

Amelioration Of Doxorubicin Induced Cardiotoxicity

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الخلاصة

اجريت الدراسة الحالية للتعرف على التأثير العلاجي لكل من فيتامين E، والكارفيدلول والتلمسارتان والايوميغا-3 في التقليل من سمية القلب الناتجة عن الدوكسوربسين في الجرذان باستخدام الطرق الكيموحيوية. جرعت الجرذان بـ (1,100,10,20) ملغم من وزن الجسم يوميا بكل من فيتامين E والكارفيدلول والتلمسارتان والايوميغا-3 على التوالي لمدة 7 ايام وحقت بعدها تحت الخلب بجرعة مفردة من الدوكسوربسين. لوحظ وجود ارتفاع في مستوى الـ (LDH) والـ (CK-MB) والـ (MAD) ولوحظ ايضا حصول انخفاض معنوي في مستوى الـ (DHH) والـ (CK-MB) بعد العلاج بفيتامين E، الكارفيدلول، التلمسارتان والايوميغا-3 بالمقارنة مع المجموعة المعاملة بالدوكسوربسين. كما لوحظ حصول انخفاض معنوي في اكسدة الدهون (MDA) بعد العلاج بفيتامين E والكارفيدلول والتلمسارتان. يمكن الاستنتاج ان المواد المستخدمة في الدراسة الحالية لها تاثيرات وقائية اتجاه التسمم الحاد للقلب في الجرذان.

Abstract

The therapeutic usefulness of doxorubicin (DXR), an anthracycline antibiotic, is limited by its cardiotoxicity. The present study investigated the effects of vitamin E, carvedilol, telmisartan and omega-3 against doxorubicin-induced cardiotoxicity in rats using biochemical approaches. Telmisartan (1 mg/kg/day) orally, vitamin E (100 mg/kg) orally, carvedilol (10mg/kg) orally and omega-3 (20 mg/kg) orally were administered orally for 7 days followed by doxorubicin (15 mg/kg) was injected intraperitoneally (ip) as a single dose. Rats treated with DXR showed cardiotoxicity as evidenced by elevation of serum lactate dehydrogenase (LDH) activity, serum creatin kinase (CK-MB); serum malondialdehyde (MDA) level, catalase activity did not change in our study after treatment with doxorubicin. Post-treatment with vitamin E, carvedilol, omega-3 and telmisartan elicited a significant decrease in the activities of LDH and CK-MB in comparison with DXR-treated group. Furthermore, post-treatment with vitamin E, carvedilol, and telmisartan also significantly decreased lipid peroxidation (MDA level) in comparison with DXR group.

These results suggest that vitamin E , carvedilol, omega -3 and telmisartan treatment provides a significant protective effect against acute-doxorubicin induced cardiotoxicity in rat.

Introduction

Doxorubicin (DXR) is a broad-spectrum anticancer drug(Blum RH, and Carter SK: ,1974). Despite its broad therapeutic effectiveness, the clinical use of DXR is limited by a dose dependent and cumulative cardiotoxicity (Hrdina R. et al ,2000.).

The exact mechanism of DXR-induced cardiotoxicity remains unclear, but most studies support the view that oxidative stress plays an important role in the pathogenesis of DXR cardiotoxicity (Kumar D, et al, 2001). Cellular damage induced by doxorubicin is mediated by the formation of an iron-anthracycline complex that generates free radicals, which then cause severe damage to the plasma membrane and interfere with the cytoskeleton structure [Billingham ME, et al 1978). Due to the presence of less developed antioxidant defense mechanisms, heart is particularly vulnerable to injury by anthracycline-induced reactive oxygen species. Because liberation of free radicals is central to the mechanism of DXR-induced damage to the myocardium (Potemski P, et al , 2006), considerable efforts have been made to use antioxidants and iron chelators to protect the heart against DXR-toxicity. Many lines of evidence have suggested that the vitamin E, prevent cell damage by binding to the free radical and neutralising its unpaired electron. For example, vitamin E binds to $OO\cdot$ or $O_2\cdot$ they form an intermediate structure that is converted to α -tocopherylquinone(Kendler, BS,1997). Renin-angiotensin system (RAS) plays an important role in the development of cardiac hypertrophy, failure and reperfusion injury (Baker KM, et al , 1992). Suppression of the RAS ameliorates the remodeling process of heart and prolongs long-term survival in animal models and humans with cardiac hypertrophy, failure and reperfusion injury (Dunn FG, et al , 1984). (Toko et al. 2002)reported the non-toxic effect of doxorubicin on cardiac muscle of angiotensin II type 1a receptor (AT1) knockout mice, indicating that AT1 mediated angiotensin II (Ang II) signaling pathway plays an important role in the doxorubicin induced cardiac impairment. Telmisartan is a nonpeptide AT1 receptor

antagonist which selectively and insurmountably inhibits AT1 receptor subtype (Maillard MP, et al 2002)without affecting other systems involved in cardiovascular regulation (Wienen W and Entzeroth M. 1994). Carvidelol is a lipophilic nonselective b-adrenoceptor antagonist with strong antioxidant effect (Yue et al., 1992a). Carvedilol, a new vasodilator and b-adrenergic blocking agents adrenoceptor antagonist, is an antioxidant and free radical scavenger. (Tanaka Y, et al 2004) assessed the Efficacy of n-3 polyunsaturated fatty acid enriched enteral nutrient solution in relieving oxidative stress in patients with severe psychophysiologic disorders. The aim of the present study was to investigate the possible effects of vitamin E ,carvedilol,omega-3 and telmisartan against DXR-induced cardiotoxicity in rats using biochemical markers of oxidative stress and cellular damage.

Materials and Methods

Animals

Thirty male adult Sprague-Dawely rats were enrolled in this study. The animals were obtained from the Animal House in Kufa Medical College. Their weight was (90-150)g and age between 3-6 months. The rats were housed in Kufa Medical College Animal House in (43 x 27 x 15)cm cages and kept at 25 °C and 12 hours light-dark cycles with 12.00 AM being the mid dark period. Rats had free access to drinking water and libitum. After two weeks of adaptation, rats were divided into five groups , each group comprise six rats .

Blood sample was taken from each one befor starting drug treatment by direct heart puncture and considered as control for each group from which the baseline value of experimental parameters was measured. After that the groups where divided as follow :-

Group1 :- received single intraperitoneal (I.P) dose of doxorubicin (15mg/kg) .After 48 hours of receiving the dose ,blood samples were taken and the value of experimental parameters was measured.

Group 2 :- received vitamin E orally by stomach tube (100 mg / kg) for seven cumulative days followed by single (I.P) dose of doxorubicin (15 mg / kg) at the seventh day, after 48 hours of receiving the dose ,blood samples were taken and the value of experimental parameters was measured.

Group 3 :- received omega 3 free fatty acid orally by stomach tube (20 mg / kg) for seven cumulative days followed by single (I.P) dose of doxorubicin (15mg/kg) at the seventh day, after 48 hours of receiving the dose ,blood samples were taken and the value of experimental parameters was measured.

Group 4 :- received carvedilol (10 mg/kg) orally by stomach tube for seven cumulative days followed by single (I.P) dose of doxorubicin (15mg / kg) at the seventh day, after 48 hours of receiving the dose ,blood samples were taken and the value of experimental parameters was measured.

Group 5 :- received telmisartan (1 mg / kg) orally by stomach tube for seven cumulative days followed by single (I.P) dose of doxorubicin (15mg / kg) at the seventh day, after 48 hours of receiving the dose ,blood samples were taken and the value of experimental parameters was measured.

Drugs and chemicals

List of Chemicals

Chemicals	Company
H ₂ O ₂	Analar
KH ₂ PO ₄	Analar
K ₂ HPO ₄	Analar
Na ₂ HPO ₄	Analar
TBA	Merck Co. Ltd.
TCA	Hopkin and Williams.

Doxorubicin was obtained from (Ebewe , Australia), carvedilol tablet was obtained from (Roche, Germany), telmisartan (micardis) from (Boehringer Ingelheim, Germany), vitamin E from (Jamieson laboratories, Canada) and omega-3 from (Jamieson laboratories, Canada) , lactate dehydrogenase (LDH) assay kit from (Biomaghreb, Morocco) , Creatine kinase (CK-MB) assay kit from (Biolabo SA, France)

Determination of lactate dehydrogenase (LDH) activity

LDH activity was estimated in serum by commercially available kit using an UV-visible spectrophotometer (Shimadzu).

Determination of lipid peroxide level

Lipid peroxide level was estimated by thiobarbituric acid (TBA) reaction with malondialdehyde (MDA), a product formed due to the peroxidation

of membrane lipids. To 0.15 ml serum sample the followings are added: 1 ml TCA 17.5 %, 1 ml of 0.6% TBA, solutions are mixed well by vortex, incubated in boiling water bath for 15 minutes, and then allowed to cool. Then 1 ml of 70% TCA is added, and the mixture is allowed to stand at room temperature for 20 minutes, centrifuged at 2000 rpm for 15 minutes, and the supernatant is taken out for scanning spectrophotometrically.

$$\text{The concentration of MDA (M)} = \frac{\text{Absorbance at 532 nm}}{\text{L} \times \text{€}} \times \text{D}$$

L: light path (1 cm).

€: extinction coefficient $1.56 \times 10^5 \text{ M}^{-1} \cdot \text{cm}^{-1}$.

$$\text{D: dilution factor} = \frac{\text{Total volume}}{\text{Volume of the sample}}$$

Determination of catalase (CAT) activity

0.05 ml serum is diluted with 5 ml phosphate buffer immediately before measurement.

reagents	sample	Blank
Phosphate buffer solution	-----	1 ml
Diluted serum	2 ml	2 ml
H ₂ O ₂	1 ml	-----

-The reaction occurs immediately after the addition of H₂O₂.

-Solutions are mixed well and the first absorbance (A1) is read after 15 seconds (t1) and the second absorbance (A2) after 30 seconds (t2).

The absorbance is read at wave length 240 nm.

Calculation

$$K = \frac{V_t}{V_s} \times \frac{2.3}{\Delta t} \times \text{Log} \frac{A_1}{A_2} \times 60$$

K= Rate constant of the reaction.

Δ t = (t2 – t1) =15 seconds.

A1= absorbance after 15 seconds.

A2= absorbance after 30 seconds.

Vt = total volume (3 ml).

Vs = volume of the sample (2 ml).

2.6: Measurement of Serum Creatine kinase (CK-MB) :- (Stein W. 1961).

activity was estimated in serum by commercially available kit using an UV-visible spectrophotometer (Shimadzu).

Results

Effect on MDA

There was statistically significant increase in serum MDA level (P<0.05) in all groups except the group 4 as shown in table (1) and figure(1) There was statistically significant differences (P<0.05) in the means of serum MDA level between the group1 and other four groups treated by different drugs as shown in table (2).

Table (1): Effect of different groups treatment on rat serum MDA in µmol/L

Groups	At zero time	After 10 days	P value
Group 1	3.27±0.52	14.8±0.45	P<0.05
Group 2	2.98±0.29	7.87±0.99	P<0.05
Group 3	3.32±0.35	17.3±0.9	P<0.05
Group 4	3.27±0.37	2.9±0.45	P>0.05
Group 5	3.17±0.29	11.1±0.37	P<0.05

The values are expressed as mean ± SEM.

Table (2) : Multiple comparison between different groups mean values of serum MDA in µmol/L by using LSD test

Groups	Group 2	Group 4	Group 3	Group 5
Group 1	6.93*	11.9*	-2.49*	3.69*
Group5	3.24*	8.22*	-6.18*	
Group 3	9.43*	14.4*		
Group 4	-4.98*			

* P<0.05

Effect on CPK

There was statistically significant increase in serum CKP level (P<0.05) in all groups except the carvedilol group and vitamine E group as shown in table (3) and figure (2)

There was statistically significant differences (P<0.05) in the means of serum CKP level between the doxorubicine group and other four groups treated by different drugs as shown in table (4).

Table (3): Effect of Doxorubicine , Vitamine E, Omega-3, Carvidilol and Telmesartan on rat serum CK-MB in IU/L

Groups	At zero time	After 10 days	P value
Group 1	685.8±115.4	4759.1± 137.7	P<0.05
Group 2	587.6±116.2	1008.7±181.9	P>0.05
Group 3	731.5±143.2	1713.6±196	P<0.05
Group 4	677.8±130.4	1247.4±225.8	P>0.05
Group 5	666.7±146.1	1926.2±155.4	P<0.05

The values are expressed as mean ± SEM.

Table (4) : Multiple comparison between different groups mean values of serum SK-MB (IU/L) by using LSD test

Groups	Group 2	Group 4	Group 3	Group 5
Group 1	3750.4*	3511.7*	3045.5*	2832.9*
Group 5	917.5*	678.8*	212.6	
Group 3	704.9*	466.2		
Group 4	238.7			

* P<0.05

Effect on LDH

There was statistically significant increase in serum LDH level (P<0.05) in all groups as shown in table (5)

There was statistically significant differences (P<0.05) in the means of serum LDH level between the doxorubicine group and other four groups treated by different drugs as shown in table (6).

Table (5): Effect of Doxorubicine , Vitamine E, Omega-3, Carvidilol and Telmesartan on rat serum LDH in IU/L

Groups	At zero time	After 10 days	P value
Group 1	578.8±72.3	4328.1±209.5	P<0.05
Group 2	585.1±51.4	1494.9±55.1	P<0.05
Group 3	555.3±48.7	1775.4±58.8	P<0.05
Group 4	564.5±66.9	1045.6±74.2	P<0.05
Group 5	570.4±31.9	3108.5±248.9	P<0.05

The values are expressed as mean ± SEM.

Table (6) : Multiple comparison between different groups mean values of serum LDH (IU/L) by using LSD test

Groups	Group 2	Group 4	Group 3	Group 5
Group 1	2833.3*	3282.5*	2552.7*	1219.6*
Group 5	1613.6*	2062.9*	1333.1*	
Group 3	280.6	729.8*		
Group 4	- 449.3*			

* P<0.05

Effect on Catalase

There was no statistically significant difference in serum catalase level (P>0.05) in all groups as shown in table (7) There was no significant differences (P>0.05) in the means of serum catalase level between the doxorubicine group and other four groups treated by different drugs as shown in table (8).

Table (7): Effect of Doxorubicine , Vitamine E, Omega-3, Carvidilol and Telmesartan on rat serum catalase in IU/L

Groups	At zero time	After 10 days	P value
Group 1	0.06±0.005	0.045±0.003	P>0.05
Group 2	0.055±0.004	0.047±0.001	P>0.05
Group 3	0.058±0.003	0.046±0.003	P>0.05
Group 4	0.058±0.006	0.049±0.004	P>0.05
Group 5	0.057±0.002	0.055±0.008	P>0.05

The values are expressed as mean ± SEM.

Table (8) : Multiple comparison between different groups mean values of serum catalase (IU/L) by using LSD test

Groups	Group 2	Group 4	Group 3	Group 5
Group 1	-0.0013	-0.0042	-0.001	-0.0097
Group 5	0.0083	0.0055	0.0088	
Group 3	-0.0005	-0.0033		
Group 4	0.0028			

* P<0.05

Discussion

Acute DXR-induced cardiotoxicity alters the organization of the cardiomyocyte and induces apoptosis, which is a potentially modifiable and preventable form of myocardial tissue loss (Arola OJ, et al 2000). This potentially novel mechanism is transient, but it may be of a key importance to the ensuing heart failure. The results of this study have confirmed that a single dose of DXR (20 mg/kg *ip*) induces acute cardiotoxicity in rats which is in agreement with previous studies (Buyukokuroglu ME, et al 2004). Existing evidence points to a multitude of molecular mechanisms involved in DXR-induced cardiac dysfunction. An important factor, which can mediate the toxic action of DXR, especially in mitochondria, is high affinity binding of DXR to cardiolipin, an anionic phospholipid in the inner mitochondrial membrane, which is essential in eukaryotic energy metabolism (Goormaghtigh E. et al, 1986). Cardiolipin-bound DXR would induce dissociation of cardiolipin-associated peripheral proteins from the inner mitochondrial membrane, like e.g. cytochrome c and mitochondrial creatine kinase (MtCK). This could affect electron transport chain and energy channeling, as well as may favor initiation of programmed cell death (Tokarska-Schlattner M, et al, 2005).

Toxicity of mitochondrial, mostly cardiolipin-bound DXR is mediated by oxidative stress, which represents particular threats to cellular energy metabolism in the myocardium and is considered to be the main mediator of DXR cardiotoxic action. Two different mechanisms of free radical formation by DXR have been described. The first implicates the formation of a semiquinone free radical by the action of several NADPH-dependent reductases that produce a one-electron reduction of the DXR to the corresponding DXR semiquinone. In the presence of oxygen, redox cycling of DXR-derived quinone-semiquinone yields superoxide radicals ($O_2^{\cdot-}$). In the second, DXR free radicals are produced by a non-enzymatic mechanism that involves reaction with iron. Iron-DXR complex can reduce oxygen to H_2O_2 and other active oxygen forms (Tokarska-Schlattner M, et al, 2005). Superoxide anion ($O_2^{\cdot-}$) generated by DXR is transformed to hydrogen peroxide by superoxide dismutase and further detoxified by catalase or glutathione peroxidase. Superoxide can also get converted to peroxynitrite ($ONOO^-$)

via reaction of ($O_2^{\bullet-}$) with nitric oxide. Hydrogen peroxide subsequently leads to the formation of hydroxyl radicals ($OH\bullet$), which is considered to be most damaging (Chattopadhyay A, and Bandyopadhyay D 2006), greatly enhances the NADH-dependent microsomal lipid peroxidation and thus initiates a lipid radical chain reaction causing oxidative damage to cell membranes.

Effect on MDA

Effect of doxorubicin on MDA

In this experiment, MDA levels were significantly elevated after a single dose of DXR as shown in table (1) and (2) and supported the hypothesis that a major role is played by free radicals in DXR cardiotoxicity (Minotti G. 1990) the effect of doxorubicin on lipid peroxidation was discussed before.

Effect of carvedilol on MDA

MDA was significantly decreased after carvedilol treatment as shown in table (1) and (2), result is in agreement with This (Yue TL et al, 1993). This result can be explained by the fact that Carvedilol inhibited superoxide ion release from activated neutrophils (Yue et al., 1992c). Carvedilol preserves the endogenous antioxidant systems (i.e., vitamin E and glutathione) that are normally consumed when tissues or organs are exposed to oxidative stress (Lysko et al., 1995), (Yue et al. (1992a) have reported that carvedilol inhibits lipid peroxidation by scavenging free radicals, while some others reported that carvedilol is not free radical scavenger but rather sequester of ferric ion (Tadolini & Franconi, 1998; Noguchi et al., 2000).

Effect of vitamin E on MDA

MDA was significantly decreased after vitamin E treatment as shown in table (1) and (2). result is in agreement with (Emma A. et al, 2001). This can be explained by that vitamin E allow free radicals to abstract a hydrogen atom from the antioxidant molecule rather than from poly unsaturated fatty acids, thus breaking the chain of free radical reaction. The resulting antioxidant radicals being a relatively unreactive species (Pascoe, et al, 1987). In many studies vitamin E neutralizes lipid peroxidation and unsaturated membrane lipids because of its oxygen scavenging effect (John 2001)

Effect of telmisartan on MDA

MDA was significantly decreased after telmisartan treatment as shown in table (1) and (2). result is in agreement with (Keun-Hwa et al, 2007). This can be explained that reactive oxygen species (ROS) are involved in many of the Ang II signalling pathways. Ang II stimulates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity via the AT1 receptor to produce the superoxide anion, hydrogen peroxide, and hydroxyl radicals, which are reported to exert effects on intracellular growth-related proteins and enzymes to mediate the final biological responses.(5)

Effect of omega-3 on MDA

MDA was significantly increase after omega-3 treatment as shown in table (1) and (2). This result is in agreement with (H Grundt et al, 2003), Dolores Parra et al 2002) found that (EPA, DHA) decrease oxidative stress markers.(Matti Narkia, 2008) found that Omega-3 PUFAs from fish and fish oil do not increase oxidative stress nor lipid peroxidation. Our result can be explained by the fact that PUFAs create oxidative stress in biological system as they undergo lipid peroxidation, forming free radicals such as peroxy and alkoxy radicals. Although these lipid hydroperoxides are relatively short-lived, their breakdown results in the formation of secondary products of lipid peroxidation (aldehydes such as malondialdehyde and the 4-hydroxyalkenals) that are longer-lived and can attack a variety of cellular targets(Kenneth A, 2002).

Effect of carvedilol on lipid peroxidation

MDA was significantly decreased after carvedilol treatment as shown in table (1) and (2). result is in agreement with (Yue et al, 1992b) Carvedilol, a new vasodilator and β -adrenoreceptor antagonist, inhibits oxygen- radical-mediated lipid peroxidation in swine ventricular membranes. this result can be explained by the fact that Carvedilol may suppress the formation of lipid radicals via two mechanisms:

- 1) by scavenging oxygen radicals in aqueous phase, therefore preventing oxygen radical-initiated lipid peroxidation and reducing formation of lipid radicals, and
- 2) by scavenging lipid radicals directly, thus breaking the chain reaction in membranes.(TL Yue et al, 1993). injury to endothelial cells Carvedilol, a new antihypertensive agent, prevents lipid peroxidation

and oxidative Carvedilol has been reported to prevent Fe²⁺/vitamin C-induced depletion of α -tocopherol from brain homogenates (Yue et al., 1992a; Lysko et al., 2000) have reported that carvedilol inhibits lipid peroxidation by scavenging free radicals, while some others reported that carvedilol is not free radical scavenger but rather sequester of ferric ion (Tadolini & Franconi, 1998).

Effect on catalase

Effect of doxorubicin on catalase

Serum catalase level did not significantly changed after doxorubicin treatment as seen in table (7) and (8) , (Hossam M.et al 2005) found that serum catalase notably lowered after doxorubicin treatment (15mg/kg).D Alessandro et al ,1981 and calaiborne A ,1985 found that catalase activity increase significantly after doxorubicin treatment.

Effect of vitamin E on catalase

Catalase did not significantly change after vitamin E treatment as seen in table (7) and (8), (S.A et al 2004) found that vitamin E has significantly decreased the level of antioxidant enzymes (CAT).

Effect of omega-3 treatment on catalase

Catalase did not significantly change after omega-3 treatment as seen in table (7) and (8). (Kukoba et al ,2005) found that omega-3 PUFAs caused the beneficial effects on activity of antioxidant enzymes in cardiac tissue (increased superoxide dismutase and catalase activity).

Effect of carvedilol on catalase

Catalase did not significantly change after carvedilol treatment as seen in table (7) and (8) Huang He et al ,2006) found that carvidilol treatment increase the activities of CAT,sod,and GSH-Px in healthy control and diabetic rat significantly.

Effect of temisartan on catalase

Catalase did not significantly change after temisartan treatment as seen in table (7) and (8) .(Muzaffar et al 2008) found that telmisartan treatment significantly decrease catalase activity after doxorubicin treatment .

Protective effects of telmisartan against acute doxorubicin-induced cardiotoxicity in rats .

Effect on LDH**Effect of doxorubicin treatment on LDH**

LDH activity significantly increased after doxorubicin treatment as shown in table (5) and (6) result is in agreement with (Muzaffar et al ,2008).the rapid cell swelling of sub-sarcolemmal bulbs and injured myocardium could facilitate the loss of intracellular enzymes in doxorubicin treated rats ,this might be the possible mechanism for increased levels of LDH in serum.

Effect of vitamin E on LDH

LDH activity significantly decreased after vitamin E treatment as shown in table (5) and (6) result is in agreement with (Ihsan Salah El Din Hedayat* & Khaled Shaban Azab,2004) ,(Sridharan & Shyamadevi 2002), attributed that the increase of LDH and CPK are due to the excessive production of free radicals and lipid peroxides that might have caused leakage of cytosolic enzymes and to membrane cell damage which are susceptible for radiation damage. (Przybyszewski. et al. 1994) reported that the application of vitamin E diminished the MDA and the activity of LDH and CPK in serum of gamma irradiated rats.

Effect of carvedilol on LDH

LDH activity significantly decreased after carvedilol treatment as shown in table (5) and (6) result is in agreement with (Zhonghua et al 2005).he order of potency for inhibition of lipid peroxidation was positively correlated with the order of potency for prevention of cell injury and death among carvedilol, suggesting that cell damage (LDH release) and loss of viability were secondary to the primary event of lipid peroxidation (Yue T. et al, (1992b). Carvedilol, a new vasodilator and b-adrenoreceptor antagonist, inhibits oxygen-radical-mediated lipid peroxidation in swine ventricular membranes.Carvedilol dose dependently inhibited xanthine-xanthine oxidase induced LDH release . (Yue T. et al, (1995).

Carvedilol, a new vasodilating b-adrenergic blocker, inhibits oxidation of low-density lipoproteins by vascular smooth muscle cells and prevents leukocyte adhesion to smooth cells.

Moreover, carvedilol produced a dose-dependent protection against cell death induced by oxygen free radicals, and the effective concentrations

were compatible with the antioxidant activity of carvedilol (Giora Feuerstein et al, 1999).

Effect of telmisartan on LDH

LDH activity significantly decreased after telmisartan treatment as shown in table (5) and (6) result is in agreement with (Muzaffar et al, 2008). The reduction in the activity of serum LDH in animals treated with telmisartan showed the suppression of cardiac injury by AT1 receptor antagonism.

Effect on CK-MB

Effect of doxorubicin on CK-MB

CK-MB activity significantly increased after doxorubicin treatment as shown in table (3) and (4) result is in agreement with (Xiuhua and Y. James 2002). Few of the plethora of mechanisms that have been characterized in the recent years in relation to the mechanisms of anthracyclines-induced cardiotoxicity including, inhibition of nucleic acid and protein synthesis (Olson & Mushlin 1990), change in adrenergic function and adenylate cyclase (Kalyanaraman et al. 2002), lipid peroxidation (Pacher et al. 2003), release of iron (II) from mitochondrial aconitase (Vasquez-Viver et al. 2000), semiquinone intermediate (daunorubicin^{••})-generated superoxide radical and hydrogen peroxide production which act as proapoptotic agents in endothelial cells and myocytes (Wang et al. 2002), depletion of intracellular antioxidants reserve (Saad et al. 2004), disruption of mitochondrial membrane potential with cytochrome C release, increased caspases activity, and peroxynitrite formation due to nitric oxide synthase induction (Mihm et al. 2002). Pritsos et al. (1992), reported that ebselen protects against doxorubicin-induced lipid peroxidation in heart and liver tissue and doxorubicin-induced toxicity in general.

Effect of vitamin E on CK-MB

CK-MB activity significantly decreased after vitamin E treatment as shown in table (3) and (4), result is in agreement with (Mukesh Nandave et al, 2007) Singh Arya. vit E showed cytoprotective activity of it as evidenced by significant restoration of myocardial CK-MB activity, preservation of myofibrils and mitochondrial morphology. Improved hemodynamics, antioxidant, anti-peroxidative and myocardial preservative effects contribute to the overall cardioprotective action of

vitamin E.(Mukesh Nandave,2007) .the administration of vitamin E to the doxorubicin treated rat showed the protective effect on the myocardium with significant decrease in the extent and severity of myocardial damage (S.A Ayaz et al 2004).this protective changes by vitamin E might be due to its antioxidant potential ,where it is known that vitamin E act as peroxy radical trapping chain breaking antioxidant along with free radical scavenging property(Tappel, 1980).

Effect of omega-3 on CK-MB

CK-MB activity significantly decreased after omega-3 treatment as shown in table (3) and (4).result is in agreement with (Oskarsson et al.1993) , Omega-3 fatty acids found in fish oil have various biological properties that may modify myocardial injury caused by severe ischemia and perfusion.

Effect of carvedilol on CK-MB

CK-MB activity significantly decreased after carvedilol treatment as shown in table (3) and (4).result is in agreement with (Santos et al.2002) .carvedilol decrease the extent of cellular vacuolization in cardiac myocytes and prevented the inhibitory effect of doxorubicin on mitochondria I respiration in both heart and liver Carvedilol also prevented the decrease in mitochondrial Ca super loading capacity and the inhibition of the respiratory complexes of heart mitochondria caused by doxorubicin (Santos et al 2002).

Effect of telmisartan on CK-MB

CK-MB activity significantly decreased after telmisartan treatment as shown in table (3) and (4) .result is in agreement with(Keun-Hwa et al, 2007) . the AT1-mediated actions of angiotensin II on blood pressure regulation, vascular reactivity, cell growth, and apoptosis (Wright et al., 2002). The relative stimulation of the AT2R, as an indirect result of AT1R antagonism, may contribute to the overall effects of the AT1R antagonists during pathophysiological processes. The inhibition of AT1R induces eNOS expression in injured tissue via the activation of AT2R (Thai et al., 2003).In addition, nitric oxide from eNOS can be protective against oxidative stress and excitotoxic damage Bright and dark sides of nitric oxide in ischemic brain injury (Agnoletti et al., 1999).

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