



Protective Nature of *Salacia Chinensis* on Isoproterenol Induced Myocardial Infarction in Wistar Albino Rats

Thilagavathi Thiruganasambandam*

Department of Biotechnology, Vel's University, Pallavaram, Chennai, Tamil Nadu, India.

*Corresponding author's E-mail: thilagavathi228@gmail.com

Accepted on: 02-12-2015; Finalized on: 31-01-2016.

ABSTRACT

Salacia chinensis plant extract evaluated the effect of isoproterenol induced myocardial infarction in rats. Myocardial infarction was induced by isoproterenol in adult male rats, as a single intraperitoneal injection at a dose of 150mg/ kg body weight / the induced myocardial infarction in rats were pre-treated by *salacia chinensis* (300mg / kg body weight / day oral) in aqueous solution orally for 11 days. At the end of experiment the rats were scarified by cervical decapitation. A portion of heart tissue was homogenized (0.1N Tris HCL buffer PH 7.4) and used for the biochemical studies. Another portion of the heart tissue was stored in formal saline for histological analysis studies. In rats pre-treated with *S. Chinensis* extract perform the activity of cardiac markers remains normal. Prior oral treatment with *S. Chinensis* extract had reduced the extent of myocardial damage induced by isoproterenol and maintained the activity of the marker enzymes at normal. The level of non enzymic antioxidant in myocardial infarction rats were almost normalized upon the pre-treated methonolic stem extract of *S.chinensis*.

Keywords: Myocardial infarction, Marker enzyme, isoproterenol, *Salacia chinensis*.

INTRODUCTION

Myocardial infarction is one of the major causes of mortality in industrially developed as well as in developing nations. Increasingly, it is claiming victims from the younger, more active middle and lower strata of the society in developing countries. Myocardial infarction or cardiovascular disease is the leading cause of death for both men and woman in all over the world.¹ Heart attack occurs when heart muscle's oxygen deprivation is severe enough to kill myocardial cells. The technical name of heart attack accurately reflects the event of myocardial (heart muscle) infarction (cell death) due to ischemia.² Two types of myocardial infarction having different morphology and clinical significance are commonly observed. The more common and serious type is the transmural infarct, in which the ischemic necrosis involves the full or nearly full thickness of the ventricular wall.³ It is associated with severe coronary atherosclerosis. Sub endocardial (non transmural) infarcts constitute areas of ischemic necrosis limited to the inner one third or at most one half of the left ventricular wall. Acute myocardial infarction is the most important form of ischemic heart disease.⁴ Myocardium, the middle layer of heart is supplied with blood by coronary arteries. When blood supply is insufficient to this myocardium, death of myocardial muscle occurs, a condition known as 'ischemia of the myocardium'. Ischemia of myocardium leading to its necrosis is referred as myocardial infarction.⁵ Myocardial ischemia occurs when an imbalance develops between myocardial oxygen supply and demand. In the presence of a fixed coronary artery stenosis, increase in heart rate, blood pressure and left ventricular wall stress can lead to an imbalance in the supply or demand ratio, resulting in

ischemia. Medicinal plant and some organic product used to important productivity effect of the treatment of heart attack.⁶ The stem of *S.chinensis* have been used as an antidiabetic drug, astringent, abortifacient and root decoction used to treat amenorrhoea, dysmenorrhoea, and venereal diseases⁷. The stems of *S.chinensis* have been extensively used for carminative, emmenagogue, blood tonic, cardio tonic, anti-inflammatory, antidiabetic purposes and the treatment of rheumatism, leukorrhoea and stimulated lochial excretion. The stem and leaves are commercially used as a gutta-a linear isomer of natural rubber.

The intravenous injection of *S. chinensis* extract (4-120 mg/kg) caused a decrease in mean arterial blood pressure and heart rate of anesthetized rats in a dose-dependent manner. These effects were not blocked by atropine, a muscarinic receptor antagonist or propranolol, β -adrenergic receptor antagonist.

The *S. chinensis* extract (0.01-0.3 mg/ml) caused vasodilatation of the thoracic aortic rings pre-constricted with phenylephrine in a dose-dependent manner.⁸ Ischemic heart disease is the generic designation for a group of closely related syndromes resulting from an imbalance between the supply and demand of the heart for oxygenated blood.⁹

The *Salacia chinensis* is an important medicinal plant belonging to the family of Hippocrateaceae and small erect or straggling tree.

S.chinensis is used to treat various diseases, therefore in this present study to investing the tissues defence system, cardio tonic properties and also isolate the



marker compound present in the methanolic stem extract of *S. chinensis*.

MATERIALS AND METHODS

Chemicals

Isoproterenol-HCL was obtained from sigma chemical company, St.Louis, MO, USA.

Administration of *salacia chinensis*

The *S. chinensis* stem extract was orally administered at a concentration of 75,150,300,600 and 1200 mg/Kg b.w./rat/day for a period of 11 days. The toxic effect of *S.chinensis* stem extract was measured by body weight gain and morphological changes. After the completion of treatment, the rats were induced the myocardial by using isoproterenol. The optimum dosage for *S.chinensis* stem extract was fixed as 300mg/kg b.w./day for 11 days.

Induction of Myocardial infarction

Myocardial infarction was induced by intraperitoneal (ip) injection of isoproterenol hydrochloride 150mg/kg body weight dissolved in physiological saline for at an interval of 24hrs/ for two days.

Experimental animal

Male Wistar albino rats (140-160g) were procured from Tamil Nadu Veterinary and Animal Sciences University, Chennai and were housed in polycarbonate cages in an animal room with a 12h day-night cycle with at temperature of 22 ± 2 °C and humidity of 45-60 %. They were fed with a commercial pelleted rats chow and water ad libitum during the experiment. The suitable optimum dosage of the drug was assessed by administering different dosages of *S.chinensis* stem extract (200, 300 and 400 mg/kg b.w./day) for different periods (7, 11 and 15 days).

Experimental method

The rats were divided into four groups with six animals for each group. **Group 1:** Normal control rats (untreated). **Group 2:** Rats administered with isoproterenol (150mg/Kg body weight / day, at an interval of 24 hrs) for 2 days. **Group 3:** Rats administered with *S.chinensis* stem extract (300mg/Kg body weight / day, oral) for 11 days prior to administration of isoproterenol and along with isoproterenol administration for subsequent two days. **Group 4:** Rats are pre-treated with *S. chinensis* stem extract (300mg/kg b.w./day) in aqueous solution orally for 11 days. At the end of the experiment period (24 hours after the second dose of isoproterenol) the animals were sacrificed by cervical decapitation. The Blood was collected and the serum was separated by centrifugation. The heart was dissected out and immediately washed with ice-cold saline. A portion of heart tissue was homogenized (0.1 N Tris HCl buffer, pH 7.4) and used for the biochemical studies. Another portion of the heart tissue was stored in formal saline for histological studies.

Preparation of Hemolysate

The animals were sacrificed by cervical decapitation. The Blood was collected and the serum was separated by centrifugation. The heart was dissected out and immediately washed with ice-cold saline. A portion of heart tissue was homogenized (0.1 N Tris HCl buffer, pH 7.4) and used for the biochemical studies.

Biochemical estimation

The lipid peroxide was assessed by method.¹⁰ LDH-Lactate dehydrogenase was assayed according to the method.¹¹ Alanine aminotransferase (ALT) Aspartate aminotransferase (AST) the enzyme activity was assayed by the method.¹² Protein was estimated.¹³ Super oxide dismutase (SOD).¹⁴ Glutathione peroxidase (Gpx).¹⁵ Glutathio- s transferase was assayed by the method.¹⁶

Statistical analysis

All data were expressed as mean \pm standard deviation for four animals in every group. Mean values were obtained by one-way analysis of variance (ANOVA), followed by post hoc Sheffe's test using 7.5 version of SPSS computer soft ware. The significance of difference between and within various groups was determined. The values were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

Cardiac marker enzymes

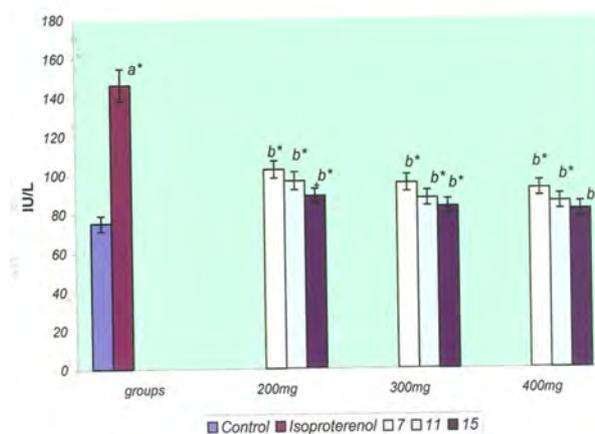


Figure 1: Lactate dehydrogenase activity

The activities of glutathione -S-transferase and glutathione reductase also decrease in isoproterenol-administered rats, which are reverted in normal in *S.chinensis* stem extract pre-treated rats.

The results also show an increase in the levels of glutathione in the heart of isoproterenol treated rats.

Glutathione is the substrate for the enzyme glutathione peroxidases, which metabolized hydrogen peroxides.¹⁷ The SOD and CAT activities in heart of control and experimental groups of rats.

A significant ($P < 0.05$) decrease in the SOD and CAT activities in heart was observed in isoproterenol-

administered rats. The methonolic stem extract of *S.chinensis* pretreated rats were tolerated against isoproterenol-administration and activities near normal.

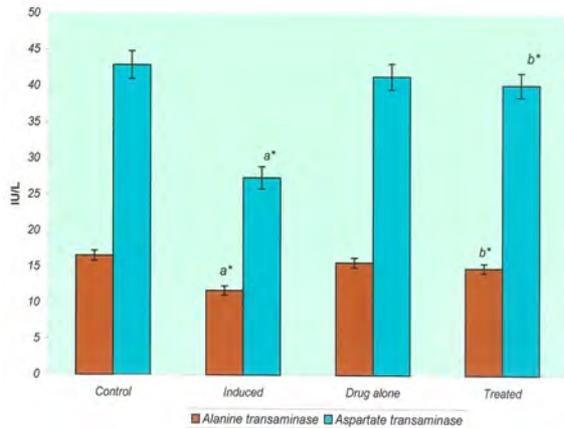


Figure 2: Alanin transaminase and aspartate transaminase

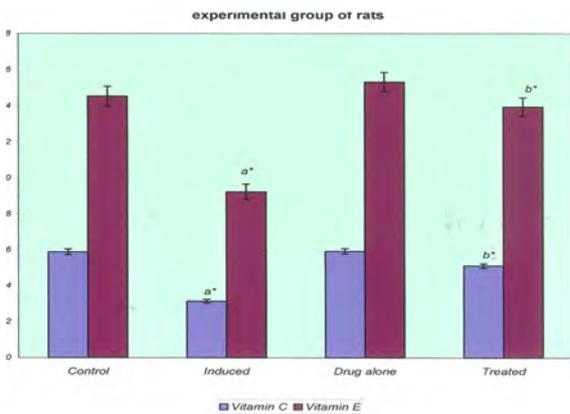


Figure 3: Level of nonenzymatic antioxidant

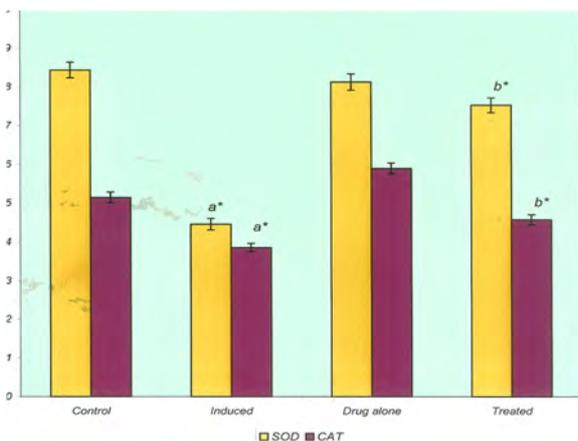


Figure 4: Activities of SOD and CAT in the experimental rat

The glutathione peroxidase and reduced glutathione activities in heart of control and experimental groups of rats. A significant ($P < 0.05$) decrease in the glutathione peroxidase and reduced glutathione activities in heart was observed in isoproterenol-induced rats. The activities were brought back in the methonolic stem extract of *S.chinensis* pretreated and isoproterenol-administered

rats near normal. The levels of non-enzymic antioxidant in myocardial-infracted rats were almost normalized upon the pretreated methonolic stem extract of *S.chinensis*. This may be due to the oxidant and free radical scavenging activity if the plant, which effectively reduced the myocardial, damage, ensuring the activities of tissues defence system in experimentally induced myocardial infarction in rats.¹⁸

The biochemical alterations leading to actual myocyte necrosis involve cellular processes. At the cellular level, several structural alterations in cardiocytes and interstitial occur in the failing heart which, acting individually or in concert, can adversely influence global left ventricular contractile performance. Such alterations include: hypertrophy of residual myocytes.¹⁹ Abnormalities of mitochondria abnormalities in myocyte contractile structure and progressive accumulation of collagen in the interstitial space.²⁰⁻²² The biochemical and physiological changes associated with naturally occurring infarction has very close resemblance with that produced by inducing with isoproterenol hydrochloride obtained from sigma chemicals. The macromolecules were to leak from the damaged tissues, enzymes; because of their damage.²³ The mechanism of isoproterenol damages the membrane is still not clearly understood. The release of cellular enzymes reflect a non- specific in the plasma membrane integrity / or permeability as a response to β -adrenergic stimulation.

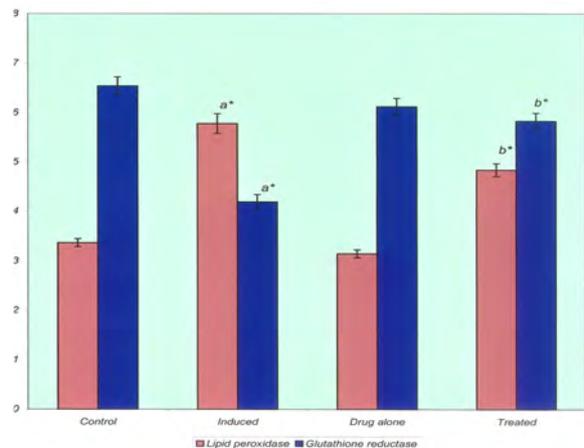


Figure 5: Activities of lipid peroxidase and glutathione reductase

Lipid peroxidation, free radicals and membrane damage

Cell membranes rich in polyunsaturated fatty acid as a part of the structure are susceptible to peroxidative injury. Lipid peroxidation reactions with subsequent disruption of both liposomal and cellular membranes may be induced by enzymatically generated free radicals. When membranes undergo peroxidative damage the products of lipid peroxidation may be released in to the extra cellular space, which influence the vascular permeability and leukocyte chemotaxis. Membrane fluidity is due to the presence of polyunsaturated fatty acid chain in many membrane lipids. Free radicals

generated during lipid peroxidation process will reduce membrane fluidity, increase membrane permeability and impair the functions of membrane bound enzymes, receptors and other proteins.²⁴

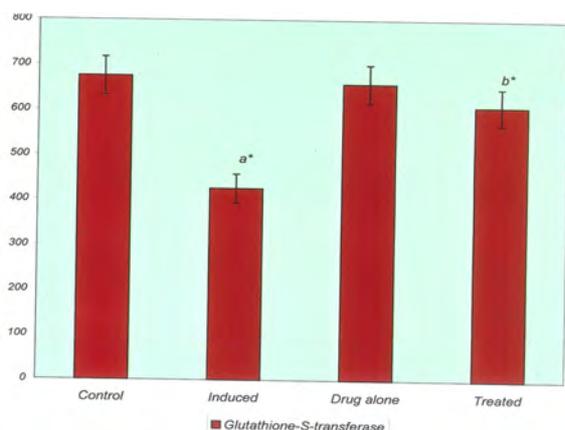


Figure 6: Activities of glutathione S- transferase in heart tissue of control and experimental rat

To investigate the biochemical events in the protective action of methonolic stem extract of *Salacia chinensis* during experimental myocardial infarction in albino rats. Histopathological studies were carried out in myocardium to reveal the protective role of plant extract on isoproterenol-induced myocardial infarction. Myocardial ischemia is associated with the information of oxygen free radicals.²⁵ The activities of antioxidant enzymes in serum and heart were assayed to show the antioxidant and free radicals scavenging potential of *Salacia chinensis*.

S.chinensis is used to treat various diseases. So far there were no reports available on tissue defence system in experimentally induced myocardial infarction in rats for *S.chinensis*. Therefore in this present study to investing the tissues defence system, cardio tonic properties and also isolate the marker compound present in the methonolic stem extract of *S. Chinensis*. Experimental models of myocardial infarction have been developed in laboratory animals to understand the morphology, biochemistry, electro physiology and mechanical properties of the damaged myocardium and to test the efficacy of various therapeutic agents. Myocardial infarction due to occlusion of coronary artery by ligation has been developed by.²⁶ Ligation of either of the two major branches of the left coronary artery has been one of the most popular techniques in smaller animals like dog, donkeys and pigs etc. however the mortality rate are fairly high in this method. Subcutaneous administration of isoproterenol – Hcl, adrenergic receptor induces myocardial necrosis in smaller animals like rats and is found to be more convenient because of the relatively small size of the coronary arteries.²⁷

Ischemia induces profound metabolic changes in the myocardium; postulated sequence of alterations involved in the pathogenesis of irreversible myocardial injury in oxygen deficiency induces metabolic changes including

decreased ATP, decreased pH and lactate accumulation in ischemic myocytes.²⁸ The altered metabolic leads to impaired membrane transport, with resultant derangements in intra cellular electrolytes. An increase in cytosolic Ca²⁺ might trigger the activation of proteases and phospholipases, with resultant cytoskeletal damage and impaired membrane phospholipid balance, lipid alteration which include increased phospholipid degradation, with release of free fatty acids and lysophospholipids and decreased phospholipid synthesis. Lipid peroxidation occurs as a result of attack by free radicals that are produced at least in part by the generation of excess electrons in oxygen-deprived mitochondria.²⁹ Free radicals might also be derived from metabolism of arachidonic acid, metabolic of adenine neutrophils. The irreversible phase of injury appears to be mediated by severe membrane damage produced by phospholipid loss, lipid peroxidation and cytoskeletal damage.³⁰

SOD and CAT activities were decreased on isoproterenol administration, which is in accordance with the observation of CAT and SOD play vital role in body defense mechanisms against the harmful effect of oxygen free radicals.^{31,32} During myocardial infarction, superoxide radicals are generated at the site of damage modulates SOD and CAT resulting in the loss of activity and accumulation super oxide anion, which damages myocardium. Pretreatment with *S.chinensis* extract increased the activity of SOD and CAT, which scavenge, super oxide radicals and reduced the myocardial damage caused by free radicals upon isoproterenol administration. The level of serum antioxidants α - tocopherol and ascorbic acid decreased significantly in isoproterenol administered rats decreases in serum antioxidants levels in rats could be due to an increase in free radical production by isoproterenol, α - tocopherol is a lipid soluble chain breaking antioxidant capable of scavenging centred free radicals. Histological findings confirm the induction of myocardial infarction by isoproterenol. The following observations were made in the heart tissue of control and experimental groups of rats. The control rat heart showed normal architecture. In the isoproterenol induced myocardial infarction rat heart showed severe cardiac damage with large areas of endocardial fibre generation with Foci of necrosis and inflammatory infiltrate in the myocardial.

The rats given *S.chinensis* extract alone did not showed any abnormal changes in the architecture of the heart. The *S.chinensis* stem extract pretreated and administered isoproterenol rats exhibited normal endocardial and myocardial fibre with occasional focus of few inflammatory cells only.

Analysis of haematological parameters could be used to finding the Effect of presence of the foreign compound like phyto- extracts on the blood of experimental animals. They are also used to confirm the suitable alteration in the metabolic level pathway.

CONCLUSION

In this, study myocardial infarction is induced in rat model following the administration of isoproterenol and it has been extensively used as a model for producing a infarction where it resembles the ischemic necrosis induced by coronary occlusion. Cardiac marker enzymes were assayed in serum and heart. The methanolic stem extract of *S.chinensis* was shown to possess antioxidative and cardio tonic properties. The results show that stem extract of *S.chinensis* was safe and highly effective in preventing cardiovascular dysfunction in rats, possibly due to both antioxidative and cardio tonic properties.

REFERENCES

- Murugesan M, Ragunath M, Nadasabapathy S, Revathi R, Manju V, Productive role of Fenugreek on isoproterenol induced myocardial infarction in rats. *Int. Res. J. pharm.* 3(2), 2012.
- Collins R and Macmohan S. Blood pressure, antihypertensive drug treatment and the risk of stroke and coronary heart disease. *Br Med Bull*, 50, 1994, 272-298.
- Celermajer DS, Sorenson KE, Georakopoulos D. Cigarette smoking is associated with dose-related and potentially reversible improvements of endothelium dependent dilation in healthy young adults. *Circulation*, 88, 1993, 2149-2155.
- Boon NA, Fox KAA and Bloodfield P. Disease of the cardiovascular system. In: principles and practice of medicine (Eds.) Haslett C, Chilvers ER, Hunter JAA and Boon NA, 18th Edn, Churchill Livingstone, London. 1999, 245-247.
- Chappel CL, Rona G, Balaz T and Gaudry R. Severe myocardial necrosis produced by isoproterenol in the rat. *Arch intl pharmacodyn ther*, 122, 1959, 123-128.
- Subashini R and Rajadurai M. Evaluation of cardioprotective efficacy of nelumbo nucifera leaf extract on isoproterenol – induced myocardial infarction in wistar rats. *Int. J. pharma. Biol. Sci.* 2(4), 2011, 34-35.
- Tissera MHA and Thabrew M. Medicinal plants and Ayurvedic preparations used in Sri Lanka for the control of diabetes mellitus. A publication of the Department of Ayurveda, Ministry of Health and indigenous medicines Lanka. 2001.
- Jansakul C, Jusapalo NS, Mahattanadul H. Hypotensive effect on n-butanol extract from stem of *Salacia chinensis* in rats. *ISHS Acta Horticulturae*, 4, 2004, 678.
- Berhard JG and Shabudin HR, In "Acute myocardial infarction" Elsevier publication, New York. 99, 1991, 49.
- Ohkawa H, Ohishi N and Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*, 95, 1979, 351-358.
- King J. Colorimetric determination of serum lactate dehydrogenase. *J Med Lab Tech*, 16, 1959, 265.
- King J. The transferase alanine and aspartate transaminase. In: Practical *Clinical Enzymology* (ed) D.Van, Nostrand Co., London, 1965, 363-395.
- Lowry SS, Rosenbrough NJ, Farr AL and Randaii R. Protein determination using Folin-Ciocalteu reagent. *Bio chem.* 193, 1951, 265-9.
- Misra HP and Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay of superoxide dismutase. *J Bio Chem*, 247, 1951, 3170-3175.
- Rotruck JT, Pope A, Ganther HE and Swanson AB, Selenium biochemical roles as a component of glutathione peroxidase. *Science*, 179, 1973, 588-590.
- Habig WH, Pabst MS and Jekpoly WB. Glutathione transferase: a first enzymatic step in mercapthric acid formation. *Biol Chem*, 249, 1974, 7130-7139.
- Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys*, 82, 1959, 70-77.
- Prabhu S and ShyamalaDevi CS, Efficacy of mangiferin on serum and heart tissue lipids in rats subjected to isoproterenol induced cardiotoxicity. *Toxicol*, 228, 2006, 135-139.
- Anversa P, Olivetti G and Capasso JM, Cellular basis of ventricular remodeling after myocardial infarction. *Am J cardiol*, 68, 1991, 7-16.
- Sabbah HN, Sharov VG, Riddle JM, Kono T, Lech M and Goldstein S. Mitochondria abnormalities in myocardium of dogs with chronic heart failure. *J Mol cell cardiol*, 24, 1992, 1333-1347.
- Sharov VG, Sabbah HN, Shimoyama H. Abnormalities of contractile structures in viable myocytes of the failing heart. *Int J cardiol*, 43, 1994, 287-297.
- Schaper J and Hein S. The structure correlates of reduced cardiac function in human dilated cardiomyopathy. *Heart failure*, 9, 1993, 95-111.
- Arthur C, Guyton MD, John E. Hall Ph.D. Text book of medical physiology, 1996, 259.
- Bie Mond P, Swack AJ, Berndsoff CM. Super oxide dependent and independent mechanisms of iron mobilization from ferritin by xanthine oxidase implications for oxygen radical induced tissue destruction during ischemia and inflammation. *Biochem J*, 239, 1986, 169-173.
- Dormandy T.L. Free radical oxidation and antioxidants. *Lancet*. 1, 1978, 647-650.
- Heimberg RF. Injection in to pericardial sac and ligation of coronary artery of the rat. *Arch Surg*, 52, 1946, 677-681.
- Ravichandran LV, Puvanakrishnan R and Joseph KJ. Influence of isoproterenol induced myocardial infarction on certain glycohydrolases and cathepsin in rats. *Biochem Med Metab Biol*, 1991, 6-15.
- Murray B. *Nutr Bull*, 6, 1985, 93.
- Maxwell SJ. Prospects for the use of antioxidants therapies drugs, 49, 1995, 345-349.
- MaxMilan L and Willerson JT. Experimental analysis of myocardial ischemic. In: Cardio vascular athology (Ed. Silver MD), Chrchill Livingstone, 1, 1991, 453-484.
- Manjula TS, Deepa R, Shyamala Devi CS. Effect of aspirin on lipid peroxidation in experimental myocardial infarction in rats. *J Nutr Biochem*. 5, 1994, 95-98.
- Sushma kumari S, Jayadeep A, Suresh kumar JS and Venugopal P, Menon. *Indian J. Exp. Biol.* 27, 1989, 134-137.

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

Corresponding Author's Biography : Ms T.Thilagavathi

Ms. Thilagavathi is graduated from T.B.M.L College in Porayar, post graduated from Bharath College of science and management in Thanjavour, master of philosophy at Vel's university and completed the M.phil thesis in "Isolation and extraction of secondary metabolite and RAPD analysis of *fusicoccum sp*". Currently doing her Ph.D at Vel's University.