratio of heart weight to body weight ( $P < 0.001$ ) as compared to the ISO-treated group.

### **Effect of *Harungana madagascariensis* on serum marker enzymes**

As shown in Table 2, there was a significant rise observed in the levels of diagnostic marker enzymes (LDH ( $P < 0.01$ ), AST ( $P < 0.05$ ) and ALT ( $P < 0.05$ )) in the serum of the ISO-treated rats. Pre-treatment with *H. madagascariensis* (200 and 400 mg/kg) as well as vitamin C (100 mg/kg) showed a significant reduction in the levels of all serum diagnostic marker enzymes compared to ISO group.

### **Effect of *Harungana madagascariensis* on lipid peroxidation and reduced glutathione level**

To determine the lipid peroxidation, MDA levels were measured in myocardial homogenates. Heart MDA levels increased insignificantly in isoproterenol alone treated rats as compared to the control group (Table 3). Pre-treatment with the *H. madagascariensis* (200 and 400 mg/kg) extract induced a dose-dependent but non-significant decrease of MDA levels of myocardium. There was a significant ( $P < 0.001$ ) decrease in GSH level and CAT activity in the heart of ISO-treated rats as compared to the control group. Pre-treatment with *H. madagascariensis* (200 and 400 mg/kg) significantly increased ( $P < 0.001$ ) the myocardial GSH level.

### **Histopathological examination of the cardiac tissue**

In the control group, myocardial fibers were arranged regularly with clear striation, without any damage (Figure 3A). Histopathological sections of the isoproterenol alone treated hearts displayed hypertrophy, degeneration of myocytes, infiltration of neutrophilic granulocytes and increased edematous inter-muscular space and myofibroblasts (Figure 3B), whereas, the rats treated with *H. madagascariensis* extract (200 and 400 mg/kg) showed protection from myocardial injury evidenced by decreased myocytes degeneration as well as edema and minimal inflammation (Figure 3C, D).

## **DISCUSSION**

The purpose of this work was to evaluate the potential cardioprotective role of *Harungana madagascariensis* aqueous extract stem bark aqueous extract in isoproterenol-induced myocardial damage model in rats. ISO in high doses, induces morphological and functional alterations in the heart which closely resembles local myocardial infarction-like pathological changes seen in human myocardial infarction<sup>27</sup>. It has been reported that auto-oxidation of excess catecholamines such as isoproterenol results in free radical mediated peroxidation of membrane phospholipids and consequently leading to permeability changes in the myocardial membrane, intracellular calcium overload and irreversible damages<sup>28</sup>.

Electrocardiogram (ECG) is considered the most important clinical tool for the diagnosis of myocardial infarction<sup>29</sup>. In the present study, subcutaneous injection of isoproterenol (85 mg/kg) for two consecutive days caused ST-segment elevation and R-amplitude depression. The elevated ST-segment reflects the potential difference in the boundary between ischemic

and non-ischemic zones and a consequent loss of cell membrane function and the depressed R-amplitude might be due to the isoproterenol-induced myocardial edema<sup>30</sup>. *Harungana madagascariensis* (200 mg/kg) pre-treatment as well as vitamin C markedly inhibited isoproterenol-induced R-amplitude depression and amended the ST-segment elevation, indicating its protective effects on cell membrane function and electrical discharges.

In the present study, we have observed a significant increase in the heart weight and the ratio of heart weight to body weight in ISO-treated rats. The observed increase in the heart weight in ISO-induced rats might be due to the increased water content, edematous intramuscular space and extensive necrosis of cardiac muscle fibers followed by the invasion of damaged tissues by the inflammatory cells<sup>30,31</sup>.

Pre-treatment with the plant extract or vitamin E did not modify this increase. These results suggest that AEHM does not affect the gain or loss of weight of this organ. However, the short duration of the preventive treatment (seven days) could be responsible for the observed result. It would be wise to consider a longer duration in future experiments to better elucidate the effects of the plant extract on this parameter.

Myocardium contains many diagnostic marker enzymes like creatinine kinase, lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Upon administration of isoproterenol, the oxygen demand of the heart increases with increase in inotropic effect in the heart, resulting in prolonged ischemia and glucose deprivation. The cells are damaged with increased muscle contractility, which results in increasing the cell membranes permeability allowing cardiac enzymes to leak out into the bloodstream<sup>32</sup>. Increased activities of these marker enzymes in the serum are indicative of cellular damage and loss of functional integrity<sup>33</sup>. In the present study, the significant increase observed in the activities of LDH, AST, ALT in the serum of ISO-induced rats may be due to the leakage of them from the heart as a result of necrosis induced by ISO. The aqueous extract of *Harungana madagascariensis* seems to preserve the structural and functional integrity and/or permeability of the cardiac membrane and thus restricting the leakage of these indicative enzymes from the myocardium, as evident from the markedly blunted levels of these enzymes in the extracts pre-treated group when compared to the ISO-treatment group, thereby establishing the cardioprotective effect of the aqueous extract of *Harungana madagascariensis*.

Malondialdehyde (MDA), a product of the oxidative degradation of unsaturated fatty acids, is a biomarker of oxidative stress. Since the major constituents of biological membranes are lipids, their peroxidation can lead to cell damage and death<sup>34</sup>. The concentration of MDA increases in response to the free radical production in myocardial infarction, and decreases by antioxidant systems<sup>34</sup>. Increased formation of MDA of 49% as compared to the control group is an indication of the severity of the cellular injury to the heart induced by ISO, and this can be linked with altered membrane structure and enzyme inactivation<sup>35</sup>. *H. madagascariensis* administration substantially reduced MDA levels in a dose-dependent manner by 35 and 42% respectively at 200 and 400 mg/kg.

Glutathione (GSH) is a tripeptide which has a direct antioxidant function by reacting with superoxide radicals, peroxy radicals and singlet oxygen followed by the formation of oxidized GSH and other disulfides. It plays an important role in the regulation of variety of cell functions and in cell protection against free radical mediated injury<sup>36, 37</sup>. Depressed GSH levels may be associated with an enhanced protective mechanism to oxidative stress in myocardial infarction. In this study, ISO administration was found to reduce the levels of GSH. This observation concurs with several earlier findings<sup>28, 31, 34</sup>. Decreased GSH levels might be due to increased utilization in protecting 'SH' containing proteins from lipid peroxides. Pre-treatment with *H. madagascariensis* (400 mg/kg) significantly improved the level of GSH. This points to the potential antioxidant and free radical scavenging activity of

*H. madagascariensis*. In previous studies, *H. madagascariensis* has been described as an antioxidant and free radical scavenger<sup>38,39</sup>. The current study showed the antioxidant activity of *H. madagascariensis* and endorses its cardioprotective effect mediated by its antioxidant effect in myocardium.

Histopathological examination of myocardial tissue in the control rats illustrated clear integrity of the myocardial cell when compared to the hearts of ISO treated rats. ISO-induced rats showed separations of cardiac muscle fibers, edema and extensive infiltration of neutrophil granulocytes. Pre-treatment with the aqueous extract of *H. madagascariensis* (200 and 400 mg/kg) considerably attenuated the edema, reduced inflammatory cell infiltration and preserved normal cardiac muscle fibers structure, further confirming the cardioprotective effect of *H. madagascariensis*.

## CONCLUSION

In conclusion, our study reveals that pre-treatment of rats with the aqueous extract of *H. madagascariensis* exerts a remarkable protective potential against damages caused by isoproterenol-induced myocardial infarction. This cardioprotective effect could be associated with the enhancement of antioxidant defense, reduction of lipid peroxidation and is confirmed by amending electrocardiographic pattern, improvement of cardiac markers and less histopathological damage following isoproterenol-induced myocardial infarction. Although this study has provided a possible new therapeutic tool for myocardial infarction, more studies are required to elucidate the precise mechanism of *H. madagascariensis* in reversing the pathogenesis of myocardial infarction.

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## CONFLICT OF INTEREST:

No conflict of interest associated with this work.

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## TABLES

**Table 1 : Effect of *Harungana madagascariensis* aqueous extract on heart weight, body weight and heart weight/body weight ratio in rats**

Groups	Body weight (g) at the end of the experimental period	Heart weight (g)	Heart weight/body weight ratio (%)
Control	193.57 ± 8.69	0.61 ± 0.03	0.31 ± 0.01
ISO	185.01 ± 7.88	0.74 ± 0.03*	0.40 ± 0.01***
HM 200+ISO	178.71 ± 4.20	0.71 ± 0.02*	0.39 ± 0.01***
HM 400+ISO	179.57 ± 2.42	0.73 ± 0.02*	0.40 ± 0.01***
Vit E+ISO	185.00 ± 8.82	0.75 ± 0.03*	0.40 ± 0.01***
HM 400	190.29 ± 6.75	0.56 ± 0.02###	0.30 ± 0.01###
Vit E	192.86 ± 4.47	0.60 ± 0.04#	0.31 ± 0.01###

Values are given as mean ± SEM (n = 7). \*P<0.05 and \*\*\*P < 0.001 as compared to the control group; #P<0.05 and ###P < 0.001 as compared to the isoprenaline (ISO)-treated group. HM 200 or HM 400:*Harungana madagascariensis* aqueous extract (200 or 400 mg/kg); Vit E: vitamin E (100 mg/kg).

**Table 2 : Effect of *Harungana madagascariensis* aqueous extract on serum marker enzymes**

Groups	AST (IU/L)	ALT (IU/L)	LDH (IU/L)
Control	88.44 ± 5.77	32.37 ± 3.68	556.50 ± 50.31
ISO	109.13 ± 2.55*	49.70 ± 4.40*	831.42 ± 55.94**
HM 200+ISO	64.79 ± 5.17###	32.05 ± 2.35##	208.29 ± 40.92###
HM 400+ISO	68.88 ± 3.26###	31.30 ± 2.40##	370.61 ± 22.82###
Vit E+ISO	72.96 ± 3.96##	31.73 ± 2.87##	373.39 ± 49.90#*
HM 400	89.24 ± 2.65	29.10 ± 3.20	418.56 ± 49.01
Vit E	90.42 ± 3.13	29.24 ± 3.90	569.06 ± 61.39

Values are given as mean ± SEM (n = 7). \*P<0.05 and \*\*P < 0.01 as compared to the control group ; #P<0.05 and ###P < 0.01 as compared to the isoprenaline (ISO)-treated group. HM 200



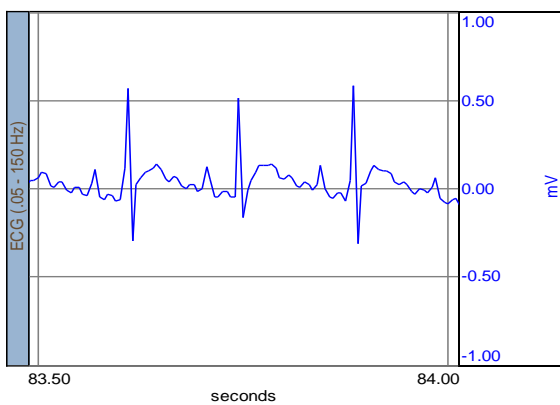
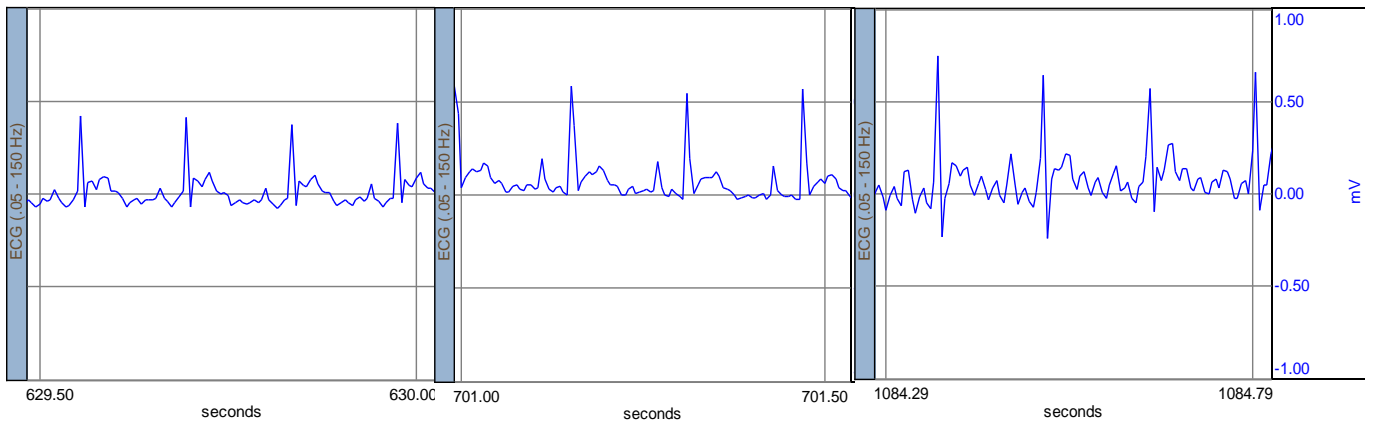
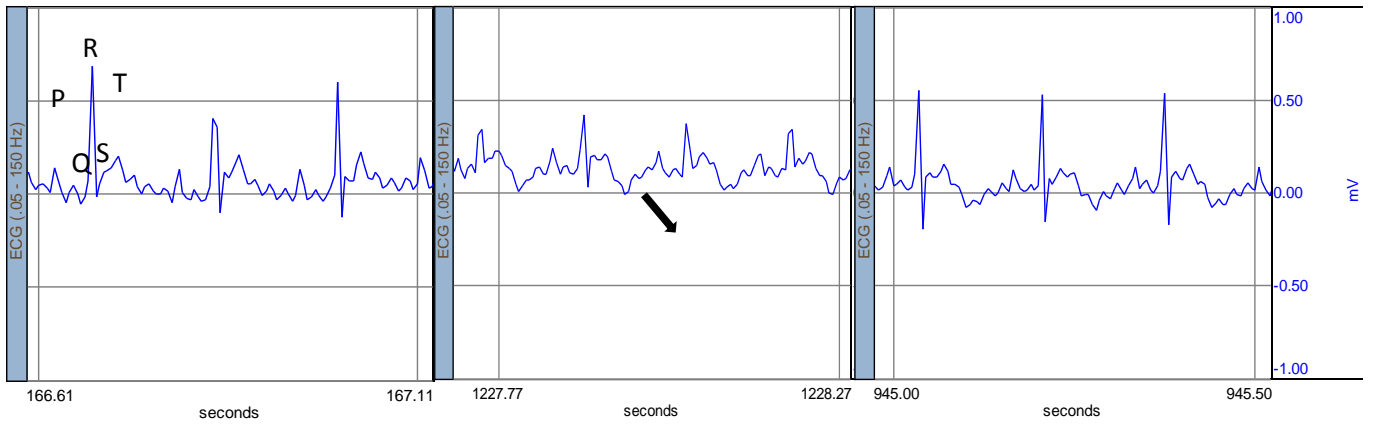
or HM 400 : *Harungana madagascariensis* aqueous extract (200 or 400 mg/kg) ; Vit E: vitamin E (100 mg/kg).

**Table 3 : The effects of *Harungana madagascariensis* treatment on reduced glutathione (GSH) and malondialdehyde (MDA) levels in the heart tissue of rats.**

Groups	GSH ( $\mu\text{mol/g tissue}$ )	MDA ( $\mu\text{mol/g tissue}$ )
Control	$0.17 \pm 0.01$	$0.42 \pm 0.08$
ISO	$0.10 \pm 0.01^{***}$	$0.85 \pm 0.16$
HM 200+ISO	$0.14 \pm 0.01^{##}$	$0.62 \pm 0.07$
HM 400+ISO	$0.21 \pm 0.01^{###**}$	$0.43 \pm 0.06$
Vit E+ISO	$0.15 \pm 0.01^{##}$	$0.50 \pm 0.07$
HM 400	$0.19 \pm 0.01^{###}$	$0.54 \pm 0.06$
Vit E	$0.18 \pm 0.01^{###}$	$0.47 \pm 0.04$

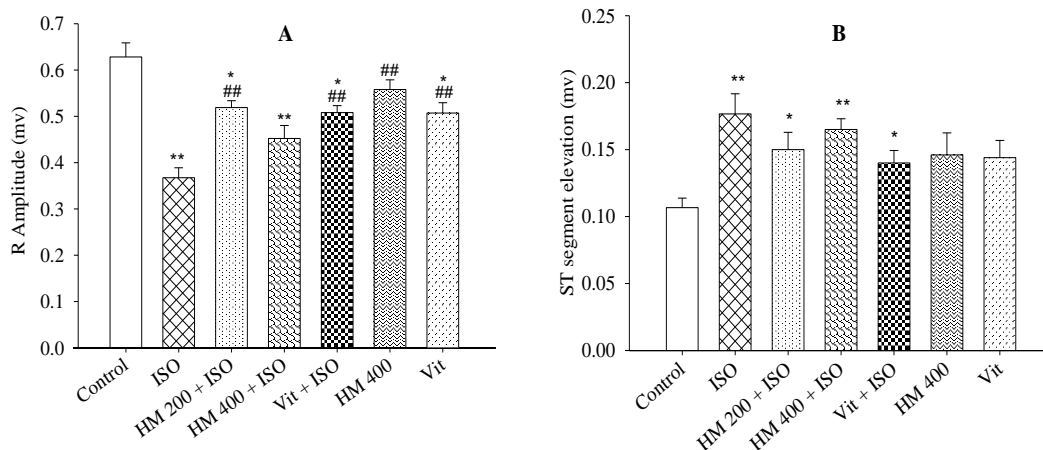
Values are given as mean  $\pm$  SEM (n = 7). \*\*P<0.01 and \*\*\*P < 0.001 as compared to the control group ; ##P<0.01 and ###P < 0.001 as compared to the isoprenaline (ISO)-treated group. HM 200 or HM 400 : *Harungana madagascariensis* aqueous extract (200 or 400 mg/kg) ; Vit E: vitamin E (100 mg/kg)

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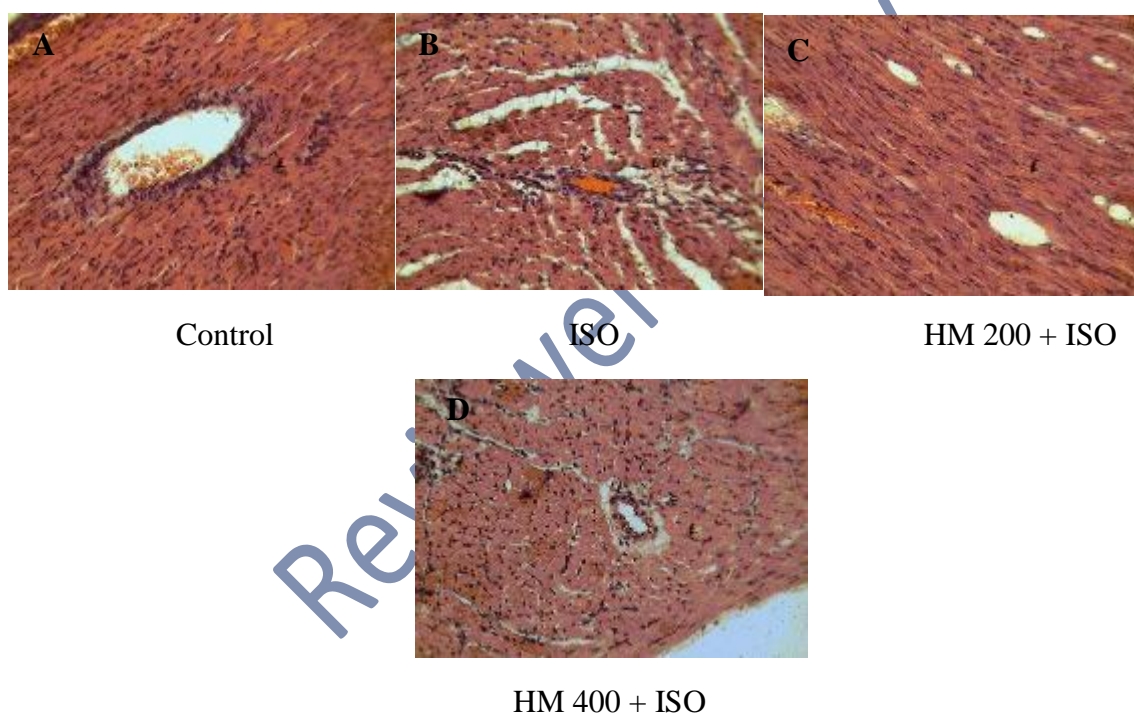


0.5 mV  
0.5 s

**Figure 1: Representative electrocardiogram tracings of control and experimental animals receiving isoproterenol (ISO), *Harungana madagascariensis* aqueous extract (200 or 400 mg/kg) + isoproterenol (HM 200 + ISO or HM 400 + ISO), Vitamin E + isoproterenol (Vit E + ISO), the extract alone at 400 mg/kg (HM 400) and vitamin E alone (Vit E). The ECG was recorded from II limb leads with recorder speed 0.5 s/div.**



**Figure 2 : Effects of oral administration of *Harungana madagascariensis* on R-amplitude (A) and ST segment (B) (recorded from limb lead II).** Data are reported as mean  $\pm$  SEM (n = 7). \*P<0.05 and \*\*P < 0.01 as compared to the control group ; ##P < 0.01 as compared to the isoprenaline (ISO)-treated group. HM 200 or HM 400 : *Harungana madagascariensis* aqueous extract (200 or 400 mg/kg) ; Vit : vitamin E (100 mg/kg).



**Figure 3. Effect of *Harungana madagascariensis* stem bark aqueous extract (HM) on histopathological changes in heart tissue.** (A) : normal group received saline showing normal structure of myocardium; (B) : diseased group received two subcutaneous injections of ISO (85 mg/kg) showing necrosis of myofibrils and edema through penetration of inflammatory cells; (C, D) : HM 100 mg/kg and 200 mg/kg treated group showing lesser myocardial necrosis and edema following ISO administration. Heart tissues were stained with hematoxylin and eosin and visualized under light microscope at x 200 magnification.