

# *Strophanthus hispidus* attenuates the Ischemia-Reperfusion induced myocardial Infarction and reduces mean arterial pressure in renal artery occlusion

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Submitted: 06-05-2014

Revised: 19-06-2014

Published: 30-08-2014

## ABSTRACT

**Background:** The myocardium is generally injured in the case of reperfusion injury and arterial damage is caused by hypertension. In reference to these statements, the present study was focused. Cardiac glycosides were said to have protective effects against myocardial infarction and hypertension. *Strophanthus hispidus* was thus incorporated in the study. **Objective:** The prime objective of the study was to investigate the protective effects of *Strophanthus hispidus* against ischemia-reperfusion myocardial infarction and renal artery occluded hypertension in rats. **Materials and Methods:** The animal model adopted was surgically-induced myocardial ischemia, performed by means of left anterior descending coronary artery occlusion (LAD) for 30 min followed by reperfusion for another 4 h. Infarct size was assessed by using the staining agent TTC (2,3,5-triphenyl tetrazolium chloride). Hypertension was induced by clamping the renal artery with renal bulldog clamp for 4 h. **Results:** The study was fruitful by the effect of *Strophanthus hispidus* on infarction size, which got reduced to  $27.2 \pm 0.5$  and  $20.0 \pm 0.2$  by 500 mg/Kg and 1000 mg/Kg ethanolic extracts which was remarkably significant when compared with that of the control group  $52.8 \pm 4.6$ . The plant extract did reduce heart rate at various time intervals. There was also a protective effect in the case of mean arterial blood pressure were the 500 mg/Kg and 1000 mg/Kg of the plant extract did reduce the hypertension after 60 minutes was  $60.0 \pm 4.80$  and  $50.50 \pm 6.80$ . **Conclusion:** The results suggest that 500 mg/Kg and 100 mg/Kg ethanolic extract of *Strophanthus hispidus* was found to possess significant cardiac protective and anti-hypertensive activity.

**Key words:** Cardio toxicity, hypertension, renin-angiotensin system

## INTRODUCTION

Hypertension is considered as the most common chronic illness among all the people in the world.<sup>[1]</sup> It is not only responsible for morbidity but also mortality. Hypertension causes considerable damage to the blood vessels leading to conditions like end organ failure.<sup>[2]</sup> In heart, mechanical stress initiates numerous pathways, including ion channels,

integrin interaction between cells and matrix, activation of various tyrosine kinases, autocrine production, and release of growth factors.<sup>[3]</sup> Elevated pressure has been one of the major issues in public health.

On the other hand, myocardial infarction is another pathophysiological condition where the heart tissue is damaged by various external factors. Myocardial ischemic reperfusion is a clinical condition similar to that of myocardial infarction.<sup>[4]</sup> Hence, in the condition of myocardial infarction management, the method of reversing the condition is by limiting the infarction size and thus decreasing the mortality.<sup>[5]</sup> Reperfusion is a condition where there is an accelerated necrosis of myocytes because

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### Access this article online

#### Website:

www.phcog.com

#### DOI:

10.4103/0973-1296.139782

#### Quick Response Code:



of cell swelling disruption of cell ultrastructure, formation of contraction bands and deposition of intramitochondrial calcium phosphate granules.<sup>[5]</sup> The major mechanism behind the pathogenesis of ischemic reperfusion injury is oxidative stress. Infact oxidative reperfusion injury was suggested to be a central mechanism of the cellular damage affecting all organs and tissues after ischemia and that is the reason why there are numerous proofs that anti-oxidants can reverse the condition of myocardial infarction.

*Strophanthus hispidus* was used since historic period. The latex and seeds were used as arrow poisons in Africa.<sup>[6]</sup> Decoctions of the root, stem and seeds were employed in the treatment of leprosy, malaria, dysentery and other sexually transmitted diseases.<sup>[6]</sup> Evidence for this statement was gained when *Strophanthus hispidus* was successful in devitalizing some pathogenic bacteria and some dangerous drug resistant strains.<sup>[7]</sup> Root extracts of *Strophanthus hispidus* were useful in reducing inflammation of paw and ear.<sup>[8]</sup> The plant was even employed in treating various medical conditions in an early period of herbal therapeutics.<sup>[9]</sup> A phenomenal hypoglycemic study was proved on this plant.<sup>[10]</sup> The study confirmed the potential of the activity due to the presence of cardiac and cyanogenic glycosides. The root and leaf extracts were found to be potent sources of antidiabetic activity.<sup>[10]</sup> Cardiac glycosides and aglycones were identified by paper chromatography.<sup>[11]</sup> This evaluation was considered as the most substantial evidence for conducting this study. The presence of cardiac glycosides would certainly aid in attenuating cardiac damage and hypertension.

## MATERIAL AND METHODS

### Plant collection and authentication

Fresh plants were imported from Mushin market, in Lagos, Nigeria from an authentic source (Ebana herbal market). However, the plant was authenticated by Dr. Madhava Chetty, Department of Botany, Sri Venkateshwara University, Tirupathi Dist. Chittoor, Andhra Pradesh, India and the voucher was preserved in herbaria the College laboratory, Malla Reddy Institute of Pharmaceutical Sciences, Hyderabad, Andhra Pradesh, India.

### Plant extract

The plant was air dried, pulverized to a coarse powder in a mechanical grinder, passed through a 40-mesh sieve, and extracted in a soxhlet extractor with ethanol for 72 hrs. The extract was decanted, filtered with Whatman No. 1 filter paper and concentrated at reduced pressure below 40°C through rota vapor to obtain dry extract. This ethanolic extract was taken up for biological screening.<sup>[12,13]</sup> The percentage (%) yield was 2.5%.

### Experimental animals

Healthy adult albino rats of Wistar strain weighing 150-200 g were used in the present study. The experiment was performed after the permission of the Institutional Animal Ethical Committee (IAEC). The animals were properly housed under natural photo periodic conditions, atmospheric conditions along with access to food and water *ad libitum* throughout the study. The IAEC of Malla Reddy Institute of Pharmaceutical Sciences, India, with College Reg. No. 1217/a/08/CPCSEA approved the study.

### Chemicals

All the chemicals used in the study were of analytical grade procured from standard sources like SIGMA®.

### Acute toxicity study

Acute toxicity study was conducted to determine the safe dose according to the OECD (Organization for Economic Co-operation and Development) guideline 420, ANNEXURE 1. The extract was administered orally to overnight fasted animals. After administration the animals were observed continuously for one hour, frequently for the next 4 hrs and then after 24 hrs. After administration, Irwin's test was conducted, where the animals were observed for gross behavioral changes. For this, the following checklist was employed, which did involve: Behavioral, neurological and autonomic profile. The dose selected for the extracts were about 1/10<sup>th</sup> of the maximum tolerated, safe dose found from acute toxicity studies.

With the reference to the toxicity studies, the doses 500 mg/Kg and 1000 mg/Kg were incorporated in the present study.

### *In vivo* evaluation of myocardial ischemia reperfusion surgical preparation

Rats were initially anesthetized by thiopentene sodium (30 mg/Kg, intra peritoneal) tracheotomized and ventilated with room air by a Techno Positive Pressure Respirator (Animal respirator, Crompton Parkinson Ltd., England). A left thoracotomy and pericardiotomy were performed and left anterior descending coronary artery (LAD) was dissected free above the first diagonal branch and the artery and was ligated below the origin of the left circumflex artery with the help of a silk thread. The artery was occluded for 30 min. The reperfusion was allowed for the succeeding 4 hrs. The sham control animals were subjected to the entire surgical procedure and the thread was passed beneath the coronary artery, but the LAD coronary artery was not ligated. A lead II electrocardiogram was monitored throughout the study by using Cardiart 408 (BPL) with sensitivity 20 mm mv<sup>-1</sup> at a paper speed 50 mm s<sup>-1</sup>. Heart rates were expressed as beats/min.<sup>[14]</sup>

### Measurement of infarction size

In all the groups after sacrificing the animal by injecting 2.56 M potassium chloride directly into the left ventricle, the heart was excised from the thorax rapidly and the greater vessels were removed. The left ventricle was separated from the heart and was weighed. It was sliced parallel to the atrioventricular groove to 2–3 mm thick sections and the slices were incubated in 1% TTC (2, 3, 5-triphenyl tetrazolium chloride) solution prepared in pH 7.4 phosphate buffer for 30 min at 37°C. The pale necrotic myocardial tissue was separated from the stained portions and weighed on an electronic balance.

### Estimation of anti-hypertensive potential

Male Wistar rats were divided into the six groups, each group had six animals. Animals in normal control and negative control groups received distilled water. Ethanolic extract of *Strophanthus hispidus* was administered orally at the dose levels of 500 and 1000 mg/kg to the treatment groups for 6 weeks. At the end day of treatment, animals were anesthetized by intraperitoneal injection of 1.25 g/kg of Urethane. A small incision was given on the left side of the peritoneal cavity of the animal to expose left kidney. The renal artery was occluded for 4 hrs by using renal bulldog clamp. The jugular vein was cannulated for the administration of the test drug. The carotid artery was cannulated to measure the blood pressure and connected to the blood pressure transducer of power lab eight channel recorder Power Lab. After stabilizing blood pressure, the renal bulldog clip was removed. Then, 1/10<sup>th</sup> of the administered dose of the *Strophanthus hispidus* ethanolic extract, that is 50 and 100 mg/kg was given respectively through the jugular vein and mean arterial blood pressure (MABP) was measured at different time intervals (5, 30, 60 min). MABP of normal control groups were recorded without clamping the renal artery<sup>[15]</sup> Ramipril 2 mg/kg, intravenous (i.v) was used as a standard. A change in blood pressure of treated groups was compared with the negative control.

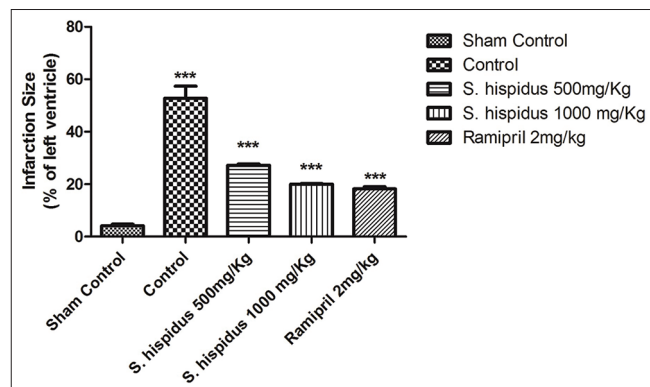
## RESULTS

Figure 1 illustrates the infarction size in experimental animals. The infarction size in the control group was found to be  $52.8 \pm 4.6$  which was significant in comparison to the sham control group animals. The infarction was reduced to  $27.2 \pm 0.5$  and  $20.0 \pm 0.2$  with the treatment of ethanolic extract of *Strophanthus hispidus*. The ramipril has shown the highest significance ( $18.2 \pm 0.8$ ). The difference was significant in comparison with the sham control group ( $P < 0.05$ ).

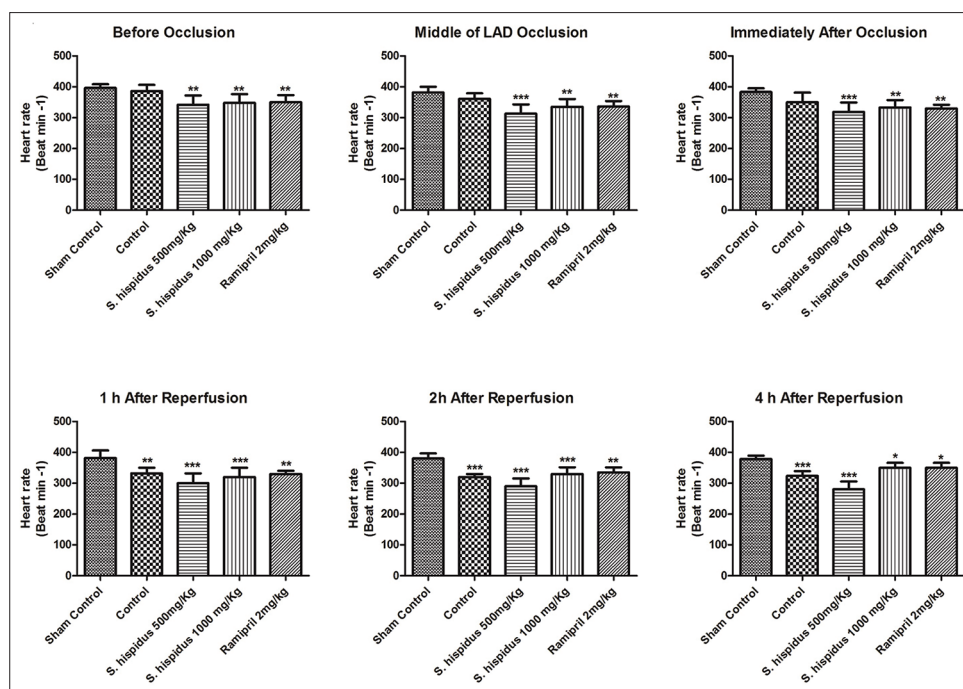
Figure 2 explains the data for heart rate recorded at various intervals of time during the experiment for all

the groups. In the control group, a continuous decrease in heart rate was observed during 30 min coronary artery ligation and throughout the reperfusion period compared to sham control group [Figure 2]. The groups treated with *Strophanthus hispidus* at doses of 500 and 1000 mg kg<sup>-1</sup> produced a slight decrease in heart rate during 30 min coronary artery ligation and there after gradually increased throughout the reperfusion period and restored to normal value at the end of the 4 hrs. The protection produced with *Strophanthus hispidus* at doses of 500 and 1000 mg kg<sup>-1</sup> depicting its protective activity against ischemia-reperfusion induced injury. The standard drug ramipril significantly decreased the infarct size, which was evidently visible, protected from fall in heart rate during ischemia-reperfusion period compared to control group. In context to ischemia reperfusion injury, 1000 mg/Kg of *Strophanthus hispidus* did show an effect similar to the standard drug ramipril.

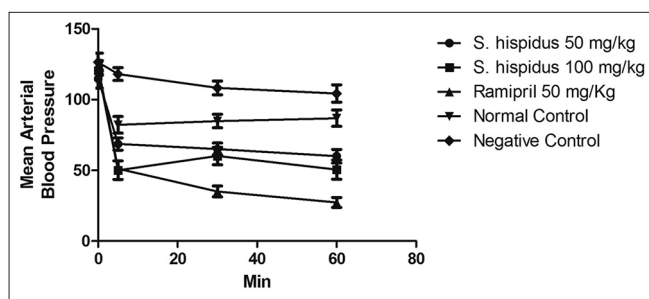
Table 1 and Figure 3 elucidates the data of mean arterial pressure in the condition of renal artery occluded hypertension. Pretreatment of animals with *Strophanthus hispidus* ethanolic extract 50 and 100 mg/kg i.v. showed significant decrease ( $P < 0.05$ ) in the MABP (mean arterial blood pressure) at different time intervals. The standard drug (ramipril) did demonstrate a significant event. The hypotensive effect was maximum after 60<sup>th</sup> minute. The mean arterial pressures recorded at the dose rate of 50 mg/Kg and 100 mg/Kg of the plant extract did reduce the hypertension after 60 minutes was  $60.0 \pm 4.80$  and  $50.50 \pm 6.80$  respectively, and the standard drug (ramipril) did show the highest significance value ( $27.30 \pm 3.50$ ) that is, it did reduce the mean arterial pressure to the maximum extent. The advantage of this experimental model was that the enhancement of mean arterial pressures in the control group in different time intervals was evidently comparable to the decreasing order of mean arterial pressures in the 50 mg/Kg, 100 mg/Kg and ramipril treated groups.



**Figure 1:** Infarction size in experimental animals. Values are expressed as mean +S.D. Significant ( $P < 0.05$ ) compared to the sham control group



**Figure 2:** Heart rate (Beat min<sup>-1</sup>) recorded at various intervals during myocardial infarction/reperfusion. Values are expressed as mean + S.D. Significant (P < 0.05) compared to the sham control group



**Figure 3:** Significance of *Strophanthus hispidus*'s ethanolic extract in lowering of MABP on renal artery-occluded hypertensive rats. Values are expressed as mean + SEM. Significant (P < 0.05) compared to the negative control group

## DISCUSSION

In the past, many natural compounds have been used in the studies of myocardial ischemia and reperfusion. The agents used to possess oxygen free radical scavenging, antioxidant ability, calcium channel blocking, inhibitors of neutrophils, nitric oxide, adenosine-related agents, inhibitors of the renin-angiotensin system, endothelin receptor antagonists, Na<sup>+</sup>/H<sup>+</sup> exchange inhibitors, and antiapoptotic agents.<sup>[16]</sup> Several affirmations have been proposed which emphasized the myocardial injury observed after ischemia and reperfusion.<sup>[17]</sup> Myocardial reperfusion generates free radicals that can damage cardiac cells and mimic the pathological features of ischemia-reperfusion injury. The treatment with free radical scavenger reduces infarct size after regional myocardial ischemia and reperfusion in a

canine preparation has been reported.<sup>[18]</sup> Such reports have stood as a firm support for this study. The presence of cardiac glycosides was also regarded as a substantial evidence<sup>[11]</sup> which aids in attenuating infarction. On the other hand, ischemia of the kidneys causes elevation of blood pressure by activation of the renin-angiotensin system. The procedure can be used for acute and chronic hypertension. Acute renal hypertension in the present study was induced in rats by clamping the left renal artery for 4 hrs. After reopening of the vessel, accumulated renin was released into circulation.<sup>[15]</sup> Renin acts as on angiotensinogen to release the decapeptide angiotensin I. This decapeptide is cleaved by angiotensin converting enzyme (ACE) to generate the active angiotensin II (octapeptide) which is a potent vasoconstrictor leading to hypertension. Angiotensin II undergoes hydrolysis by an aminopeptidase to yield the heptapeptide angiotensin III which is also active. Further cleavage yields to peptides with little activity.<sup>[19,20]</sup> The protease renin catalyzes the first and rate-limiting step in the formation of angiotensin II, leading to acute hypertension. Initially, the toxicity studies were performed in which no mortality was recorded even at 10 gm/Kg dose. 500 mg/Kg and 1000 mg/Kg were selected as the ideal doses.

## CONCLUSION

The present findings suggest that the *Strophanthus hispidus* ethanolic extract possesses a dose dependent



**Table 1: Effect of ethanolic extract of *Strophanthus hispidus* renal artery-occluded hypertensive rats**

Groups	Treatment	Mean arterial blood pressure (after removing the clip)	5 min	30 min	60 min
Normal control	Distilled water (p. o)	-	82.2±5.92	84.82±4.82	86.82±5.82
Negative control	Distilled water (p. o)	126.42±6.36	118.12±4.56 <sup>@</sup>	108.3±4.84 <sup>@</sup>	104.30±6.16 <sup>@</sup>
<i>Strophanthus hispidus</i>	50 mg/Kg (i.v)	114.72±6.52	68.66±4.32*	65.12±4.2*	60.0±4.80*
<i>Strophanthus hispidus</i>	100 mg/Kg (i. v)	120.52±3.50	50.10±6.60*	60.15±6.20*	50.50±6.80*
Ramipril	2 mg/Kg (i. v)	122.84±4.8	51.2±2.34*	35.1±3.84*	27.30±3.50*

Values in the results are expressed as mean±SEM, (n=6), <sup>@</sup>P<0.05 significantly different in comparison with Normal control, \*P<0.05 significantly different in comparison with Negative control

cardio protection against ischemia-reperfusion induced myocardial injury. The groups treated with *Strophanthus hispidus* at doses of 500 and 1000 mg kg<sup>-1</sup> produced a slight decrease in heart rate during 30 min coronary artery ligation and thereafter gradually increased throughout the reperfusion period and restored to normal value at the end of the 4 h. The protection produced with *Strophanthus hispidus* at doses of 500 and 1000 mg kg<sup>-1</sup> depicting its protective activity against ischemia-reperfusion induced injury. *Strophanthus hispidus* was also fruitful in reducing the mean arterial blood pressure. There was a significant ( $P < 0.05$ ) decrease in the elevated blood pressure in a dose dependent manner. The drastic fall in MABP after 1 h may precipitate reflex tachycardia and a compensatory increase in sympathetic tone. The extract appears to be free from such hypertensive effect. The anti-hypertensive action of the ethanolic extract of *Strophanthus hispidus* was found to be pragmatic and could be due to the action on renin-angiotensin system.

## ACKNOWLEDGEMENT

We are very much thankful to Dr. Rajashekar who has helped us in identifying the plant source from some of the ancient homeopathic literature. Corresponding author Dr. Kim Kah Hwi (Associate Professor at department of Physiology) is thankful to the University of Malaya for the University of Malaya research grant (UMRG 357/11 HTM). Author Rajeev K Singla is also thankful to Science and Engineering Research Board, Government of India for providing SERB-Young Scientist/Principal Investigator fellowship vide project no. SR/FT/LS-149/2011. Author Rohit Gundamaraju is receiving Bright Spark scholarship from University of Malaya. Dr. ARR thanks University of Malaya Research Grant (UMRG RP001i-13SUS), University of Malaya for providing financial support to this project.

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**Cite this article as:** Gundamaraju R, Vemuri RC, Singla RK, Manikam R, Rao AR, Sekaran SD. *Strophanthus hispidus* attenuates the Ischemia-Reperfusion induced myocardial Infarction and reduces mean arterial pressure in renal artery occlusion. *Phcog Mag* 2014;10:557-62.

**Source of Support:** Nil, **Conflict of Interest:** None declared.