The Protective Effect of Metformin Against Doxorubicin Induced Cardiotoxicity in Rabbits

Mohammed Hussein shaty *, Inam Sameh Arif **, Muthanna I. Al-Ezzi **, Dalya Basil***

Department of Pharmacology and Toxicology, College of Pharmacy, Mustansiriyah University, M.Sc. program, Iraq.

****Department of Pharmacology and Toxicology, College of Pharmacy, Mustansiriyah University, Iraq.***

*****Department of Clinical Laboratory Sciences, College of Pharmacy, Mustansiriyah University, Iraq.***

DOI: https://doi.org/10.32947/ajps.19.01.0393

Article Info:

Received 23 Oct 2018
Accepted 29 Jan 2019
Published 1 Mar 2019

Corresponding Author email:
pharm.dr.muthanna@uomustansiriyah.edu.iq
orcid: https://orcid.org/0000-0002-5622-958

Abstract:

Back ground: Doxorubicin is a very effective anticancer therapy of the anthracycline's family used in many pediatric and adult cancers. However, due to severe cardiotoxicity adverse effect, the uses of doxorubicin are limited. Metformin reducing basal and postprandial glucose levels. Metformin has a good treatment efficacy and safety profile in treatment of T2DM in conjunction with lifestyle modification. Metformin have a cardioprotective effect in addition to reducing basal and postprandial levels of glucose by decreasing the production of reactive oxygen species, maintaining energy homeostasis and apoptosis regulation by its activation of adenosine monophosphate-activated protein kinase.

Method: Thirty-six white male rabbits randomly divided to six groups, each comprising of six rabbits. 1- Control group injected 2 ml saline single dose intraperitoneally. 2- Metformin group 300 mg/kg/daily for 14 days orally. 3- Acute doxorubicin induction group 16 mg/ kg intraperitoneally as a single dose. 4- Chronic doxorubicin induction group 4mg/kg intraperitoneally twice a week for two weeks. 5- Metformin+ acute doxorubicin induction group 16 mg/kg intraperitoneally single dose and Metformin 300 mg/kg/daily for 14 days orally, three days before doxorubicin treatment. 6- Metformin + chronic doxorubicin induction group 4 mg/kg intraperitoneally, twice a week for two weeks and Metformin 300 mg/kg/daily for 14 days orally, three days before doxorubicin treatment.

Result: our results revealed the treatment with metformin significantly ($p < 0.05$) reduced the serum level of troponin I and MMP2 in Metformin+ acute doxorubicin induction and Metformin + chronic doxorubicin induction groups in comparison with the acute doxorubicin and chronic doxorubicin groups.

Conclusion: From these results in this study, we can conclude that metformin has a cardioprotective effect against doxorubicin induced cardiotoxicity in acute and also the chronic induction by decreasing serum level of troponin I and MMP2.

Key words: Cardiotoxicity, Doxorubicin, Metformin, Troponin, Matrix Metalloproteinases.

تأثير الحماية للميتفورمين ضد سمية القلب الناجمة عن الدوكسوروبسين في الأرانب

محمد حسين شاطي*، أ.م.د. إنعام سامح عارف، أ.م.د. مثنى إبراهيم العزي**، داليا باسل حنا*****

الدراسات العليا، فرع الأدوية والسموم، كلية الصيدلة، الجامعة المستنصرية
*** فرع الأدوية والسموم، كلية الصيدلة، الجامعة المستنصرية***
***** فرع العلوم المختبرية السريرية، كلية الصيدلة، الجامعة المستنصرية.***

الخلاصة:

الدوكسوروبسين هو علاج مضاد للسرطان فعال للغاية لعائلة الانثراسيكلين المستخدمة في العديد من سرطانات الأطفال والبالغين. ومع ذلك، بسبب التأثير السلبي الشديد لأمراض القلب، فإن استخدام الدوكسوروبسين محدود. الميتفورمين يعمل على خفض مستويات الجلوكوز . يتمتع المتفورمين بفعالية جيدة في علاج وسلامة المرضى المصابين بمرض السكري نوع 2 بالتزامن مع تعديل نمط الحياة. للميتفورمين تأثير حماية للقلب بالإضافة إلى تقليل مستويات الغلوكوز عن طريق تقليل إنتاج أنواع الأوكسجين التفاعلية، والحفاظ على توازن الطاقة وتنظيم الاستموات من خلال تنشيط البروتين كيناز الادينوسين المفعل.

طريقة العمل: ستة وثلاثين من الذكور البيض الذكور ينقسمون عشوائيا إلى ست مجموعات، كل واحدة تضم ستة أرانب. 1- المجموعة الضابطة حقنت 2 مل محلول ملحي داخل الغشاء البريتوني. 2 - مجموعة المتفورمين أعطيت 300 مغ/كغ/يومياً لمدة 14 يوماً عن طريق الفم. 3- مجموعة الدوكسوروبسين الحاد حقنت 16 مجم/كجم بالغشاء البريتوني كجرعة وحيدة. 4- مجموعة دوكسوروبسين مزمنة حقنت 4 مجم بالغشاء البريتوني مرتين في الأسبوع لمدة أسبوعين. 5- ميتفورمين + مجموعة الدوكسوروبسين الحاد حقنت 16 مجم/كجم كجرعة واحدة و أعطيت المتفورمين 300 مجم/كغ/يومياً لمدة 14 يوماً فمويا، قبل العلاج بالدوكسوروبسين بثلاثة أيام. 6- ميتفورمين + مجموعة دوكسوروبسين مزمنة 4 مجم/كجم في الغشاء البريتوني، مرتين في الأسبوع لمدة أسبوعين والمتفورمين 300 مجم/كيلوغرام يومياً لمدة 14 يوماً فمويا ، قبل 3 أيام من العلاج بالدوكسوروبسين.

النتائج: أظهرت النتائج أن المعالجة بإستخدام المتفورمين قللت من مستوى مصل تروبونين I و MMP2 في مجموعات المتفورمين + الدوكسوروبسين الحاد و المتفورمين + الدوكسوروبسين المزمنة بالمقارنة مع مجموعات الدوكسوروبسين الحاد و الدوكسوروبسين المزمنة.

الخلاصة : من خلال نتائج في هذه الدراسة، يمكننا أن نستنتج أن المتفورمين له تأثير حماية ضد تسمم القلب الناجم عن الدوكسوروبسين الحاد وكذلك المزمن عن طريق خفض مستوى المصلي للتروبونين I و MMP2.

الكلمات المفتاحية: سمية القلب، دوكسوروبسين، ميتفورمين، تروبونين، مصفوفة ميتيلوبروتينوز.

Introduction:

The National Cancer Institute defined Cardiotoxicity as the toxicity which affects the heart. The definition comprises both a direct effect of the medication on the heart and indirect effect due to thrombotic events or induction of haemodynamic flow alterations [1].

Chemotherapy is used as a primary or as an adjuvant for cancer treatment, although it has a risk of adverse effects that might leave an unfavorable damage to patients. Cardiotoxicity-induced by chemotherapy is a dangerous complication that limits the use of chemotherapeutic agents, especially the anthracyclines [2].

The severity of cardiotoxicity of many anticancer drugs, as an adverse effect, occurs by a cumulative, dose-dependent toxicity for both patients and healthcare workers during the manipulation of anticancer drugs. Cardiovascular disease is the second cause of long-term mortality and morbidity in people cancer survivors [3]. Nearly about ten million of cancer survivors undergo cardiomyopathy in USA with the same number in Europe [4]. Doxorubicin cardiotoxicity including cardiomyopathy, ECG alterations (e.g. ST-T alterations, elongation of QT interval and decreased QRS voltage) and congestive heart failure (CHF) [5]. There are many risk factors for doxorubicin cardiotoxicity including the cumulative dose, age, ethnicity, and irradiation therapy, combination therapy with other chemotherapeutics, hypertension, chromosomal deformities and liver disease [6].

Doxorubicin-induced cardiotoxicity is divided into subacute or acute toxicity which is infrequent and occur during or instantly after infusion, is ordinarily transient (e.g. ECG abnormalities such as

ST-T alterations and QT elongation, pericarditis–myocarditis syndrome and ventricular dysfunction with congestive heart failure and it will reduce after stopping the treatment[5] and the chronic cardiotoxic effects which occur with early cardiac defects that can develop to heart disease. Chronic cardiotoxic effects continue after stopping the treatment and the clinical symptoms comprise all signs of cardiomyopathy such as electrophysiological alterations, left ventricular dysfunction, variations in exercise stress capacity and signs of congestive heart failure [7]. Several mechanisms are indicated in doxorubicin induced cardiotoxicity. Doxorubicin-induced cardiomyopathy is largely associated with increasing of oxidative stress that induced destruction, e.g. lipid peroxidation, in addition to decreased antioxidants levels. Another important mechanism is deterioration of myofibrillar and intracellular calcium level dysregulation, changes in endothelin-1 levels, the high energy phosphate pool and apoptosis [8]. Understanding the mechanisms which are responsible for toxicity could help to reduce the unfavorable effect on normal tissues and improve of the cancer treatment regimen [2]. The cardioprotective properties of several pharmacologic drugs have been confirmed through anticancer therapy in a laboratory, however the most of these drugs are not proven as cardioprotective for cancer related cardiotoxicity. Several drugs such as dexrazoxane, beta-blockers such as carvedilol, Angiotensin-converting enzyme inhibitors, angiotensin antagonists, statins and aldosterone antagonists such as spironolactone have been revealed to be potentially protective in patients receiving anthracycline or trastuzumab [9]. Metformin also may have a cardioprotective effect. In experimental animal models of isolated myocardial infarction and heart failure have revealed that metformin rises the tolerance of the cardiac muscle to ischemia-reperfusion injury and declines the incidence of heart failure [10]. Metformin is a biguanide (1,1-dimethyl-biguanide). Metformin is commonly used in type 2 diabetes mellitus (T2DM) patients. Metformin absorbed primary by plasma membrane monoamine transporter on the luminal side of enterocyte. Metformin is widely distributed into the body tissue. Metformin is not metabolized and is excreted unchanged in urine by active tubular secretion, with a half- life 5 hours [10]. Metformin has a good treatment efficacy and safety profile, low cost, and consider as the first-line oral treatment in T2DM in conjunction with lifestyle modification [11]. Metformin have cardioprotective effect in addition to reducing basal and postprandial levels of glucose by decreasing the production of reactive oxygen species, maintaining energy homeostasis and apoptosis regulation by its activation of adenosine monophosphate-activated protein kinase (AMPK). Metformin has also the ability to increase the cardiac adiponectin and its receptors (adipoR1 and adipoR2), increases NO production, prevent intracellular Ca^{2+} overload and finally reduce inflammatory responses by attenuating toll-like receptor 4 (TLR) activity [12-15]. Troponins are medium sized regulatory proteins act to regulate the contractile elements, myosin and actin. While it is usually undetectable protein, troponins can increase after 2-3 hours when the cardiac injury occurs [16]. Many studies demonstrated that troponins can detect cardiotoxicity at a preclinical period, even before any reduction in left ventricular ejection fraction occurs in patients using anticancer drugs [17]. Matrix metalloproteinase 2 (MMP-2) are proteolytic enzymes responsible for degradation of extracellular matrix component and it is important for remodeling of normal tissue and growth process. Several myocardial injury models demonstrated that MMP-2 play an important role in cardiac remodeling and ventricular dilation which can be increasing in both acute and chronic doxorubicin therapy [18,19].

This study aimed to assess the metformin cardioprotective effect against cardiotoxicity induced by acute and chronic doxorubicin therapy.

Materials and Methods

Study Design

Thirty-six white male rabbits randomly divided to six groups, each comprising of 6 rabbits:

1- Control group: injected 2 ml saline single dose intraperitoneally.
2- Metformin group: 300 mg/kg/daily for 14 days orally.
3- Acute doxorubicin induction group: 16 mg/ kg intraperitoneally as a single dose.
4- Chronic doxorubicin induction group: 4mg/kg intraperitoneally twice a week for two weeks.
5- Metformin+ acute doxorubicin induction group: 16 mg/kg intra-peritoneally single dose and Metformin 300 mg/kg/daily for 14 days orally, three days before doxorubicin treatment.
6- Metformin + chronic doxorubicin induction group: 4 mg/kg intraperitoneally, twice a week for two weeks and Metformin 300 mg/kg/daily for 14 days orally, three days before doxorubicin treatment.

Induction of Cardiotoxicity

Cardiotoxicity inducted by doxorubicin injected in a dose of 16 mg /kg intraperitoneally single dose in acute doxorubicin induction and in a dose of 4 mg/kg intraperitoneally twice a week for two weeks in chronic induction [9].

Sample Collection and Preparation

The fife milliliters blood sample collected by using heart puncture, put in plain gel tube and centrifugation at 3000x for fifteen minutes. The serum stored at -80°C for ELISA analysis where troponin I was measured in serum according to manufacturer instruction (Elabscience - china), then sacrificed the rabbits by using di-ethyl ether for anesthesia, then removed the heart and washed by distilled water, then the heart tissue kept in a sterile normal saline tube and stored at -80 º C for DNA extraction and RT- PCR to measure MMP-2 expression in heart tissues by using gSYNC ™ DNA Extraction Kit (Geneaid – UK).

Statistical Analysis

Statistical analysis was done by SPSS version 16.0. The results calculated as mean ± standard deviation (SD). The results compared among the groups by using One-way ANOVA test followed by a post hoc Tukey test. The statistically significant differences were considered when the $p < 0.05$.

Results

Cardiac Troponin I

The statistical analysis for cardiac troponin I levels that is represented as mean ± SD was increased significantly in both acute and chronic doxorubicin induction group (252.12 ± 40.79, 295.33 ± 144.12 pg/ml respectively) when compared with the control and Metformin group (126.32 ± 30.91, 136.42 ± 32.06 pg/ml respectively; $P < 0.05$). The cardiac troponin I level was reduced significantly in Metformin +acute doxorubicin induction and Metformin +chronic doxorubicin induction groups in comparison with the acute doxorubicin induction and chronic doxorubicin induction groups (130.37 ± 22.30, 124.48 ± 49.09 pg/ml respectively; $P < 0.05$). The cardiac troponin I level was non-significantly differences in both Metformin +acute doxorubicin induction and Metformin +chronic doxorubicin induction groups in compare with the control and Metformin group as shown in (table 1).

Table-1: Effect of metformin on serum troponin I level in acute and chronic doxorubicin cardiotoxicity.

	Group	Troponin I (pg/ml)
1	Control (2ml saline)	126.32 ± 30.91*#
2	MET (300 mg for 14 day orally)	136.42 ± 32.06 *#
3	Acute DOX (16 mg single dose)	252.12 ± 40.79
4	MET +Acute DOX (300 mg for 14 day +16mg single dose)	130.37 ± 22.30 *
5	Chronic DOX (4mg twice wk for 2 wks)	295.33 ± 144.12
6	MET+Chronic DOX (300 mg for 14 day orally +4mg twice wk for 2 wks)	124.48 ± 49.09 #

Each value expressed as mean ±SD. The statistical analysis done by using one-way ANOVA followed by Tukey test.

*Significant difference ($p < 0.05$) when compared among acute DOX with control, MET and MET+ Acute DOX groups

#Significant difference ($p < 0.05$) when compared among chronic DOX with control, MET and MET+ Chronic DOX groups.

Matrix metalloproteinase 2 (MMP2)

The statistical analysis for DNA load of MMP2 concentration that is represented as mean ±SD was increased significantly in both acute and chronic doxorubicin induction groups (2280±717.77, 8341.66± 359.03copies/gm respectively) when compared with control and Metformin group (423.33±123.55, 765±296.36 copies/gm respectively; (P>0.05). The DNA load of MMP2 level was reduced significantly in both Metformin + acute doxorubicin induction and Metformin + chronic doxorubicin induction groups in comparison with the acute and chronic doxorubicin induction groups (190±56.56, 716±98.31copies/gm respectively; P> 0.05). Matrix metalloproteinase 2 level was non-significantly differ in both Metformin + acute doxorubicin induction and Metformin + chronic doxorubicin induction groups when compared with the control and Metformin groups, as shown in table (2).

Table-2: Effect of Metformin on DNA Load of MMP2 Level in Acute and Chronic Doxorubicin Induction Cardiotoxicity.

	Group	MMP2(copies/gm)
1	Control (2ml saline)	423.33±123.55 *#
2	MET (300 mg for 14 day orally)	765±296.36 *#
3	Acute DOX (16 mg single dose)	2280±717.77
4	MET+Acute DOX (300 mg for 14 day +16mg single dose)	190±56.56 *
5	Chronic DOX (4mg twice wk for 2 wks)	8341.66±359.09
6	MET+Chronic DOX (300 mg for 14 day orally +4mg twice wk for 2 wks)	716±98.31 #

Each value expressed as mean ±SD. The statistical analysis done by using one-way ANOVA followed by Tukey test.

*Significant difference ($p < 0.05$) when compared among acute doxorubicin induction with control, MET and MET+ Acute doxorubicin induction groups

#Significant difference ($p < 0.05$) when compared among chronic doxorubicin induction with control, MET and MET+ Chronic doxorubicin induction groups**.**

Discussion

Troponins are medium size proteins that released to circulation after damage of cardiac myocyte [20]. Cardiac troponins are the first biomarkers for the detection of heart damage which may increase after 2-3 hours. Troponins are important in regulating the cardiac muscle contractile elements (actin and myosin) [16]. Troponin levels increased in the blood stream have become a well-established biomarker with large sensitivity and specificity for cardiac infarction or necrosis in both animals and human. Myocardial troponin level increased proportionally to the size and extent of myocardial injury in many animal models of cardiotoxicity [21].

Doxorubicin may cause a significant elevation in serum troponin concentration which may be due to oxidative stress that lead to cardiac muscle cell damage [22]. From the result of this study, Metformin led to a significant reduction ($P < 0.05$) in serum troponin I level in both Metformin + acute doxorubicin induction and Metformin + chronic doxorubicin induction groups.

Basnet *et al* (2015), revealed that cardiac troponin I levels are significantly reduced by Metformin may be by the activation of AMPK [23]. Authors support that Metformin has a significant cardioprote-ctive effects on heart injury in many studies by ricing the tolerance tissues to ischemic injury by reducing the cardiotoxicity induced by doxorubicin through activation of AMPK [24].

The AMPK regulate the cardiac metabolic pathway by regulation of catabolic and anabolic processes, which reserve the cardiac energy homeostasis. The AMPK also plays an important role in mitochondrial protection by regulation of gene transcription and increased the endogenous antioxidant system [25].

Matrix Metalloproteinases are proteolytic enzymes synthesized as inactive zymogens, which are activated after cleaved of the propeptide domain. The MMP-2 is responsible for the degradation of extracellular matrix components and is important for normal tissue remodeling and growth process. It's become over activated during cardiac injury such as ischemia, toxic injury, and also during oxidative stress [26]. Doxorubicin has been shown to chronically and acutely increase oxidative stress [8]. The MMP-2 activation in cardiac myocyte involved in degradation of cytoskeletal and sarcomeric proteins such as alpha-actinin, troponin I, and myosin light chain, inducing left ventricular dysfunction [27].

Ivanova *et al* (2012), showed increased MMP-2 gene expression and activity after chronic doxorubicin treatment [18]. Polegato *et al* (2015) showed increased MMP-2 activity after acute doxorubicin treatment [19]. Cha *et al* (2010), and Esfahanian *et al* (2012), showed that Metformin treatment significantly decrease the MMP-2 level [28,29].

This study revealed that Metformin led to significant decrease ($P < 0.05$) in MMP-2 level in both Met + acute doxorubicin induction and Metformin + chronic doxorubicin induction groups when compared with acute and chronic doxorubicin induction groups. The MMP-2 suppressive effect of Metformin may be related to decreasing the oxidative stress that occurs by increase the activation of AMPK [30].

Conclusions

From these results, we can conclude that metformin has a cardioprotective effect against doxorubicin induced cardiotoxicity in acute and also the chronic induction. Metformin exhibited a cardioprotective effect by suppression of serum troponin I and the expression of MMP2 may be by the activation of AMPK.

Acknowledgments: The investigator would like to thank Al-Mustansiriyah University (www.uomustansiryiah.edu.iq) for its support in this study.

References

1- Albini A, Pennesi G, Donatelli F, Cammarota R, De Flora S, Noonan DM. Cardiotoxicity of anticancer drugs: the need for cardio-oncology and cardio-oncological prevention. J Natl Cancer Inst 2010; 102: 14–25

2- Angsutararux P, Luanpitpong S, Issaragrisil S. Chemotherapy-induced cardiotoxicity: overview of the roles of oxidative stress. Oxidative medicine and cellular longevity. 2015 Sep 29; 2015.

3- Bodai BI, Tuso P. Breast cancer survivorship: a comprehensive review of long-term medical issues and lifestyle recommendations. Perm J. 2015; 19: 48-79.

4- Carver JR, Shapiro CL, Ng A, Jacobs L, Schwartz C, Virgo KS, Hagerty KL, Somerfield MR, Vaughn DJ. American Society of Clinical Oncology clinical evidence review on the ongoing care of adult cancer survivors: cardiac and pulmonary late effects. Journal of Clinical Oncology. 2007 Sep 1;25(25):3991-4008.

5- Schimmel KJ, Richel DJ, van den Brink RB, Guchelaar HJ. Cardiotoxicity of cytotoxic drugs. Cancer treatment reviews. 2004 Apr 30;30 (2): 181-91.

6- Jones RL, Swanton C, Ewer MS. Anthracycline cardiotoxicity. Expert Opin Drug Saf 2006; 5: 791–809.

7- Lipp. Cardiotoxicity of cytotoxic drugs. Anticancer drug toxicity. Prevention, management and clinical pharmacokinetics. New York: Marcel Dekker; 1999. p. 471-488.

8- Octavia Y, Tocchetti CG, Gabrielson KL, Janssens S, Crijns HJ, Moens AL. Doxorubicin-induced cardiomyopathy: from molecular mechanisms to therapeutic strategies. Journal of molecular and cellular cardiology. 2012 Jun 30;52(6):1213-25.

9- Seicean S, Seicean A, Plana JC, Budd GT, Marwick TH. Effect of statin therapy on the risk for incident heart failure in patients with breast cancer receiving anthracycline chemotherapy: an observational clinical cohort study. J Am Coll Cardiol. 2012; 60:2384-2390.

10- Gong L, Goswami S, Giacomini KM, Altman RB, Klein TE. Metformin pathways: pharmocokinetics and pharmacodynamics. Pharmacogenet Genomics 2012; 22: 820-7

11- Nathan DM, Buse JB, Davidson MB, Ferrannini E, Holman RR, Sherwin R, Zinman B. Medical management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy. Diabetes care. 2009 Jan 1;32(1):193-203.

12- Russell III RR, Li J, Coven DL, Pypaert M, Zechner C, Palmeri M, Giordano FJ, Mu J, Birnbaum MJ, Young LH. AMP-activated protein kinase mediates ischemic glucose uptake and prevents post ischemic cardiac dysfunction, apoptosis, and injury. Journal of Clinical Investigation. 2004 Aug 16;114(4):495.

13- Adamia, N.; Virsaladze, D.; Charkviani, N.; Skhirtladze, M.; Khutsishvili, M. Effect of metformin therapy on plasma adiponectin and leptin levels in obese and insulin resistant postmenopausal females with type 2 diabetes. Georgian Med. News 145:52–55; 2007.

14- Ren J, Dominguez LJ, Sowers JR, Davidoff AJ. Metformin but not glyburide prevents high glucose-induced abnormalities in relaxation and intracellular Ca2+ transients in adult rat ventricular myocytes. Diabetes 1999; 48: 2059–2065.

15- Vaez H, Rameshrad M, Najafi M, Barar J, Barzegari A, Garjani A. Cardioprotective effect of metformin in lipopolysaccharide- induced sepsis via suppression of toll-like receptor 4 (TLR4) in heart. Eur J Pharmacol 2016, 772:115-123.

16- Cardinale D, Sandri MT, Colombo A, Colombo N, Boeri M, Lamantia G, Civelli M, Peccatori F, Martinelli G, Fiorentini C, Cipolla CM. Prognostic value of troponin I in cardiac risk stratification of cancer patients undergoing high-dose chemotherapy. Circulation. 2004; 109:2749-2754.
17- Ky B, Putt M, Sawaya H, French B, Januzzi JL, Sebag IA, Plana JC, Cohen V, Banchs J, Carver JR, Wiegers SE. Early increases in multiple biomarkers predict subsequent cardiotoxicity in patients with breast cancer treated with doxorubicin, taxanes, and trastuzumab. J Am Coll Cardiol. 2014; 63:809-816.
18- Ivanova M, Dovinova I, Okruhlicova L, Tribulova N, Simoncikova P, Barteková M, Vlkovičová J, Barančík M. Chronic cardiotoxicity of doxorubicin involves activation of myocardial and circulating matrix metalloproteinases in rats. Acta Pharmacol Sin 2012; 33:459-469.
19- Polegato BF, Minicucci MF, Azevedo PS, Carvalho RF, Chiuso-Minicucci F, Pereira EJ, Paiva SA, Zornoff LA, Okoshi MP, Matsubara BB, Matsubara LS. Acute doxorubicin-induced cardiotoxicity is associated with matrix metalloproteinase-2 alterations in rats. Cellular Physiology and Biochemistry. 2015;35(5):1924-33.
20- Daubert MA, Jeremias A. The utility of troponin measurement to detect myocardial infarction: review of the current findings. Vasc Health Risk Manag. 2010; 6: 691-699.
21- O'brien PJ, Smith DE, Knechtel TJ, Marchak MA, Pruimboom-Brees I, Brees DJ, Spratt DP, Archer FJ, Butler P, Potter AN, Provost JP. Cardiac troponin I is a sensitive, specific biomarker of cardiac injury in laboratory animals. Laboratory animals. 2006 Apr 1;40(2):153-71.
22- Mitry MA, Edwards JG. Doxorubicin induced heart failure: Phenotype and molecular mechanisms. IJC Heart & Vasculature. 2016 Mar 31; 10:17-24.
23- Basnet S, Kozikowski A, Makaryus AN, Pekmezaris R, Zeltser R, Akerman M, Lesser M, Wolf-Klein G. Metformin and Myocardial Injury in Patients with Diabetes and ST-Segment Elevation Myocardial Infarction: A Propensity Score Matched Analysis. Journal of the American Heart Association. 2015 Oct 27;4(10): e002314.
24- Apaijai N, Chinda K, Palee S, Chattipakorn S, Chattipakorn N. Combined vildagliptin and metformin exert better cardioprotection than monotherapy against ischemia-reperfusion injury in obese-insulin resistant rats. PloS one. 2014 Jul 18;9(7): e102374.
25- Ronnebaum SM, Patterson C, Schisler JC. Minireview: hey U (PS). metabolic and proteolytic homeostasis linked via AMPK and the ubiquitin proteasome system. Molecular Endocrinology. 2014 Aug 6;28(10):1602-15.
26- Spinale FG. Myocardial Matrix Remodeling and the Matrix Metalloproteinases: Influence on Cardiac Form and Function. Physiol Rev 2007; 87:1285-1342.
27- Kandasamy AD, Chow AK, Ali MA, Schulz R. Matrix metalloproteinase-2 and myocardial oxidative stress injury: beyond the matrix. Cardiovasc Res 2010; 85:413-423.
28- Cha HN, Choi JH, Kim YW, Kim JY, Ahn MW, Park SY. Metformin inhibits isoproterenol-induced cardiac hypertrophy in mice. The Korean Journal of Physiology & Pharmacology. 2010 Dec 1;14(6):377-84.
29- Esfahanian N, Shakiba Y, Nikbin B, Soraya H, Maleki-Dizaji N, Ghazi-Khansari M, Garjani A. Effect of metformin on the proliferation, migration, and MMP-2 and-9 expression of human umbilical vein

endothelial cells. Molecular medicine reports. 2012 Apr 1;5(4):1068-74.

30- Li L, Mamputu JC, Wiernsperger N, Renier G. Signaling pathways involved in human vascular smooth muscle cell proliferation and matrix metalloproteinase-2 expression induced by leptin. Diabetes. 2005 Jul 1;54(7):2227-34.