Comparative study of cardio protective effect between enalapril and dexrazoxane against doxorubicin induce toxicity

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Abstract: -

Doxorubicin (DOX)is one of the anthracycline family. It is a highly effective anticancer drug. Several studies focused on the role of DOX in anticancer activities, but the cardiotoxicity is the most dangerous side effect. The experimental-based finding on heart injury reduced by dexrazoxane(DXZ) treatment. DXZ is a first-line drug for the treatment of iron poisoning, which is a chelating agent considered as an antidot for DOX cardiotoxicity, and also enalapril, which is an angiotensin converting enzyme (ACE) inhibitor used to reduce cardiotoxicity. In the present study, 48 male rats were included. The DXZ was given in a dose of 200mg/kg intraperitoneal (IP) three times weekly for seven days. And compare with enalopril given at a dose of 2 mg/kg administered orally via oral gavage for 7 days. On day 7, DOX at a dose of 20 mg/kg IP was administered. At the end of the experiment, biomarkers such as troponin-I(TN-I) and glutathione(GSH) were measured. Necrosis on cardiac tissue was also measured. As a result, coadministration of enalapril with DOX and the coadministration of DXZ with DOX reduced the DOX cardiotoxicity but in different ways. Finally, campers the cardioprotective effect of enalapril and DXZ against DOX cardiotoxicity. In conclusion, DXZ was seen as more effective than enalapril against DOX cardiotoxicity. Since DXZ produces dangerous side effects in the same case, enalapril can be used instead of it.

Keywords: - doxorubicin, dexrazoxane, cardiotoxicity, cardioprotective, enalopril.

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Introduction: -The heart is a complex organ that pumps blood through the body with an intricate system of muscle layers, chambers, valves, and nodes. It has its own circulation system and receives electric impulses that make it contract and relax, which triggers a sequence of events that form the cardiac cycle. Pathologic conditions, including exposure to toxicants, result in pathology of cardiac myocytes. (1)

Cardiotoxicityis defined as a reduction in left ventricular ejection fraction (LVEF) of 5% to 55% with symptoms of heart failure (HF) or asymptomatic with LVEF reduction of 10% to 55% in patients receiving chemotherapy. [2]

1-Oxidative stress (OS) is particularly harmful to the heart because the heart simply requires a large and consistent supply of ATP, making it extremely susceptible to poisoning. And because the heart's antioxidant capacity is low, it is prone to the effects of OS. The glutathione GS-peroxidase and catalase levels in cardiac muscle, for example, are quite low. [4]

2- Mitochondrial dependent reactive oxygen species (ROS): -Mitochondria is the most commonly injured organelles. DOX poisoning has an effect on subcellular organelles. DOX is a cationic drug that is retained in the mitochondrial inner membrane by forming an irreversible complex with cardiolipin. Because the electron-transport chain proteins require cardiolipin binding to function properly, more superoxide union (O2-.) formation occurs because the cardiolipin–protein interface is disrupted by DOX. [5]

3-Iron–Doxorubicin complex: Iron DOX compounds (recycled) and doxorubicinol (a DOX metabolite) are known to interact with the thiol groups of proteins, promoting cell damage. According to research, DOX has no effect on iron metabolism and its interactions with the iron enzyme aconitase-1 protein regulator. The cell releases free iron as a result of this contact, preventing the iron from being translated. [6,7].

4- Apoptosis: -Doxorubicin appeared to cause apoptosis in cardiomyocyte by a number of mechanisms. It can activate intrinsic mitochondrial pathway by disrupting the

molecular mechanism of DOX- induced cardiotoxicity*:*Several mechanisms are involved in DOX cardiotoxicity, including oxidative stress, reactive oxygen species(ROS)
formation and intracellular calcium dysregulation. [3]
cardiolipin, an importantcomponent in the membrane of mitochondria.[8]

Cardiotoxicity caused by doxorubicin is classified as follows:

A-Acute cardiotoxicity occurs at or shortly after the start of treatment. This is usually temporary and self-limiting. [9]

B-Chronic cardiotoxicity is the most prevalent and serious form of anthracycline cardiotoxicity. This manifests as LV systolic dysfunction, which is subtle at first and asymptomatic, but can develop into dilated cardiomyopathy and overt CHF, which is often irreversible. [10]

Prevention of doxorubicin cardiotoxicity: 1-Administration of Cardioprotective Drugs:

A-DXZ*:-the* iron chelator ethylenediaminetetraacetic acid (EDTA), which is used to treat a variety of conditions, is potentially protective against anthracycline-related cardiotoxicity. [11]

B-ACE inhibitorsare blood pressure-lowering medications. ACE, which converts angiotensin I to angiotensin II, is inhibited by ACEI. And it effects on oxidative mechanism. [12]

2-Changes in Chemotherapy Administration*:* adjusting the dose, pharmacological structure, and chemotherapy administration timing.[12]

3-exercise Physical activity at various intensities is conducted before, during, and or after chemotherapy treatment improvescirculatory reserve and lowers cardiotoxicity in doxorubicin-treated individuals. [13]

Materials and methods: -

Chemicals and reagents

doxorubicin (Hubei vanz pharm.china), enalapril maleat (changsha.china) ,dexrazoxane(USA) distilled water (PDPL; India).

Experimental animals

Forty-eight previously untreated male rats were used. (140-200 gm) weight. In well ventilated conditions, food, water, as well as a regular light-dark cycle. The animals were housed at the animal house of the College of Pharmacy and Mustansyriah University. This study began in November 2021 and finished in February 2022.

treatments and animal grouping

Induction of heart toxicity by DOX According to drug instructions, each 1ml of solution contains

2mg of DOX. The rats were divided randomly into six groups, each consisting of eight rats:

Group I: The control group; rats in this group were treated with a single IP of distill water (DW). for 7 days (eight rats).

Group II: The DOX group was treated with DOX (20 mg/kg) IP in a single dose. [14] (eight rats).

Group III: The enalopril group was treated with enalopril (2 mg/kg/day, every day for 7 days) orally. [15] with gavage and on day 7 was given DW IP in a single dose. (8 rats).

Group IV (treatment 1) rats were given enalopril (2 mg/kg/day, every day for 7 days) orally via gavage, and on day 7, they were given DOX (20 mg/kg) IP in a single dose.*(8 rats).*

Group V*:*-DXZ group were treated with DXZ (200mg/kg) IP three times weekly. [16] (eight rats) and on day seven were given DW IP in a single dose. (8 rats).

Group VI: The (treatment 2) rats were given dexrazoxane (200mg/kg) IP three times weekly and DOX (20mg/kg) IP in a single dose on day seven.(8 rats).

Blood samples were collected directly from the heart by-heart puncturing. S. TN-I level and oxidative stress biomarkers (glutathione, GSH) were measured for each sample by the ELISA technique (Enzyme Linked Immunosorbent Assay).

The heart is immediately removed and washed with distilled water, and finally 10% formalin (neutral buffer formalin) is added for preservation for histopathology study.

Statistical analysis:

Group namber	**Type of treatment**	**S. troponin I(Mean±SD) (nmol/ml)**
Group I	DW	301.65 ± 14.375
GroupII	DOX	1918.33 ±77.84
GroupIII	enalapril	303.8 ±10.48
GroupIV	Enalapril+DOX	543.33 ±48.44
GroupV	DXZ	303.166 ±8.998
GroupVI	DXZ +DOX	520±26.076

Each value expressed as mean ± stander deviation SD. The statistical analysis done by using one wayANOVA followed by Tukey test. To fined P- value .

Result :

table -1-Effect of dexrazoxane and enalapril on doxorubicin induced changes on serum cardiac troponin-I level of rats:-

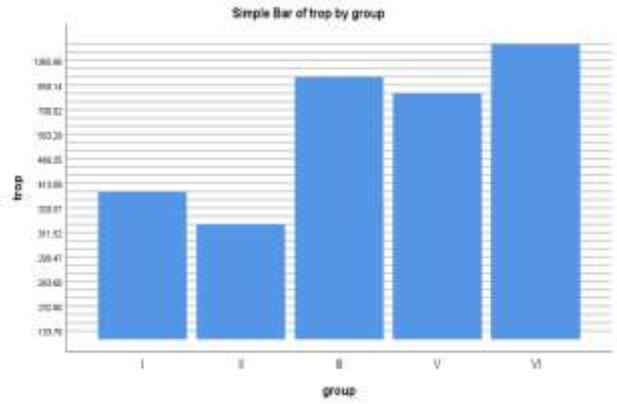


Fig -1- The bar chart of disparity shows the serum level of troponin effect of enalapril and DXZ on DOX cardiotoxicity

In table 1and figure1 result group II (DOX) showed significant increased (p-value < 0.05) of troponin-I levels in serum; in comparison to group IP-value=0.001 . In contrast, group IV showed a significant
decrease in serum levels of TN-I when compared to group II (*P value=0.019*< 0.05), and also group VI showed a significant decrease in serum levels of TN-I when compared to group II (*P value=0.004*< 0.05) . non-significant differences in TN-I level when compared group IV with group VI (*P*-value > 0.05)

In table 2and figure2 results in group II showed significant decreased (p-value=0.001<0.05) in GSH levels when compared to group I. while GSH level in group IV was a significantly increased when compared with group II (*p-value=0.012*< 0.05) and also GSH level in group VI was a significantly increased when compared with group II (*p-value=.0003*< 0.05).

Table (2): Effect of enalapril and DXZ on serum GSH level against DOX cardiotoxicity

Group namber	Type of treatment	S. GSH (M± SD)(nmol/ml)
Group I	**DW**	**49.833 3.256**
GroupII	**DOX**	**13.735 ± 1.7421**
GroupIII	**enalapril**	**48.666± 5.125**
GroupIV	**Enalapril+DOX**	**24.5 ± 2.81**
GroupV	**DXZ**	**50.33 ± 4.6332**
GroupVI	**DXZ +DOX**	**31 ± 1.414**

Histopathological changes before and after cardioprotective therapy.

Enalapril and DXZ improved the cardioprotective effect against DOX by histopathology change of cardiac tissue. The result for all groups is illustrated below:

In group I ,V, and III There were no discernible difference. The cardiac myocyte in there group

is normal . Striation in a normal myofibrillar structure

Group II: cardiac myocyte swelling, nuclear change (some nuclei undergo pyknosis, while others are lost through karyolysis) that leads to cell necrosis or apoptosis, cytoplasmic vacuoles, eosinophilic infiltration, oedema, and striation loss.

In comparison to group II, group IV showed mild necrosis, cytoplasmic vacuoles, mild oedema, and myofibrillar structure with relatively well preserved striation. And also, group VI showed mild necrosis, cytoplasmic vacuoles, mild oedema, and myofibrillar structure with striation relatively well preserved in comparison with group II .

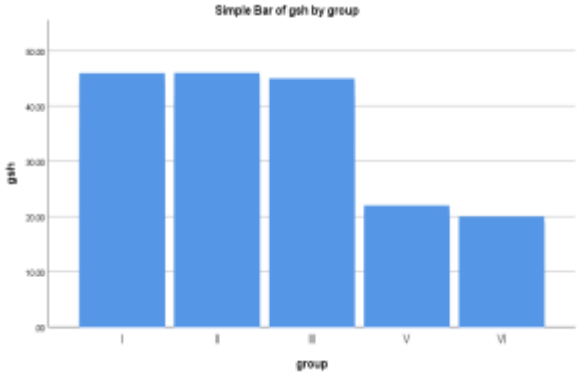
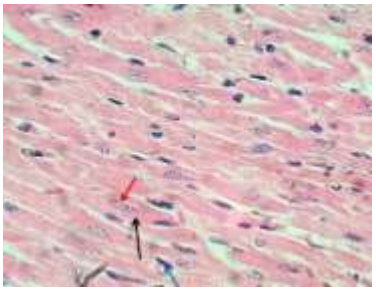
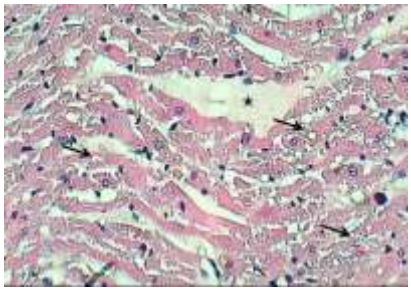


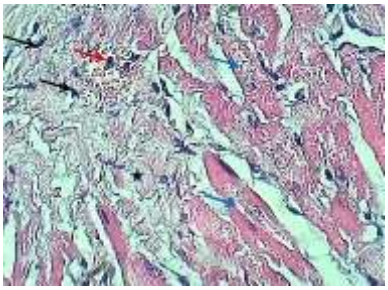
Fig-2- The bar chart of disparity shows the serum level of GSH effect of enalapril and DXZ on DOX cardiotoxicity



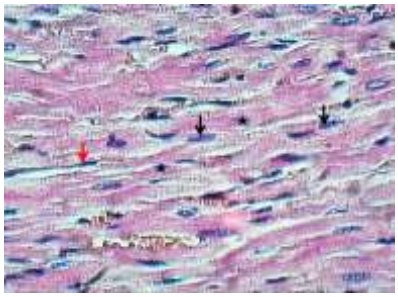
Group I



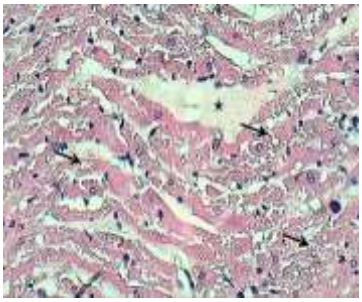
Group II



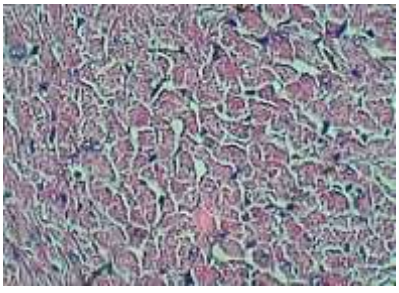
group III



Group IV



Group V



Group VI

Figure -3-Histopathological result of heart tissue for all study groups

DISCUSSIOIN

Doxorubicin is one of the most frequently utilized anticancer drugs in clinical practice. The cardiotoxicity side effects severely limit its use as the main mechanism for creating ROS. Cardiotoxicity raises the risk of death and morbidity in patients [17].

to reduce cardiotoxicity from DOX, several investigations have revealed. Pharmacological medicines can protect you against the effects of DOX cardiotoxicity[18].

Enalapril is one of the ACE inhibitor drug classes. It also has a protective effect againstmyocardial ischemia-reperfusion injury. The result of the present study clarifies the effect of enalopril on cardiac enzyme TN-I level and oxidative enzyme as well as on heart histology study in DOX induced cardiac damage [19].

Dexrazoxane is a chelating medication with cytoprotective characteristics that is used to prevent and treat cardiomyopathy caused by DOX therapy. The current study's findings also shed light on the effect of DXZ on cardiac enzyme levels and oxidative enzymes.

as well as in a heart histology study in which DOX induced cardiac damage.

DXZ is the most perfect DOX antidot. According to several studies [20],

This study revealed that enalapril and DXZ led to a significant decrease (P 0.05) in plasma TnI level. Enalapril and DXZ significantly reduce cardiac TnI levels by enalapril and DXZ by activation of endothelial NO [21].

Authors support that enalapril and DXZ have significant cardioprotective effects on cardiac injury in animal model studies through increasing the tolerance to ischemic damage by decreasing the DOX induced-cardiotoxicity [22].

After exposure to a toxic dose of DOX in campers taking enalapril and DOX, the p value was 0.004.

The results obtained in the present study are consistent with other studies which showed DXZ is a more effective antidot for DOX cardiotoxicity than other studies. Whereas the level of GSH was found to be significantly reduced in the DOX treated group as in Table 2 and Figure 2 and then returned to normal in the cardiac GSH serum level was significantly reduced in group IV compared with group II. P = 0.012 (P = 0.05).

and also significantly reduced in group IV in comparison with group II. P value = 0.003,

This could occur as a result of the oxidative stress induced by DOX, which may lead to the production of free radicals (such as O2**.**NO**)** [23].

Reactive oxygen metabolism related to DOX may overcome the heart limited capacity in order to detoxify free radicals, which result in myocardial

cells oxidative damage. These effects suggested that the reduced GSH levels contributed partly to the reduction in the capacity to defense oxidative stress decreased in GSH level, which leads to cellular destruction [23].

The treatment with enalapril or DXZ enhanced the antioxidant defense system independently of glycemic control. DXZ has a direct scavenging effect against ROS, reestablished the antioxidant system, and therefore improves the level of oxidative stress. Earlier studies have confirmed the protecting effect of DXZ in reducing OS, and restoration of ferric reducing antioxidant power. [24].

The primaryeffect of enalapril or DXZ is the inhibition of mitochondrial complex I and the mitochondrial complex I might contributed substantially to the production of cellular ROS. It is well recognized that the inhibition of this complex by enalapril or DXZ leads to decreased ROS production leading to increase in GSH level. [25].

Histopathological changes before and after cardioprotective therapy:

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The cardiac myocyte in the control and enalapril or DXZ groups of this study is normal, showed centrally located nuclei, normal myofibrillar structure.

with striation. While in DOX groups, swelling of cardiac myocyte, necrosis, cytoplasmic vacuoles, eosinophilic infiltration, oedema, and loss of striation were observed. DOX causes changes in the normal morphology of cardiomyocytes, including necrosis.

myofibrillar loss, vacuolization, and mononuclear cells infiltration due to the action of oxidative stress that considered as an indication of cardiac injury and

DOX 20mg/kg (cumulative dose) causes myocyte edema, myocyte vacuolization and loss of myofibrils[26].

In this study, both DOX + enalapril groups show mild necrosis, cytoplasmic vacuoles, mild oedema and myofibrillar structure with striation relatively well preserved in comparison with DOX groups. It is reported that oral therapy of enalapril (10 mg/kg) reduces histopathological changes produced by DOX (20 mg/kg cumulative dose), thus exerting a cardioprotective effect[27]

And also, in this study, both DOX + DXZ groups showed mild necrosis, cytoplasmic vacuoles, mild oedema and myofibrillar structure with striation relatively well preserved in comparison with DOX groups. It was reported that IP therapy of DXZ (200 mg/kg) removed histopathological changes produced by DOX (20 mg/kg cumulative dose), so exerting a cardioprotective effect [27].

The DXZ treatment reduced DOX-induced myocardial alterations, reported by normal appearance of muscle fibers, less widening of interstitial space, and moderate

interstitial cellular infiltration, no myocytic degeneration, and normal nuclear location [28].

The histopathology change of group IV and group VI against group II was not a wide difference, which may predict display DXZ with enalapril in some cases.

Conclusion :-

Enalapril and DXZ co-administration markedly have a good cardioprotective effect against doxorubicin cardiotoxicity by many mechanisms.DXZ is more effect than enalapril and act as antidot for DOX .but have side effect may by limiting use.Enalapril co-administration markedly reduced the size of necrosis

and other histopathological changes in myocardial tissue. these effects similar to DXZ effect and no extremely differ

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