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Research Article

Protective efficacy of Thymol on Glycoproteins in Isoproterenol induced myocardial Infarcted rats: An *in- vivo* and *in- vitro* study

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Abstract

This study evaluates the protective efficacy of thymol on glycoproteins in isoproterenol induced myocardial infarcted rats. Male albino Wistar rats were pre and co-treated with thymol (7.5 mg/kg body weight) daily for a period of 7 days. Isoproterenol (100 mg/kg body weight) was injected subcutaneously into rats at an interval of 24 h for two days (6^{th} and 7^{th} days). Decreased activity/level of creatine kinase in the heart, protein in the plasma with a marked increase in the levels of plasma uric acid and glycoprotein components in the serum and heart were observed in isoproterenol induced myocardial infarcted rats. Pre and co-treatment with thymol (7.5 mg/kg body weight) showed significant protective effects on all the biochemical parameters studied. Furthermore, the in vitro study on hydrogen peroxide confirmed the potent free radical scavenging activity of thymol. Thus, thymol protects isoproterenol induced myocardial infarction by its antilipid peroxidative, antioxidant and antiglycative properties. This scientific study will be useful for the prevention of myocardial infarction.

Keywords: Cardiac marker enzyme; Isoproterenol; Glycoprotein components; Myocardial infarction; Thymol.

Introduction

Myocardial infarction is a clinical syndrome arising from sudden and persistent curtailment of myocardial blood supply which results in the necrosis of the myocardium (Sunmonu andAfolayan, 2010). It is the most dreaded sequel among ischemic heart diseases invariably followed by several biochemical alterations such as hyperlipidemia, lipid peroxidation, free radical damage, thrombosis etc. leading to qualitative and quantitative alteration of myocardium. The model of isoproterenol (ISO) 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 (Stanely Mainzen Prince, 2011).

Isoproterenol (ISO) is a synthetic catecholamine and beta-adrenergic agonist, which causes severe stress in the myocardium, resulting in infarct like necrosis of the heart muscle (Sushama Kumari *et al*., 1989). It has been reported that ISO generates free radicals and stimulates lipid peroxidation, which is a causative factor for irreversible damage to the myocardial membrane (Sushama Kumari *et al*., 1989). Changes in glycoprotein levels are

characteristic of many pathological conditions. Morphological changes in the necrotic cell surface may alter the pattern of glycoprotein synthesis. The appearance of an abnormal level of different proteins in the blood reflects myocyte damage and helps to identify myocardial necrosis (Alpert *et al*., 2010). By studying the biochemical alterations that takes place in an animal model, it is possible to gain more insight in to the mechanism leading to the altered metabolic process in human MI (Rajadurai and Stanely Mainzen Prince, 2007).

Recently, much attention has been focused on the protective effects of natural products on myocardial infarction. Thymol is a dietary monoterpene phenol which is found in the oils of thyme and plants such as *Thymus vulgaris*, *Thymbra spicata*, *Thymus ciliates*, *oregano*, *Trachyspermum ammi* species commonly known as ajwain in Indian subcontinent, *Monarda fistulosa* and *Nigella sativa* seeds. It exhibits multiple biological activities such as antibacterial, antifungal, anti-inflammatory, and radioprotective (Nagoor Meeran and Stanely Mainzen Prince, 2012). Recently, there has been an upsurge of interest to explore the cardio protective potential of natural products. Natural products have lesser side effects than synthetic drugs. There are no scientific reports available on the effects of monoterpenes on MI. Lipid peroxidation and glycoprotein components play an important role in the pathology of MI. One of the treatment strategies aims to prevent MI is to maintain the levels of lipid peroxidation and glycoprotein components. Hence, in view of the above facts, we made an attempt to evaluate the the effects of thymol on glycoprotein components in ISO induced myocardial infarcted rats. In addition to this, in vitro free radical scavenging effect of thymol on hydrogen peroxide was carried out. To our best knowledge, this is the first study carried out on the effects of thymol on glycoprotein components in ISO induced MI.

Materials and Methods

Chemicals

Thymol, orcinol, galactosamine, periodic acid, cysteine hydrochloride and fucose were obtained from S.D fine Chemicals, Mumbai, India. Isoproterenol hydrochloride was purchased from Sigma Chemical Company, St. Louis, MO. All other chemicals used were of analytical grade.

Experimental Animals

The experiment was done with male albino Wistar rats weighing 160–180 g, aged 7-8 weeks old, obtained from Central Animal House, Rajah Muthiah Institute of Health Sciences, Department of Experimental Medicine, Annamalai University, Tamil Nadu, India. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi, India. All the experimental protocols were approved by the Animal Ethical Committee of Annamalai University (Proposal No.835; Approval Date: September. 28, 2011). Rats were housed in polypropylene cages (47 × 34 × 20 cm) lined with husk (replaced every 24 h) in a 12 h light dark cycle at around 22°C. Rats were fed on standard pelleted diet (Pranav Agro Industries Ltd, Pune, Maharashtra, India) and water ad libitum.

Induction of MI in Wistar Rats

ISO (100 mg/kg body weight) dissolved in saline was subcutaneously injected into rats at an interval of 24 h for 2 days (Nagoor Meeran *et al*., 2012). ISO induced MI was confirmed by elevated activity of serum creatine kinase in rats.

Experimental Design

The animals were grouped into four groups of six rats each. Group I: normal control rats; Group II: rats were orally treated with thymol (7.5 mg/kg body weight) dissolved in 0.5% dimethyl sulfoxide (DMSO) daily for a period of 7 days by an intragastric tube; Group III: rats were subcutaneously injected with ISO (100 mg/kg body weight) at an interval of 24 h for 2 days (on 6th and 7th day); Group V: rats were orally pre- and co-treated with thymol (7.5 mg/kg body weight) dissolved in 0.5% DMSO daily for a period of 7 days by an intragastric tube and were subcutaneously injected with ISO at an interval of

24 h for two days (6th and 7th days). Normal control and ISO control rats were given 0.5% DMSO alone orally daily for a period of 7 days by an intragastric tube. Twelve hours after the second dose of ISO injection (on 8th day), all the rats were anesthetized by pentobarbital sodium (60 mg/kg body weight) and then sacrificed by cervical decapitation. Blood was collected, serum and plasma were separated by centrifugation and used for the estimation of various biochemical parameters.

Biochemical Estimations

The activity of creatine kinase in the heart was assayed by a standard commercial kit obtained from Agappe Diagnostics, Kerala, India. The level of plasma uric acid was estimated by a standard reagent kit (Ranboxy Laboratories, United Kingdom).The levels of glycoprotein components in the serum and heart such as hexose, hexosamine, fucose and sialic acid were estimated (Dubois and Gilles, 1956; Wagner, 1979; Dische and Shettles, 1948 and Warren, 1959 respectively). The content of protein in the heart tissue homogenate was also determined (Lowry *et al*., 1959).

The hydrogen peroxide scavenging of thymol was determined in vitro by the method of Ruch (Ruch *et al*., 1984)

Statistical Analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) using Statistical Package for the Social Science (SPSS) software package version 12.00. Results were expressed as mean ± SD for six rats in each group. P-values < 0.05 were considered significant.

Results

Rats induced with ISO (Group-III) showed significant ($P < 0.05$) decreased activities of creatine kinase in the heart compared to normal control rats (Group-I). Pre and co-treatment with thymol (7.5 mg/kg body weight) near normalized the activity of creatine kinase in the heart (Figure-1) of ISO induced rats (Group-IV) compared to ISO alone induced rats (Group-III).

Isoproterenol induced rats (Group-III) showed a significant ($P<0.05$) increase in the levels of plasma uric acid with a significant ($P<0.05$) decrease in the level of plasma total protein compared to normal control rats (Group-I). Pre and co-treatment with thymol (7.5mg/kg body weight) to ISO induced rats (Group-IV) near normalized the levels of plasma uric acid and total protein compared to ISO alone induced rats (Group-III) (Figure-2).

Figure-3 and 4 reveals significant ($P < 0.05$) increase in the levels of hexose, hexosamine, fucose and sialic acid in the serum and heart of ISO induced myocardial infarcted rats (Group-III) compared to normal control rats (Group-I).

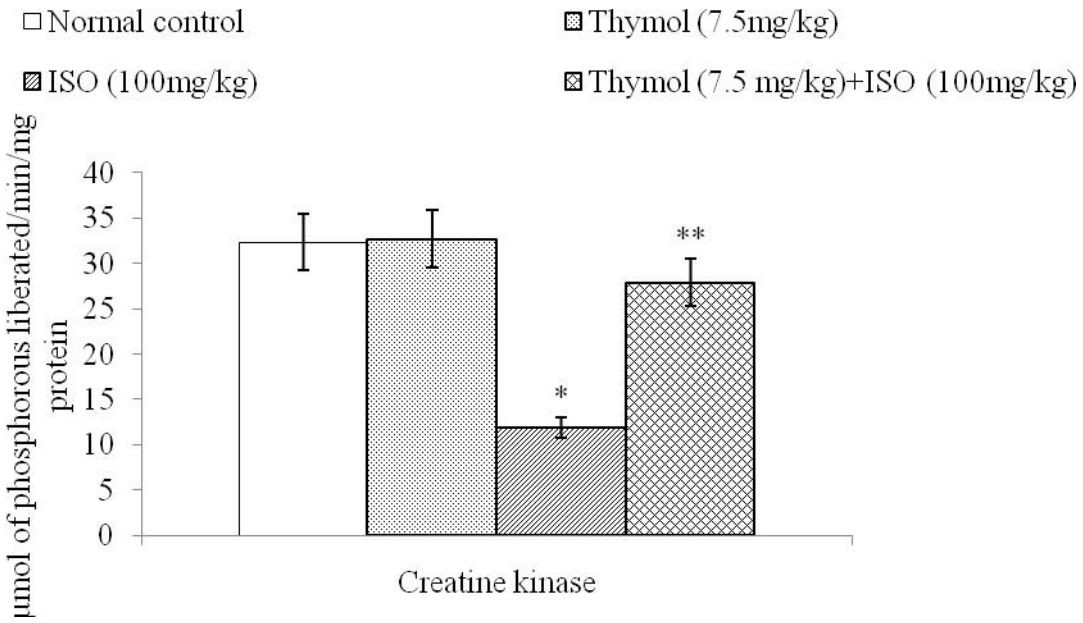
Figure 5 presents the percentage *in vitro* scavenging effects of thymol on hydrogen peroxide. Thymol scavenged these free radicals *in vitro* in a concentration-dependent manner (10, 20, 30, 40 and 50 µM). The percentage-scavenging activity of thymol increased with increasing concentration. The percentage scavenging effects of thymol on hydrogen peroxide at various concentrations (10, 20, 30, 40 and 50 µM) were found to be 13.45, 27.81, 40.79, 57.55 and 71.34% respectively. The percentage scavenging of thymol on hydrogen peroxide at the concentration of 50 µM was found to be 71.66% respectively.

For all the biochemical parameters studied, pre and co-treatment with thymol (7.5 mg/kg body weight) showed significant ($p<0.05$) effects in ISO induced myocardial infarcted rats (Group-IV). Rats treated with thymol (7.5 mg/kg body weight) daily for a period of 7 days had no signifiant effect on all the biochemical parameters studied (Group II).

Discussion

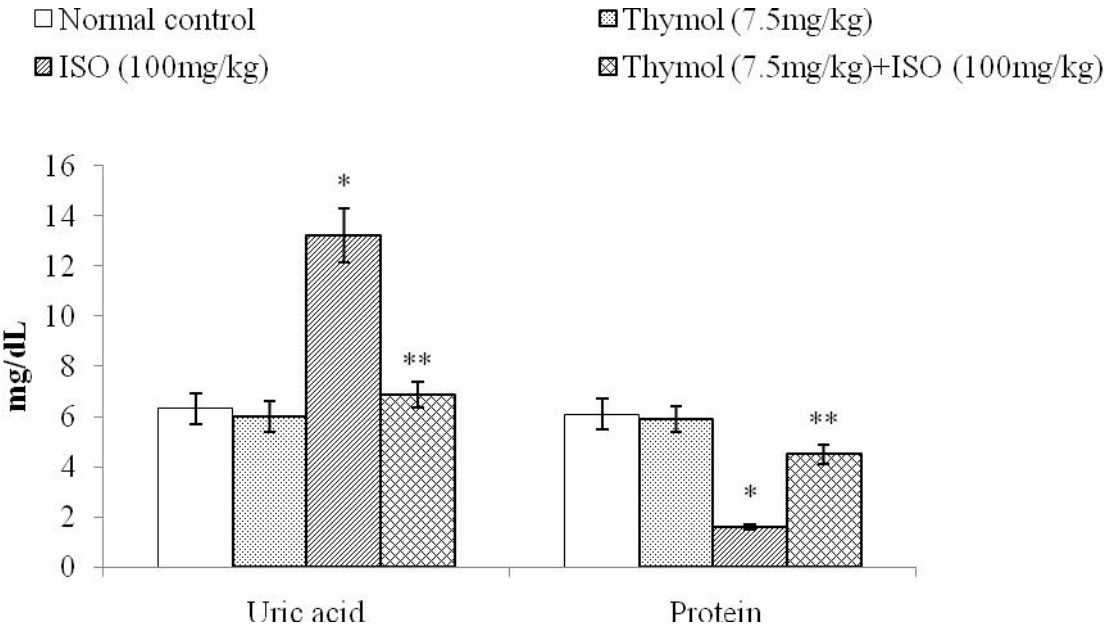
The diagnostic marker enzyme like CK is present in the myocardium that is used as a predictor for pathological changes. This enzyme is released into the extracellular fluid during myocardial injury (Suchalatha and Shyamala Devi, 2004). Our experimental data showed a decrease in activity of

Figure 1. Activity of creatine kinase in the heart



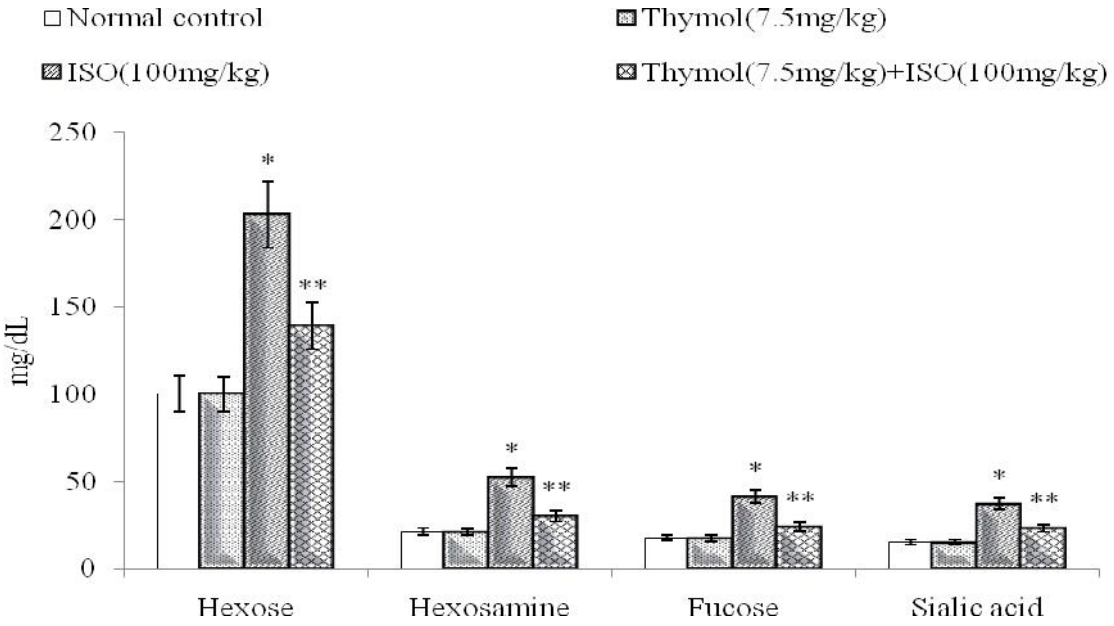
Each column is mean ± standard deviation for six rats in each group; *P<0.05 compared to normal control (Group-I), **P<0.05 compared to isoproterenol control (Group-III) (DMRT).

Figure 2. Levels of plasma uric acid and total protein



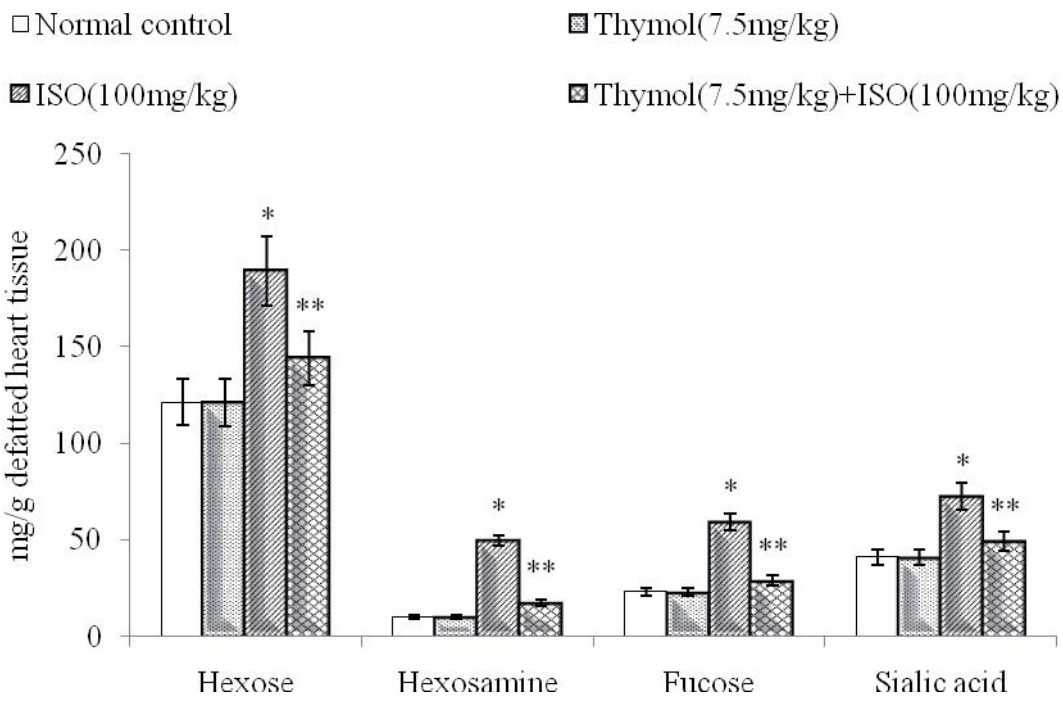
Each column is mean ± standard deviation for six rats in each group; *P<0.05 compared to normal control (Group-I), **P<0.05 compared to isoproterenol control (Group-III) (DMRT).

Figure 3. Levels of glycoprotein components in the serum and heart



Each column is mean ± standard deviation for six rats in each group; *P<0.05 compared to normal control (Group-I), **P<0.05 compared to isoproterenol control (Group-III) (DMRT).

Figure 4. Levels of glycoprotein components in the serum and heart



Each column is mean ± standard deviation for six rats in each group; *P<0.05 compared to normal control (Group-I), **P<0.05 compared to isoproterenol control (Group-III) (DMRT).

CK in the heart of ISO induced rats indicates necrotic damage of the myocardial membrane. Pre and co-treatment with thymol (7.5 mg/kg body weight) near normalized the activity of CK in the heart tissue following ISO insult and thus exhibited the protective effect on the myocardium cell membrane integrity due to its antioxidant effect.

Lipid peroxidation is a well established mechanism of cellular injury and has been used as an indicator of oxidative stress that leads to pathogenesis of MI (Saravanan and Prakash, 2004). Uric acid is the most abundant aqueous antioxidant in humans, and contributes as much as two-thirds of all free radical scavenging capacity in plasma. It is particularly effective in quenching hydroxyl, superoxide and peroxynitrite radicals, and may serve a protective physiological role by preventing lipid peroxidation (Squadrito *et al*., 2000). Uric acid concentrations increase during acute oxidative stress and ischaemia, and the increased concentrations might be a compensatory mechanism that confers protection against increased free radical activity (Nieto *et al*., 2000). A substantial body of experimental evidence suggests that plasma uric acid is a strong risk factor for MI (Weir *et al*., 2003). Increased levels of plasma uric acid indicate increased production of free radicals in MI. Pre- and co-treatment with thymol near normalized plasma uric acid levels. We have already reported that thymol near normalized the plasma lipid peroxidation products in ISO-induced rats (Nagoor Meeran and Stanely Mainzen Prince, 2012). The near normalized levels of lipid peroxidation observed in thymol pre- and co-treated ISO induced rats resulted in near normalized levels of uric acid. This effect shows the antioxidant potential of thymol.

Administration of isoproterenol causes excessive production of free radicals. The observed decrease in the level of plasma protein is due to increased production of free radicals by ISO. The near normalized level of plasma total protein observed in this study in thymol pre- and co-treated ISO induced rats is due to its ability to inhibit lipid peroxidation.

The function of glycoprotein components in stabilizing the tissue may be involved in maintaining the structural stability of collagen fibrils. Glycoprotein components are important components of intracellular matrix, cell membrane and membranes of the subcellular organelles (Zachariah and Basu, 1993). During cardiovascular diseases, significantly increased levels of the glycoprotein components were observed and it is related to the severity and existence of degenerative vascular diseases (Mathew *et al*., 1982). There is a report showing that serum sialic acid reflects the progression of ischemic disease (Masuda and Wakabayashi 1998). Increased levels of sialic acid in the serum and heart of ISO induced MI were observed in this study. Gokmen *et al*. (2000) suggested that either shedding or secretion of sialic acid fromthe cell membrane surface may be partly responsible for increased serum sialic acid concentration following MI.

Increased levels of hexose, hexosamine and fucose were observed in the serum and heart of ISO-induced rats. The increased levels of glycoprotein components may be due to glycosylation of proteins or biosynthesis of glycoproteins in liver or due to release of preformed proteins (Mukesh *et al*., 2005). Furthermore, increased levels of glycoprotein components may also be due to increased deposition of macromolecular components, which is a physiological adjustment to the pathological process. Pre and co-treatment with thymol near normalized the levels of serum and heart glycoprotein components in ISO induced rats. Thus, thymol protected the myocardium against ISO induced toxicity and maintained the levels of glycoprotein components by its antiglycative effect.

Hydrogen peroxide is a weak oxidizing agent that inactivates a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. It can cross cell membranes rapidly; once inside the cell, it can probably react with Fe^{2+} and possibly Cu^{2+} ions to form hydroxyl radicals and this may be the origin of many of its toxic effects (Kumaran and Karunakaran, 2007). In this study, thymol 50 µM *in vitro* exhibits 71.34% of hydrogen peroxide scavenging activity. Thus, the present findings

evidenced that thymol is a potent free radical scavenger depicted by *in vitro* hydrogen peroxide scavenging assay. Thus, thymol scavenges the free radicals produced in excess by the ISO metabolism.

In conclusion, pre and co-treatment with thymol (7.5mg/kg body weight) exhibits protective effects in ISO (100mg/kg body weight) induced myocardial infarcted rats by modulating lipid peroxidation thereby maintaining the activity/levels of cardiac marker enzyme and glycoprotein components. The observed effects in this study are due to the antilipid peroxidative and antiglycative properties of thymol. Thus, thymol prevents the accumulation of free radicals and protects the heart from the deleterious effects of lipid peroxidation and glycoprotein components in ISO induced MI. The present study as well as the previous studies on thymol further strengthened the protective effects of thymol in ISO-induced myocardial infarcted rats.

References

Alpert. J.S, Thygesen. K, Antman. E and Bassand. J.P. 2000. Myocardial infarction redefined--a consensus document of The Joint European Society of Cardiology/ American College of Cardiology Committee for the redefinition of myocardial infarction. *J. American Coll. Cardiol.* 36:959-969.

Dische. Z and Shettles. L.B. 1948. Specific colour reactions of methylpentoses and spectrophotometric micromethod for their determination. *J. Biol. Chem*. 175:595–603.

Dubois. M and Gilles. K.A. 1956. Methods in Enzymology: Academic Press, NewYork; pp 83–85.

Gokmen. S.S, Kilicli. G, Ozcelik. F and Gulen. S. 2000. Serum total and lipid bound sialic acid levels following acute myocardial infarction. *Clin. Chem. Lab. Med*. 38:1249-1255.

Kumaran. A and Karunakaran. R.J. 2007. *In vitro* antioxidant activities of methanol extracts of *Phyllanthus* species from India. *Lebensm. Wiss. Technol*. 40: 322-352.

Lowry. O.H, Rosebrough. N.J, Farr. A.L and Randall. R.J. 1951. Protein measurement with the Folin's phenol reagent. *J. Biol. Chem.* 193:265–275.

Masuda. H and Wakabayashi. I. 1998. Serum sialic acid and ankle versus brachial arterial pressure ratio in NIDDM. Scand. *J. Clin. Lab. Invest*. 5:433-439.

Mathew. S, Menon. P.V and Kurup. PA. 1982. Changes in glycoproteins in isoproterenol induced myocardial infarction in rats. *Indian J. Biochem. Biophys.* 19:41-43.

Mukesh. N, Ojhal. S and Ranjit. K. 2005. Changes in levels of serum glycoproteins in major depressive disorders. *Indian J. Clin. Biochem.* 20:154-157.

Nagoor Meeran. M.F and Stanely Mainzen Prince. P. 2012. Protective effects of thymol on altered plasma lipid peroxidation and nonenzymic antioxidants in isoproterenol-induced myocardial infarcted rats. *J. Biochem. Mol. Toxicol,* 26:368-73.

Nagoor Meeran. M.F, Stanely Mainzen Prince. P and Hidhayath Basha. R. 2012. Preventive effects of N-acetyl cysteine on lipids, lipoproteins and myocardial infarct size in isoproterenol induced myocardial infarcted rats: an *in vivo* and *in vitro* study. *Eur J Pharmacol.* 677:116-22.

Nieto, F.J, Iribarren. C, Gross. M.D, Comstock. G.W and Cutler. R.G. 2000. Uric acid and serum antioxidant capacity: a reaction to atherosclerosis? Atherosclerosis 148:131–139.

Rajadurai. M and Stanely Mainzen Prince. 2007. Preventive effect of naringin on isoproterenol induced cardiotoxicity in wistar rats: an *in vivo* and *in vitro* study. *Toxicology,* 232:216-225.

Ruch. R.T, Cheng. S.J and Klaunig. J.E. 1984. Methods enzymol.105:198-209.

Saravanan. G and Prakash. J. 2004. Effect of garlic (*Allium sativum*) on lipid peroxidation in experimental myocardial infarction in rats. *J. Ethanopharmacol.* 94:155–158.

Squadrito. G.L, Cueto. R, Splenser. A.E, Valavanidis. A, Zhang. H, Uppu. R.M and Pryor. W.A. 2000. Reaction of uric acid with peroxynitrite and implications for the

mechanism of neuroprotection by uric acid. Arch. Biochem. Biophys. 376:333–337.

Stanely Mainzen Prince. P. 2011. A biochemical, electrocardiographic, electrophoretic, histopathological and *in vitro* study on the protective effects of (−) epicatechin in isoproterenol-induced myocardial infarcted rats. *Eur. J. Pharmacol,* 671:95-101.

Suchalatha. S and Shyamala Devi. C.S. 2004. Protective effect of *Terminalia chebula* against experimental myocardial injury induced by isoproterenol. *Indian J. Exp. Biol.* 42:174–178.

Sunmonu. T.O and Afolayan. A.J. 2010. Protective effect of *Artemisia afra Jacq*. on isoproterenol-induced myocardial injury in Wistar rats. *Food Chem, Toxicol*, 48:1969-1972.

Sushama Kumari. S, Jayadeep. A, Kumar. J.S and Menon. V.P. 1989. Effect of carnitine on malondialdehyde, taurine and glutathione levels in heart of rats subjected to myocardial stress by isoproterenol. *Indian J. Exp. Biol*, 27:134-137.

Wagner. W.D. A more sensitive assay discriminating galactosamine and glucosaminein mixtures. *Anal. Biochem*. 1979; 94:394–396.

Warren, L. 1959. The thiobarbituric acid assay of sialic acids. *J. Biol. Chem*. 234:1971–1975.

Weir. C.J, Muir. S.W, Walters. M.R and Lees. K.R. 2003. Serum urate as an independent predictor of poor outcome and future vascular events after acute stroke. Stroke 34:1951-1956.

Zachariah. B and Basu. D. 1993. Surface carbohydrates in cell biology. Indian J. Biochem. Biophys. 30: 422-425.

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