Int J Ayu Pharm Chem
ISSN 2350-0204
www.ijapc.com

An Experimental Analysis on the Cardio Protective Action of *Vrikshamla*

Author: Sruthi Mohan[1]

Co Authors: Shrikanth P H[2] and Sudhakar Bhatt[3]

[1-3]Department of PG studies in Samhita and Siddhanta, Sri Dharmasthala Manjunateshwara College of Ayurveda, Kuthpady, Udupi, KA, India

ABSTRACT

BACKGROUND

HRUDAYA is the most important vital organ and any damage to this organ can lead to a number of serious complications and even death, so improving its functional quality is very much essential. The term cardiovascular disease is familiar which refers to a group of diseases that affects heart and its parts. Among all cardio vascular diseases, myocardial infarction is considered as one of the most dangerous disease. There is a scope for natural therapy in preventing cardiac ailments with the help of medicinal plants. Cardio protection includes all mechanisms and means that contribute to the preservation of the heart by reducing or even preventing myocardial damage. Traditional medicine is a field that is still much in demand for research. In Charaka Samhita a group of drugs termed as Hrudya Dashemani possessing Amla Rasa is explained. These drugs are useful in maintaining cardiac health.

Vrikshamla (Garcinia indica) is one of the Dravyas explained under Hrudya Dashemani. In the present study, cardio protective effect of Vrikshamla fruit rind's decoction in isoprenaline-induced myocardial infarction was evaluated in Wistar albino rats.

METHODS:

In vivo effect of Vrikshamla (Garcinia indica) decoction was evaluated in Wistar albino rats by isoprenaline-induced myocardial injury model. Cardiac injury markers (SGOT, SGPT, Urea, LDH, CK-MB etc.), oxidative stress markers and histopathological changes were evaluated in each group and compared using appropriate statistical tests.

RESULTS AND INTERPRETATION:

Observations after the study showed that the test drug Vrikshamla has protective effects on Heart. Blood parameters, histopathology as well as anti-oxidant study supported the same.

CONCLUSION:

Results of the study revealed that the study drug gave a statistically significant result which confirms that Vrikshamla from Charakokta Hrudya Dashemani possess cardioprotective qualities.

Key Words: *Hrudya, Amlam, Dashemani, Anti-oxidant, Vrikshamla*

INTRODUCTION

Ischemic heart disease is a serious non communicable disease and it has become a major problem worldwide. The damage to heart tissues because of ischemia eventually causes irreversible ardiac injury which can even lead to death. Among Ischemic heart disease, acute myocardial infarction is the most alarming one and it occurs due to imbalance between coronary blood supply and myocardial demand. Several preclinical studies suggest that during myocardial ischemia, the level of oxidative stress is significantly

enhanced which influences the development of myocardial infarction. Consequently, there is a great need for a defensive antioxidant therapeutic approach to safely avert ischemic heart complications. Ayu is described as the conjugation of Shareera, Indriya, Satwa and Atma[1]. This conjugation is the reason for life and the place for conjugation is Hrudaya[2]. So, Hrudaya is the most important vital organ and improving its functional quality is very much essential for healthy as well as diseased individuals. Hrudaya is Moolasthana of two important Srotas i.e. Rasavaha Srotas and Pranavaha Srotas3. Rasavaha Srotas is responsible for circulation of Rasa Dhatu and nourishment to all body constituents. A group of drugs termed Hrudya Dashemani is found mentioned in Charaka Samhita which acts on Hrudaya. Hrudya Gana includes: Amra, Amrataka, Lakucha, Karamarda,Vrikshamla, Amlavetasa, Kuvala, Badara, Dadima, Matulunga[4].

It is mentioned Amlam Hrudyanam in the context of Agrya Dravyas5, which means Amla Rasa is more Hrudya in nature among all Rasas. But for the present era, it is important to validate or confirm by experimentation. Hence, this study is focused on assessing and proving the Hrudya action of an Amla Rasa Pradhana Dravya (Vrikshamla), commonly known as Kokum (Garcinia indica). The use of herbal supplements is gaining popularity in recent years. The role of plant based bioactive compounds or phytochemicals has attracted much attention due to their unique cardio protective activity. Cardiotonic drugs are substances that increase the contracting mechanism within the heart, thereby causing more blood to be pumped throughout the circulatory system.

Vrikshamla is an easily available dietary fruit and its dried rinds are used to make cool drinks during summer and also used in dishes instead of tamarind. This fruit appears to have more potential as a health supplement rich in natural anti-oxidants.

Vrikshamla is illustrated as Hrudya and Hrudrogajit. In the present study, somatic protection of Hrudaya is given prime importance as thestudy is concentrated on the cardio-tonic action of the drug Vrikshamla. The changes in the histopathology of cardiac tissues and biochemical alterations are considered ashallmark for the study.

AIM

To evaluate the *Hrudya* effect of *Vrikshamla* in isoprenaline induced cardiotoxicity through animal experimentation.

REVIEW OF LITERATURE:

This section is separated into two segments:

1. Drug Review:

Botanical name: *Garcinia indica*[6]

Family: *Guttiferae*

Genus: *Garcinia*

Chemical Composition[7]:

The fruit rind contains polyiosoprenylated phenolic pigment, Garcinol and its isomer isogarcinol along with hydroxycitric acid, cyaniding – glycoside and cyaniding

sambubioside. L-Leucine and DNP - L- Leucine hydrochloride have been reported from the leaves. Heartwood – Cuxanthone, Volkensiflovone, Morelloflavone, Comboginol[8]

Seed – Neutral lipids, glycolipids

Ayurvedic pharmacodynamic:

Guna –Ruksha[9]

Rasa-Amla,Katu,Kashaya

Veerya-Ushna

Vipaka-Katu

Doshagnata- Vatakaphanashaka

Karma- Sangrahi, Rochana, Deepana, Shoolagna

Prabhava – Hrudya

AmayikaPrayoga:

Trishnachikitsa[10], *Rakthasravi Arshas, Atisara, Gulma, Pleeha, Shoola*[11].

Garcinia species are important group of plants, being used for different purposes, especially as fruit crops, source of edibleoils and fats, and nutraceuticals in different parts of the world. Edible fruits are life enhancing medicines packed with vitamins, minerals, antioxidants and many phytonutrients. There are plenty of underutilized fruit crops which possess immense nutraceutical value. Garcinia is one such underutilized group of fruit bearing plants. *Garcinia indica* has many culinary, pharmaceutical and industrial uses. It has an agreeable flavour, a sweetish acid – taste, and is used as a souring agent in drinks and food. Fresh and dry Garcinia fruit rinds are used as spice, condiment and garnish in several cuisines to impart an acidic flavour to the food and to enhance shelf life. In Travancore, Malabar and Konkan region of south India, the fruits of *Garcinia combogia* and *Garcinia indica* are used in garnishing curries and also as a substitute for tamarind.

Fruit and syrup of *Garcinia indica* is very popular in Konkan region as a refreshing

and rejuvenating drink.

MATERIALS AND METHOD:

Drug source - Market sample of dried *Vrikshamla* rinds were collected.

Sample source – Wistar albino rats of either sex weighing between 150-200 gm bred in animal house, of S.D.M. Centre of Research in Ayurveda and Allied Sciences, Udupi was selected for the study. Eighteen rats were selected, and separated into 3 groups, each group consisting of 6 rats (Table 1).

Inclusion criteria:

- Healthy albino rats of either sex
- Weighing about 150-200 gm

Exclusion criteria:

- Wistar strain albino rats weighing less than 150 and more than 200 gm.
- Pregnant and diseased rats
- Rats which were under trial of other experiments

Preparation of the drug:

Daily fresh *Kwatha* was prepared from 3-4 dried rinds of *Vrikshamla* by boiling it

in 20 ml of water and then reducing to half.

Dose fixation of the drug:

The dose was calculated by extrapolating ‘human dose’ to ‘rat dose’ based on ‘body

surface area ratio' by referring to the table of Paget and Barne's Standard dose conversion formula.

Human dose × 0.018× 5/kg body weight

50 ml × 0.018 × 5 = 4.5 ml/kg body weight

= 0.0045/gm body weight

For the present study, in one group 6 rats were kept. So daily 10 ml *Kwatha* was needed for one group.

Route of drug administration:

The drugs were administered by oral route with the help of feeding tube.

Duration of study:21 days

Animal Grouping:

Each group having 6 rats were kept in separate metabolic cages.

Table 1 Information about group formation

Sl.No	Group	Drug	No. of animals
I	Control	No medicine	6
II	Test Group	*Vrikshamla* + Isoprenaline	6
III	Positive Control	Isoprenaline	6

EXPERIMENTAL METHODOLOGY

Three groups were made and cardio toxicity was induced with 80 mg/kg body weight of Isoprenaline (ISO) subcutaneously (Table 2).

On 21st day, Mild anesthetic agent was given and blood was withdrawn from Retro-orbital plexus.

Table 2 Experimental methodology details

Days	Test Group	Positive Control
From 1st to 18th day	*Vrikshamla* + normal diet and water	Normal diet and water
On 19th day	1st Dose ISO (80mg/kg body weight)	1st Dose ISO (80mg/kg body weight)
On 20th day	2nd dose of ISO	2nd dose of ISO
On 21st day	Mild anesthesia, blood withdrawal, sacrifice	Mild anesthesia, blood withdrawal, sacrifice

Followed by sacrifice, and the heart was sent for anti-oxidant as well as histopathological study.

Statistical analysis:

All the values were expressed as Mean ± SEM (Standard error of mean). The data were analysed by One-way ANNOVA followed by Dunnett's Multiple Comparison 't' test with $p < 0.05$. A level of significance was noted and interpreted accordingly, using GraphPad InStat 3 software.

Assessment criteria:

Assessment criteria was made on the basis of

- Biochemical parameters such as SGOT, SGPT, Urea, Creatinine, Cholesterol, Tri-glycerides, CK-MB, LDH (Table 3)
- Heart weight (Table 4)
- Histopathology of Heart (Table 6)
- Anti-oxidant study of Heart (Table 5)

OBSERVATION & RESULTS

Table 3 Evaluation of Serum Biochemical Parameters

Parameters	Normal control	Isoprenaline control	*Vrikshamla* group
Sugar (mg/dl)	93±2.03	99.16±5.26	115.4±4.30 *
Urea (mg/dl)	51±2.81	45.16±4.74	40.2±5.46
SGOT (U/L)	116.83±5.01	124.83±10.73	159.4±19.39
SGPT (U/L)	55.5±4.03	79.16±5.82**	60±0.94*
Creatinine (mg/dl)	0.36±0.02	0.5±0.03**	0.52±0.02
Cholesterol (mg/dl)	70.33±4.73	58.5±6.27	94.6±11.24**
Triglycerides (mg/dl)	89.6±5.220	118.3±10.70	77.6±14.19*
CKMB (U/L)	199.4±73.38	208.54±30.53	154.64±31.17
LDH (U/L)	226.4±37.21	644.02±59.12**	562.74±94.77

Data: MEAN±SEM ** P<0.01 * P<0.05

Table 4 Evaluation of Heart Weight

Organ	Normal control	Isoprenaline control	*Vrikshamla* group
Heart	0.81±0.01	0.96±0.05	0.75±0.06*

Data: MEAN±SEM * P<0.05

Table 5 Evaluation of Anti-Oxidant Parameters

Anti-oxidant parameters	Normal control	Isoprenaline control	*Vrikshamla* group
Lipid Peroxidation (mmoles of MDA formed/ g wet tissue)	1.47±0.087	2.67±0.63	1.14±0.02*
Protein estimation (mg/dl)	0.054±0.054	0.04±0.001*	0.033±0.003
catalase activity (mmoles/min/mg protein)	0.928±0.071	1.648±0.189*	1.617±0.229

Data: MEAN±SEM * P < 0.05

Table 6 Histopathological study result

Groups	Changes observed	Remarks
Normal	Heart The sections consist of cardiac muscle, the myocardium. The myocardium consists of muscle cells with centrally placed nuclei	Normal tissue architecture
G1	All tissue sections showed severe degeneration of muscle fibers with inflammatory cells. There were many areas of necrosis, edema also	Severe toxic changes
G2	Compared with G1 there was no reduction in degenerative changes. Necrosis and inflammation present in all sections	Mild to moderate toxic changes. Moderate protection seen when compared to G1 group

DISCUSSION

Biochemical Parameters:

Total nine serum biochemical parameters were measured as a part of analysing the cardio protective activity (Table 3). Among them significant changes were observed in ISO control in the following parameters- Increase in SGPT, Serum creatinine, LDH, then moderate but statistically non significant increase was observed in SGOT, Serum triglycerides, CK-MB, Blood sugar level. Statistically non-significant decrease was observed in Serum urea and Serum cholesterol (Table 7).

Table 7 Consolidated statement of the result obtained during the study

Parameter	ISO control	ISO plus T_1
SGOT	NSI	NSI
SGPT	SI	SD
Serum urea	NSD	NSD
Serum creatinine	SI	NSI
Serum cholesterol	NSD	SI
Serum triglycerides	NSI	SD
CK-MB	NSI	NSD
LDH	SI	NSD
Sugar	NSI	SI
Heart weight	NSI	SD
Lipid Peroxidation	NSI	SD
Protein Estimation	SD	NSD
Catalase Activity	SI	NSD

SI – Significant increase, NSI – Non significant increase, SD – Significant decrease, NSD – Non significant decrease.

Analysis of the results obtained from these parameters form the main basis for this discussion. The above changes can be considered as indications of isoprenaline induced pathology in the heart. SGOT is widely distributed in tissues with the highest concentration found in liver, heart, skeletal muscles and kidneys. Damage or disease involving any of these tissues can lead to elevated levels of SGOT in serum. This is one of the enzymes which get elevated in myocardial infarction. However, its elevation is also seen in cirrhosis, metastatic carcinoma and viral hepatitis. Muscular dystrophy, dermatomyositis and acute pancreatitis are the other condition where elevation is observed. If AST (Aspartate Amino Transferase) elevation is the index of myocardial injury then it is natural to expect its reversal by drugs effective as cardio-protectives. In the present study results, further elevation though statistically insignificant was observed in the test group. This may indicative of in effectiveness of the drug, but the trend observed in case of other parameters presents a complex type of action. It is possible that non-cardiac tissue like skeletal muscle may also be contributing significantly to the observed elevation; hence in-effectiveness is employed. SGPT/ALT (Alanine Aminotransferase) also referred to as Glutamate pyruvate transaminase (GPT) is an enzyme involved in amino acid metabolism. It is found in many tissues like cardiac tissue, kidney etc. but highestlevels are found in liver. Tissue destruction leads to release of intracellular enzyme into circulating blood. Markedly elevated SGPT levels may be found in variety of diseases which involve the liver such as hepatitis, mononucleosis and cirrhosis. ALT is regarded as a reasonably specific indicator in liver disease. SGPT is frequently normal in the face of elevated SGOT in less extensive infractions, but may rise in the presence of large infarcts. In the present study a significant elevation is observed in SGPT level in the ISO control group, whereas a significant decrease of this parameter is observed in the test drug group. This may point to the effectiveness of the drug in cardio protection. Creatine Kinase-MB/CKMB isoenzyme activity is a useful biomarker for the diagnosis of myocardial injury due to different reasons. CK is dimeric composed of M and B subunits, which is immunologically distinct. It exists as three main iso-enzymes i.e, CK-MM, CK-MB, and CK-BB. The CK-MM is found in muscles while CK-MB is found mainly in myocardial cells. The CK-BB found mainly in the brain and lungs, enters the blood stream only on injury to these organs like cerebrovascular accidents or pulmonary infarction. CK-MB level increases significantly 4-6 hours following a myocardial infarction and a peak at around 12 to 24 hours after the infarct. The levels return to normal in case of no further myocardial damage, after 24 to 48 hours. Hence the increased levels of CK-MB along with elevated levels of total CK is good indicator of myocardial infarction. In the present study an increase in CK-MB though non-significant was observed in ISO control group indicating marked damage to the myocardium. Whereas, a non-significant decrease is observed in

the test drug administered group to which ISO was injected. This can be considered as an index of myocardial protection. This may be indicative of mild to moderate myocardial membrane integrity protection through different mechanisms. LDH is found in all organ cells especially plentiful in cardiac and skeletal muscle, liver, kidney and RBC's. It is found in the form of iso enzymes. Elevated LDH are found in MI, liver diseases, haemolytic anemia, pernicious anaemia, Leukemia and pulmonary diseases. LDH elevation in MI occurs in acute (48-72hr) as well as delayed (10-14 days) stages. Along with CK-MB it is considered as an important bio-marker for the assessment of myocardial injury. In the present study significant elevation of LDH activity was observed after ISO injection which is indicative of disruption of myocardial membrane integrity. This was reversed to moderate extent by test drug group as a non-significant decrease is observed there. This result along with CK-MB results may be considered to indicate moderate myocardial protection in test group/*Vrikshamla* administered group. Measurement of different lipid parameters is carried out during evaluation of test drug for cardioprotective activity. In the present study significant decrease was observed in serum triglyceride level and increase in serum cholesterol level in the test drug group while, in ISO control group a non-significant increase was observed for serum triglyceride level and a non-significant decrease in cholesterol level. This indicates that serum triglyceride was reversed in test drug administered group. This may be considered as moderate reversal of the toxicant induced change. In the present study a non-significant decrease in serum Urea was observed in ISO control group and a non-significant decrease is seen in the test drug administered group as well. It is found that isoprenaline caused a significant increase in the level of serum creatinine in case of ISO control group. Which indicates pre-renal kidney damage and not an intrinsic renal damage as urea level is not showing much changes. And in the test drug administered group; a non-significant increase was observed in the level of creatinine, so compared to ISO control group, there is a noticeable change observed and it shows moderate reversal of the toxicant induced change. Isoprenaline enhances the heart weight due to cardiac hypertrophy, in the present study a non-significant increase is observed in ISO control group and a significant decrease is observed in test group. This states that the test drug attenuated the myocardial hypertrophy induced by isoprenaline.

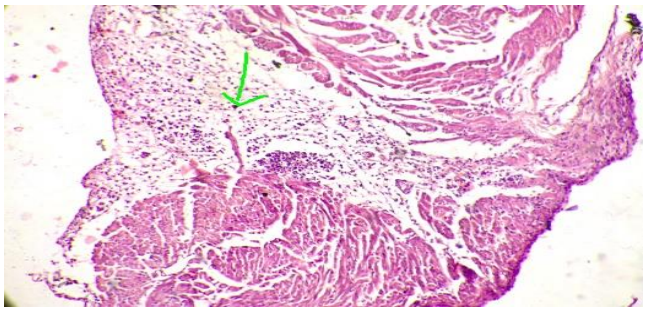
ANTI-OXIDANT STUDY:

Isoprenaline induced MI in animals shows excessive generation of free radicals followed by production of oxidative stress as a result of reduced endogenous antioxidant activity. In the present study, lipid peroxidation, protein estimation and catalase activity were analysed for finding the anti-oxidant property of *Garcinia indica*. Catalase was found to be moderately decreased in non-significant manner in test group compared to Isoprenaline control group. The lipid peroxidation has shown significant decrease in test group whereas a non-significant increase was

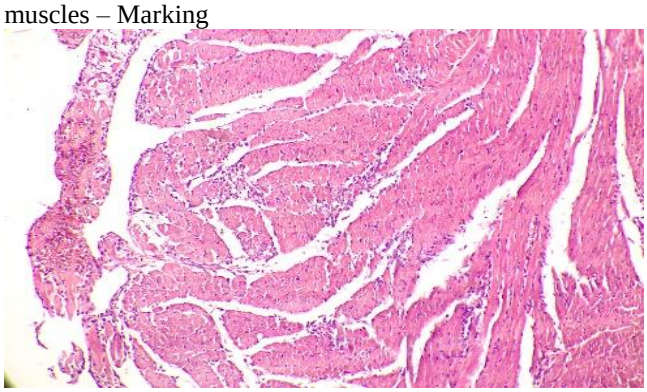
observed in Isoprenaline control group (Table 5). This may contribute to the lessening of the oxidative stress. Thus, the effect of treatment on anti-oxidant parameters were ascertained.

HISTOPATHOLOGY:

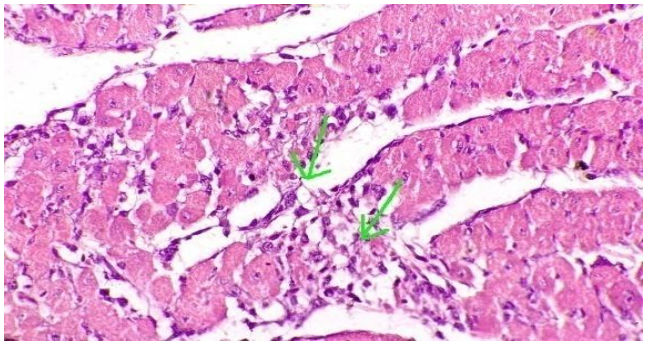
Histopathological examination of the heart sections from Isoprenaline control group revealed drastic pathological changes in the form of severe degeneration in cardiac muscle fibers with loss of striations, necrosis, edema and inflammation with loss of muscle fibers in some parts (Figure 1).



(a) 10x showing edema and inflammatory cells in the muscles – Marking



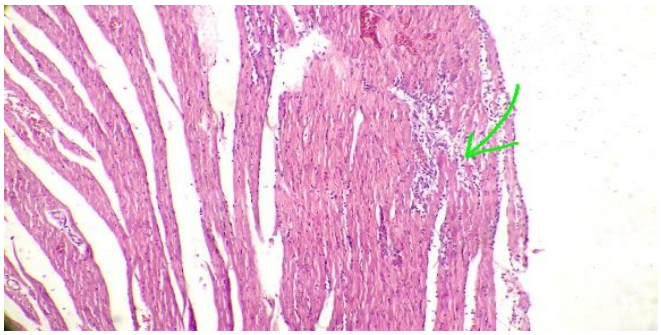
(b) 10x showing inflammatory infiltrates and degenerated muscles



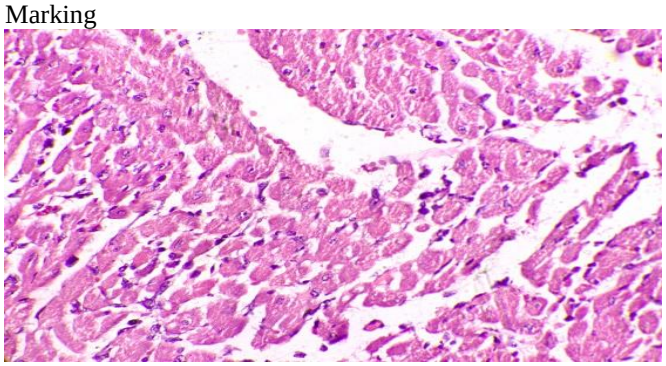
(c) 40x showing inflammatory infiltrate and degenerated muscle fibers– Marking

Figure 1 G_1(Isoprenaline control) results:

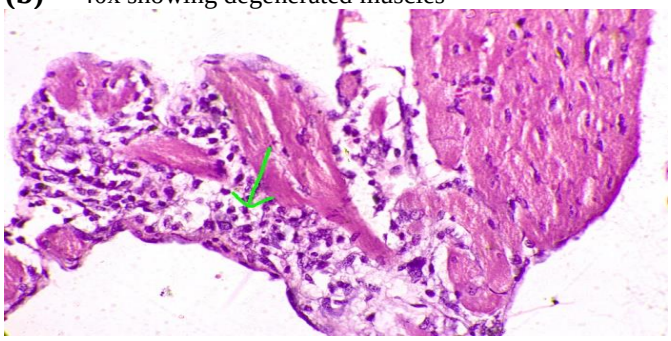
These changes were attenuated to moderate extent in the *Vrikshamla* group / test drug group (Figure 2), as only mild to moderate toxicity changes were observed compared to Isoprenaline control group (Table 6). Hence, histopathological examination provides strong evidence for the presence of cardioprotective activity in drug *Vrikshamla.*



(a) 10x showing inflammatory infiltrate – Marking



(b) 40x showing degenerated muscles



(c) 40x showing inflammatory infiltrate – Marking

Figure 2 G_2 (*Vrikshamla* group) results

CONCLUSION

Analyzing the study in total will suggest that, injecting Isoprenaline leads to alterations in biochemical parameters as well as histopathology

which indicates features of marked myocardial perturbations stimulating changes found in myocardial infarction. Production of highly cytotoxic free radicals are responsible for the observed myocardial injury reflected in the form of changes in the biomarkers and infarct like necrosis in the cytoarchitecture. In the present study, the key biomarkers especially CK-MB, LDH, Creatinine, SGPT etc. showed results supporting the cardio protective action of the drug *Vrikshamla*. Evaluation of heart weight showed significant decrease in test group when compared to the control group, this points that the study drug attenuated the myocardial hypertrophy induced by isoprenaline. The anti-oxidant study results contribute to lessening the oxidative stress induced by the chemical (Isoprenaline) and the histopathological results also showed mild to moderate toxic changes and moderate protection of heart. So, the results obtained proves that the *Amla Rasa Pradhana* drug *Vrikshamla* has an effective cardioprotective action.

REFERENCES

1. Acharya Agnivesha, Charaka Samhita, Ayurveda Deepika Teeka of Chakrapanidatta, Sutrasthana, Chapter 1, Verse no: 42, edited by Yadavji Trikamji Acharya, Chaukambha Prakashan, Varanasi, Reprint 2013; 8.

2. Acharya Agnivesha, Charaka Samhita, Ayurveda Deepika Teeka of Chakrapanidatta, Sutrasthana, Chapter 30, Verse no: 4, edited by YadavjiTrikamji Acharya, Chaukambha Prakashan, Varanasi, Reprint 2013; 183.

3. Acharya Agnivesha, Charaka Samhita, Ayurveda Deepika Teeka of Chakrapanidatta, Vimanasthana, Chapter 5, Verse no: 8, edited by Yadavji Trikamji Acharya, Chaukambha Prakashan, Varanasi, Reprint 2013; 240.

4. Acharya Agnivesha, Charaka Samhita, Ayurveda Deepika Teeka of Chakrapanidatta, Sutrasthana, Chapter 4, Verse no: 10, edited by YadavjiTrikamji Acharya, Chaukambha Prakashan, Varanasi, Reprint 2013; 32.

5. Acharya Agnivesha, Charaka Samhita, Ayurveda Deepika Teeka of Chakrapanidatta, Sutrasthana, Chapter 25, Verse no: 40, edited by YadavjiTrikamji Acharya, Chaukambha Prakashan, Varanasi, Reprint 2013; 131.

6. K.M.Nadkarni, Indian Plants and drugs with their Medicinal properties and uses, Chaukambha Publications, Print 2010; 167.

7. Prof: K Nishteswar, Lifestyle diseases and ayurvedic herbal drugs; 138.

8. Dr JLN Shastry, Illustrated dravyagunavijnana, Vol.2 9. Mayarama Aniyaala, Prayogatmaka Abhinava Dravyaguna Vijnanam, Chaukambha publications, Print 2009; 72.

10. Acharya Agnivesha, Charaka Samhita, Ayurveda Deepika Teeka of Chakrapanidatta, Chikitsasthana, Chapter 22, Verse no: 34, edited by Yadavji Trikamji Acharya, Chaukambha Prakashan, Varanasi, Reprint 2010; 569.

11. Acharya Sushrutha, Sushrutha Samhita, Nibandhasangraha Teeka of Dalhana, Uttaratantra, Chapter42, Verse no: 29, edited by YadavjiTrikamji Acharya, Chaukambha Prakashan, Varanasi, Reprint 2017; 719.