ISSN : 0974 - 7532 *Volume 6 Issue 4,5*

Trade Science Inc.

Research & Reviews in

BioSciences

Regular Paper

RRBS, 6(4,5), 2012 [127-133]

Correlation study on oxidant and antioxidant levels in plasma of acute myocardial infarction among male patients

Ashok Narasimhan[1], Anbarasan Chakrapani[2], Sheela Sasikumar Changam*[1], Soma Guhathakurta[3], Kotturathu Mammen Cherian[3]

[1]Department of Biochemistry, Frontier Lifeline Pvt Ltd, R 30 C Ambattur Industrial Estate Road, Mogapair, Chennai 600 101 (INDIA)

[2]Department of Molecular Biology, Frontier Lifeline Pvt Ltd, R 30 C Ambattur Industrial Estate Road, Mogapair, Chennai 600 101 (INDIA)

[3]Department of Cardiothoracic Surgery, Frontier Lifeline Pvt Ltd, R 30 C Ambattur Industrial Estate Road, Mogapair, Chennai 600 101 (INDIA)

E-mail : sheelsasic@yahoo.co.in

Received: 21st May, 2012 ; Accepted: 21st June, 2012

ABSTRACT

Aims: To evaluate and correlate the plasma concentration of oxidants and antioxidants between control and within Acute Myocardial Infarction (AMI) patients. **Settings and Design**: Subjects (mean age of 45-60yrs) were divided into two equal groups with evidence of AMI (n=25) and control (n=25). The proposed molecules were estimated using standard protocol from blood samples. **Methods and Material**: The levels of antioxidants like vitamin E (α tocopherol), vitamin C (Ascorbic acid), reduced Glutathione (GSH), total protein thiols (TPT) were estimated in the plasma. Lipid peroxides, ceruloplasmin, ischemia modified albumin and protein carbonyls levels were also measured in the plasma of control and AMI patients using standard procedures. **Statistical analysis used**: Data from patients and controls was compared using Student's't'-test. Values were expressed as mean ± standard deviation (SD). SPSS software version 16.0 was used for statistical analysis. 'p' value of less than 0.05 was considered to indicate statistical significance. **Results**: Among the comparison studies, Vitamin E antioxidant and Lipid peroxides oxidant were found to be more significantly associated with AMI patients when compared against control. Vitamin E showed strong positive relationship between ejection fraction in myocardial infarcted patients and reduced Glutathione in diabetic AMI patients. **Conclusions**: Plasma vitamin E and Reduced Glutathione are found to be key players in MI events which could be used as an effective biomarker and also in the treatment of cardiac events in acute myocardial infarcted individuals.

KEYWORDS

Acute myocardial Infarction;
Vitamin E;
Reduced glutathione.

Regular Paper

INTRODUCTION

Acute myocardial infarction (AMI) is one of the leading causes of mortality and morbidity in the world[1]. The deaths due to Cardiovascular Vascular Disease (CVD) in India were 32% of all deaths in 2007 and are expected to rise from 1.59 million in 2000 to 2.03 million in 2010[2]. The generation of free radicals due to physiological imbalance in oxygen supply to the cardiac tissue caused by Coronary Artery Disease (CAD) which will predispose the patients to AMI resulting in myocardial damage which in turn deteriorates the myocardial contractile function and alters the Ca^{2+}homeostasis[3,4].

Free radicals remain to play a critical role in the pathogenesis of many human diseases including CVD like atherosclerosis, ischemic heart disease, cardiac hypertrophy, hypertension, shock and trauma[5]. The human biological system possesses good extrinsic (alpha-tocopherol, ascorbic acid) and intrinsic (GSH, SOD, GPx) antioxidant defence mechanism but the disparity between oxidant and antioxidant molecules determines the health conditions[6,7].

Many research papers have elaborated the oxidants and antioxidants role in AMI patients in detail but oxidant and antioxidant relationship are not elucidated with respect to anthropometric and clinical parameters. Hence this study was performed not only to estimate the plasma concentrations of oxidants and antioxidants in AMI and control population but also tried to establish its correlation. Comprehensive availability of Lipid Profile data in Indian population made us exclude those parameters for this current study[8,9].

SUBJECTS AND METHODS

Patients who were admitted to Coronary Care Unit (CCU) in our unit between November 2010 and December 2010 for AMI treatment were selected as the study population. Inclusion criteria were presence of Chest Pain, ST changes of more than 2mm in one or at least in two leads in ECG elevation of serum CPK-MB and Troponin I level and free from any other disease. Healthy volunteers who were not under any medication were selected as controls. Institutional Ethical clearance was obtained for the proposed study. Age and sex matched population recruited for the study consisted of 50 male subjects (mean age of 45-60yrs) divided into two equal groups with evidence of AMI (n=25) and control (n=25). The study population were classified as:

1. **Control subjects**: Populations with no evidence of AMI (n=25)
2. **AMI patients** : Patients with the evidence of AMI with or without hypertension or diabetes (n=25).
3. **Hypertensive AMI patients (HAMI)**: AMI patients with hypertension with or without diabetes (n=18).
4. **Diabetic AMI patients (DAMI)**: AMI patients with diabetes with or without hypertension (n=19).

All the studied populations were non smokers and normolipidemic. Patients were considered diabetic if the random blood glucose level was more than 140 mg/dl and hypertensive when systolic pressure was more than 140 and diastolic pressure more than 90 mm/Hg. Ejection fraction (EF) in the range of 40-49 and below 40 was considered as patients with mild and severe Left Ventricular dysfunction (LVD) respectively**.**

Blood samples were collected by venous puncture method in heparinized tubes and plasma was separated by centrifugation at 1500 g for 15 min. Informed consent was obtained from all the subjects involved. All the chemicals and reagents were procured from Sigma. The levels of reduced Glutathione (GSH) were estimated using Tietze *et al*[10] method. Total protein thiols (TPT) were estimated using Ellman method[11]. vitamin E (á tocopherol) and vitamin C (ascorbic acid) was estimated using Desai *et al*[12] and Omaye *et al*[13] methods respectively. Lipid peroxides was estimated by measuring the amount of thiobarbituric acid reactive substances in plasma using Okhawa *et al* method[14] (1990). Ceruloplasmin, ischemia modified albumin and protein carbonyls levels were measured by using Ravin *et al*[15], Chawla *et al*[16] and Levini *et al*[17] methods respectively.

Statistical analysis

Data from patients and controls was compared using Student's't'-test. Values were expressed as mean ± standard deviation (SD). SPSS software version 16.0 was used for statistical analysis. 'p' value of less than 0.05 was considered to indicate statistical significance. Correlations among the parameters were studied using regression analysis.

Regular Paper

RESULTS

The control and AMI patients are age and sex matched as shown in TABLE 1. Systolic blood pressure was significantly high in AMI patients as compared with controls ($p<0.001$). TABLE 2 reflects the status of plasma oxidants and antioxidants between control and AMI population. Antioxidants Vitamin E (Vit E),Vitamin C (Vit C),Total Protein Thiols (TPT) and Reduced glutathione (GSH) were significantly decreased ($p<0.001$) with radical increase in oxidants Lipid Peroxides (LP), Protein Carbonyls (PC),Ischemia Modified Albumin (IMA)and Ceruloplasmin (CP) of AMI patients when compared with controls ($p<0.001$).

Figures 1 and 2 explain the impact of age, Body Mass Index (BMI), Diabetes Mellitus (DM) Hypertension (HTN) on the levels of antioxidants and oxidants respectively. The studied population age was divided in the range of 40-55 yrs (n=13) and 56-75 yrs (n=12). Significant difference was observed in Vit E, Vit C ($p<0.01$), LP (not shown in the figure), IMA and PC ($p<0.001$) in the age group of 56-75 yrs. There was a notable difference in the plasma levels of Vit C ($p<0.01$), LP (not shown in the figure), PC and IMA ($p<0.001$) when compared between overweight (Body Mass Index (BMI) in the range of 25-29) AMI and control. Both DAMI and HAMI conditions revealed that the levels of Vit E, Vit C and GSH were affected ($p<0.001$) with concomitant increase in LP (not shown in the figure), IMA and PC ($p<0.001$)

TABLE 1: Demographic data in AMI and control.

Parameter	Control(n=25)	AMI Patients (n=25)
Age	56.12 ± 6.5315	56.92±8.948
BMI	25.405±3.2707	24.37±2.9130
DM NDM	-	68% 32%
Systolic Blood Pressure (SBP)	128.4± 12.54	168.2± 27.16*
Diastolic Blood Pressure (DBP)	83.04± 8.31	101.4± 15.30
Single Vessel Disease (SVD)	-	48%
Double Vessel Disease(DVD)		52%
MI		
Anterior wall	-	68%
Inferior Wall		32%

Values expressed as Mean ±SD; *-- p<0.001

TABLE 2 : Antioxidant and oxidant status in AMI and control patients.

Parameter	Control (n=25)	AMI Patients (n=25)
Ascorbic Acid(mg/dl)	0.3936 ± 0.0119	0.2944 ± 0.0843
α Tocopherol(mg/dl)	1.3924±0.0514	0.8220±0.2742
Total Protein Thiols(µm/l)	0.0615±0.0503	0.3868±0.0090 hs
Glutathione(µmole/l)	0.7668±0.1730	0.4990±0.1208 hs
Ceruloplasmin(mg/dl)	37.336±7.3899	39.456±9.623
Lipid Peroxides(nm/ml)	0.3024±0.0012	0.7020±0.046
IMA(mg/dl)	39.7020±13.52	108.16±32.48
Protein Carbonyls(mg/dl)	2.425±0.8934	25.65±1.594

Ascorbic acid and α Tocopherol, Total protein thiols, reduced glutathione –p<0.001
Lipid peroxides, Ceruloplasmin, IMA, Protein carbonyls - p<0.001 Values expressed as Mean ±SD

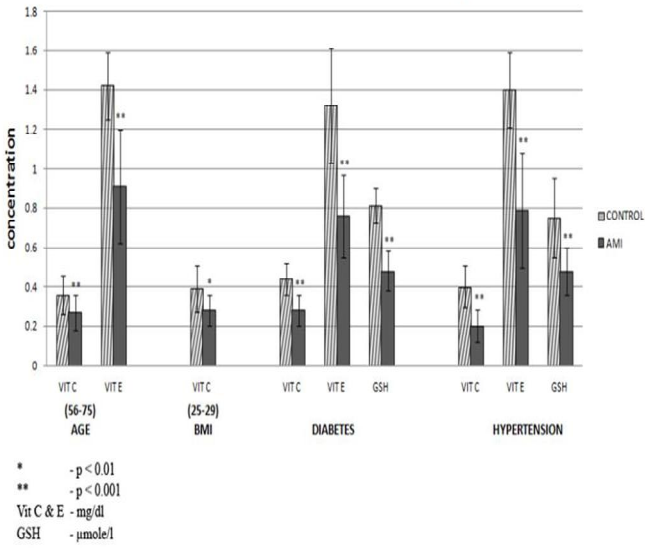


Figure 1 : Antioxidant status-Control and AMI patients.

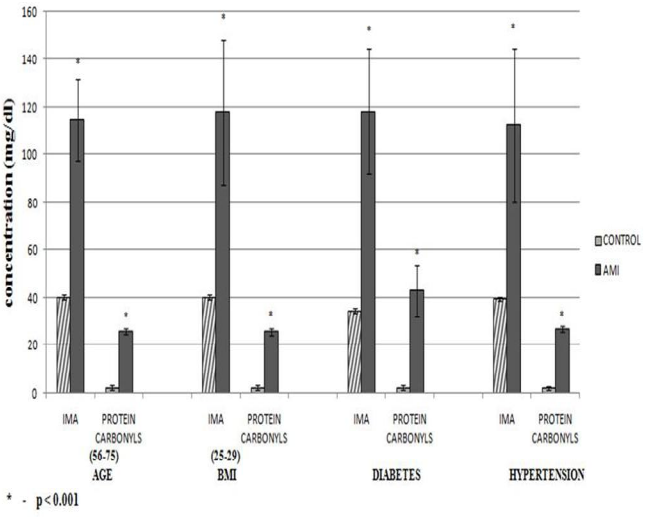


Figure 2 : Oxidant status-Control and AMI patients.

Regular Paper

when compared with control. No other significance was observed in other parameters of oxidants and antioxidants.

Figures 3 and 4 represent the antioxidants, oxidants and Ejection Fraction (EF) status of AMI patients with normal Left Ventricular Function (LVF) (n=10) and severe (n=10) LVD. The level of oxidants was significantly increased with LP, PC, IMA ($p<0.001$) with concurrent decrease in antioxidants (VitC, VitE, GSH), ($p<0.01$) within AMI patients against severe LVD and normal LVF. No significant change was observed in CP and TPT.

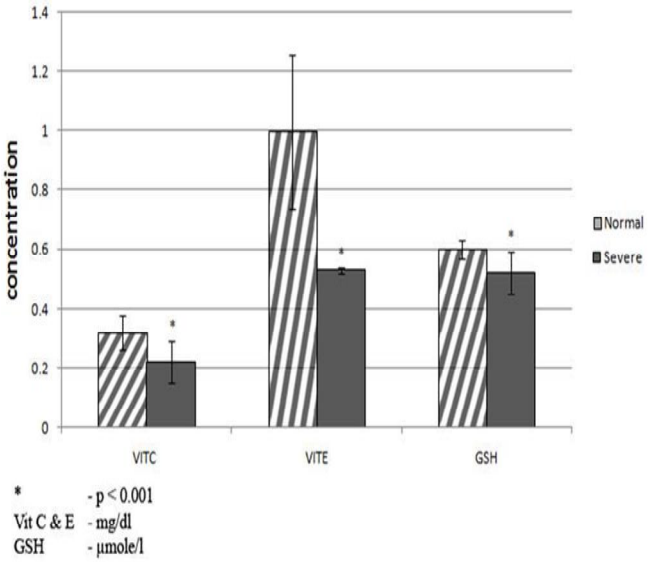
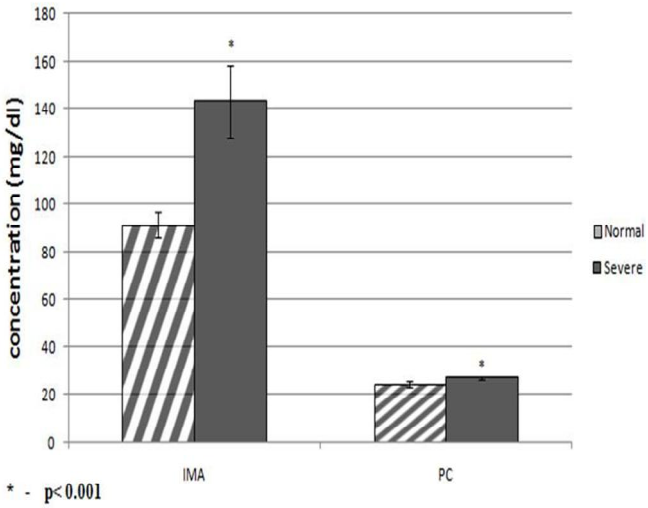


Figure 3 : Antioxidant status in AMI patient with severe LV dysfunction vs. normal LV function.



Values expressed as Mean ±SD

Figure 4 : Antioxidant status in AMI patient with severe LV dysfunction vs normal LV function.

Correlation analysis based on EF, HAMI, vessels and wall segments

Vitamin E and GSH levels were positively correlated in populations possessing severe LVD

(r-0.61; $p<0.001$), normal LVF (r-0.7; $p<0.001$), Hypertensive AMI (HAMI) (r-0.56;$p<0.05$) and anterior wall infarction patients (r-0.83; $p<0.0001$). Severe LVD population among HAMI expressed positive correlation between EF and GSH (r-0.61; $p<0.001$), IMA and CP (r-0.63; $p<0.01$) and inverse relationship between Vit C and PC (r-0.78; $p<0.01$).

Patients having Single Vessel Disease (SVD) with poor EF (Severe LVD) showed positive association of values in PC (r-.65; $p<0.05$), CP (r-0.43; $p<0.05$) and IMA (r-0.41; $p<0.05$). Similarly, Double Vessel Disease (DVD) population with poor EF revealed a positive relationship in the levels of PC (r-0.57; $p<0.01$), CP (r-.51; $p<0.01$) and IMA (r-0.43; $p<0.01$).

AMI patients with anterior wall segment involvement showed strong positive relationship between Vit C and Vit E (r-0.79; $p<0.0001$), Vit C and GSH (r-0.659; $p<0.01$). Inverse relationship was obtained between Vit E and IMA (r-0.81; $p<.0001$) and Vit C and IMA (r-.48; $p<0.05$). Moderate correlation was observed between severe LVD and IMA (r-0.43; $p<0.001$). Effective correlation of Vit E and GSH (r-0.60and 0.70) ($p<0.05$) was found between Diabetic AMI (DAMI) and AMI patients.

DISCUSSION

Age

As age increases, there was a significant decrease in the level of Vit E ($p<0.0001$) was found when compared to Vit C ($p<0.01$). A significant increase was seen in the level of oxidants particularly in PC ($p<0.001$) in similar to other earlier reports[18]. Carnitine is responsible for transporting long chain fatty acids across the mitochondrial membrane, required for beta oxidation and subsequent fat oxidation[19,20] and decreased levels of carnitine causes the accumulation of fat in tissues. This mechanism could have had an impact on Vit C levels in obese AMI patients when compared against controls. No significance was found in either GSH or TPT. Increase in oxidants was

found to be more significant in LP ($p<0.001$).

Diabetes

Similar to the work of Singh *et al*[21] and Kharb *et al*[22] our DAMI patients had decreased level of Vit C, Vit E and GSH (n=19), ($p<0.001$) and a considerable decrease in the level of TPT ($p<0.001$). This indicated a severe damage to antioxidant system which would have otherwise combatted oxidative stress and inflammation effectively. The level of all oxidants in AMI patients (n=16) was comparatively higher ($p<0.001$) than in control (n=25). Our results highly correlate with the study of Maxwell *et al*[23] which explains about the oxidative stress increase in diabetic patients due to the prolonged exposure to hyperglycaemia. In addition, our study also exemplifies the existence of an abnormal balance between the oxidative and protective mechanisms in AMI patients, particularly in Type II Diabetes.

Hypertension

The levels of Vit E, PC and LP was found to be more significant in HAMI patients (n=18) ($p<0.001$) when compared against control (n=25). Vit C, GSH and Ceruloplasmin were also found to have a significant difference between HAMI and control ($p<0.001$). Comparison amongst HAMI patients (n=7) showed elevated levels of LP, IMA and PC ($p<0.001$). It is well established that circulating GSH protects the cells from oxidative damage by neutralizing the free radicals through maintaining the active form of Vit C and Vit E. Literatures have shown that there exists an inverse relationship between ascorbic acid and protein thiols with LP in animal models[24,25]. Excessive free radical generation causes inactivation of enzymes and introduction of carbonyl groups into amino acid side chain of proteins which suggests an increased protein oxidation. Authors have reported that HTN has an impact on plasma levels of Vit C, Vit E, lipid peroxides and protein carbonyls which is similar to our findings[26,27]. Between control and HAMI, among antioxidants and oxidants, Vit E and LP possess a strong correlation than their counterparts respectively.

Ejection fraction

The levels of Vit E was significantly decreased in AMI patients ($p<0.001$) with severe left ventricular (LV) dysfunction (n=9) when compared to AMI patients with normal LV function (n=10) (not shown in graph or table). In oxidants, the level of IMA was significantly increased ($p<0.001$). Though the levels of Vit E is highly significant when compared to other antioxidants, the fact that a multiplicity of antioxidant compounds with overlapping activities exist in the organism, might cause difficulties in the identification of one single antioxidant over others in terms of pathophysiological importance. However, the observation of a statistically significant correlation between the left ventricular ejection fraction of patients with normal and severe AMI and plasma levels of Vit C and E is of potential clinical interest and warrants further studies in this field. The study population was divided based on the area of infarction: Anterior (n=17) and Inferior walls (n=8) MI. The level of oxidants and antioxidants was compared and a significant difference was found in IMA ($p<0.01$) and CP ($p<0.05$). There was no significance found between the cardiac enzymes (Troponin, CKMB and CK NAC) and the studied parameters.

Many articles have discussed the combination of Vit E with Glutathione peroxidase 1 (GPX-1) [28], L-carnitine[29], quercetin[30] via membrane stabilizing property and this property might be due to decreased lipid peroxidation. Vit C[31,32] is also used as a valuable marker for monitoring and treating cardiovascular events but till date but none has reported the key molecular correlation. Our data is supported by other reports which suggest that vitamin E could be used as an antihypertensive agent[33] in post reperfusion hypertensive patients. Similar to our study, Sood R *et al*[34] have also confirmed that decreased levels of Vit E are more significant than Vit C in reperfused MI patients.

Among the oxidants, PC, CP, IMA showed a negative relationship with Vit E and Vit C, with predominant results in Vit E. Most important observation is that Vit E levels were affected by PC, IMA and CP sequentially. Vit C levels were reduced less significantly by IMA, CP and PC in order. LP showed significant difference between AMI patients and control but subgroup comparison with antioxidants does not reveal any association with any others which reveal that PC, CP, IMA could be a more specific oxidant than LP. Vit E and Vit C showed strong relationship with GSH, which is unclear to our present knowledge

Regular Paper

and opens our mind for the question of possibility that there could be a possible mechanism explaining the rise of GSH along with Vit E and Vit C.

When physiological imbalances occur in human system there occurs multiple molecular interactions of which some are known and several are unknown. Patient's risk factors and multiple diseases often complicate the key molecule identification. Hence, understanding of molecule involvement, relationship within and between study population parameters, oxidants and antioxidants in MI patients becomes very important as the future focus on biological or regenerative medical treatment. It is essential to perform extensive correlation study so that critical molecule can be pointed and targeted for better monitoring and treatment therapeutic strategy in order to control every year raising MI mortality.

This paper hypothesize the fact that changes might take place in EF with Vit E and Vit E with GSH levels which in turn show that GSH may be indirect and Vit E may be a direct determinant of Cardiac function in MI patients. Vit E and GSH give maximum relevance with compared parameters than any other. But, the basic mechanism behind the relationship is unknown and beyond our knowledge. Thereby we conclude that plasma Vit E and GSH levels assay can be included as cardiac biochemical screening test for elderly patients as their levels are related to AMI, can be used as a potential biomarker and also for treating cardiac events in reperfusion individuals. This preliminary work stresses on the importance of molecular relationship study and has to be validated with a larger population and more oxidants and antioxidant molecules should be included in the study to provide definitive results.

REFERENCES

[1] S.K.Ojha, M.Nandave, S.Arora, R.Narang, A.K.Dinda, D.S.Arya; Int.J.Pharmacol., **4**, 1-10 **(2008)**.

[2] A.Ghaffar, K,S.Reddy, M.Singhi; BMJ, **328 (7443)**, 807-810 **(2004)**.

[3] S.Kasap, A.Gonenc, D,E.Sener, I.Hisar; J.Clin.Biochem.Nutr., **41(1)**, 50-57 **(2007)**.

[4] J.G.Murphy, J.D.Marsh, T.W.Smith; Circulation, **75**, 15-24 **(1987)**.

[5] M.Valko, D.Leibfritz, J.Moncol, M.T.D.Cronin, M.Mazur, J.Telser; The International Journal of Biochemistry & Cell Biology, **39**, 44-84 **(2007)**.

[6] R.A.Jacob; The Integrated Antioxidant System. Nutrition Research, **15**, 755-766 **(1995)**.

[7] E.D.Harris; The Journal of Nutrition, **122**, 625-626 **(1992)**.

[8] Arun Kumar, Ramiah Sivakanesan, Susil Gunasekeran; Indian J.of Biochem., **23**, 296-298 **(2008)**.

[9] P.K.Nigam, V.S.Narain, M.Hasan; Serum Lipid Profile in Patients with Acute Myocardial Infarction, **19(1)**, 67-70 **(2004)**.

[10] F.Tietze; Anal.Biochem., **27**, 502-522 **(1969)**.

[11] G.L.Ellman; Arch.Biochem.Biophys., **82**, 70-77 **(1959)**.

[12] I.D.Desai; Methods Enzymol., **105**, 138-143 **(1984)**.

[13] S.T.Omaye, T.P.Turbull, H.C.Sauberchich; Methods Enzymol., **6**, 3-11 **(1979)**.

[14] Ç.Okhawa, Í.Onishi, Ê.Yagi; Anal.Biochem., **95**, 351 **(1979)**.

[15] H.A.Ravin; J.Lab.Cin.Med., **58(1)**, 161-168 **(1961)**.

[16] R.Chawla, N.Goyal, R.Calton, S.Goyal; Indian J.of Clinical Biochemistry, **21(1)**, 77-82 **(2006)**.

[17] R.L.Levini, D.Garland, C.N.Oliver, A.Amici, I.Climent, A.Lenz; Meth.Enzymol., **186**, 464-478 **(1990)**.

[18] S.Leutner, K.Schindowski, L.Frölich, K.Maurer, T.Kratzsch, A.Eckert, W.E.Müller; Pharmacopsychiatry, **38(6)**, 312-315 **(2005)**.

[19] N.Siliprandi, L.Sartorelli, M.Ciman, Di Lisa F.Carnitine; Clin.Chim.Acta., **183**, 3-12 **(1989)**.

[20] E.Reda, S.D'Iddio, R.Nicolai, P.Benatti, M.Calvani; Acta.Diabetol., **40**, S106-S113 **(2003)**.

[21] R.B.Singh, M.A.Niaz, J.P.Sharma, R.Kumar, I.Bishnoi, R.Begom; Acta.Cardiol., **49**, 441-452 **(1994)**.

[22] S.Kharb; Ind.J.Med.Sci., **57(8)**, 335-337 **(2003)**.

[23] S.R.J.Maxwell, H.Thomason, D.Sandler, C.Leguen, M.A.Baxter, G.H.G.Thorpe, A.F.Jones, A.H.Barnett; Eur.J.Clin.Invest. **27**, 484-490 **(1997)**.

[24] T.H.Aulinskas, D.R.Van Der Westhuyzen, G.A.Coetzee; Atherosclerosis., **43**, 159-171 **(1983)**.

[25] M.Santillo, Mondola, A.Milone; Ascorbate Administration to Normal and Cholesterol Fed Rats Inhibits in Vitro Formation in Liver Homogenates., **58**, 1101-1108 **(1996)**.

[26] H.U.Nwanjo, G.Oze, M.C.Okafor, D.Nwosu, P.Nwankpa; African Journal of Biotechnology., **6 (14)**, 1681-1684 **(2007)**.

[27] D.V.Simic, J.Mimic-Oka, M.Pljesa-Ercegovac, A.Savic-Radojevic, M.Opacic; J.Hum.Hypertens, **20**, 149-155 **(2006)**.

[28] M.L.Cheng, C.M.Chen, H.Y.Ho, J.M.Li, D.T.Chiu; Am.J.Cardiol., **103(4)**, 471-475 **(2009)**.
[29] A.R.Gaby; Altern.Med.Rev., **15(2)**, 113-123 **(2010)**.
[30] V.R.Punithavathi, P.S.Prince; Life Sci., **86(5-6)**, 178-184 **(2010)**.
[31] M.D.Bagatini, C.C.Martins, V.Battisti, D.Gasparetto, C.S.Da Rosa, R.M.Spanevello, M.Ahmed, R.Schmatz, M.R.Schetinger, V.M.Morsch; Oxidative Stress Versus Antioxidant Defenses in Patients with Acute Myocardial Infarction, Heart Vessels., **(2010)**.
[32] T.Jaxa-Chamiec, B.Bednarz, K.Herbaczynska-Cedro, P.Maciejewski, L.Ceremuzynski; Cardiology, **112(3)**, 219-223 **(2009)**.
[33] R.Raghuvanshi, M.Chandra, A.Mishra, M.K.Misra; Exp.Clin.Cardiol., **12(2)**, 87-90 **(2007)**.
[34] Z.Serdar, A.Serdar, A.Altin, U.Eryilmaz, S.Albayrak; Acta.Cardiol., **62(4)**, 373-380 **(2007)**.