International Journal of Pharmaceutical Research and Applications
Volume 5, Issue 2, pp: 741-747 www.ijprajournal.com ISSN: 2249-7781

Cardioprotective activity of ethanolic leaf extract of Terminalia catappa against isoproterenol induced myocardial infarction in albino rats

Karthika.K[*1], Mallika.J[2], Senthilkumaran.P[3]
[1]*Assitant Professor,Department of Biochemistry, Sengunthar Arts and Science College Tiruchengode – 637 205. Tamil Nadu, India.*
[2]*Assitant Professor,Department of Biochemistry, Sengunthar Arts and Science College Tiruchengode – 637 205. Tamil Nadu, India.*
[3]*Assitant Professor,Department of Biochemistry, Sengunthar Arts and Science College Tiruchengode – 637 205. Tamil Nadu, India.*

Date of Submission: 15-12-2020 Date of Acceptance: 22-12-2020

ABSTRACT: To evaluate the cardioprotective activity of ethanolic leaf extract of Terminalia catappa against isoproterenol induced myocardial infarction in albino rats.Wister albino rats comprising a six rats in five Groups. Group I served as a control,Group II rats were given isoproterenol,Group III (500mg/kg),Group IV (500mg/kg), and Group V (1000mg/kg), were pretreated with ethanolic extract of Terminalia catappa dissolved in water and administered to rats orally for a period of 21days and then induced with isoproterenol (30mg/kg subcutaneously) for consecutive days.The blood was collected by cervical decapitation under anesthesia.Heart was removed for histopathological studies. The results indicated that the decreased pretreatment of TCEE in serum the enzyme markers level (AST,ALT,LDH and CPK) in all treatment groups as compared to ISO injected rats and also increased levels in heart.Sigficantly observed decrease levels of GSH,SOD and GAT in ISO treated group.In ISO induced rats levels of cholesterol,TG,LDL were increased significantly with the decreased in HDL.The pretreated group animals increased in HDL levels and decreased in cholesterol,TG and LDL levels.On the basis of results obtained to suggest that the extracts of Terminalia catappa have prodection of heart against myocardial infarction by isoproterenol.

Keywords: Terminalia catappa, Cardioprodective,Isoproterenol,myocardial infarction.

I. INTRODUCTION

Myocardial infarction (MI) or acute myocardial infarction (AMI), commonly known as a heart attack, results indicates the heart cells to die due to the interruption of blood supply to the heart[1]. Diseases of the heart and blood vessel are collectively known as cardiovascular disease (CVD), which are the leading cause of death in India. Risk factors for CVD include obesity, diabetes, hypertension, elevated blood cholesterol levels and oxidative stress [2]. Due to changing lifestyles in developing countries in particularly in urban areas, MI is making an increasingly important contribution to mortality statistics [3]. The World Health Organization (WHO) estimates that 17 million people die of CVD annually, of which 7.2 million deaths are attributable to coronary heart disease (CHD). Epidemiological studies indicate that ischemic heart diseases, especially myocardial infarction (MI), will be the major disease-burden worldwide in the year 2020[4].Antioxidant compounds, highly present in plants have shown protective effects against diseases without reducing their therapeutic efficacy[5].Medicinal plants continue to provide valuable therapeutic agents, both in modern and traditional medicines[6]. Antioxidants are substances that delay or prevent the process of oxidation by scavenging the free radicals in body cells and may reduce potential mutations and thereby help prevent cancer and heart disease[7].The chemo-therapeutic agents, which inhibit the free radical formation and can reduce the medicines having antioxidant properties, may therefore, have a protective role in cardiovascular diseases [8]. Most important secondary metabolites like alkaloids, steroids, polyphenols, saponins etc., have great importance due to their interesting biological and pharmacological actions [9].

Isoproterenol (ISO), a β-adrenergic agonist, has been found to produce stress in the myocardium due to the generation of free radicals by its auto-oxidation. Some of the mechanisms proposed to explain ISO-induced damage to cardiac myocytes include hypoxia, coronary hypotension, calcium overload, energy depletion and excessive production of free radicals as a result of catecholamine autoxidation [10].The model of isoproterenol – induced myocardial ischemia is considered as one of the most widely used experimental model to study the beneficial effects of many drugs and cardiac function [11]. Administration of large amount of catecholamines, particularly isoproterenol to experimental animals constitutes a rapid and reproducible means of provoking myocardial ischemia. Isoproterenol – induced MI serves as a well-standardized model because of pathophysiological changes following isoproterenol administration are comparable to those taking place in human MI[12].Herbal medicine is increasingly gaining acceptance from the public and medical professionals due to advances in the understanding of the mechanisms by which herbs positively influence health and quality of life[13]. Many medicinal plants have been found to possess beneficial effects in pathological conditions like cancer, liver diseases, cataract and myocardial ischemia [14].Terminalia catappa is a large tropical tree in the family Combretaceae. Common name include Indian almond, Bengal almond, Singapore almond etc. Indian almond tree is presenting throughout any region of Thailand. The plant reach up to 30m height with a thick brood trunk, the leaves cluster and turn red before turning brown and failings. It is a medium size tree, and the leaves are clustered towards the ends of the branches. Various extracts of the leaves of this plant have been reported to have anti-cancer, anti-HIV reverse transcriptase, hepatoprotective, anti-inflammatory, anti-hepatitis and aphrodisiac effects. Previously, we found that the T. catappa leaf extract contains phytochemicals such as phenol, flavonoids, and steroidal glycosides [15]. In this study to evaluate the cardioprotective effects of the ethanolic extract leaves of Terminalia catappa against isoproterenol induced in cardiotoxicity in albino rats.

II. MATERIALS AND METHODS

2.1. Preparation of extract

Dried leaves of Terminalia catappa were coarsely powdered and 1 kg of this powdered plant material was extracted with the help of the Soxhlet apparatus using ethanol as a solvent. The solvent from the ethanol extract was removed under vacuum distillation; dried material was kept in a desiccators. This ethanol extract was dissolved in distilled water for further experiments.

2.2. Experimental animals

In the present study, Adult male albino rats of Wistar strain weighing 150 – 200g were used. The animals were kept in animal house polyprolene cages lined with husk, renewed every 12 hours day and had free access to tap water and food.

The experimental protocol duly approved by the Institutional Animal Ethical Committee and care of animals was carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India. (Reference No. Reg. No – 1416/a/11/ CPCSEA).

2.3. Induction of Experimental Myocardial Infarction

Isoproterenol (85mg/kg) was dissolved in normal saline and injected subcutaneously to rats at an interval of 24 hours for two days [16].

2.4. Experimental design

Animals were divided into five groups, each group contains six animals.

Group I: Normal control rats

Group II: ISO control rats

Group III: Terminalia catappa ethanol extract (TCEE) alone

Group IV: Terminalia catappa ethanol extracts - TCEE (500mg/kg) +ISO

Group V: Terminalia catappa ethanol extracts - TCEE (1000mg/kg) +ISO

TCEE dissolved in water and administered to rats orally for a period of 21days.At 22nd & 23rd day isoproterenol was injected by subcutaneously.

At the end of the experimental period the rats were fasted overnight, and sacrificed by cervical decapitation. Blood was taken from the external jugular vein. The blood plasma and serum were separated by using centrifugation at 3000rpm in 15 minutes and used for various biochemical estimations.

Heart was isolated and stored in ice – chilled physiological saline for histopathological studies. The homogenate was centrifuged and the supernatant was used for the estimation of various biochemical parameters.

2.5 Biochemical estimation:

The activities of Creatine kinase(CK)[17], Lactate dehydrogenase (LDH)[18],Asparatate transaminase (AST)[19], Alanine transaminase (ALT)[20] levels were estimated in serum and heart tissue.Troponin T was estimated by the method of Bhaskar and Rao[21]. The blood protein content was estimated by the method of Lowry's et al [22]. The activities of Total Cholesterol,Triglycerides(TG)[23],High density lipoprotein (HDL)[24] and Low density

lipoprotein(LDL)[25] were estimated in serum and heart tissue. The endogenous antioxidant enzyme superoxide dimutase's (SOD), Catalase (CAT),Glutathione were estimated in heart tissue by(GSH) thiobarbituric acid method[26].

2.6 Histopathological examination

Isolated organ [Heart] from normal and experimental rats were fixed in buffered formalin [10%] and processed for paraffin sectioning [27].

2.7 Statistical analysis

All the data were expressed as mean +_SEM. The one – way analysis of varience followed by Tukey's multiple comparison tests or by unpaired Student's test using GraphPad Prism 5.0. The values are considered statistically significant when $p<0.005$[28].

III. RESULTS AND DISCUSSION

The analysis of biochemical parameters were tabulated.Cardiovascular disease (CVD) is directly or indirectly related to oxidative stress that shares a common mechanism of molecular and cellular damage. Administration of isoproterenol (ISO) to rats mediated its toxic effect through β1 and β2 adrenoceptors. Both β1 and β2 adrenoceptors are responsible for the positive inotropic and chronotropic effects [29]. Free radicals by ISO metabolism could initiate lipid peroxidation process, which leads to both functional and structural abnormalities of the myocardium [30]. Biochemical alterations in ISO-induced cardiomyopathy represent a complex pattern of changes in cardiac markers enzymes, lipid profile, lipid metabolizing enzymes antioxidants levels, glycoprotein levels, decrease in ATP store and changes in electrolyte levels in the blood as well as in the myocardial tissue [18, 31].In this present study, similar results were observed in case of the parameters taken for analysis.

Troponin is a protein, it consist of three subunits Troponin I, Troponin C and Troponin T. Troponin I or Troponin T has consistently been than traditional markers due to its sensitivity and specificity for the diagnosis of myocardial injury. When the myocardial damage occurs thecytosolic troponin reach the blood stream quickly resulting the rapid peak of serum troponin observed during first few hours[32]. In this study we have observed an increase in the levels of cardiac troponin T (cTnT) in ISO – induced rats. Increased levels of Troponin T indicates the damaged heart tissue into the blood stream as a result of necrosis. Pretreatment with Terminalia catappa rats indicate decreased levels of troponin T, its clearly emphasize the beneficial action cardioprotective agents. Results are shown in table 1.

Cardiac markers or cardiac enzymes are protein from cardiac tissue found in the blood. The amount of enzymes released depends on the degrees or cellular damage, the intracellular concentration of the enzyme and the mass of affected tissue. The cause of the damage the enzyme released reflects the severity of the damage[33].Heart contains an abundant concentration of diagnostic marker enzymes like CPK, LDH, and transaminases (AST &ALT) and once the heart is metabolically damaged, it releases its content into the extra cellular fluid(ECF).In the earlier report, shows the decline activities of cardiac markers such as CK,LDH,SGPT,SGOT in heart tissue of ISO-treated rats and also noted that increased activities of serum [34].Decreased activities of theses enzymes were due to the leakage from the damaged heart tissues into the blood stream as a result of necrosis induced by isoproterenol in rats .Significant increased activities were noticed in serum enzyme markers due to enhanced susceptibility of myocardial cell membrane to the isoproterenol mediated peroxidative damage, resulting in increased release of these diagnostic markers enzymes into the systemic circulation [35].In the present study, the prior administration of Terminalia catappa group of rats significantly increased levels of enzyme markers in heart and decreased in serum compared to ISO-induced rats. It was indicating the Terminalia catappa may also have the viability of myocardial cell membrane stabilizing action. The results are shown in Table 2.

Lipid peroxiadtion in vivo has been identified as one of the basic deteriorative reactions in cellular mechanisms of myocardial ischemia. It is already known that lipids are the most susceptible macromolecules to oxidative stress[36]. Endogenous enzymes such as catalase and superoxide dismutase are the first line cellular defense free radical scavenging enzymes against oxidative injury [37].GSH status is a highly sensitive indicator of cell functionally and viability.It is a ubiquitous thiol-containing tripeptide, which plays a central role in cell biology. It is implicated in the cellular defences against xenobiotics and naturally occurring deleterious compounds such as free radicals and hydroperoxides.GSH depletion is linked to a number of disease states including cancer, neurodegenerative and cardiovascular diseases. Glutathione not only protects cell membranes from oxidative damage,but also helps to maintain the sulphydryl groups of many proteins in their normal function[38].In the present study, the reduction noticed in the levels of GSH in heart of ISO-induced myocardial infarction was either due to increased degradation or decreased sysnthesis of glutathione. Pretreatment with Terminalia catappa group of rats prevented the ISO-induced lipid

peroxidation and maintained the level of reduced glutathione near normal level in heart. Like wise, the levels of SOD and CAT significantly elevated. This is due to antioxidant activity of Terminalia catappa.These results are shown in the table 3.

The lipid profile is a group of tests comprising total cholesterol, Triglycerides,HDL and LDL Cholestrol.The lipid profile is used together with other risk factors, to assess a persons risk of cardiovascular disease[39].In this study,significant myocardial infarction was indicated by the elevated levels of total cholesterol, Triglycerides LDL Cholestrol and decreased levels of HDL in ISO-induced rats.Pertreatment with Terminalia catappa group of rats result were significantly comparable with the normal group of rats.These results are shown in Table 4.

Terminalia catappa leaves proved to be effective in reducing the extent of myocardial damage,associated lipid peroxidation as suggested by biochemical indices the structure and function of the myocardium.The potential cardioprotective activity of Terminalia catappa leaves may be due to the presence of therapeutic phytochemicals such as flavonoids and natural polyphenols.

Table 1. Effect of TCEE on plasma Troponin T in normal and isoproterenol (ISO) – induced myocardial infarction (MI) in rats.

Troponin T	GroupI	GroupII	GroupIII	GroupIV	GroupV
	0.65±0.06	2.43±0.9	0.55±1.2	1.56±1.02	1.19±1.21

Results are mean±SEM for six animals.Values expressed in mg/dl.P<0.005 significantly different compared with Group I control animals.

Table 2. Effect of TCEE on serum and heart creatine kinase (CK), lactate dehydrogenase (LDH), aspirate transaminase (AST) and alanine transaminase (ALT) in normal and isoproterenol (ISO) – induced myocardial infarction (MI) in rats.

	CK		LDH		AST		ALT	
Groups	Serum	Heart	Serum	Heart	Serum	Heart	Serum	Heart
GroupI	168.5±2.92	14.7±0.78	65.62±3.53	94.2±4.52	19.73±1.15	33.9±2.12	15.5±0.68	27.5±1.24
GroupII	316.5±1.7	7.22±0.37	109.1±3.5	66.97±2.11	37.23±2.8	28.8±1.47	25.1±1.29	17.01±0.87
GroupIII	179.2±3.32	15.1±0.71	64.14±3.43	88.65±3.55	19.51±1.18	34.2±2.71	16.3±0.61	26.5±1.47
GroupIV	287±4.3	10.11±0.48	89.25±2.8	79.52±0.38	30.15±1.49	29.9±1.78	20.6±0.52	20.2±1.32
GroupV	235±1.52	12.15±0.79	74.5±3.38	66.97±0.23	25.53±0.87	31.8±2.02	17.8±0.58	23.8±1.19

Results are mean±SEM for six animals.Values expressed in IU/L.P<0.005 significantly different compared with Group I control animals.

Table 3. Effect of TCEE on the activities of superoxide dismutase (SOD), catalyse and glutathione (GSH) in the heart of normal and isoproterenol (ISO) – induced myocardial infarction (MI) in rats.

Groups	SOD	CAT	GSH
GroupI	13.21±0.94	5.91±0.39	3.89±0.14
GroupII	6.92±0.45	3.25±0.27	1.24±0.05
GroupIII	14.87±0.89	5.86±0.34	1.24±0.05
GroupIV	11.54±0.92	4.32±0.42	2.98±0.14
GroupV	13.82±0.37	5.13±0.18	3.57±0.24

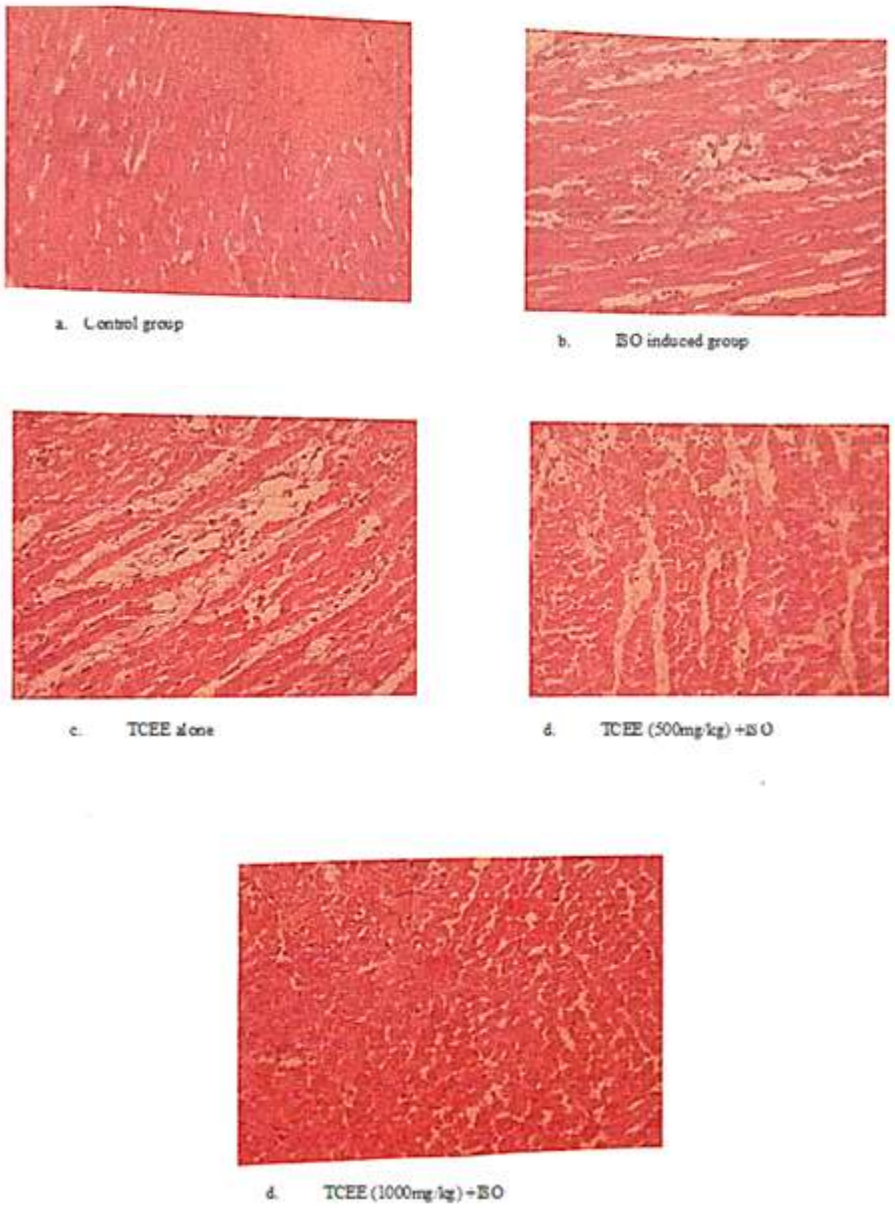
Results are mean±SEM for six animals.Values expressed in U/mg.P<0.005 significantly different compared with Group I control animals.

Table 4. Effect of TCEE on the activities of serum and heart total cholesterol,Triglycerides (TG), and Free fatty acids (FFA) in normal and isoproterenol (ISO) – induced myocardial infarction (MI) in rats.

Groups	Total cholestrol	TG	LDL	HDL
GroupI	112.4±6.28	29.3±1.35	73.4±4.7	21.6±1.48
GroupII	208.2±4.32	55.6±1.48	137.5±7.9	13.9±0.89
GroupIII	117.4±3.67	30.1±0.32	78.2±5.7	22.3±1.38
GroupIV	129.5±5.41	40.7±1.56	99.5±5.5	18.2±1.23
GroupV	118.2±4.76	32.9±1.76	85.5±3.5	19.1±0.98

Results are mean±SEM for six animals.Values expressed in mg/dl.P<0.005 significantly different compared with Group I control animals.

Fig.1 Histopathology section of heart after a treatment of period



IV. CONCLUSION

In conclusion, the results of the present study indicate that the prior administration of Terminalia catappa extract attenuates ISO induced MI in rats. The cardioprotective effect of TCEE is probably related to a strengthening of the myocardical membrane by its membrane stabilizing action, or to a counteraction of free radicals by its antioxidant property.Further experiments and detailed phytochemical analyses are underway to determine the phytoconstituents responsible for the as well as the cardioprotective mechanisms involved.

REFERENCES

[1]. Marcus GM, Cohen J, Varosy PD, " The utility of gestures in patients with chest discomfort".Am.J. Med., 2007,120(1):83-9.

[2]. Stephanie DW, Yanwen W, Stan K, Peter JHJ. Effect of a medium chain triglyceride oil mixture and alpha-lipoic acid diet on body composition, antioxidant status, and

plasma lipid levels in the Golden Syrian hamster. J Nutr Biochem.,2004,15:402-10.

[3]. Levy RI, Feinleib M. Risk factors for coronary artery disease and their management. In: Brawnwald E, ed. Heart Disease: A Textbook of Cardiovascular Medicine.Philadelphia: WB Saunders.,1984,2(2):1205- 34.

[4]. Lopez AD, Murray CCJL. The global burden of disease, 1990-2020.Nat Med., 4:1241-43, 1998.

[5]. You JS, Pan TL, Lee YS. Protective effects of Danshen (Salvia militiorrhiza) on adriamycin-induced cardiac and hepatic toxicity in rats. Phytotherapy Research 2007., 21:1146-52.

[6]. Krentz A.J. and Bailey C.J. Oral antidiabetic agents: current role in type 2 diabetic mellitus. Drug 2005., 65:385-411.

[7]. Senthilkumaran P,Mallika J,Ranjitha R, Karthika K, Balaji N.Phytochemical,Antioxidant activity and Invitro and diabetic study of Eucalyptus globoidea leaf extracts on ethylacetate and ethanol.Journal of emerging technologies and innovative research.,2019:635-644.

[8]. Viswanatha GLS, Vaidya SK, Ramesh C, Krishnadas N, Rangappa S. Antioxidant and antimutagenic activities of bark extract of Termminalia arjuna. Asian Pac J. Trop Med 2010., 3:965-70.

[9]. Cragg G.M, Newman DJ and Sander K.M. Natural products in drug discovery and development. J. Nat. Prod,1997.,60(1):52-58.

[10]. Rona G. Catecholamine cardiotoxicity. J Mol Cell Cardiol 1985; 17:291-300.

[11]. Punitha R. and Manohar S., Antihyperglycemic and antilipid-peroxidative effects of Pongamia pinnata (Linn) . Pierre flowers in alloxan induced diabetic rats. J Ethnopharmacol .,2006,11:786-91.

[12]. Sasikumar CS, Shyamala Devi CS. Protective effect of Himalaya Abana, a poly-herbal formulation, on isoproterenol-induced myocardial infarction in rats. Ind J Pharmacol 2000.,32:198-201.

[13]. Vandana S Panda, Suresh R Naik. Evaluation of Cardioprotective Activity of Ginkgo biloba and Ocimum sanctum in Rodents. Alternative Medicine Review .,2009,14(2):161-171.

[14]. Shalini VK, Srinivas L. Lipid peroxide induced DNA damage protection by turmeric (Curcuma longa). Molecular Cell Biochemistry 1987., 77:3-10.

[15]. C.C Lin, Y.L. Chen, J.M.Lin, T.Ujiie. Evaluation of the antioxidant and hepatoprotective activity of Terminalia catappa.Am.J.Chinese Med.,1997,25:153-161.

[16]. Rajadurai,M.and Stanely Mainzen Prince, P. Preventive effect of naringin on isoproterenol –induced cardiotoxicity in Wistar rats: an in vivo and in vitro study.Toxicol.2007,232:216-225.

[17]. Okinaka S, Kumaggi H, Ebashi S, Sugita H, Momoi H, Toyokura Y, Fujie Y. Serum creatine phophokinae. Activity in progressive muscular drytrophy and neuromuscular disease. Arch Neurol., 1961, 4:520-525.

[18]. Lehr D. Healing of myocardial necrosis caused by sympathomimetic amines. Recent Adv. Stud. Cardiac.Struct. Metab. 1972,1:526-550.

[19]. Szkudelska K, Nogowski L, Szkudelski T. Resveratrol, a naturally occurring diphenolic compound, affects lipogenesis,lipolysis and the antilipolytic action of insulin in isolated rat adipocytes. J Steroid Biochem Mol Biol.,2009,1-2:17-24.

[20]. Ren Y, Li Y, Zhao Y, Yu F, Zhan Z, Yuan Y, Yang J. Effect of resveratrol on lipid etabolism in C57BL/6J mice.Wei Sheng Yan Jiu.,2011.40(4):495-97.

[21]. Bhaskar I,Rao SB.New, simple and cheap alternative to troponin test for diagnosis of acute myocardical infarction. Indian J Exper Biol 2002., 40: 628-630.

[22]. Lowry OH, Rosenbrough NJ., et al. Protein measurement with the Folin's reagent. J Biol Chem 1951.,193:265-276.

[23]. Barger JL. Kayo T, Vann JM, Arias EB, Wang J, Hancker TA, Wang Y, Raederstorff D, Morrow JD, Leeuwenburgh C, Allison DB, Saupe KW, Cartee GD, Weindruch R, Prolla TA. " Alow dose of dietary resveratrol partially mimics caloric restriction and retards aging parameters in mice." 2008,4:3(6):2264-14.

[24]. Mc Gowam MW, Artiss JD, Strandbergh DR & Zak BA. Peroxidase – coupled method for the colorimetric determination of serum cholesterol, triglycerides, HDL. Clin Chem.,1972,18:538-40.

[25]. Friedwald MW, Artiss JD, et al., Estimation of concentration of LDL in serum without use of preparative ultracentrifuge, Clin Chem.,1972,18:499.

[26]. Hagan TM, IngerSoll RT, Laykkesfeld J, Liu J, Wehr CM, Vinarsky V, Bartholomew JC, Ames BN, (R)-2- Liponic acid supplement old rats have improved mitochondrial function,decreased oxidative damage and increased metabolic rate. FASEB J.,1999,13:411-18.

[27]. Pearse AGE. Histochemistry: theoretical and applied.1985. 4th edition.Edinburgh: Churchill Livingstone. Volume 2: Chapter 16.
[28]. Harvey J, Paige SM. The Instat Guide to choosing and interpreting statistical tests: A manual for Graph pad Instat.,San Diego, CA USA. 3:1998.
[29]. Yeager JC, Iams SG. The hemodynamics of isopeoterenol – induced cardiac failure in the rat. Circ Shock., 1981,8: 151-163.
[30]. Thompson J, Hess ML. The oxygen free radical system: a fundamental mechanism in the production of myocardial necrosis. Pro Cardiovac Dis., 1986, 28(6):449-62.
[31]. Gnanapragasam A, Yogeera S, Subhashini R. Adriamycin induced myocardial failure in rats protective role of Centella asiatica. J Mol. Cell Biochem.,2007, 294:55-63.
[32]. Nigam PK. Biochemical markers of myocardial injury. Indian J Clin Biochem., 2007,22(1): 10-17.
[33]. Vasudha KC, Nirmal Kumar A, et al., Studies on the age dependent changes in serum adenosine deaminase activity and its changes in hepatitis. Indian J Clin Biochem 2006,21:116-120.
[34]. Kurian GA, Philp S, Varghese T. Effect of aqueous extract of Desmodium gangeticum DC root in the severity of myocardial infarction.J Ethanopharmacol 2005,97:457-461.
[35]. Senthil S, Sridevi M, Pugalendi KV. Protective effect of Ursolic acid against myocardial ischemia induced by isoproterenol in rats. Toxicology Mechm Methd., 2007,17:57-65.
[36]. 36. Handforth CP. Isoproterenol – induced myocardial infarction in animals.Arch Pathol 1962, 73:161-65.
[37]. Sharma M, Kishore K, Gupta SK, Joshi S, Arya DS. Cardioprotective potential of Ocimum sanctum in isoproterenol induced myocardial infarction in rats. Mol Cell Biochem 2001,225:75-83.
[38]. Pastore A, Federici G, Bertini E, Piemonte F.Analysis of glutathione: Implication in redox and detoxification. Clinical chimica Acta., 2003,333:19-39.
[39]. Velavan S, Aegil I, Gokulakrishnan K. Protective effect of Vitis vinifera against myocardial ischemia induced by isoproterenol in rats.2008,3:958-967.