EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 7
513-520
Year: 2017

HESPERETIN AMELIORATES ISOPROTERENOL INDUCED CARDIAC HYPERTROPHY: ROLE OF NFKB PATHWAY

Velusamy Prema, Mohan Thangarajeswari, Ravi Divya Bhavani, Srinivasan Ashokkumar, Chakrapani Lakshmi Narasimhan, Singh Abhilasha, Narasimhan Kishore Kumar, Arun Shruthi, Biswas Sapna, Periandavan Kalaiselvi*

India.

***Corresponding Author: Dr. Periandavan Kalaiselvi**
India.

Article Received on 10/05/2017 **Article Revised on 30/05/2017** **Article Accepted on 20/06/2017**

ABSTRACT
Latest epidemiological data has revealed that cardiac hypertrophy is a major predictor of heart failure, with a mortality as high as 80% for men and 70% for women within 8 years. Therefore, it is inevitable to develop therapeutic strategies that aim at modulating the hypertrophic remodeling of the heart by modulating inflammatory pathways. Cardiac hypertrophy was induced by subcutaneous injections of isoproterenol (5 mg/kg body weight) for seven days. Rats were pre-treated with hesperetin 30mg/kg body weight suspended in 0.5% methyl cellulose orally for 30 days. The HW/BW ratio, fetal gene expression and macromolecular damage were found to be increased in the isoproterenol administered rats, whereas, hesperetin treated rats showed a decline in the HW/BW ratio and fetal gene expression. The protein expression of inflammatory marker NF-κB was found to be decreased in the hesperetin treated rats when compared to the isoproterenol administered rats. This study suggests NF-κB as a potential target for anti-inflammatory therapy for cardiac hypertrophy and hesperetin modulated NF-κB expression, it therefore could be useful as an anti-inflammatory agent against cardiac hypertrophy.

KEYWORDS: Cardiac hypertrophy, Hesperetin, NF-κB, Inflammation, TNF-α.

INTRODUCTION
Cardiac hypertrophy is one of the main ways in which cardiomyocytes respond to mechanical and neurohormonal stimuli (Carreño et al., 2006). Hypertrophic growth accompanies many forms of heart disease, including ischemic disease, hypertension, heart failure, and valvular heart disease. Cardiac hypertrophy has been considered as an important risk factor for cardiac morbidity and mortality whose prevalence has increased during the last few decades (Rohilla et al., 2012). Although hypertrophy in response to pathological signaling has traditionally been considered as an adaptive response, prolonged hypertrophy is associated with an increased risk of progression to heart failure and sudden death (Katholi and Couri, 2011). In cardiovascular research, animal models have allowed the study of cardiovascular disease in the early stages, as well as the investigation of the mechanisms of the pathogenesis of cardiovascular disease and the effects of drug intervention. Nevertheless, despite considerable research efforts, the complex signaling events regulating the cardiac hypertrophy are not fully understood.

Eminence of inflammation in cardiac hypertrophy
There is a considerable variety of transcription factors that appear to play a role in the regulation of the events that lead to the activation of the genetic program involved in cardiac hypertrophy (Babu et al., 2000; Liang et al., 2000; Morimoto et al., 2000). The role of inflammation in cardiac hypertrophy is not to be disregarded as high expression levels of cytokines such as interleukins (IL)-6, IL-1β, IL-1RA, and tumor necrosis factor-alpha (TNF-α) and activation of inflammatory signaling pathways such as nuclear factor kappa B (NF-κB) are all characteristic hallmarks of a pathologically hypertrophied heart.

TNF-α mediated pathological myocardial hypertrophy, which is maladaptive has also been associated with angiotensin II, tumor necrosis factor-α (TNF-α), and catecholamines (Dorn and Brown, 1999) and seems to involve downstream signaling pathways, c-Jun N-terminal kinase and p38, which promote cardiac fibrosis and apoptosis (Liang et al., 2003). Nuclear factor-κB (NF-κB) is a pleiotropic transcription factor that is found to be elevated during cardiovascular disease, and its signaling is strongly implicated in the development of cardiac remodeling (fibrosis), hypertrophy, and heart failure (Li et al., 2004, Zelarayan et al., 2009).

Over the past decade, polyphenols, abundant in fruits and vegetables, have gained recognition for their antioxidant

anti-cancer, chemo-preventive, anti-inflammatory properties (Pietta, 2000) and their roles in protecting against chronic diseases such as cancer and cardiovascular diseases (Hertog et al., 1993; Kroon and Williamson, 2005). Hesperetin, an abundant bioflavonoid shows a wide spectrum of pharmacological effects such as anti-inflammatory, anti-carcinogenic, anti-hypertensive, anti- atherogenic effects and anti-oxidant properties (Yang et al., 2011). Studies have shown that supplementation with hesperidin and hesperetin on the cardiovascular system include anti-hypertensive, anti-coagulant, cardio-protective effects against oxidative stress and ischemia induced by drugs, hypolipidemic and hypoglycemic effects and promotes cellular antioxidant defense-related enzyme activity (Pollard et al., 2006).

MATERIALS AND METHODS

Source of chemicals

Hesperetin, Isoproterenol hydrochloride and bovine serum albumin were procured from Sigma- Aldrich, USA. All other chemicals used were of analytical grade obtained from Merck Chemical Supplies (Darmstadt, Germany), Sisco Research (SRL, India) and S.D. Fine Chemicals, Mumbai, India.

Animals

Healthy male Wistar young rats were used throughout the study. The animals were procured from Central Animal House Facility, Dr. ALM PGIBMS, University of Madras, Taramani Campus, Chennai – 600 113, India. All experiments with animals approved by the Institutional Animal Ethical Committee (IAEC No. 01/23/12) were performed in compliance with the relevant laws and institutional guidelines. The animals were housed under conditions of controlled temperature (25 ± 2°C) with 12/12 h light/dark cycle and were given food and water *ad libitum*. The experimental animals were divided into three groups of six animals each: Group I- Control (Received vehicle alone), Group II- Hypertrophy induced rats (Received isoproterenol hydrochloride 5mg/kg body weight, subcutaneously for 7 days), Group III- Rats pre-treated with hesperetin (received hesperetin 30mg/kg body weight suspended in 0.5% methyl cellulose orally for 30 days) and given isoproterenol 5mg/kg body weight, subcutaneously for 7 days.

At the end of the experimental period, rats were anesthetized with ketamine (22mg/kg bw/ip) and hearts were excised, washed in ice cold saline, blotted dry, weighed and part of the heart was homogenized in ice-cold tissue lysis buffer. A 10% tissue homogenate was prepared by using Tris-HCl buffer (0.01M, pH 7.4) followed by centrifugation at 12,000 rpm for 10 min. The supernatant was used for the analysis of various parameters.

Estimation of total collagen by hydroxyproline assay

Hydroxyproline assay was performed to measure total collagen content of heart (Stegemann and Stalder, 1967).

Oxidative Stress Markers

The level of lipid peroxides was assayed by the method of Devasagayam and Tarachand (1987) and the protein carbonyl was estimated by the method of Levine et al. (1990).

Assessment of antioxidant defense system

The enzyme superoxide dismutase was assayed according to the method of Marklund and Marklund (1974). The activity of catalase was assayed by the method of Sinha (1972) and the activity of GPx was determined by the modified method of Rotruck et al. (1973).

Western blotting

Western blotting was carried out as per the standard protocols (Townbin et al., 1992). The samples were solubilized in the sample solubilizing buffer and electrophoresed in 10% SDS polyacrylamide gels. Then transferred on to PVDF membranes. The membranes were incubated with specific primary antibody against NFκB and TNF-α (Santa Cruz Biotechnology). After washing with PBS containing 0.05% Tween 20, the membranes were incubated with horseradish peroxidase (HRP)- conjugated goat anti-rabbit or goat anti-mouse (BioRad, USA) secondary antibody. Immunoreactive bands were developed with Immobilon Western-Chemiluminescent HRP substrate (Millipore Corporation, Billerica, MA, USA) and visualized by using an enhanced chemiluminescence system (Chemi-Doc, BioRad, USA) and presented in comparison to β-actin expression.

Statistical Analysis

The results are expressed as mean ± Standard Deviation (SD). Differences between groups were analyzed by One-way Analysis of Variance (ANOVA) using the SPSS software package for Windows (Version: SPSS 20.0). Post hoc testing was performed for inter-group comparisons using the least significant difference (LSD) test; significance at p values <0.05 has been given by respective symbols in tables and figures.

	Group 1	Group 2	Group 3

Results Table 1: Effect of hesperetin on the heart to body weight ratio

Heart Weight (mg)	**560±44**	**790±60**	**595±47**
Body Weight (g)	**157±14.2**	**153±13.9**	**154±12.7**
HW/BW Ratio	**3.56**	**5.16**	**3.86**

Group 1- Control Rats; Group 2- Isoproterenol administered rats; Group 3- Isoproterenol + Hesperetin pre-treated rats. Values are expressed as mean ± SD for six animals in each group. Values are statistically significant at the level of p< 0.05, where 'a' - compared with Group 1, 'b' –compared with Group 2.

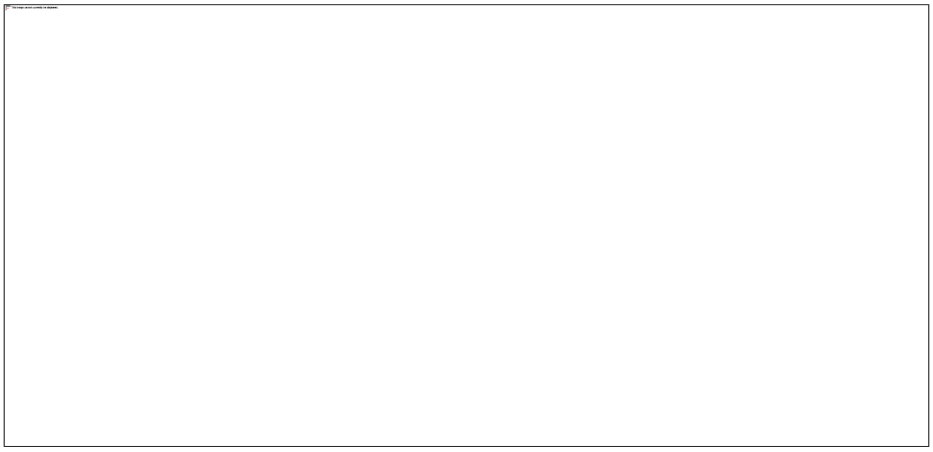


Figure 1: Effect of hesperetin on the collagen content in the heart

Group 1- Control Rats; Group 2- Isoproterenol administered rats; Group 3- Isoproterenol + Hesperetin pre-treated rats. Values are expressed as mean ± SD for six animals in each group. Values are statistically significant at the level of p< 0.05, where 'a' - compared with Group 1, 'b' –compared with Group 2.

Figure 2: Effect of hesperetin on the oxidative stress markers in cardiac hypertrophy

Group 1- Control rats; Group 2- Isoproterenol administered rats; Group 3- Isoproterenol + Hesperetin pre-treated rats. Values are expressed as mean ± SD for six animals in each group. Values are statistically significant at the level of p< 0.05, where 'a' - compared with Group 1, 'b' –compared with Group 2.

Figure 3: Effect of Hesperetin on enzymatic antioxidants in control and experimental rats

Units: SOD - amount of enzyme required to prevent 50% auto-oxidation of pyrogallol/min/mg protein; Catalase - µmoles of H_2O_2 consumed/min/mg protein. GPx - µmoles of GSH oxidized/min/mg protein.

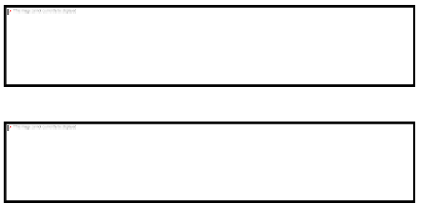
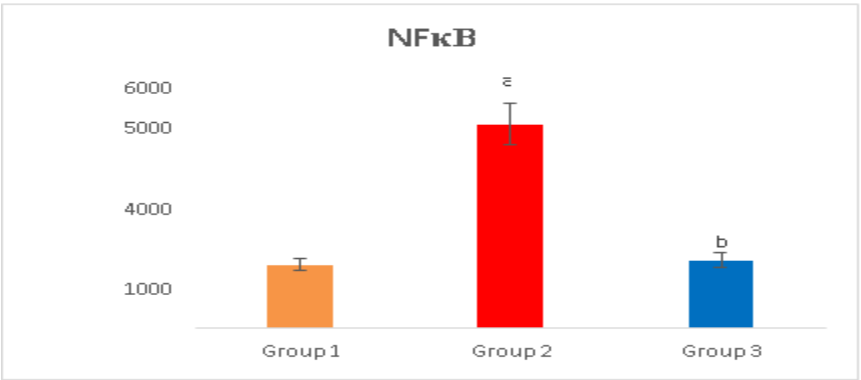


Figure 4: Effect of hesperetin on the protein expression of NFκB



Group 1- Control rats; Group 2- Isoproterenol administered rats; Group 3- Isoproterenol + Hesperetin pre-treated rats. Values are expressed as mean ± SD for three independent observations. Values are statistically significant at the level of p< 0.05, where 'a' - compared with Group 1, 'b' –compared with Group 2.

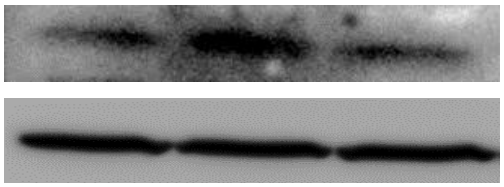


Figure 5: Effect of hesperetin on the protein expression of TNFα

Group 1- Control rats; Group 2- Isoproterenol administered rats; Group 3- Isoproterenol + Hesperetin pre-treated rats. Values are expressed as mean ± SD for three independent observations. Values are statistically significant at the level of $p < 0.05$, where 'a' - compared with Group 1, 'b' –compared with Group 2.

DISCUSSION

The heart to body weight ratio, is a useful index of ventricular hypertrophy at the organ level (Brooksby et al 1998). The HW/BW ratio as evident from **Table.1** was found to be increased by 44.94% in the isoproterenol administered rats (Group 1) when compared to control rats (Group 2), demonstrating an increase in the heart size. However, hesperetin pre-treated rats (Group 3) showed a 25.19% reduction in the HW/BW ratio when compared to isoproterenol administered rats. The heart to body weight ratio of the hypertrophy induced animals was found to be significantly increased when compared to the control rats. In contrast, the heart to body weight of the hesperetin pretreated animals was found be significantly decreased when compared to the hypertrophy induced rats.

Figure 1 shows the collagen content as a measure of cardiac fibrosis. This assay revealed that isoproterenol administration elevated the levels of collagen in the heart by 2-fold when compared to that of the control rat heart. Whereas, on hesperetin pre-treatment, a marked decrease in the collagen content (1.56 fold) was observed when compared to the hypertrophied rat heart. Increased deposition of collagen proteins has been observed in patients with hypertension, dilated cardiomyopathy and end stage heart failure (Daniels *et al*., 2009). Consistent with these previous studies, we also observed increased levels of total collagen in the isoproterenol administered rats when compared to the control rats. The present study obviates that the levels of total collagen as evident from the hydroxyproline assay is significantly lowered in the hesperetin treated rats when compared to the isoproterenol group.

Lipid peroxidation is a free radical process acting as a source of secondary free radical, which further can act as second messenger or can directly react with other biomolecule, enhancing biochemical lesions (Lobo et al., 2010). Lipid peroxidation leads to the formation of a number of compounds, for example, alkenes, malondialdehyde, and isoprostanes. These compounds are used as markers in lipid peroxidation assay to measure the levels of lipid peroxides (Yin et al., 2011).

The TBARS levels were found to be increased in the hypertrophied heart samples when compared to control rat hearts **(Figure 2).** This was in corroboration with the studies by Kannan & Quine (2013) who have reported that isoproterenol administered rats show increased levels of lipid peroxidation products.

The mechanisms underlying the isoproterenol induced cardio toxicity have not been fully understood, however, several investigators have shown that ROS are closely related to the oxidative stress and cardio toxicity induced by isoproterenol (Stanely Mainzen et al., 2009; Arya et al., 2010). However, hesperetin treatment was found to mitigate the protein carbonyl content to a considerable extent.

The activity of the enzymatic antioxidant SOD was found to be decreased by 45.45% in the isoproterenol administered rats when compared to the control rats. Whereas, hesperetin treated rats showed a significant increase (63.33%) in the activity of SOD when compared to group 2 rats **(Figure 3).** Superoxide released by processes such as oxidative phosphorylation is first converted to hydrogen peroxide and then further reduced to give water. This detoxification pathway is the result of multiple enzymes, with superoxide dismutases catalyzing the first step and then catalases and various peroxidases removing hydrogen peroxide (Ho et al., 1998; Johnson and Giulivi, 2005). Superoxide dismutases (SODs) are a class of closely related enzymes (Zelko et al., 2002) that play a vital anti-oxidant role in human health, conferred by their scavenging of one of the reactive oxygen species, superoxide anion (Johnson and Giulivi, 2005). We found a decline in the SOD activity in isoproterenol administered rat hearts when compared to that of the control rats. Isoproterenol administration has been shown to increase the myocardial lipid peroxidation and deplete the activity of SOD, and catalase (Maulik et al., 2012). Similarly, Balta and his co-workers (1999) have reported that that administration of high doses of isoproterenol, induced oxidative stress and decreased superoxide dismutase (SOD) activity in the heart and the results obtained are considered an outcome of oxidative stress conditions imposed by isoproterenol auto oxidation. The decrease observed in the activity of SOD due to isoproterenol administration is shown to be augmented by hesperetin treatment. This is in corroboration with Choi and Ahn (2008) who have reported that hesperetin significantly enhances the total superoxide dismutase (SOD) activity in mice and Aranganathan and Nalini (2009) who have reported that hesperetin increase the

activity of SOD in a model of rat colon carcinogenesis.

Catalase is a common enzyme found in nearly all aerobic organisms, where it functions to catalyze the decomposition of hydrogen peroxide to water and oxygen. This hypothesis was supported by the results wherein we found a significantly decreased (2.6 fold) activity of catalase in the isoproterenol administered rats compared to the control rats. Whereas, hesperetin treated rats showed a significant increase (2.1 fold) in the activity of catalase when compared to the isoproterenol group. These results can be attributed to the fact that hesperetin has been shown to increase the activity of catalase in various studies and under various experimental conditions (Agrawal et al., 2014; Wang et al., 2013; Aranganathan and Nalini, 2009; Choi, 2008).

Glutathione peroxidase (GPx), a selenium containing enzyme protects cellular and sub cellular membrane from peroxidative damage by catalyzing the removal of H_2O_2 and other non-reactive products (Ojha et al., 2010; Ursini et al., 1995). In the current study, isoproterenol administered rats show a decline (40%) in the activity and protein levels of GPx when compared to the control rats. Similar results have been obtained by El-Demerdash et al. (2005) and Suchalatha et al. (2005) wherein they have observed a decrease in GPx in the cardiac tissue of rats administered with isoproterenol.

Cardiac hypertrophy is correlated with aninflammatory process, suggesting that inflammation could be a key event in cardiovascular complications in hypertensive animals (Simko et al, 2009). The close relationship between hypertrophy and inflammation is also from a clinical point of view of great importance, because chronic low-grade inflammation isthought to play a significant role in cardiac hypertrophy and failure (Yndestad et al, 2006).

Nuclear factor-B (NF-κB), a ubiquitous transcription factor, isknown for its role in immunity, inflammation, regulation of cell growth, apoptosis and embryonal development (Ghosh and Karin, 2002). In most resting cells, NF-κB is sequestered in the cytoplasm by interaction with its inhibitory proteins, the IκBs, which are degraded on stimulation. Cytokines have been implicated in the development of cardiac hypertrophy; most likely via downstream activation of pro-inflammatory transcription factors such as NF-κB (Purcell et al, 2001). Also, the reports stated by Freund et al (2005) firmly establish that activation of NF-κB in cardiomyocytes is a requisite to the development of cardiac hypertrophy by Ang II and Isoproterenol in vivo. NF-κB plays an active role in the development of cardiac hypertrophy when induced with a beta adrenergic stimulant isoproterenol. It is proposed that inhibition of pro-inflammatory pathways, in general, and NF-κB, in particular, could be beneficial for the diseased heart (Ogato et al, 2004; Kawano et al, 2006).

The prohypertrophic and inflammatory signaling pathways in the cardiomyocytes appear to converge on NF-κB. These observations point to a close relationship between the hypertrophic and inflammatory signaling pathways in the cardiac muscle cell and suggest that anti-inflammatory interventions could be therapeutically effective. Hesperetin is one such compound with profound anti- inflammatory action (Chen et al, 2010). Moreover, hesperetin was shown to modulate NF-κB activation through the NIK/IKK, ERK, p38, and JNK pathways (Kim et al, 2006). Accordingly, the ability of hesperetin to attenuate inflammation and thereby hypertrophy was studied. Western blot analysis from the present study showed an increased expression of NF-κB in the hypertrophy induced rats **(Figure 4)**. Pretreatment with hesperetin inhibited the increase in NF-κB expression upon isoproterenol induction.

TNF-α, a proinflammatory cytokine with a broad range of pleiotropic effects, is expressed de novo by cardiac myocytes after certain forms of stress (Giroir, 1994). Both basic and clinical studies strongly support the hypothesis that myocardial expression of TNF alpha is an important step in the pathophysiologic pathway leading to progressive cardiac dilatation and failure (Feldman et al., 2000). Growing evidence implicates TNF-α in the pathogenesis of heart failure. It is well known that the healthy heart does not produce TNF-α but the insufficient myocardium does. Experimentally, it has been demonstrated that transgenic mice that chronically overexpress myocardial TNF-α develop cardiac hypertrophy, fibrosis, dilated myocardiopathy, and premature death (Garza et al., 2002).

In the present study, the expression of TNF-α was significantly increased (2.2 fold) in the isoproterenol administered rat hearts when compared to that of the control rat heart **(Figure 5)**. Whereas, hesperetin treated rats showed a significant decline (2.1 fold) in the expression of TNF-α when compared to that of the isoproterenol alone administered rats**.**

From the above findings, it can be concluded that the anti-hypertrophic action of hesperetin are at least in part mediated by the reducing the expression of NF-κB. Since NF-κB is associated with the development of cardiac hypertrophy and its progression to heart failure, the inhibition of NF-κB is therefore necessary. This study suggests NF-κB as a potential target for anti-inflammatory therapy for cardiac hypertrophy. Since hesperetin modulated NF-κB and its gene expression, it therefore could be useful as an anti-inflammatory agent against cardiac hypertrophy.

Conflict of interest

The authors declare that they have no potential conflict of interest, including any financial, personal or other relationships, with other people or organizations.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the financial support offered by UGC in the form of BSR fellowship, University of Madras, and Chennai, India for conducting this study.

REFERENCES

1. Agrawal, Y. O., Sharma, P. K., Shrivastava, B., Ojha, S., Upadhya, H. M., Arya, D. S., & Goyal, S. N. (2014). Hesperidin produces cardioprotective activity via PPAR-γ pathway in ischemic heart disease model in diabetic rats. PloS one, 9(11): e111212.
2. Aranganathan S, Nalini N. Efficacy of the potential chemopreventive agent, hesperetin (citrus flavanone), on 1,2-dimethylhydrazine induced colon carcinogenesis. Food Chem Toxicol. 2009 Oct; 47(10): 2594-600. doi: 10.1016/j.fct.2009.07.019. Epub 2009 Jul 24.
3. Arya, D. S., Arora, S., Malik, S., Nepal, S., Kumari, S., & Ojha, S. (2010). Effect of Piper betle on cardiac function, marker enzymes and oxidative stress in isoproterenol-induced cardiotoxicity in rats. Toxicology mechanisms and methods, 20(9): 564-571.
4. Babu GJ, Lalli MJ, Sussman MA, Sadoshima J, Periasamy M. Phosphorylation of elk-1 by MEK/ERK pathway is necessary for c-fos gene activation during cardiac myocyte hypertrophy. J Mol Cell Cardiol. 2000; 32: 1447-57.
5. Balta N, Stoian I, Petec C, Petec G. Decreased SOD activity and increased nitrates level in rat heart with left ventricular hypertrophy induced by isoproterenol. Romanian. J Physiol. 1999; 36(3-4): 175–8.
6. Brooksby P, Levi AJ, Jones JV. The electrophysiological characteristics of hypertrophied ventricular myocytes from the spontaneously hypertensive rat. J Hypertens. 1993 Jun; 11(6): 611-22.
7. Carreño, J. E., Apablaza, F., Ocaranza, M. P., & Jalil, J. E. (2006). Cardiac hypertrophy: molecular and cellular events. Revista Española de Cardiología (English Edition), 59(5): 473-486.
8. Chen, Mingcang, et al. "Protective effects of hesperidin against oxidative stress of tert-butyl hydroperoxide in human hepatocytes." Food and Chemical Toxicology, 2010; 48.10: 2980-2987.
9. Choi EJ, Ahn WS. Neuroprotective effects of chronic hesperetin administration in mice. Arch Pharm Res. 2008 Nov; 31(11): 1457-62. doi: 10.1007/s12272-001-2130-1. Epub 2008 Nov 21.
10. Devasagayam TP, Tarachand U. Decreased lipid peroxidation in the rat kidney during gestation. Biochem Biophys Res Commun. 1987 May 29; 145(1): 134-8.
11. Dorn, G. W., & Brown, J. H. (1999). Gq signaling in cardiac adaptation and maladaptation. Trends in cardiovascular medicine, 9(1): 26-34.
12. El-Demerdash, E., Awad, A. S., Taha, R. M., El-Hady, A. M., & Sayed-Ahmed, M. M. (2005). Probucol attenuates oxidative stress and energy decline in isoproterenol-induced heart failure in rat. Pharmacological research, 51(4): 311-318.
13. Feldman, A. M., Combes, A., Wagner, D., Kadakomi, T., Kubota, T., Li, Y. Y., & McTiernan, C. (2000). The role of tumor necrosis factor in the pathophysiology of heart failure. Journal of the American College of Cardiology, 35(3): 537-544.
14. Freund C, Schmidt-Ullrich R, Baurand A, Dunger S, Schneider W, Loser P, El-Jamali A, Dietz R, Scheidereit C, Bergmann MW. Requirement of nuclear factor-kappaB in angiotensin II- and isoproterenol-induced cardiac hypertrophy in vivo. Circulation. 2005 May 10; 111(18): 2319-25. Epub 2005 May 3.
15. Garza, E. H. H., Garza, J. L. H., González, H. R., Treviño, A. T., Flores, M. I., & Amione, G. T. (2002). Importance of tumor necrosis factor-alpha in the pathogenesis of heart failure. Revista Española de Cardiología, 55(01): 61-66.
16. Ghosh S, Karin M. Missing pieces in the NF-kappaB puzzle. Cell. 2002 Apr; 109 Suppl: S81-96.
17. Review. Giroir, B. P., Horton, J. W., White, D. J., McIntyre, K. L., & Lin, C. Q. (1994). Inhibition of tumor necrosis factor prevents myocardial dysfunction during burn shock. American Journal of Physiology-Heart and Circulatory Physiology, 267(1): H118-H124.
18. Hertog MG, Hollman PC, Katan MB, Kromhout D. Intake of potentially anticarcinogenic flavonoids and their determinants in adults in The Netherlands. Nutr Cancer. 1993; 20(1): 21-9.
19. Ho YS, Magnenat JL, Gargano M, Cao J. The nature of antioxidant defense mechanisms: a lesson from transgenic studies. Environ Health Perspect. 1998 Oct; 106 Suppl 5: 1219-28. Review.
20. Johnson, F., & Giulivi, C. (2005). Superoxide dismutases and their impact upon human health. Molecular aspects of medicine, 26(4): 340-352.
21. Kannan MM, Quine SD. Ellagic acid inhibits cardiac arrhythmias, hypertrophy and hyperlipidaemia during myocardial infarction in rats. Metabolism. 2013 Jan; 62(1): 52-61. doi: 10.1016/j.metabol.2012.06.003.
22. Katholi, R. E., & Couri, D. M. (2011). Left ventricular hypertrophy: major risk factor in patients with hypertension: update and practical clinical applications. International journal of hypertension, 2011.
23. Kawano, Shunichi, et al. "Blockade of NF-κB improves cardiac function and survival after myocardial infarction." American Journal of Physiology-Heart and Circulatory Physiology, 2006; 291.3: H1337- H1344.
24. Kim, J.Y., Jung, K.J., Choi, J.S., Chung, H.Y., 2006. Modulation of the age-related nuclear factor-kappaB (NF-kappaB) pathway by hesperetin. Aging Cell, 5: 401– 411.
25. Kroon, Paul and Gary Williamson. "Polyphenols:

dietary components with established benefits to health?." Journal of the Science of Food and Agriculture, 2005; 85.8: 1239-1240.
26. Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, Ahn BW, Shaltiel S, Stadtman ER. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol. 1990; 186: 464- 78.
27. Li, Y., Ha, T., Gao, X., Kelley, J., Williams, D. L., Browder, I. W., and Li, C. (2004). NF-kappaB activation is required for the development of cardiac hypertrophy in vivo. American journal of physiology. Heart and circulatory physiology, 287(4): H1712-20.
28. Liang F, Lu S, Gardner DG. Endothelin-dependent and • independent components of strain-activated brain natriuretic peptide gene transcription require extracellular signal regulated kinase and p38 mitogen-activated protein kinase. Hypertension. 2000; 35: 188-92.
29. Liang, Q., Bueno, O. F., Wilkins, B. J., Kuan, C. Y., Xia, Y., & Molkentin, J. D. (2003). c-Jun N-terminal kinases (JNK) antagonize cardiac growth through cross-talk with calcineurin–NFAT signaling. The EMBO journal, 22(19): 5079-5089.
30. Lobo, V., Patil, A., Phatak, A., & Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. Pharmacognosy reviews, 4(8): 118.
31. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1974 Sep 16; 47(3): 469-74.
32. Maulik SK, Prabhakar P, Dinda AK, Seth S. Genistein prevents isoproterenol-induced cardiac hypertrophy in rats. Can J Physiol Pharmacol. 2012 Aug; 90(8): 1117-25. doi: 10.1139/y2012-068. Epub 2012 Jul 18.
33. Morimoto T, Hasegawa K, Kaburagi S, Kakita T, Wada H. Phosphorylation of GATA-4 is involved in alpha 1-adrenergic agonist-responsive transcription of the endothelin-1 gene in cardiac myocytes. J Biol Chem. 2000; 275: 13721-6.
34. Ojha S, Nandave M, Arora S, Arya DS. Effect of isoproterenol on tissue defense enzymes, hemodynamic and left ventricular contractile function in rats. Indian J Clin Biochem. 2010 Oct; 25(4): 357-61. doi: 10.1007/s12291-010-0070-6. Epub 2010 Sep 14.
35. Pietta PG. Flavonoids as antioxidants.(2000). J Nat Prod. Jul; 63(7): 1035-42. Review.
36. Pollard SE, Whiteman M, Spencer JP. Modulation of peroxynitrite-induced fibroblast injury by hesperetin: a role for intracellular scavenging and modulation of ERK signalling. Biochem Biophys Res Commun. 2006 Sep 8; 347(4): 916-23.
37. Purcell NH, Tang G, Yu C, Mercurio F, DiDonato JA, Lin A. Activation of NF-kappa B is required for hypertrophic growth of primary rat neonatal ventricular cardiomyocytes. Proc Natl Acad Sci USA, 2001; 98: 6668-73.
38. Rohilla, A., Kumar, P., Rohilla, S., & Kushnoor, A. (2012). Cardiac hypertrophy: a review on pathogenesis and treatment. Int of Pharmac Sci Drug Res, 4: 164-167.
39. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: biochemical role as a component of glutathione peroxidase. Science. 1973 Feb 9; 179(4073): 588-90.
40. Simko, Fedor, and Olga Pechanova. "Potential roles of melatonin and chronotherapy among the new trends in hypertension treatment." Journal of pineal research, 2009; 47.2: 127-133.
41. Sinha AK. Colorimetric assay of catalase. Anal Biochem. 1972 Jun; 47(2): 389-94.
42. Stanely Mainzen Prince P, Priscilla H, Devika PT. Gallic acid prevents lysosomal damage in isoproterenol induced cardiotoxicity in Wistar rats. Eur J Pharmacol. 2009 Aug 1; 615(1-3): 139-43. doi: 10.1016/j.ejphar.2009.05.003. Epub 2009 May 15.
43. Stegemann H, Stalder K. Determination of hydroxyproline. Clin Chim Acta. 1967 Nov; 18(2): 267-73. Review.
44. Suchalatha S, Devi CSS. Protective effect of Terminalia chebula against lysosomal enzyme alterations in isoproterenol induced cardiac damage in rats. Exp. Clin. Cardiol., 2004; 10: 91-95.
45. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci. 1979; 76(9): 4350-4354.
46. Ursini F, Maiorino M, Brigelius-Flohé R, Aumann KD, Roveri A, Schomburg D, Flohé L. Diversity of glutathione peroxidases. Methods Enzymol. 1995; 252: 38-53.
47. Wang J, Zhu H, Yang Z, Liu Z. Antioxidative effects of hesperetin against lead acetate-induced oxidative stress in rats. Indian J Pharmacol. 2013 Jul-Aug; 45(4): 395-8. doi: 10.4103/0253-7613.115015.
48. Yang, H. L., Chen, S. C., Senthil Kumar, K. J., Yu, K. N., Lee Chao, P. D., Tsai, S. Y., ... & Hseu, Y. C. (2011). Antioxidant and anti-inflammatory potential of hesperetin metabolites obtained from hesperetin-administered rat serum: an ex vivo approach. Journal of agricultural and food chemistry, 60(1): 522-532.
49. Yin, H., Xu, L., & Porter, N. A. (2011). Free radical lipid peroxidation: mechanisms and analysis. Chemical reviews, 111(10): 5944-5972.
50. Yndestad, A., Kristian Damås, J., Øie, E., Ueland, T., Gullestad, L., & Aukrust, P. (2006). Systemic inflammation in heart failure–the whys and wherefores. Heart failure reviews, 11(1): 83-92.
51. Zelarayan, L., Renger, A., Noack, C., Zafiriou, M. P., Gehrke, C., van der Nagel, R., ... & Bergmann, M. W. (2009). NF-κB activation is required for adaptive cardiac hypertrophy. Cardiovascular

research, 84(3): 416-424.
52. Zelko, I. N., Mariani, T. J., & Folz, R. J. (2002). Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2) and EC-SOD (SOD3) gene structures, evolution and expression. Free Radical Biology and Medicine, 33(3): 337-349.