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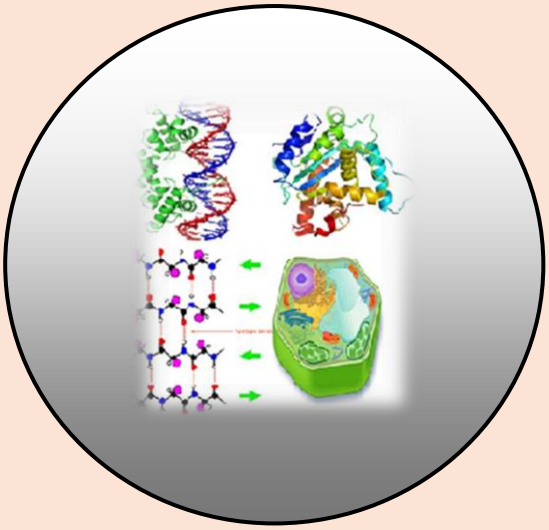

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Modulation of the Cardiac Biomarkers by Resveratrol: An *in vitro* and *in silico* Molecular Modeling Approach

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ABSTRACT

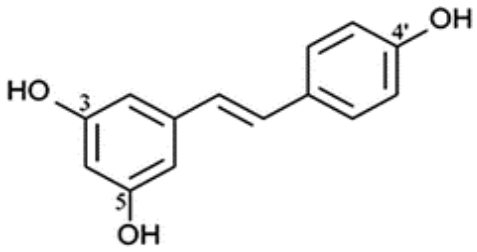
Peripheral blood mononuclear cells (PBMC's) isolated from healthy as well subjects having myocardial infarction (MI) were employed in culture studies (with and without Resveratrol). The 24 hr cultures of PBMCs were utilized for the assessment of cardiac injury markers, oxidative stress and inflammation. The patient's Res treated cultures exhibited a significantly decreased level of cardiac enzyme markers like CK-MB, cTnI and AST, increased glutathione peroxidase (GPx) levels and suppressed levels of TNF-α as compared to cultures of untreated patients. Also, the in-silico molecular docking studies supported our wet lab results. Thus, Res which is a polyphenol act as an antioxidant and reduces cardiac injury. It can be used as an adjuct which in turn may be helpful in the better management of cardiac diseases.

Keywords: Peripheral mononuclear cells; Resveratrol; Cardiac troponin I and Creatine kinase.

INTRODUCTION

Ischemic heart disease is one of the reasons for Myocardial Infarction (MI) which results in numerous deaths worldwide. When myocardial ischemia go beyond the critical threshold level for prolonged time that usually leads to irreversible damage to the myocardium. Although improvements in clinical care and public awareness are widely made, still MI is the main the cause of mortality worldwide [Aronow, 2006]. In India, the number of patients being hospitalized for myocardial infarction, commonly known as heart attack, is increasing over the past 35 years and male patients have shown a more striking increase as compared to women [Krishnaswami, 1998].

However, it has been reported that the number of mortalities due to heart ailments are considerably lower in the population of France than in peoples of other countries with comparable diets. This trend so-called “French paradox” may be due to the fact that French consume high levels of red wine [Renaud and Delorgeril, 1992]. It was proposed that after fungal infection and ultraviolet light exposure in grapevines production of trans-resveratrol (3,4’,5trihydroxystilbene) occurs [Langcake and Pryce, 1976] which is considered to be the main biologically active ingredient of red wine [Siemann and Creasy, 1992]. There are numerous physiological effects of resveratrol that have been reported like anti-inflammatory and antioxidant activity [Das et al., 2010, Lekli et al., 2010]; cancer chemoprevention [Sun et al., 2010, Jang et al., 1997]; neurodegenerative diseases [Holme, and Pervaiz, 2007] and cardioprotection [Das and Maulik, 2006]. It has been shown that resveratrol improves the recovery of ventricular function after ischemia reperfusion injury [Ray et al., 1999].



trans-resveratrol
(3,5,4'-trihydroxy-*trans*-stilbene)

Cardiac biomarkers are protein components of cell structures that are released into circulation when myocardial injury occurs. Cardiac markers are central to the new definition of acute myocardial infarction (AMI) put forward by the American College of Cardiology and the European Society of Cardiology [Alpert et al., 2000, Joint European Society of Cardiology, 2002].

Cytoplasmic CK (creatine kinase) exists as a dimer and it is mainly composed of M and/or B subunits [Bessman and Carpenter, 1985]. In cardiac muscles, CK -MB isozyme predominates (about 15±40% of the total CK activity), and thus considered to be more specific for myocardial damage comprising, and the remainder is CK-MM [Lott et al., 1996].

Cardiac troponins are one of the specific markers of Myocardial Infarction. Its complex consists of 3 components: troponin C (TnC), troponin I (TnI) and troponin T (TnT) which regulates cardiac and striated muscle contraction. TnC denotes the Ca^{2+}-binding subunit [Alpert et al., 2000] while TnI and TnT stands for the inhibitory and the tropomyosin-binding subunit respectively. Three separate genes encode for 3 isoforms of TnI [Lott et al., 1996]; two of them are skeletal while the cardiac isoform is specific for cardiac muscle. Cardiac Troponin I is released into the blood as early as 4 hours after myocardial ischemia. The serum levels reach the peak at 14-24 hours and remains elevated for 3-5 days after infarction [Sia et al., 1997].

Thus, our aim in the present study was to probe the beneficial effects of resveratrol in 24 hr culture PBMC’s from patients with MI, which also corroborate with in-silico docking studies that may provide better insights in the molecular mechanisms involved.

Methods

Study subjects: For the current study 6 healthy subjects and 20 consecutive patients of both sex having cardiovascular disease, who gave informed consent, were enrolled from the outpatient department of J.N. Medical College, Aligarh. Prior clearance from the Institutional Ethics Committee was taken. PBMC's were then isolated from the blood samples for culture studies.

In the present study, all the experiments with resveratrol with the dose of 20µg/ml were carried. This dose was selected based on our preliminary studies (data not shown). Furthermore, some evaluations were carried out in serum whereas other were in culture supernatants.

Isolation of peripheral blood mononuclear cells (PBMCs)

As described by us earlier Ficoll density gradient method was used to isolate PBMCs from both normal and healthy individuals.

Determination of Cardiac Injury biomarkers in culture PBMCs

For the determination of AST (Aspartate transaminase) Reitman-Frankel Colorimetric test was used. The test rely on the principle that enzyme glutamic oxalacetic (GOT), catalyze the transfer of the amino group of glutamic acid to oxalacetic acid in reversible reactions. The transaminase activity is proportional to the amount of oxaloacetate formed over a definite period of time and is measured by a reaction with 2.4-dinitrophenylhydrazine (DNPH) in alkaline solution. The levels of AST were analyzed both in the serum as well as culture PBMCs in healthy individuals and MI patients with and without resveratrol treatment [Reitman and Frankel, 1957].

Determination of TNF-α levels in cell culture supernatants

24 hr cultured PBMCs from healthy individuals as well as MI patients were subjected to treatment with resveratrol (20µg/ml), and the levels of TNF-α was assessed by the method as described by us earlier [Fearon and Faux, 2009, Calabrese et al., 2010].

Glutathione peroxidise (GPx activity)

Activity of glutathione peroxidase (GPx) in 24 hr resveratrol treated / untreated cells was measured as described by us earlier and elsewhere [Bostanghadiri et al., 2017, Fernández-Mar et al., 2010].

***In silico* molecular docking studies**

Molecular docking studies were performed to determine the binding site, as well as the free energy of the interaction of CK, GPx, TNF-α and troponin with resveratrol.

The 3D structures for TNF-α (PDB ID: 1A8M) and troponin (PDB ID: 1J1E), GPx (PDB ID: 2p31), CK (PDB ID: 1I0E) and AST (PDB ID: 3WZF) were obtained from the RCSB protein database (http://www.rcsb.org). 3D structure of resveratrol was obtained from PubChem (PubChem CID: 445154). All heteroatoms were removed prior to docking. Docking was performed using Hex 8.0 software and the results were analyzed using UCSF Chimera 1.01, Accelrys Discovery Studio 4.5 and PyMOL. Hex 8.0.0 works on FFT corelations using Spherical Polar Cordinates Fourier Correlations. The parameters used for the docking process were correlation type-shape only, calculation device- GPU, number of solutions-100, FFT mode-3D fast lite, grid dimension-0.6, receptor range–180, ligand Range–180, twist range-360, distance Range-40.

RESULTS

Effect of Resveratrol on Myocardial injury markers:

The activity of cardiac biomarkers that is cardiac troponin I (cTnI), creatine kinase (CK) and aspartate transaminase (AST) in both the serum and PBMCs of the patients were markedly increased when compared to the normal healthy individuals.

The serum levels of cTnI were found to be (66.25 ng/ml). However, cTnI levels in the PBMCs were (57.11 ng/ml) which after the resveratrol treatment significantly decreased to (20.16 ng/ml). Similarly, the levels of CK in serum and PBMCs were 1340 IU/L and 1232 IU/L respectively. Upon treatment

Effect of Resveratrol on Glutathione peroxidase:

The levels of GPx n healthy individuals were found to be 88.23pg/ml while it significantly decreases to 39.47pg/ml in PBMCs of patients with MI. Upon treatment with resveratrol, the GPx levels were raised considerably to 63.88 pg/ml.

Effect of Resveratrol on TNF-α:

Untreated control 24hrs cultured PBMCs isolated from blood of MI patients exhibit augmented levels of TNF-α (91.44 pg/ml).

However, treatment with resveratrol resulted in significant suppression in the same (13.32 pg/ml).

Table 1. Metabolic and biochemical parameters of subjects under study.

SUBJECTS	SEX	AGE	FASTING BLOOD SUGAR (mg/dl)
Healthy	F	Below 60	70-90
Healthy	M	Below 60	70-90
Patients	F	Below 60	80-90
		Above 60	100-130
Patients	M	Below 60	90-110
		Above 60	90-180

TABLE 2. REPRESENTS THE BINDING OF RES WITH THE RESIDUES OF CREATINE KINASE.

TYPE OF BOND	INTERACTING RESIDUES	BOND LENGTH (Å)
Conventional H- Bond	Res:H-A:GLU231:O	2.10
Pi interaction	Res- A:TRP228	4.22
Pi interaction	Res- A:ILE188	5.16
Pi interaction	Res- A:LEU193	4.78

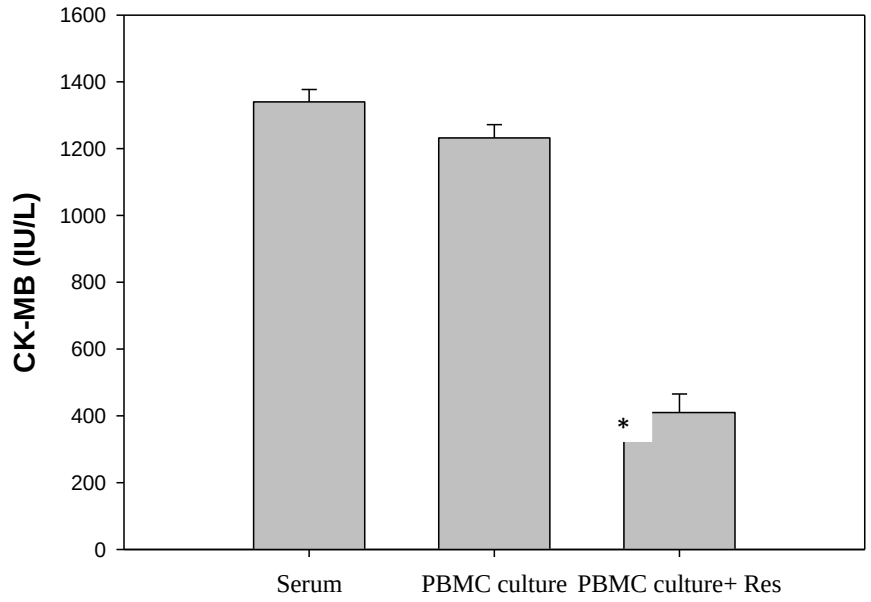
TABLE 3. REPRESENTS THE BINDING OF RES WITH THE RESIDUES OF GLUTATHIONE PEROXIDASE.

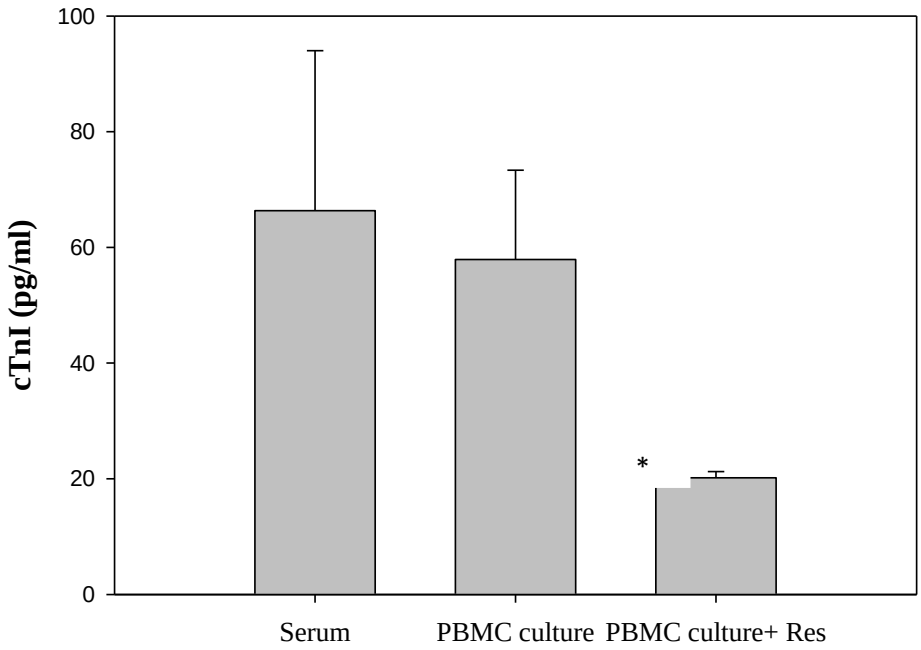
TYPE OF BOND	INTERACTING RESIDUES	BOND LENGTH (Å)
Pi interaction	B:ARG106:CG-Res	3.18
Conventional H-Bond	Res:H-B:ARG106:O	2.93
Pi interaction	Res:H-A:PHE103	2.93
Pi Stacked interaction	A:SER55:C,O;GLU56:N:Res	4.69
Pi interaction	A:PHE103:Res	5.01
Pi interaction	A:GLU93:O:Res	3.80
Pi interaction	Res-A:ILE100	5.39
Pi interaction	A:ASP95:O:Res	3.19

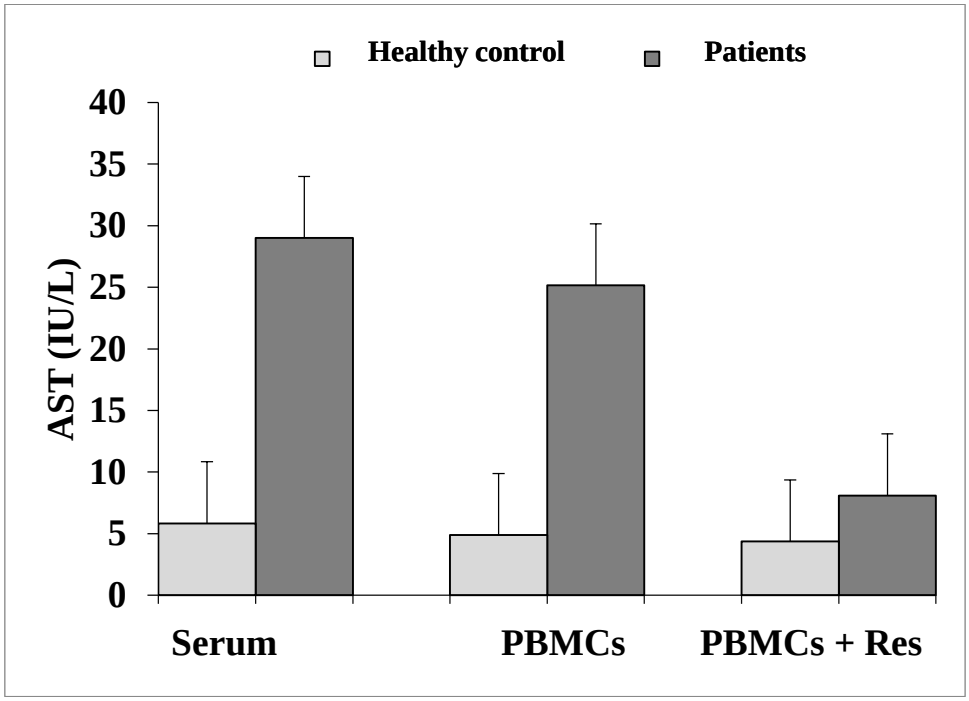
TABLE 4. REPRESENTS THE BINDING OF RES WITH THE RESIDUES OF ASPARTATE TRANSAMINASE.

TYPE OF BOND	INTERACTING RESIDUES	BOND LENGTH (Å)
Conventional H-Bond	Res: H-A: ASP 355:O	2.85
Conventional H-Bond	A:ASP 199:HN-: Res-O	1.96
Pi interaction	Res-A:PRO 202	3.83
Pi interaction	Res- A:ILE 198	5.17

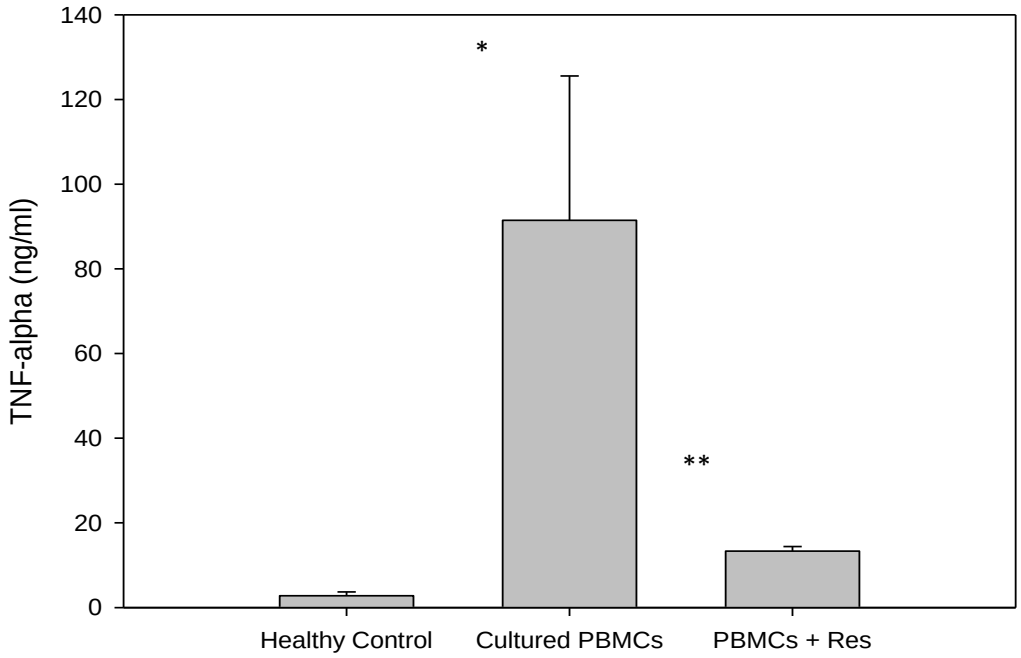
Effect of Resveratrol on Cardiac enzyme biomarkers

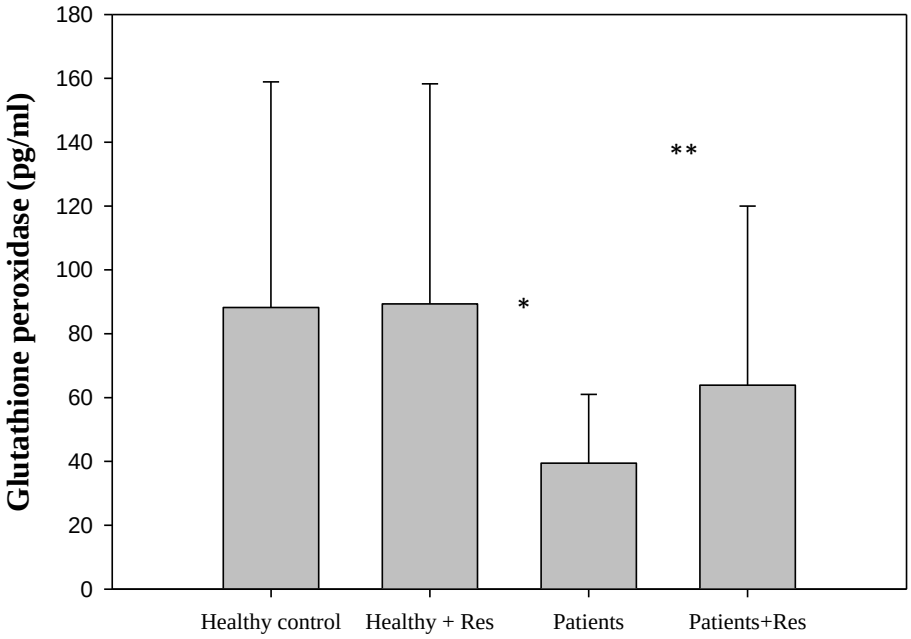




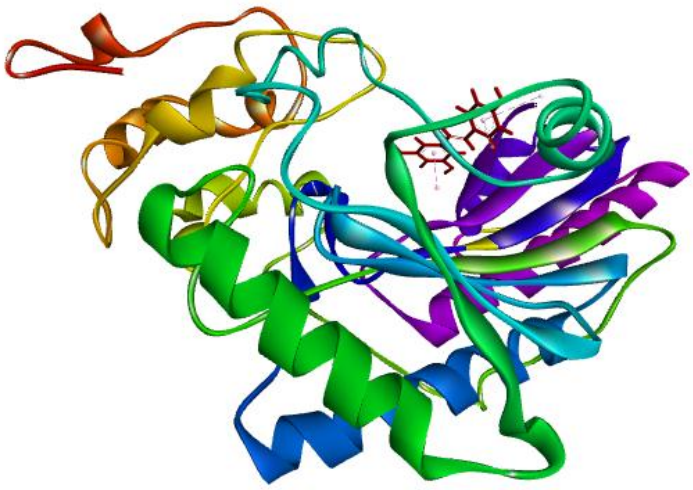


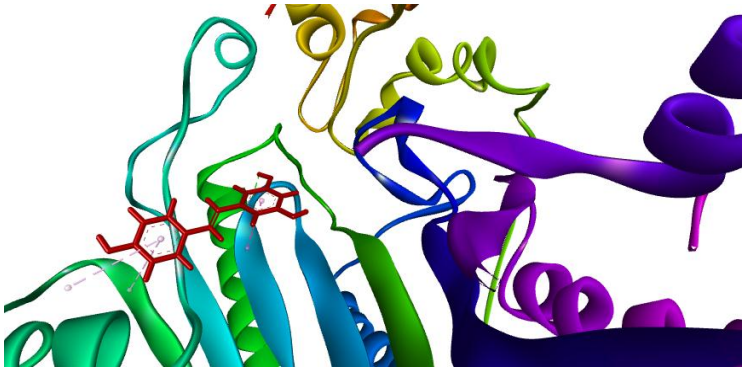
Effect of Resveratrol on Proinflammtory and Anti-inflammatory parameters:

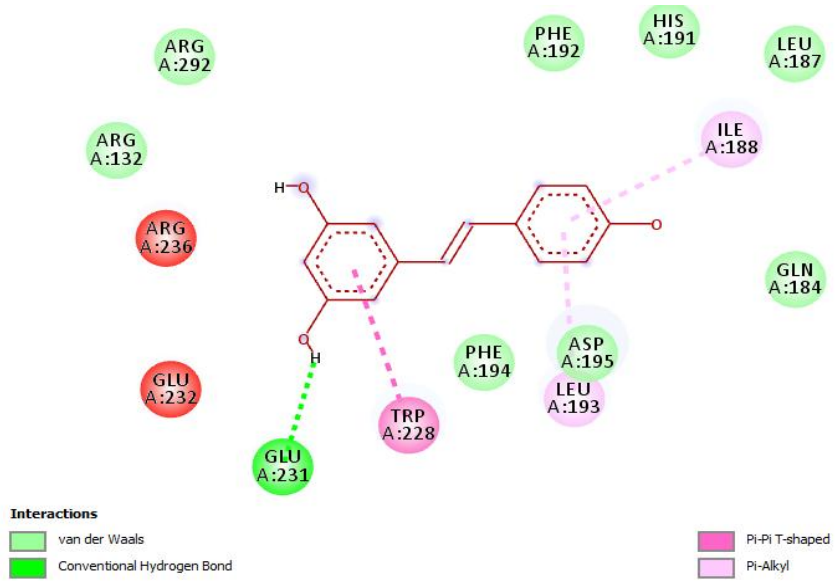




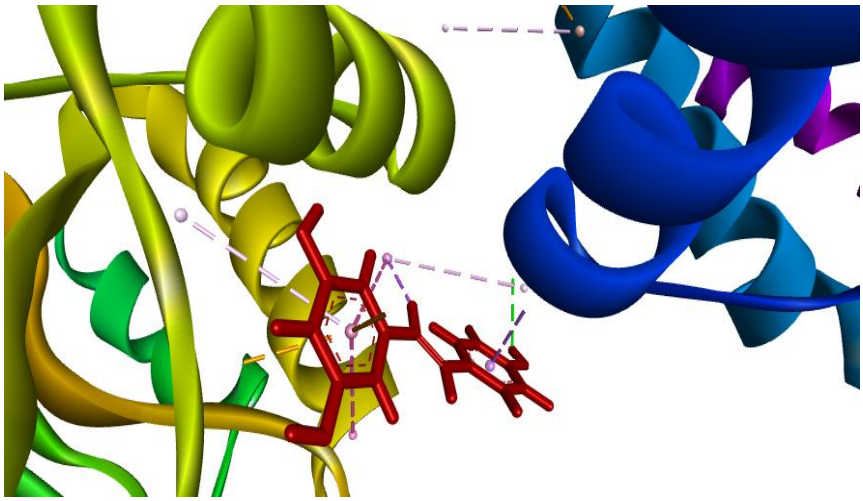
MOLECULAR DOCKING OF CK WITH RES

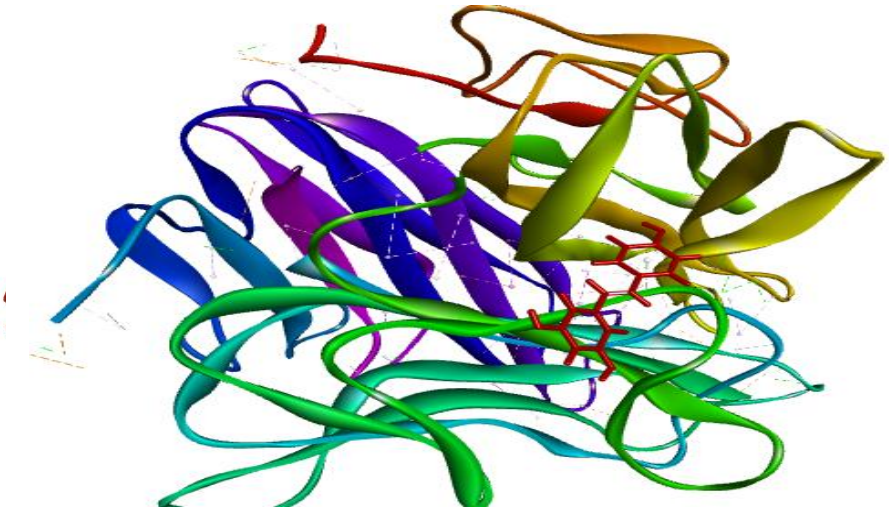


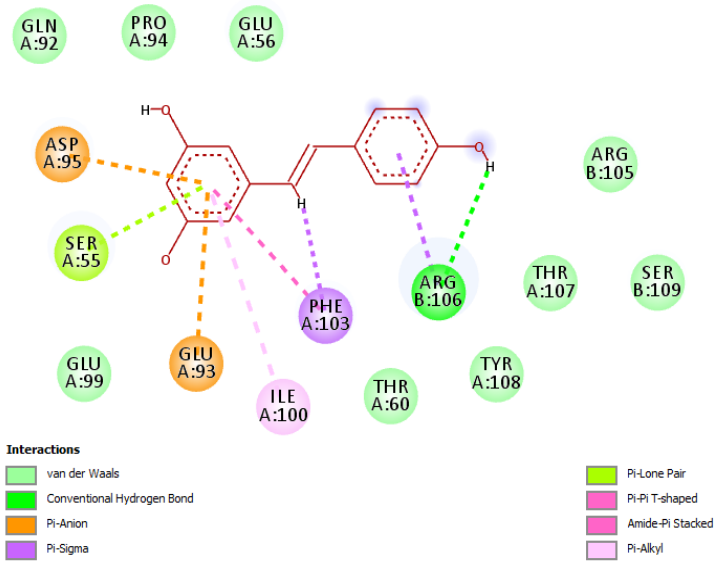




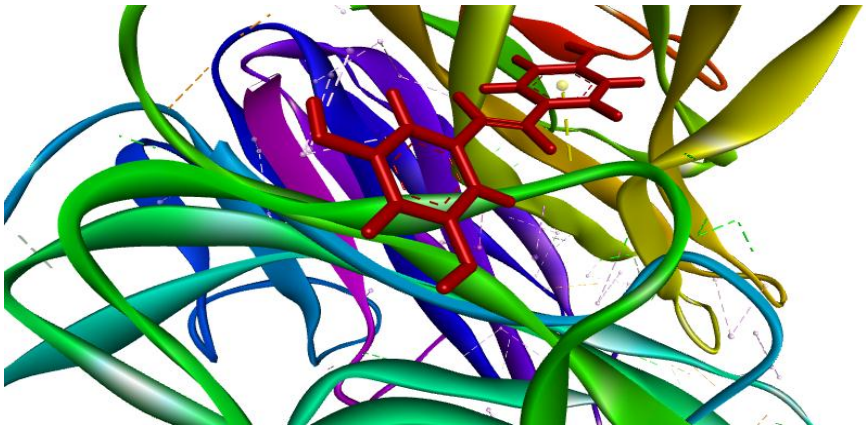
MOLECULAR DOCKING OF GPx with res

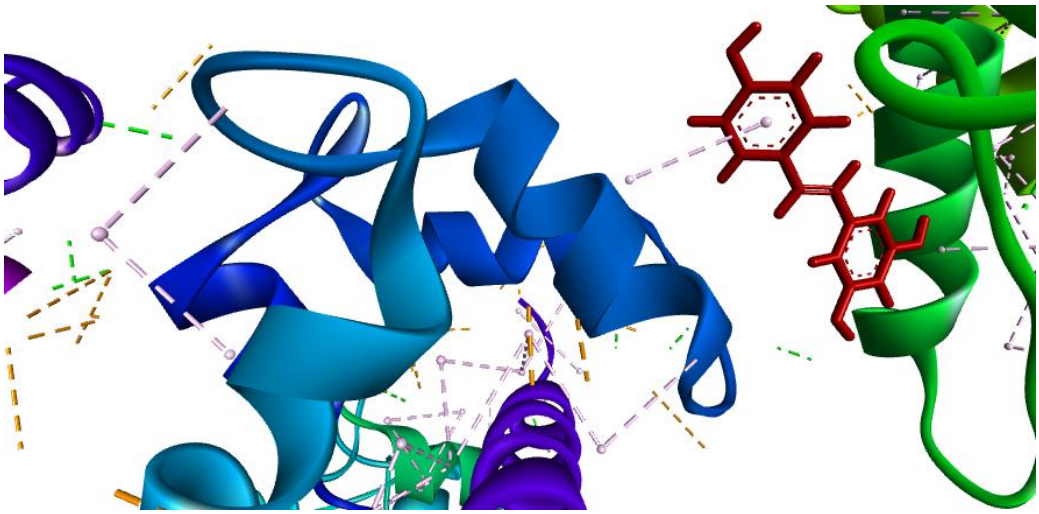


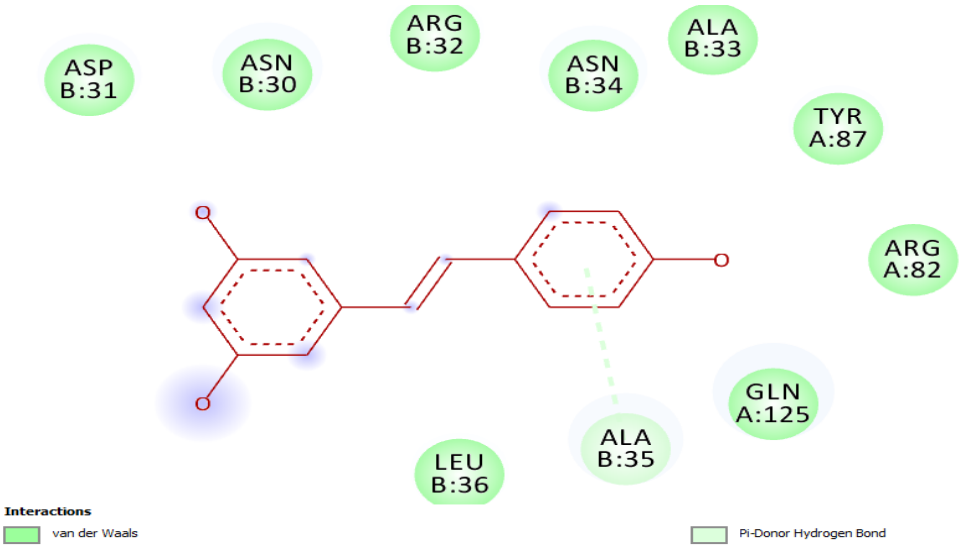




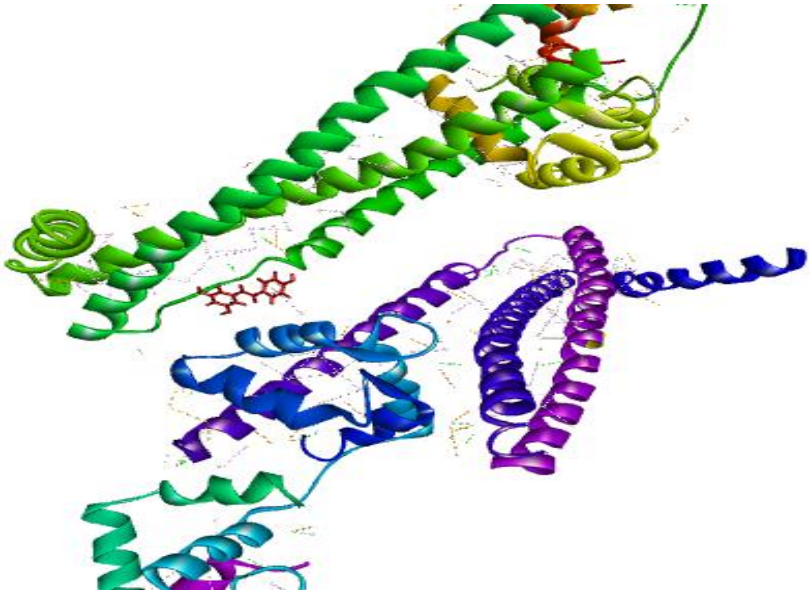
MOLECULAR DOCKING OF TNF WITH RES:

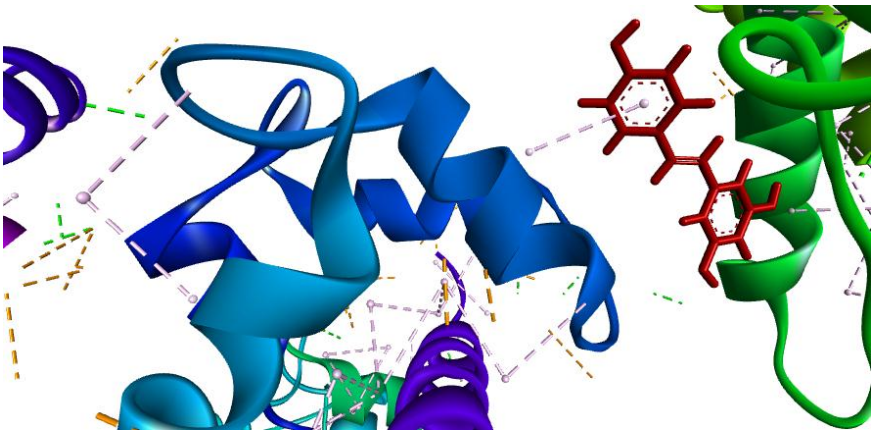


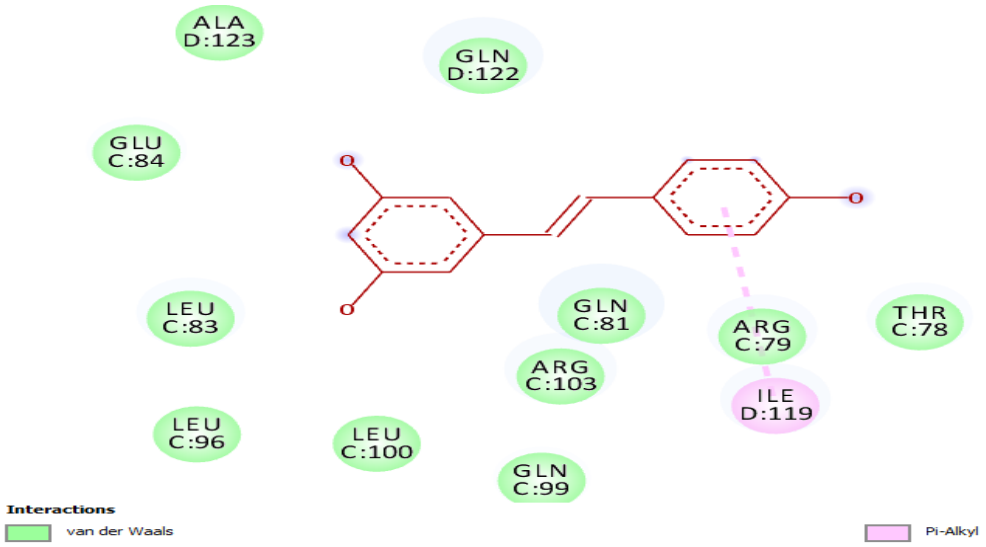




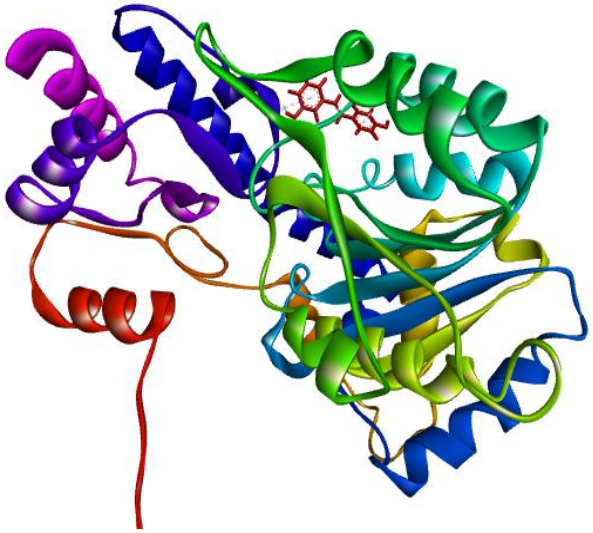
MOLECULAR DOCKING OF TROPONIN WITH RES:

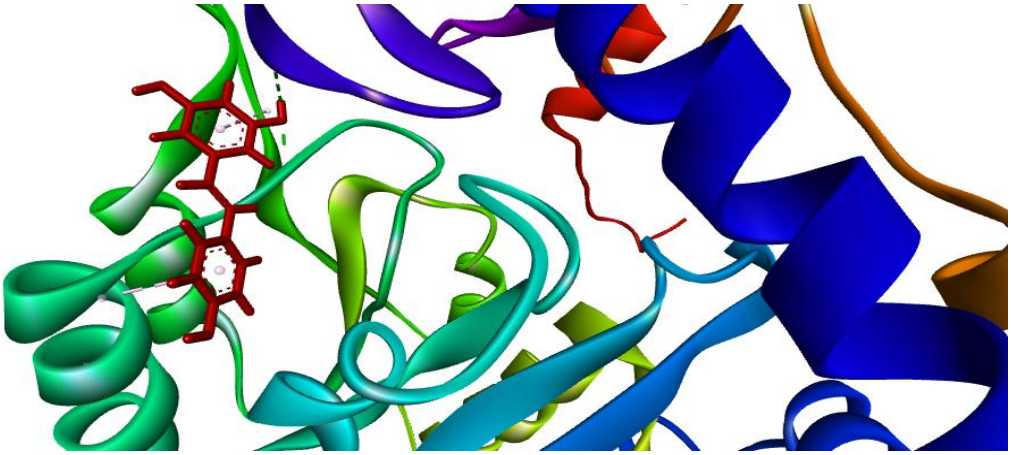


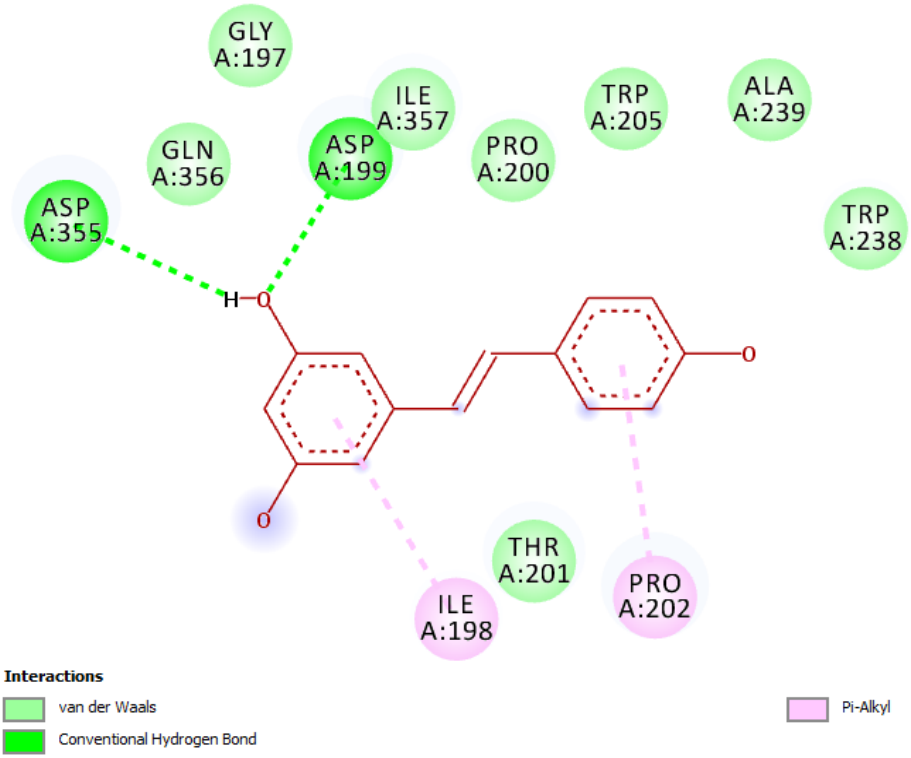




MOLECULAR DOCKING OF AST with res







DISCUSSION

Molecular docking studies of cardiac biomarker enzymes with resveratrol:

The 2D plot of the interaction of CK with resveratrol depicts a hydrogen bond between 3 OH group of Res and GLU 231. Other interactions such as van der Waal force as well as pi interactions also stabilized the complex. Table 2 represents the bond length of all the residues involved in the interaction. The free energy of the interaction was found to be -5.155Kcal/mol.

Similarly, the 2D plot of the interaction of AST with resveratrol depicts a hydrogen bond between 5 OH group of Res both with ASP 199 and ASP 355. Other forces such as pi interactions also stabilized the complex. Table 4 represents the bond length of all the residues involved in the interaction. The free energy of the interaction was found to be -4.958 Kcal/mol.

Molecular docking studies between GPX and Res also indicate the presence of a conventional hydrogen bond between 4'OH group of Res and ARG 106 of GPx. Moreover, the presence of van der Waals, as well as pi interactions, further stabilized the complex. The bond length of each interaction, as well as the residues involved, are enlisted in Table 3. The free energy of the interaction was obtained to be -4.62 Kcal/mol.

The interaction between TNF-α and Res do not indicate the existence of hydrogen bonds, however, the complex was observed to be stabilized by Van der Waal forces as well as pi interactions. The free energy of the interaction was -4.76 Kcal/mol.

Similarly, the interaction between troponin and Res also lacked hydrogen bonds but was stabilized by Van der Wal and pi interactions. The free energy of the interaction was obtained to be -4.94Kcal/mol.

Since the binding energy serves as a measure of the affinity of interaction, therefore, among all four parameters with RV, the binding affinity follows the order CK>AST>Troponin>TNF-α> GPx.

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REFERENCES

Aronow, W.S. (2006). Epidemiology, pathophysiology, prognosis, and treatment of systolic and diastolic heart failure. Cardiol. Rev. 14, 108–124.

Krishnaswami, S. (1998). Observations on serial changes in coronary artery disease in Indians. Curr. Sci. 74, 1064–1068.

Renaud, S., and Delorgeril, M. (1992). Wine, Alcohol, Platelets, and the French Paradox for Coronary Heart Disease. Lancet 339, 1523– 1526.

Langcake, P., and Pryce, R. J. (1976). Production of Resveratrol by Vitis-Vinifera and Other Members of Vitaceae as a Response to Infection or Injury. Physiol. Plant Pathol. 9, 77–86.

Siemann, E.H. and Creasy, L.L. (1992). Concentration of the Phytoalexin Resveratrol in Wine. Am. J. Enol. Vitic. 43, 49–52.

Das, D. K., Mukherjee, S., and Ray, D. (2010). Resveratrol and red wine, healthy heart and longevity. Heart Failure Rev. 15, 467–477.

Lekli, I., Ray, D., and Das, D. K. (2010). Longevity nutrients resveratrol, wines and grapes. Genes Nutr. 5, 55–60.

Sun, A. Y., Wang, Q., Simonyi, A., and Sun, G.Y. (2010). Resveratrol as a Therapeutic Agent for Neurodegenerative Diseases. Mol. Neurobiol. 41, 375–383.

Jang, M. S., Cai, E. N., Udeani, G. O., Slowing, K. V., Thomas, C. F., Beecher, C. W. W., Fong, H. H. S., Farnsworth, N. R., Kinghorn, A. D., Mehta, R. G., Moon, R. C., and Pezzuto, J.M. (1997). Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science 275, 218–220.

Holme, A.L. and Pervaiz, S. (2007). Resveratrol in cell fate decisions. J. Bioenerg. Biomembr. 39, 59–63.

Das, D.K. and Maulik, N. (2006). Red wine and heart: A cardioprotective journey fromgrape to resveratrol. Alcohol. Clin. Exp. Res. 30, 84a.

Ray, P.S., Maulik, G., Cordis, G.A., Bertelli, A.A. E., Bertelli, A., and Das, D.K. (1999). The red wine antioxidant resveratrol protects isolated rat hearts from ischemia reperfusion injury. Free Radical Biol. Med. 27, 160–169.

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 Am Coll Cardiol*; 36:959–969.

Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. A consensus document — myocardial infarction redefined. *Eur Heart J* 2002; 21:1502–1513.

Bessman, S.P. and Carpenter, C.L. (1985). The creatine ± creatine phosphate energy shuttle. *Ann Rev Histochem*; 54: 831-62

Lott, J.A. and Nemesanszky, E. (1996). Creatine kinase. In: Lott JA, Wolf PL, eds. Clinical Enzymology: a Case-orientated Approach. New York: Field and Rich/Yearbook, 1996; 166.

Sia, S.K., Li, M.X., Spyracopoulos, L., Gagne, S.M., Liu, W. Putkey, J.A. and Sykes, B.D. (1997). Structure of cardiac muscle troponin C unexpectedly reveals a closed regulatory domain. J. Biol. Chem. 272, 18216–18221.

Reitman, S. and Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology, 28, 56–63.

Fearon, I.M. and Faux, S.P. (2009). Oxidative stress and cardiovascular disease: Novel tools give (free) radical insight. J. Mol. Cell. Cardiol. 2009, 47, 372–381.

Calabrese, E.J.; Mattson, M.P. and Calabrese, V. (2010). Resveratrol commonly displays hormesis: Occurrence and biomedical significance. Hum. Exp. Toxicol. 2010, 29, 980–1015

Bostanghadiri, N.; Pormohammad, A.; Chirani, A.S.; Pouriran, R.; Erfanimanesh, S. and Hashemi, A. (2017). Comprehensive review on the antimicrobial potency of the plant polyphenol Resveratrol. Biomed. Pharmacother. 2017, 95, 1588–1595.

Fernández-Mar, M.I.; Mateos, R.; García-Parrilla, M.C.; Puertas, B. and Cantos-Villar, E. (2012). Bioactive compounds in wine: Resveratrol, hydroxytyrosol and melatonin: A review. Food Chem. 2012, 130, 797–813

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