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STUDIES ON PHARMACODYNAMIC INTERACTION OF GARLIC AND METOPROLOL IN RATS WITH CHONIC MYOCARDIAL DYSFUNCTION AND METABOLIC DERANGMENTS

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ABSTRACT

Chronic oral administration of aqueous extract of garlic and metoprolol are reported to possess cardioprotection during ischemia-reperfusion injury in rat when they were administered individually. However, no scientific evidence is available for their combined use. Therefore the present study was designed to investigate the interactive effect aq. extract of garlic and metoprolol on the ISO induced chronic M.I. in rat. Isoprenaline (3 mg/kg, s.c) was administered for 30 days to Sprague dawley rats while treated with garlic aq. extract (250 and 500 mg/kg, *p.o* 30 days) and metoprolol (150 mg/kg, *p.o.* seven days) in separate groups. When myocardial cells damaged or destroyed due to Isoprenaline, which results in the leakage of enzymes. This is the reason for increase activities of LDH, CKMB, SOD and Catalase in serum of rats with M.I. induced by Isoprenaline. In serum there was significant reduction in LDH, CKMB in groups treated with GLD and GHD. Introduction of metoprolol during therapy of GLD and GHD of garlic aq. extract caused reduction of LDH, CK-MB, activities in serum effectively. In HTH enhancement of LDH, CK-MB, SOD and Catalase activities in GLD and GHD, Introduction of metoprolol during chronic therapy of garlic aq. extract, it potentially increase the activity of LDH, CK-MB, SOD and Catalase in HTH. The elevated TBARS levels were ameliorated in all treated groups. The best results were found in-group subjected to high dose of garlic aq. extract along with metoprolol. In conclusion, combined therapy of garlic aq. extract and metaprolol possess synergistic potential as cardioprotective remedy for combating myocardial stress.

Keywords: Aq. extract of Garlic, Myocardial infarction, Isoprenaline, Metoprolol.

INTRODUCTION

Herb-drug interactions are an enigmatical work for the healthcare professionals. The administration of herbal medicines alongside cardiovascular pharmacotherapy is ubiquitous globally. Concomitant administration of herbs and drugs either may enhance or oppose the pharmacological effects of each other [1]. In view of the enormously use of herbal remedies by the general public and subsequent interest by the physicians, it is imperative to promote credible research on the safety of herbal products including the possibility of interactions with concurrent cardiovascular pharmacotherapy.

The therapeutic uses of garlic (*Allium sativum*) for treatment of various disorders have been known for centuries. In the past decade, several studies suggested that garlic has a protective effect against cardiovascular diseases [2]. Recent epidemiologic and experimental studies have been shown to reduction in cardiovascular complication on garlic consumption [3]. Garlic and its preparations have been dissemenatly recognized as agents for prevention and treatment of cardiovascular and other metabolic diseases such as atherosclerosis, arrhythmia, hyperlipidemia, thrombosis, hypertension and diabetes .It has also been found that an aqueous extract of raw garlic scavenges hydroxyl radicals (OH) and superoxide anion (O_2), Furthermore an aqueous extract of garlic is also able to inhibit lipoprotein oxidation and to scavenge OH and O free radicals.

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Beta-blockade has been traditionally used in a number of diseases including hypertrophic cardiomyopathy, systemic hypertension, vasovagal syncope, and supraventricular and ventricular arrhythmias. Based on the pathogenesis of enhanced SNS activation however, beta-adrenergic blockade has gained favor in recent years as a therapeutic modality for treatment of people with heart failure, particularly due to results of clinical trials demonstrating its efficacy [4,5]. Studies using metoprolol, carvedilol, and bisoprolol have documented benefits that accrue from chronic treatment with beta-blockers [6-8]. The use of beta-blockers significantly improves hemodynamics, cardiac function, immune function, clinical signs, exercise capacity, quality of life, and survival in patients with heart failure [9]. One recent study showed a 34% reduction in mortality in people with heart failure treated with metoprolol [10]. For most human cardiologists, beta-blockers are now considered part of a standard care protocol for people with heart failure who can tolerate them.

Garlic has remarkable ability to strengthen the cardioprotective nature of concurrently administered drugs during myocardial damage in rats. Earlier reports on the drug interaction studies of garlic with calcium channel blockers (CCBs) indicate that it produces concentration dependent synergistic effect due to its own calcium channel blocking effect [11]. Recently, we reported improved survival and cardiac function by add-on captopril [12,13] and propranolol [14] during garlic therapy in rats with myocardial infarction. Earlier reports on the drug interaction studies of garlic with calcium channel blocker indicate that it produces concentration dependent synergistic effect by its calcium blocking property [15]. As stated above both metoprolol and garlic possesses strong cardioprotective properties, however, there is no reported interaction between these two medicaments when they are taken together. Hence, the present study was designed to explore the pharmacodynamic interaction of garlic with metoprolol in Isoprenaline induced acute myocardial injury in rats.

MATERIALS AND METHODS

Chemicals

Biochemical kits were acquired from standard and authentic companies. Biochemical kits like LDH, CKMB KIT were purchased from CORAL lab (Goa, India), Isoprenaline from SIGMA –ALDRICH Lab, metoprolol(Cipla), ketamine, xylazin

Experimental animals

Sprague Dawley rats, weighing 150–200 g, were obtained from the animal house of Krupanidhi College of pharmacy Bangalore. They were fed with standard laboratory chow (Amrut laboratory feed, Maharashtra India) and provided with water ad libitum. The animals were housed at temperature 27 ±2◦C, humidity 55%, and a 12-light/12-h dark cycle. All experiments were carried out according to Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Plant material

Garlic bulbs purchased from S.K.R. Market, Bangalore, India. Garlic cloves were peeled off, weighed, chopped, and homogenized with distilled water. This homogenate was centrifuged at 20,124 · g for 10 min at 4° C. The supernatant was recovered and used at the indicated final concentrations [16].

Phytochemical evaluation

To check the constituent of garlic in aq. extract phytochemical studies was carried out. Like test for alkaloids(Hager's Test, Mayer's Test, Dragendroff's Test, Wagner's Test), test for carbohydrates (Molisch Test, Felhing's Test, Barfoed's Test, Benedictt's Test), test for protein(Ninhyrin Test Millon's Test Biuret Test),test for flavonoids (Ferric Chloride Test, Lead Acetate Test) and test for steroids (Salkowski Test), saponins and tannins were carried out.

Experimental protocol

The animals were divided into seven groups consisting of six animals each. Group I was treated with normal saline for 21 days, Group II III, IV, V, VI, VII were administered Isoprenaline (3 mg/kg/day), Group III and VI were administered with GLD, Group IV and VII were administered GHD, Group V, VI, VII were administered Metoprolol (150 mg/kg, seven days).

Experimental procedure

At the end of treatment period, under anesthesia by retro-orbital vein blood samples was withdrawn and serum was separated by centrifugation. In serum biological markers like Lactate dehydrogenase (LDH) and creatine Phosphokinase-MB (CK-MB) measured. The heart was immediately isolated from each animal under ketamine (70 mg/kg, i.p.) and xylazine (10 mg/kg, i.p) anesthesia. In each group consisting of six animals, three excised hearts were homogenized to prepare heart tissue homogenate (HTH) using sucrose (0.25 M) [17].

The activity of LDH, CK-MB, superoxide dismutase (SOD) [18] and catalase [19] were measured in heart tissue homogenate (HTH). From remaining three animals heart tissue Microscopic slides were prepared for histopathological studies.

Histological analysis

The myocardial damage was determined by giving scores depending on the intensity as follows [20] score 0-normal cardiac muscle architecture, Score 1 (mild focal necrosis/degeneration), Score 2 (mild diffuse/moderate necrosis+/- mild inflammation), Score 3 (moderate diffuse necrosis+/- mild inflammation and fibrosis) and Score 4 (sever necrosis+/-inflammation+ /-fibrosis).

Statistical analysis

The statistical significance will be assessed using one-way analysis of variance (ANOVA) followed by Dunnet comparison test. The values will be expressed as mean ± SEM and $p < 0.05$ will be considered significant.

RESULTS

Preliminary phytochemical investigation

The preliminary phytochemical investigation of the aq. extract of garlic showed the presence of Carbohydrates protein, flavonoids, steroids, saponins and tannins.

Effect on LDH and CK-MB activities

The LDH and CK-MB activities were significantly increased and decreased in serum and in heart tissue homogenate (HTH) respectively in ISO treated group when compare to Normal group. Groups treated with GLD, GHD, METO, GLD+METO, GHD+METO showed significant fall in LDH and CK-MB activities in serum and elevation in HTH compared to ISO control. The decline in LDH and CKMB activity in serum of animals pretreated with GLD+METO was significantly compared to GLD, GHD &METO alone. Similarly, CK-MB activity was raised significantly in animals treated with GLD+METO compared to GLD, GHD &METO alone. There were increased in CKMB level in HTH in GHD+METO when compare to GLD, GHD, METO, GLD+METO treated groups and there was also elevation of LDH activity in GLD+METO when compare to rest of the groups except Normal group.

Effect on SOD and Catalase

In ISO control group there were decreased in SOD and catalase activity when compared to normal control. Administration of METO, GLD, GHD and their combinations resulted in significant rise in SOD and catalase activities compared to ISO control. Concurrent administration of GLD+METO, GHD+METO resulted in significant elevation in SOD and catalase activities respectively compared to METO & GLD, GHD alone.

Effect on TBARS

A significant elevation in TBARS levels were found in ISO control compared to normal control. Treatment of animals with GLD, GHD, METO and their combination demonstrated significant fall in TBARS levels compared to ISO control. Treatment with GLD+METO, GHD +METO significantly effective when compare to GLD, GHD respectively & more effective than METO alone also.

Effect on histological score

Administration of ISO caused necrosis of cells with degeneration of myofibril and increased interstitial space. In ISO induced chronic M.I. by administration of isoprenaline for 21 days (3mg/kg/day) myocardial integrity was disturbed evident with increased interstitial space and necrosis of cells with degeneration of myofibril (Figure 2). Pre-treatment of GLD, GHD with or without Metoprolol to rats before subjected to isoprenaline induced myocardial damage showed significant fall in histological scores compared to ISO control.

ISO treated group shows moderate diffuse necrosis+/- mild inflammation and fibrosis, Groups treated with GLD and GHD has shown less diffuse necrosis, mild inflammation and fibrosis as compare to ISO group. Group treated with metoprolol alone shows more effective than GLD and GHD Recovery from necrosis, mild inflammation with fibrosis was observed more effective in the groups treated with GLD + metoprolol and GHD + metoprolol (Table 1).

Table 1. Effects of Aq. extract of garlic and metoprolol on SOD, catalase and TBARS in heart tissue homogenate against ISO induced chronic myocardial infarction

Treatment	Heart tissue homogenate			
	SOD (unit/mg protein)	Catalase (unit/mg protein)	TBARS (µmol/g wet wt)	Histopathological Score
Normal control	5.4±0.2	35.2±1.8	2.3±0.7	0
ISO control	0.2±0.20 ^{c}	8.8±0.2 ^{c}	38.7±0.7^{c}	3
GLD	0.5±0.2 ^{c}	10.4±0.4 ^{c}	22±0.5^{c3}	2.5
GHD	1.7±0.4 ^{c1}	12.6±0.5 ^{c}	16.3±1.4^{c3}	2.5
METO	2.9±0.3 ^{c3}	14.8±0.5 ^{c2}	10.4±1.1^{c3}	2
GLD+ METO	8.9±0.2 ^{c3}	19.0±0.8 ^{c3}	9.6±0.4^{c3}	1.5
GHD + METO	7.3±0.1 ^{b3}	19.3±0.2 ^{c3}	7.3±0.4^{a3}	1.5

All values are mean ± SEM, n=7, ^{a} $P<0.05$, ^{b} $P<0.01$, ^{c} $P<0.001$ when compared to normal control; $^{1}P<0.05$, $^{2}P<0.01$, $^{3}P<0.001$ compared to ISO control.

Table 2. Effects of GLD, GHD and METO on LDH and CKMB level in serum and heart tissue homogenate against isoprenaline induced chronic myocardial infarction

Treatment	CK-MB activity		LDH activity	
	Serum (unit/lit)	HTH (unit/ g wet wt)	Serum (unit/lit)	HTH (unit/ g wet wt)
Normal control	164±7.5	73.7±3.1	38.8±0.8	18.7±1.4
ISO CONTROL	705.8±20.1 [c]	9.9±2.4 [c]	737.8±12.3 [c]	0.8±0.4 [c]
GLD	508.5±32.3 [c2]	30.9±1.8 [c3]	475.2±2.8 [c3]	4.5±0.4 [c]
GHD	436±5.4 [c3]	34.9±1.7 [c3]	518.4±12.5 [c3]	9.2±0.7 [c3]
METO	445±11.0 [c3]	17.8±1.2 [c]	273.7±15.6 [c3]	7.6±0.6 [c3]
GLD+ METO	171.5±17.8 [3]	30.2±2.6 [c2]	171.6±19.7 [c3]	13.6±0.5 [b3]
GHD + METO	190.3±53.7 [3]	65.7±3.5 [3]	247.4±10.6 [c3]	9.4±0.5 [c3]

All values are mean ± SEM, n=7, [a] $P<0.05$, [b] $P<0.01$, [c] $P<0.001$ when compared to normal control; [1] $P<0.05$, [2] $P<0.01$, [3] $P<0.001$ compared to ISO control.

Histological analysis: Haematoxylin and eosin (H&E) stained section of heart in isoproterenol induced myocardial damage. Photographed at magnification 400X

Figure 1. Normal control: Heart tissue of normal group. Normal texture of cell

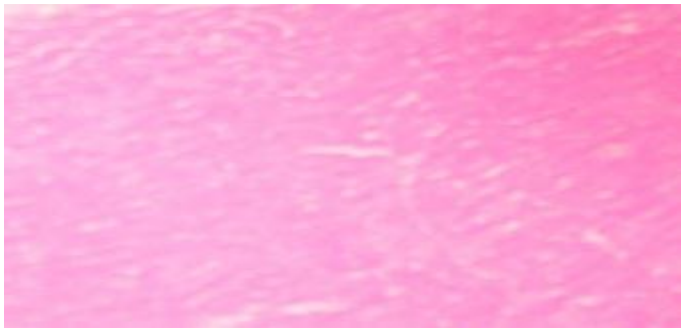

Figure 2. ISO control : Heart tissue of Isoprenaline treated group

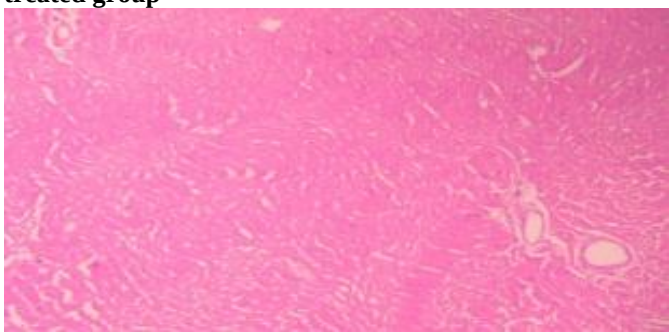

Figure 3. GLD: Heart tissue of GLD (250 mg/kg treated group

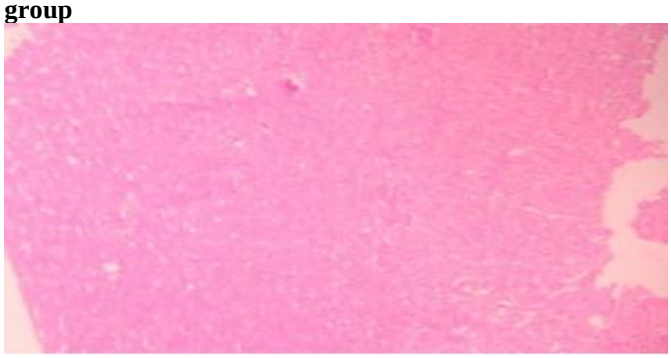

Figure 4. GHD: Heart tissue of GHD (500 mg/kg) treated group

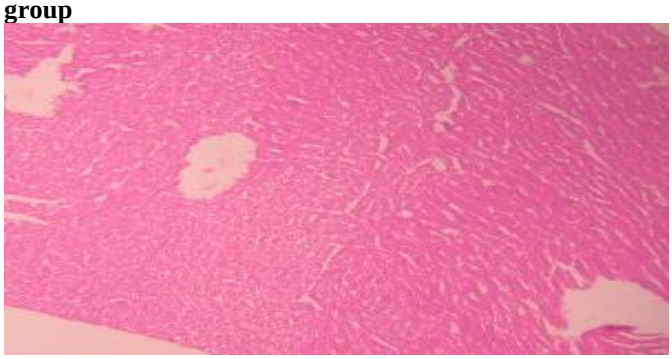

Figure 5. METO: Heart tissue of Metoprolol (150 mg/kg) treated group

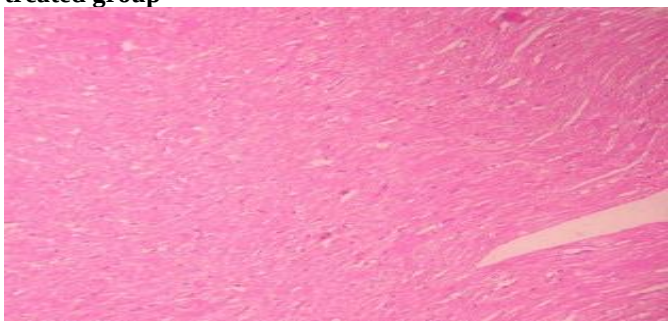

Figure 6. GLD+METO: Heart tissue of GLD (250mg/kg) + Metoprolol (150 mg/kg) treated

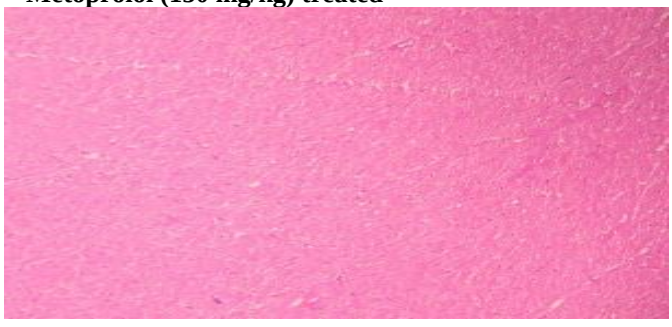

Figure 7. GHD+METO: Heart tissue of GHD (500mg/kg) + Metoprolol (150) treated

DISCUSSION AND CONCLUSION

In the present study, the interactive effects of aq. extract of garlic in presence or in the absence of metaprolol on the chronic myocardial infarction induced by Isoprenaline in rats were studied. The use of herbs and other natural products has gained popularity, and the increase in their consumption is backed by solid scientific evidence. The most studied and reported health promoting effect of garlic is cardioprotection [21,22]. Several studies have been reported that the allicin is responsible for most biological activities of garlic. When the garlic clove is crushed/chopped alliin and alliinase intract, to form allicin which is the principal bioactive compound present in aqueous garlic extract [21]. Hence, allicin is probably responsible for cardioprotective effect of aq. extract of garlic on M.I. induced by ISO in rat hearts. The aqueous extracts of raw garlic induced the increase in plasma antioxidant activity in rats suggesting that the antioxidant properties of garlic were unaffected by boiling because the OH scavenging properties of garlic were essentially preserved when garlic extracts were heated [16]. Chronic dose of isoprenaline induce myocardial ischemia, hypoxia, necrosis, and finally fibroblastic hyperplasia with decreased myocardial compliance and inhibition of diastolic and systolic function, which closely resembles local myocardial infarction-like pathological changes seen in human myocardial infarction, so it is widely used as a model of evaluating cardioprotective drugs and studying myocardial consequences of ischemic disorders [23]. The release of cellular enzymes reflects a non-specific alteration in the plasma membrane integrity and/or permeability as a response to β-adrenergic stimulation. When myocardial cells damaged or destroyed due to deficient oxygen supply or glucose, the cell membrane becomes permeable or may rupture, which results in the leakage of enzymes. This accounts for the increase activities of LDH, CKMB, SOD and Catalase in serum of rats with myocardial infarction induced by administration of isoprenaline, which may be due to excessive production of free radicals induced by Isoprenaline administration [23,24]. Administration of Isoprenaline induces subendocardial myocardial ischemia, hypoxia, necrosis, and finally fibroblastic hyperplasia with decreased myocardial compliance and inhibition of diastolic and systolic function, which induce myocardial infarction [23]. The increased generation of reactive oxygen species and/or depletion of the antioxidants in the defense system may contribute to oxidative stress and affect the pathogenesis of myocardial infarction. Free radical scavenging enzymes such as superoxide dismutase, catalase and glutathione peroxidase are the first line cellular defense against oxidative stress, eliminating reactive oxygen radical such as superoxide and hydrogen peroxide (H2O2), and preventing the formation of more reactive radical of hydroxyl radical.

REFRENCES

1. Fugh BA. Herb-drug interaction. *Lancet*, 355, 2000, 134.
2. Alnaqeeb MA, Thomson M, Bordiab T & Ali M. Histopathological effects of garlic on liver and lung of rats. *Toxicology Letters*, 85, 1996, 157.
3. Rahman K & Lowe GM. Garlic and cardiovascular diseases: a critical review. *J Nutr* 136, 2006, 736.
4. Packer M, Coats AJS, Fowler MB, Katus HA, Krum H & Mohacsi P. Effect of carvedilol on survival in severe chronic heart failure. *N Engl J Med*, 344, 2001, 1651.
5. Packer M, Bristow MR, Cohn JN, Colucci WS, Fowler MB & Rouleau J. The effect of carvedilol on morbidity and mortality in patients with chronic heart failure. *N Engl J Med*, 334, 1996, 1349.
6. Packer M, Colucci WS, Sackner-Bernstein JD, Liang C, Goldscher DA & Freeman IV. Double blind, placebo-controlled study of the effects of carvedilol in patients with moderate to severe heart failure. *The PRECISE trial. Circulation*, 94, 1996, 2793.
7. Bristow MR, O'Connell JB, Gilbert EM, French WJ, Leatherman G & Kantrowitz NE. Dose-response of chronic ß-blocker treatment in heart failure from either idiopathic dilated or ischemic cardiomyopathy. *Circulation* 89, 1994, 1632.
8. Bristow MR, Gilbert EM, Abraham WT, Adams KF, Fowler MB & Hershberger RE. Carvedilol produces dose-related improvements in left ventricular function and survival in subjects with chronic heart failure. *Circulation*, 94, 1996, 2807.

9. Lechat P, Packer M, Chalon S, Cucherat M, Arab T & Boissel JP. Clinical effects of ßadrenergic blockade in chronic heart failure, A meta-analysis of double-blind, placebo-controlled, randomized trials. *Circulation*, 98, 1998, 1184.
10. Merit HF. Study Group, Effect of metoprolol CR/XL in chronic heart failure: metoprolol CR/XL randomized intervention trial in congestive heart failure (MERIT-HF). *Lancet*, 353, 1999, 2001.
11. Martin N, Bardisa L, Pantoja C, Barra E, Demetrio C & Valenzuela J. Involvement of calcium in the cardiac depressant actions of a garlic dialysate. *J Ethnopharmacol*, 55, 1997, 113.
12. Asdaq SM &Inamdar MN. Pharmacodynamic interaction of captopril with garlic in isoproterenol induced myocardial damage in rat. *Phytother Res*, 24(5), 2010, 720-5.
13. Asdaq SM & Inamdar MN, Pharmacodynamic interaction of garlic with captopril in Ischemia-reperfusion induced myocardial damage in rats. *Pharmacologyonline*, 2, 2008, 875.
14. Asdaq SM, Inamdar MN, Asad M & Nanjundan PK. Interaction of propranolol with garlic in isoproterenol induced myocardial infarction in rat. *J Pharmacol Toxicol*, 3, 2008, 414.
15. Aqel MB, Gharaibah MN & Salhab AS. Direct relaxant effects of garlic juice on smooth and cardiac muscles. *J Ethnopharmacol*, 33, 1991, 13.
16. Jose PC, Omar NM & Sabina SM. Effect of heating on peroxynitrite scavenging capacity of Garlic. *Food and Chemical Toxicology*, 45, 2007, 622.
17. Buerke I, Prufer D, Dahm M, Oelert H, Meyer J, & Darius H. Blocking of classical complement pathway inhibits endothelial adhesion molecule expression and preserves ischemic myocardium from reperfusion injury. *J. Pharmacol. Exp. Ther*, 286, 1998, 429.
18. Erich F, & Elastner M. Inhibition of nitrite formation from hydroxyl ammonium chloride. A simple assay of super oxide dismutase. *Anal. Chem*., 70, 1976, 616.
19. Eva ML. Mechanism of pH dependent hydrogen peroxide cytotoxicity in- vitro. *Arch. Biochem. Biophys*, 365, 1988, 362.
20. Karthikeyan K, SaralaBai BR & Devaraj N. Cardioprotective effect of grape seed proanthocyanidins on isoproterenol-induced myocardial injury in rats. *Int. J. Cardiol*., 115, 2007, 326.
21. Jose PC, Omar N, Medina C, Rosaura AL, Alma BZ and Marisol OI. Reactive oxygen species scavenging capacity of different cooked garlic preparations. *Life Sciences*, 78, 2006, 761.
22. Shela G , Maria L, Hanna L, Katarzyna N, Jacek N, Yong S P,Soon T J, Seong G K and Simon T. Supplementation of garlic lowers lipids and increases antioxidant capacity in plasma of rats. *Nutrition Research*, 26, 2006, 362.
23. Ru Z, Qingbin X, Ping Z, Lin Y, Jie Z and Guidong D. Cardioprotective effect of fluvastatin on isoproterenol-induced myocardial infarction in rat. *European Journal of Pharmacology*, 586 (2008) 244.
24. Kesavarao KE, Venkatachalem S & Thiruvengadam D. Effect of arginine and lysine on mitochondrial function during isoproterenol induced myocardial infarction in rats. *Nutrition Research*, 23, 2003, 1417.