



CARDIOPROTECTIVE STUDY OF AMINOACIDS –L-ARGININE AND L-LYSINE ON ISOPROTERENOL INDUCED MYOCARDIAL STRESS IN RATS

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ABSTRACT

Heart is the vital organ responsible for the purification of blood and it transports to all organs through the circulatory system. Toxic effects of drugs on the heart may mimic almost all the qualities of the heart diseases. Cardiotoxicity or myocardial infarction was induced for the experimental findings in rats by the administration of Isoproterenol. The serum enzymes such as CPK, LDH and SGOT and the serum cholesterol were elevated and they were brought back to near normal levels by the pre-treatment of Arginine and Lysine, which acts as a putative coronary vasodilator and possess negative inotropic and chronotropic effects. L-Arginine and L-Lysine antagonize the effect of calcium and alters the important cellular processes. Due to its potent vasodilator property, L-Arginine and L-Lysine are known to prevent and relieve coronary artery spasms associated with acute myocardial infarction. The present study showed the effect of L-Arginine and L-Lysine on isoproterenol induced myocardial infarction in rats significantly.

KEYWORDS: Cardioprotective activity, amino acids, Arginine, Isoproterenol,

INTRODUCTION

Lysine is one of eight essential amino acids building blocks of protein that the body cannot manufacture on its own. L-Lysine is a specific configuration of the lysine molecule often used dietary supplements. It is an essential amino acid that cannot be manufactured in the bodies of animals. Only plants and bacteria are equipped with the ability of synthesize this crucial amino acid from aspartic acid. L-Lysine appears to help symptoms of the heart condition angina pectoris. It has also been used to treat sores caused by the herpes simplex and herpes Zoster Viruses. L-Lysine is a safe, nontoxic, natural substance when used within recommended amounts. As an indispensable component of proteins, lysine plays many significant roles in the human body. It is crucial for the proper absorption of calcium from the gastro-intestinal tract. Lysine is often used in treating herpes viral infections, especially those caused by herpes simplex virus. It has been observed that lysine is effective in accelerating the process of recovery of reducing the frequency of both cold sores and genital herpes. It is also helpful in another viral disease known as shingles, which is characterized by skin rashes. L-Arginine is an amino acid that has numerous functions in the body. It helps the body get rid of ammonia (a waste product), is used to make compounds in the body such as creatine, L-proline and

L-glutamate and can be converted to glucose and glycogen if needed. It is also involved in protein formation. In larger amounts, L-arginine stimulates the release of hormones growth hormone and prolactin. L-Arginine is a precursor of nitric oxide and other metabolites, a component of collagen, enzymes and hormones (ex. Vasopressin), ejaculate (seminal fluid and sperm), skin and connective tissues. It plays important role in the synthesis of various protein molecules (ex. Creatine and insulin). It may have antioxidant property and reduces accumulation of compounds such as ammonia and plasma lactate, byproducts of physical exercise. It inhibits platelet aggregation and can also decrease blood pressure. The applications of arginine are such as Anti-aging by natural production of Human Growth Hormone, Restful sleep and instant morning alertness, Greater vitality, lasting stamina Increased metabolism leading to effortless weight loss, Reduction in PMT and menopause symptoms, Improved sports performance at the gym and during physical exercise. L-Arginine and L-Lysine are the effective amino acids which do not have side effects in clinical experience and is relatively low costs. The cardioprotective activity of Arginine and Lysine has been demonstrated against a variety of cardiotoxins such as Isoproterenol, Doxorubicin etc. The present study was carried out to investigate the possible protective effect of L-Arginine

and L-Lysine on Isoproterenol induced cardiotoxicity. Some phytochemicals used for cardioprotective activity are bio-chemicals involved for the healing of heart diseases are flavonoids and carotenoids of *Anacardium occidentale*, *Eugenia uniflora* and *Desmodium gangeticum*^[1-3], Friedelin, stigmasterol, ursolic acid, hispidulin, scutellarein, choline, phenolic acids of *Stachytarpheta jamaicensis*^[4], alkaloid constituents, including berberine; bitter principles, including columbin, chasmanthin, palmarin and tinosporon, tinosporic acid and tinosporol of *Tinospora cordifolia*^[5], Lycopene, rubixanthin, zeaxanthin, quercetin, kaempferol and cyanidin Of *Rosa damascene*^[6], Alkaloids including cocaine, tropacocaine, Cinnamoylcocaine of *Erythroxylon coca*^[7], Protein, lecithin, saponins of *Glycine max*^[8], Vitamin C, β -glucan-enriched fraction of *Hordeum vulgare* and *Nelumbo nucifera*^[9] aloin (*Aloe vera*); alkaloids (*Premna serratifolia*); bromelain (*Ananas comosus*); curcuminoids (*Curcuma longa*); crocin (*Crocus sativus*); ginkgolides (*Ginkgo biloba*); flavonoids (*Camellia sinensis*, *Muntingia calabura*, *Orthosiphon stamineus*); emblicanin (*Embllica officinalis*); glycosides (*Cynodon dactylon*); mangiferin (*Mangifera indica*); neriine (*Nerium oleander*); piperine (*Piperlongum*)^[10-26] In this present study amino acid L-Arginine and L-Lysine are used for cardioprotective activity.

MATERIALS AND METHODS

Animals

Albino rats (wister strain) of male sex weighing about 150 – 200 g obtained from King Institute, Chennai were used in the present investigations. The animals were grouped and housed in polyacrylic cages with not more than 6 animals per cage and maintained under standard laboratory conditions. All animal procedures were carried out under Local Ethical Committee guidelines and approval, and followed the Home Office (1989) "Code of Practice for the Housing and care of Animals used in scientific procedure".

Experimental design

A total number of about 30 male albino rats were taken for the present study while selecting the rats considered being existing with similar age sex and weight of each rats. These 30 rats are divided into groups

Group 1: About 6 rats were taken and they are caged with the balanced food and water. They are not administered with isoproterenol.

Group 2: Animals received Isoproterenol (Isoprenaline) (2mg/kg body wt) as a single dose intravenously.

Group 3: Animals received the standard Arginine alone (1 mg/Kg body wt).

Group 4: Animals received the isoproterenol intravenously (2mg/Kg body wt) followed by Arginine alone for treatment.

Group 5: Animals received Isoproterenol intravenously (2mg/Kg body wt) followed by the combination of Arginine (250mg/K.g body wt) and Lysine (5 mg!Kg body wt) used for treatment.

Sample collection

Rats were sacrificed after 48 h of administration of drugs from each group by anaesthetized with chloroform and the blood was removed from the carotid vein and collected into serum separate tube and clotted at room temperature for 2 h and centrifuge it at 2500 rpm for 10 min and the serum was harvested. and the biochemical estimations of cardiac enzymes like LDH, CPK & SGOT were done with semi-autoanalyser kits. The heart was harvested, washed in normal saline and pour it in 10% formalin solution. It was subjected to histopathological examinations.

Histopathological examination

One animal from the treated groups showing maximal activity as indicated by improved biochemical parameters from control, isoproterenol induced and L-Arginine and L-Lysine were utilized for this purpose. The animals were sacrificed and the heart was removed. Then, 5 mm thick pieces of heart was fixed in appropriate fixative for 12 hand then embedded in paraffin, using conventional methods and cut into 5mm thick sections and stained using H E dye and finally mounted in Diphenyl xylene. Then, the sections were observed under microscope for histopathological architecture and their photomicrographs are taken.

Statistical analysis

The different biochemical parameters were measured using the statistical method of Analysis of Variance (ANOVA). Analysis of Variance refers to the examination of differences among the samples. It is a statistical technique specially designed to test whether the means of more than the quantities population are equal. All data were expressed as mean \pm SD of the number of experiments. The statistical significant was evaluated by one-way Analysis of Variance (ANOVA) using SPSS version 16.0 obtained the individual comparisons.

RESULTS AND DISCUSSION

Heart is the vital organ which plays essential in the purification of blood all over the body through the circulatory system. Cardiovascular diseases are most common threat for sudden deaths as worldwide. Studies involving the action of various drugs in treating cardiovascular diseases are employed by drug-induced cardiotoxicity. Drugs such as Isoproterenol, a potent β -blocker was used which release the calcium from the myocardial cells and damage the myocardium may leading to elevated levels of serum enzymes. Herbal medicines offer a variety of remedies that may have a beneficial effect on coronary artery diseases, but its action is time-consuming. L-Arginine and L-Lysine are naturally available and has been used by mankind since

before recorded history, which is safe, effective and affordable. L-Arginine and L-Lysine antagonizes the effect of calcium and alters important cellular processes.^[16, 17] Results of various biochemical parameters are presented in the tables. Table - I shows

S. No	Group 1 (Control) (IU/L)	Group2 (Isoproterenol Induced) (IU/L)	Group3 L-Arg Alone (IU/L)	Group 4 Isoproterenol + L-Arg (IU/L)	Group 5 L-Arg+ L- Lys (IU/L)	Level of Significance
1.	141.7 ± 1.86	182.0 ± 2.99	140.2 ± 0.74	148.4 ± 1.03	145.0 ± 2.68	p < 0.05

Group - II and Group - V rats, when compared with that of the normal group showed the alteration of biochemical parameters. In the assessment of cardiac damage by isoproterenol, the determination of enzyme levels i.e., CPK was largely used. Myocardial damage releases the enzyme into the circulation. therefore, it can be measured in serum. High levels of CPK indicated due to myocarditis. As CPK is one of the isoenzyme, CK - MB is more specific to heart whose. Level gets increased in myocardial infarction. It is not present in red blood cells and is not affected by hemolysis. Pre-treatment with L-Arginine and L-Lysine at a dose of 250 mg/kg body wt/day and 5 mg/kg body wt/day in Group V rats

the level of CPK of normal and experimental rats. A single dose of administration of isoproterenol produced a marked elevation of the serum levels of CPK in experimental rats.

significantly prevented the increased levels of enzymes towards the respective normal value. This was an indication of stabilization of plasma membrane as well as repair of the myocardial tissue damage caused by isoproterenol administered after I Omin's of L-Arg + L-Lys Infusion.^[11-14]

Table - 2 shows the activities of the enzymes such as LDH of experimental rats. It is well known that isoproterenol has cardiac protective effect on myocardium due to its positive chronotropic and isotropic properties. High release and leakage of LDH into serum.

Table-2: Changes in the level of LDH in different experimental groups Values are expressed as mean ± SD for six animals in each group

S. No	Group 1 (Control) (IU/L)	Group2 (Isoproterenol Induced) (IU/L)	Group3 L-Arg Alone (IU/L)	Group 4 Isoproterenol + L-Arg (IU/L)	Group 5 L-Arg+ L- Lys (IU/L)	Level of Significance
1.	85.78 ± 1.70	157.5 ± 4.96	87.6 ± 0.55	89.77 ± 0.73	83.11 ± 1.83	p < 0.03

LDH - 1 and LDH - 2 isoenzymes gets elevated in isoproterenol induced myocardial damage and caused the myofilament hypercontraction, high energy phosphate deficiency and decreased influx of macromolecules into myocardial interstitium. RBC cells are rich in LDH and so hemolysis should be avoided. Pre-treatment of L-Arginine and L-Lysine prevents myocardial damage partially by; L-Arginine was used to make nitric oxide, which reduces blood vessel stiffness, increases blood flow and improves blood vessel function and also improves the metabolic indices. L-Lysine reduces the

levels of LDL ("Bad cholesterol") in the blood stream.^[14-17]

Table - 3 shows the levels of SGOT on normal and experimental rats. The rats administered with isoproterenol (Group - II) shows the increased level of SGOT. SGOT is located in higher concentration in heart myocardium. In acute myocardial infarction, serum activity rises sharply within the first 12 h, with a peak level at 24 h and its level rise depends on the size of infarction.^[11-13]

Table-3: Changes in the level of SGOT in different experimental groups Values are expressed as mean + SD for six animals in each groups

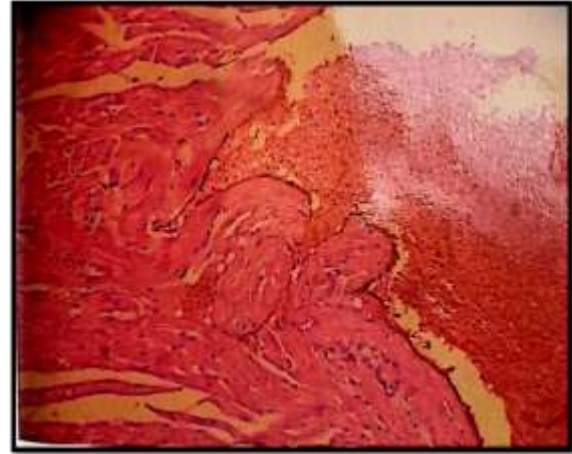
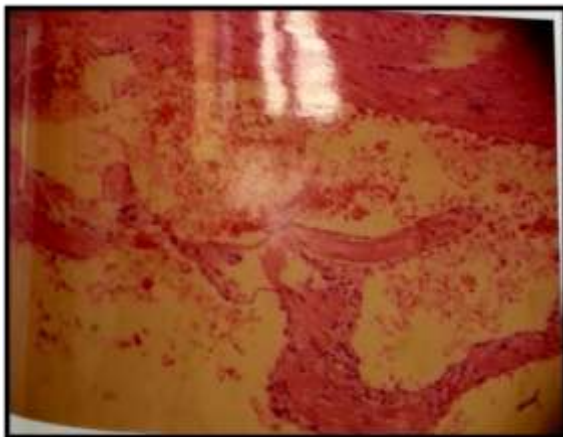
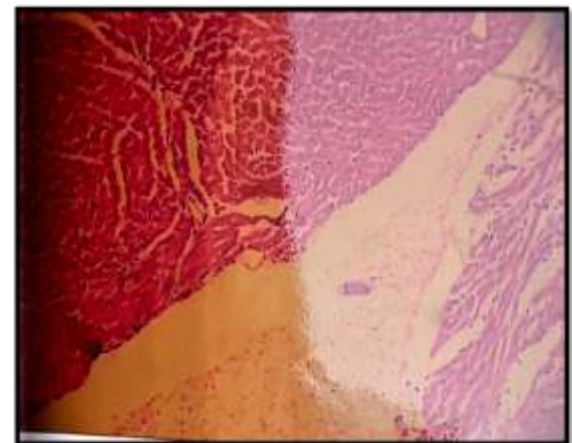
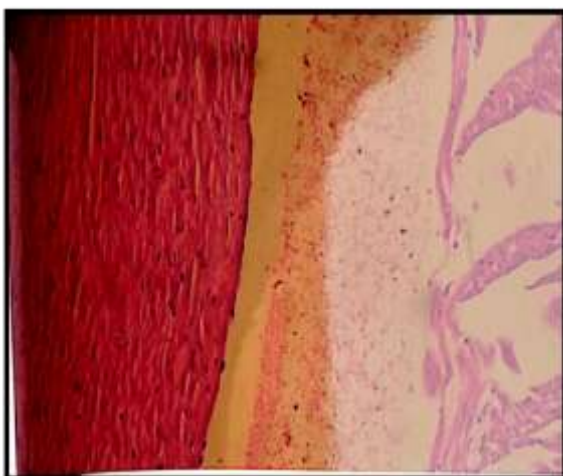
S. No	Group 1 (Control) (IU/L)	Group2 (Isoproterenol Induced) (IU/L)	Group3 L-Arg Alone (IU/L)	Group 4 Isoproterenol + L-Arg (IU/L)	Group 5 L-Arg+ L- Lys (IU/L)	Level of Significance
1.	58.31 ± 1.79	80.01 ± 0.91	57.53 ± 1.79	64.50 ± 1.98	60.88 ± 2.04	p < 0.05

As an indispensable component of proteins, L-Lysine plays many significant roles in the human body. It is crucial for the proper absorption of calcium from the gastro-intestinal tract. It also helps in conserving Ca²⁺. L-Lysine stimulates the production of creatinine, which

is responsible for converting fatty acids into energy. During this process, the level of cholesterol, especially the LDL (harmful) cholesterol (elevated level of LDL is associated with increased risk of coronary heart disease and atherosclerosis) reduces in the body.

Table-4: Changes in the level of Cholesterol in different experimental groups Values are expressed as mean + SD for six animals in each groups

S. No	Group 1 (Control) (IU/L)	Group2 (Isoproterenol Induced) (IU/L)	Group3 L-Arg Alone (IU/L)	Group 4 Isoproterenol + L-Arg (IU/L)	Group 5 L-Arg+ L- Lys (IU/L)	Level of Significance
1.	151 + 1.21	358.2 ± 13.06	153.50 ± 1.87	180.4 ± 1.74	170 ± 16.70	0.05

Histopathological studies**Figure 1: Group I Control****Figure 4: Group IV Isoproterenol + L-Arg L-Arg+ L- Lys****Figure 2: Group II Isoproterenol Induced****Figure 5: Group V L-Arg+ L- Lys****Figure 3: Group III L-Arg Alone****CONCLUSION**

The present study on the cardioprotective activity of L-arginine and L lysine were found to be very much effective on the isoproterenol induced myocardial infarction in experimental rats. As the amino acids, L arginine and L-lysine belong to essential or indispensable amino acids, they scores maximum in treating the damaged heart. Economically, both of them are less expensive and easily metabolised in the body system. Added advantage is that they have no side effects as it is proven in the present study. It has paved a new era of utilization of simple drugs for few major disorders. Further investigation is essential to understand the versatile usage of such simple drugs in other medicinal areas.

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