

PROTECTIVE EFFECT OF EXOGENOUS REDUCED NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE (NADPH) ON DOXORUBICIN-INDUCED CARDIOTOXICITY IN ADULT ALBINO RATS

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ABSTRACT

Background: Doxorubicin (DOX) is a potent chemotherapeutic drug that was widely used for the treatment of various types of cancer. It produces free radicals which result in extreme dose-limiting cardiotoxicity. **Objective:** the present study aimed to investigate the potential cardioprotective effect of exogenous NADPH, against doxorubicin-induced cardiotoxicity in albino rats. **Material and Methods:** 36 rats were divided into five groups: negative control (group I), positive solvent control (group II), which were subdivided into subgroup IIa (saline): they received (0.9% NaCl) (solvent of Doxorubicin), and subgroup IIb (modified saline) (solvent of exogenous NADPH): they received modified saline with PH: 8 (normal saline +10% NaOH). Group III (exogenous NADPH): the given dose was (8 mg/kg/day) iv/day for 7 days. Group IV (Doxorubicin): a single dose was (25 mg/kg), and it was given i.p. on the 7th day. Group V (Doxorubicin + exogenous NADPH): rats received a combination of Doxorubicin given in a single dose (25 mg/kg) i.p. on the 7th day and exogenous NADPH given in a dose (8mg/kg/day); iv/day for 7 days. **Results:** ECG analysis was done before and after treatment. On the 8th day, besides ECG analysis, cTnI, CK-MB, LDH, IL1 β , TNF α , Caspase-3, MDA and GSH were analyzed. Also, histopathological evaluation of isolated hearts was performed by light microscopy. DOX had significantly altered ECG, cTnI, CK-MB, LDH, IL1 β , TNF α , Caspase-3, MDA and GSH. Pre-treatment with NADPH significantly alleviated DOX-induced ECG changes and also guarded against DOX-induced rise of cTnI, CK-MB, LDH, IL1 β , TNF α , and Caspase-3 levels. NADPH also alleviated histopathological alteration in DOX-treated rats. Moreover, it significantly inhibited DOX-induced GSH depletion and elevation of MDA. **Conclusion:** It can be mentioned that exogenous NADPH alleviates doxorubicin-induced cardiotoxicity. NADPH exerts these cardioprotective effects through different mechanisms of antioxidant, antiapoptotic, and anti-inflammatory effects.

Keywords: Cardiotoxicity, Doxorubicin, Exogenous NADPH and Rats

INTRODUCTION

Doxorubicin (DOX) is an anthracycline chemotherapy drug used in the treatment of various types of cancer. However, short-term and long-term cardiotoxicity limits the clinical

application of DOX (Saleh et al., 2020).

Currently, dexrazoxane is the only approved treatment by the United States Food and Drug Administration to prevent DOX-induced

cardiotoxicity. However, a recent study found that pre-treatment with dexrazoxane could not fully improve the cardiac toxicity of DOX (Lisa et al., 2019).

Therefore, further targeted cardioprotective prophylaxis and treatment strategies are an urgent requirement for cancer patients receiving DOX treatment to reduce the occurrence of cardiotoxicity. (Wang et al., 2021)

Dose-dependent cardiotoxicity caused by DOX may occur early at the onset of treatment and even up to many years after completion of treatment (McGowan et al., 2017).

There are several mechanisms proposed for the cardiotoxic effects of doxorubicin including free radical-induced myocardial injury, lipid peroxidation (Renu et al., 2018), mitochondrial damage, decreased activity of Na⁺ K⁺ATPase, vasoactive amine release, impairment in myocardial adrenergic signaling/regulation, increase in serum triglyceride, total cholesterol and low-density lipoproteins. The generation of reactive oxygen species (ROS) like superoxide anion and hydrogen peroxide by doxorubicin leads to the impairment of cell functioning and cytolysis (Wang et al., 2021).

Since the discovery of doxorubicin, among all mechanisms, oxidative stress is the most frequently proposed mechanism to explain the complex pathophysiology of DOX Induced Cardiotoxicity (Qi et al., 2020). The myocardial injury is evidenced by lipid peroxidation which occurs as a result of the increase of ROS production, including superoxide (O₂⁻) and hydroxyl radicals (OH[·]) as well as other non-radicals such as hydrogen peroxide (H₂O₂), singlet oxygen (O₂), etc. (Henriksen, 2018).

As a result of the presence of less developed antioxidant defense

mechanisms, the heart is particularly vulnerable to injury by DOX-induced ROS. Liberation of free radicals is the main mechanism of doxorubicin-induced damage to the myocardium; it also causes the elevation of serum enzymes like lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) (Zare et al., 2019).

Inflammation plays a crucial role in both the initiation and progression of cardiovascular diseases, where several immune cells and chemokines are involved in the inflammatory pathway (Ding et al., 2015). Tumor necrosis factor (TNF) in particular induces ROS formation, cardiomyocyte hypertrophy, and apoptosis (Ping et al., 2017).

So, to counteract the harmful consequences of ROS generation, several types of pharmacologic agents, including antioxidants, hematopoietic cytokines, and iron-chelating agents, were used in conjunction with DOX during treatment (Zhao and Tong, 2012).

Reduced nicotinamide adenine dinucleotide phosphate (NADPH), also known as reduced coenzyme II, is a phosphorylated derivative of the 2'-position of the ribose ring attached to adenine in nicotinamide adenine dinucleotide (NAD⁺) (Ying, 2007).

Reduced (NADPH) is the main reducing power in the cell. It forms two separate reducing pools in the cytosol and mitochondria (Lewis et al., 2014).

The principal biological function of NADPH is to provide redox capacity for the antioxidant system such as the thioredoxin system, the glutathione system and the catalase enzyme (Xiao et al., 2018). Moreover, NADPH is the main hydrogen and electron donor in reductive biosynthesis reactions involved in protein and lipid synthesis also, it shares indirectly in DNA synthesis (Agledal et al., 2010). Recent studies proved that exogenous NADPH could be beneficial in treating acute

pathological conditions, such as myocardial injury and renal ischemia-reperfusion injury (I/R/I) (Zhu et al., 2020; Weng et al., 2018). The objective of this study is to evaluate the effect of exogenous reduced NADPH on doxorubicin-induced cardiotoxicity.

AIM OF THE WORK

The present work was conducted to assess the possible protective effect of exogenous NADPH against doxorubicin-induced cardiotoxicity in adult albino rats.

MATERIAL AND METHODS

I- Material:

1- Animals:

This study was carried out in the medical research center, pharmacology department, Faculty of Medicine, Benha University.

The present experimental work was performed on 36 adult albino rats of both sexes (body weight 180 - 200 gm.) which were obtained from the experimental animal center of Helwan (Egypt). Every six animals were kept in each cage in a fully ventilated room (at room temperature) and were allowed free access to food (balanced diet) and water without any medications for one week of adaptation in their new environment before the experiment.

After taking the specimens, the remains of the animals were incinerated in Benha University Hospital's Incinerator.

This research was accepted by the local ethical committee for scientific research, Benha Faculty of Medicine, Benha University.

2- Chemicals:

Exogenous NADPH and Doxorubicin (DOX) chemical powder were purchased from Sigma Chemical Company, USA.

Assay commercial kits for cardiac Troponin I, creatine kinase-MB (CK-MB), and lactate dehydrogenase (LDH) estimation were obtained from Spin lab (Spin react Company, Spain), and (Guangzhou Wondfo Biotech Co., Ltd, China).

ELISA kits of the proinflammatory cytokines (TNF- α , IL- 1β) and apoptotic marker caspase-3 estimation were obtained from commercially available stores (Cloud-clone Corp., USA). Reagents for biochemical and histopathological analysis were obtained from Sigma-Aldrich Chemical Company, USA.

II- Methods:

Experimental Design:

Dose, route and duration of study:

The widely used therapeutic dose of doxorubicin is 60–75 mg/m² 1V once every 21 days to treat varieties of cancers; this dose is equivalent to 20–25 mg/kg in rats (Hajra et al., 2018)

Doxorubicin was given with a dose (25 mg/kg) (single dose); intraperitoneal (i.p.) On the 7th day. It was prepared immediately before use by dissolving it in normal saline (0.9% NaCl) (Ahmed et al., 2021)

Exogenous NADPH was given with a daily dose (8 mg/kg) intravenous injection (i.v) in the tail vein for 7 days. NADPH is unstable in an acidic solution; so saline was first adjusted to pH 8.0 with 10% NaOH. It was prepared immediately before use by dissolving it in modified saline with PH: 8 (normal saline +10% NaOH) (Zhu et al., 2020).

Groups

Rats were randomly divided into 5 groups and treated as follows: **Group I (negative control):** (6 rats) left without intervention to measure the basic parameters, free access to water and food was allowed for 7 days.

Group II (positive solvent control): (12 rats) animals of this group were subdivided into 2 subgroups

Subgroup IIa (saline): (6 rats) received normal saline (0.9% NaCl) (solvent of Doxorubicin), 1 ml/kg (single dose); i.p. on the 7th day (Ahmed et al., 2021).

Subgroup IIb (modified saline): (6 rats) received modified saline with PH: 8 (normal saline +10% NaOH) (solvent of exogenous NADPH); 1 ml i.v/day for 7 days (Zhu et al., 2020).

Group III (Exogenous NADPH): (6 rats) rats of this group received exogenous NADPH; it was given in a dose of 8 mg/kg/day dissolved in 1 ml modified saline; it was given iv/day for 7 days (Zhu et al., 2020).

Group IV (Doxorubicin): (6 rats) rats of this group received Doxorubicin; Doxorubicin was given in a dose of 25 mg/kg dissolved in 1 ml normal saline (0.9% NaCl), was given (a single dose); i.p. on the 7th day (Ahmed et al., 2021).

Group V (Doxorubicin + Exogenous NADPH): (6 rats) rats of this group received a combination of Doxorubicin which was given in a dose (25 mg/kg) (a single dose) i.p. on the 7th day and exogenous NADPH which was given in a dose (8mg/kg/day); iv/day for 7 days.

On the 8th day (24 hours after the administration of doxorubicin), rats were anesthetized with ether inhalation at the end of the experiment, an ECG examination was done, the thoracic cavity was opened, and blood samples were collected from the heart and processed for biochemical measurements.

A) Electrocardiographic Examination (ECG):

The cardiac screening was done for all the experimental animals using ECG to avoid the inclusion of any animal having a cardiac abnormality.

On the 8th day, rats in all groups and subgroups were anesthetized and led on their back. ECG records were done using needle electrodes. The four limbs electrodes were fixed to the animal's four limbs and records were done using an ECG electrocardiograph KIT-03M for recording electrocardiograms (Medexport-USSR). The standard lead II at a rate of 25mm/min was used for recording electrocardiograms. Lead II was used because it is more informative in rats than other leads (Chan et al., 1987)

B) Biochemical study:

1- Cardiac Enzymes

Blood samples were obtained from rats' hearts. Samples were centrifuged for 10 min at 3000 r.p.m. Sera were put in the tubes to be tested for:

a. Cardiac troponin I (cTnI): Cardiac troponin I (cTnI) level was calculated by fluorescence immunochromatographic analyzing system using the commercial test of troponin I (Guangzhou Wondfo Biotech Co., Ltd), China.

b. Creatine kinase-MB (CK-MB): Creatine kinase-MB (CK-MB) was calculated spectrophotometrically using the commercial test of CK-MB with spin lab (Spinreact company), Spain

c. Lactate dehydrogenase (LDH): Lactate dehydrogenase (LDH) level was measured spectrophotometrically using the commercial test of LDH with spin lab (Spinreact company), Spain

2- Oxidative stress indices:

The malondialdehyde (MDA) concentration was determined using the method described by Chattopadhyay et al., (2003).

3- Antioxidants:

Reduced glutathione (GSH) was measured using the method of Beutler et al., (1963).

4- Estimation of proinflammatory cytokines and apoptotic marker:

The pro-inflammatory cytokines (TNF- α , IL-1 β) and apoptotic marker caspase-3 were determined in cardiac tissues using commercially available ELISA kits (Cloud-clone Corp., USA).

C) Histopathological study:

The heart was divided into two parts; one part was placed in 10% formalin for histopathological examination, and the 2nd part was used fresh frozen. Heart specimens were fixed in 10% formaldehyde buffer then embedded in paraffin and cut into 5 μ m thickness sections according to the routine procedure. The sections were stained with Hematoxylin and Eosin (H&E) for routine histopathological examination, and examined under a light microscope (BX-50; Olympus) according to **Lamberg and Rothstein, (1978)**.

Histopathological examination of heart samples was done at the pathology Department, Faculty of Medicine, Benha University

D) Statistical Analysis

The collected data were tabulated and analyzed using SPSS version 16 software (SpssInc, Chicago, ILL Company). Data were expressed as mean \pm standard deviation. They were tested for normality using the Shapiro-Wilks test assuming normality t $P > 0.05$. Differences among normally distributed variables were tested using a one-way analysis of variance (ANOVA) test. Significant ANOVA tests were followed by post hoc multiple comparisons using Bonferroni tests to detect significant pairs. The accepted level of significance in this work was stated at 0.05 ($P < 0.05$ was considered significant). Data are presented as mean (M) \pm SD

RESULTS

A. Electrocardiographic (ECG) changes:

The results of the ECG examination obtained from all groups and subgroups are illustrated as follows

Groups I, IIa, IIb, III and V show regular, sinus rhythm and normal heart rate.

Group IV (Doxorubicin) shows tall T wave, prolonged P-R interval, prolonged Q-T interval, depressed S-T segment and tachycardia.

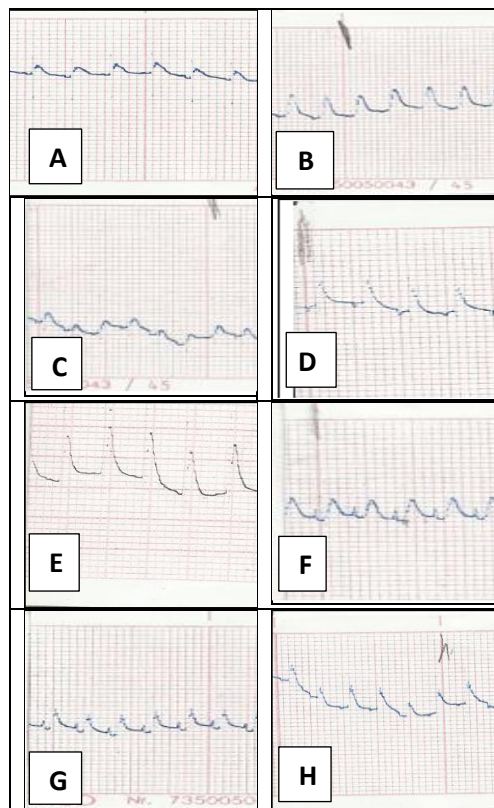


Fig. (1): ECG trace of all studied groups showing:

A: Regular, sinus rhythm and normal rate (300-333/min) of group I (negative control).

B: Regular, sinus rhythm and normal rate of subgroup IIa (saline) (positive solvent control)

C: Regular, sinus rhythm and normal rate of subgroup IIb (modified saline) (positive solvent control)

D: Regular, sinus rhythm and normal rate of group III (Exogenous NADPH)

E: Regular, sinus rhythm, and tall T wave of group IV (Doxorubicin).

F: Regular, sinus rhythm, prolonged P-R interval, prolonged Q-T interval and

depressed S-T segment of group IV (Doxorubicin).

G: Regular, sinus rhythm and tachycardia of group IV (Doxorubicin)

H: Regular, sinus rhythm and normal rate of group V (Doxorubicin+Exogenous NADPH).

B. Biochemical study:

In the present work, negative control and positive solvent control groups showed non-significant differences ($p>0.05$) as regard biochemical parameters (cardiac enzymes, MDA, GSH, TNF- α , IL-1 β and caspase-3), as shown in Table 1. So, the mean of all control groups was chosen as a representative group for the three control groups to be compared with the results of the treated groups.

As regard the levels of cardiac enzymes, MDA, GSH, TNF- α , IL-1 β and caspase-3, a significant difference ($p<0.05$) between studied groups was observed, as illustrated in tables 2-4.

The present work detected a significant ($p< 0.05$) increase in serum

cardiac enzymes, MDA, TNF- α , IL-1 β and caspase-3 levels and a significant ($p< 0.05$) reduction in serum GSH level in group IV (Doxorubicin group) as compared to the control group.

A significant ($p< 0.05$) reduction in serum cardiac enzymes, MDA, TNF- α , IL-1 β and caspase-3 levels and a significant ($p< 0.05$) increase in serum GSH level in Group V (Doxorubicin + Exogenous NADPH group) as compared to group IV (Doxorubicin group).

A significant ($p< 0.05$) reduction in serum cardiac enzymes, MDA, TNF- α , IL-1 β and caspase-3 levels in Group V (Doxorubicin + Exogenous NADPH group) as compared to controls but there is a non-significant increase in serum GSH level in Group V (Doxorubicin + Exogenous NADPH group) as compared to controls.

Table (1): Comparison between control groups regarding (cardiac enzymes, MDA, GSH, TNF- α , IL-1 β and caspase-3)

	Troponin I (ng/L)		CK-MB (ng/ml)		LDH (U/L)		MDA (nmol/ml)		GSH (mg/ml)		TNF- α (pg/mg)		IL-1 β (pg/mg)		caspase-3 (ng/mg)	
	Mean	\pm SD	Mean	\pm SD	Mean	\pm SD	Mean	\pm SD	Mean	\pm SD	Mean	\pm SD	Mean	\pm SD	Mean	\pm SD
-Group I (negative control)	1.414		61.75	3.546	112	4.817	5.57	0.309	4.75	0.44	48.5	6.83	106.8	4.87	0.52	0.03
-Group IIa (solvent saline control)	1.966		60.5	4.505	116.3	2.066	5.36	0.469	5.058	0.217	53.3	4.54	115	11.8	0.57	0.06
-Group IIb (solvent modified-saline control)	1.169		61.66	3.266	116.8	3.371	5.44	0.413	5.08	0.64	54.5	5.01	115.8	6.24	0.58	0.02
P	>0.05 (NS)		>0.05 (NS)		>0.05 (NS)		>0.05 (NS)		>0.05 (NS)		>0.05 (NS)		>0.05 (NS)		>0.05 (NS)	

NS: non-significant

Table (2): Comparison between studied groups regarding cardiac enzymes (troponin I, CK-MB, and LDH)

Groups	Troponin I (ng/L)		CK-MB (ng/ml)		LDH (U/L)	
	Mean	± SD	Mean	± SD	Mean	± SD
Controls	16.94	1.62	61.30	3.62	115.06	4.05
Group III (Exogenous NADPH)	17.5	2.66	60.16	3.97	120.83	2.64
Group IV (Doxorubicin)	48.62 [^] #	1.366	184.83 [^] #	6.33	179.17 [^] #	8.77
Group V (Doxorubicin+ Exogenous NADPH)	27.33 ^{*+}	2.42	77.83 ^{*+}	7.08	131.17 ^{*+}	1.94
P	<0.05 (S)		<0.05 (S)		<0.05(S)	

(S): significant with Doxorubicin [^]: significant with control #: significant with Exogenous NADPH *: significant with Doxorubicin +: significant with control

Table (3): Comparison between studied groups regarding serum MDA and serum GSH levels

Groups	MDA (nmol/ml)		GSH(mg/ml)	
	Mean	± SD	Mean	± SD
Controls	5.45	0.387	4.96	0.46
Group III (Exogenous NADPH)	5.36	0.779	4.57	0.508
Group IV (Doxorubicin)	11.95 [^] #	0.705	1.1 [^] #	0.34
Group V (Doxorubicin+ Exogenous NADPH)	7.33 ^{*+}	0.742	4.57 ^{*\$}	0.346
P	<0.05 (S)		<0.05 (S)	

(S): significant [^]: significant with control #: significant with Exogenous NADPH *: significant with Doxorubicin +: significant with control \$: non-significant with control

Table (4): Comparison between studied groups regarding (TNF- α , IL-1 β and caspase-3)

Groups	TNF- α (pg/mg)		IL-1 β (pg/mg)		caspase-3 (ng/mg)	
	Mean	\pm SD	Mean	\pm SD	Mean	\pm SD
Controls	52.11	5.86	112.56	8.76	0.56	0.056
Group III (Exogenous NADPH)	62.33	6.80	129.5	5.95	0.55	0.035
Group IV (Doxorubicin)	349.67 ^{^#}	74.210	446.33 ^{^#}	64.95	1.57 ^{^#}	0.43
Group V (Doxorubicin+ Exogenous NADPH)	207.67 ^{*+}	34.74	215 ^{*+}	20.43	0.87 ^{*+}	0.033
P	<0.05 (S)		<0.05 (S)		<0.05(S)	

(S): significant ^: significant with control #: significant with Exogenous NADPH
*: significant with Doxorubicin +: significant with control

C. HISTOPATHOLOGICAL RESULTS:

Histopathological results supported the evidence of biochemical parameters analyzed in this study, as sections of the rat heart show the following:

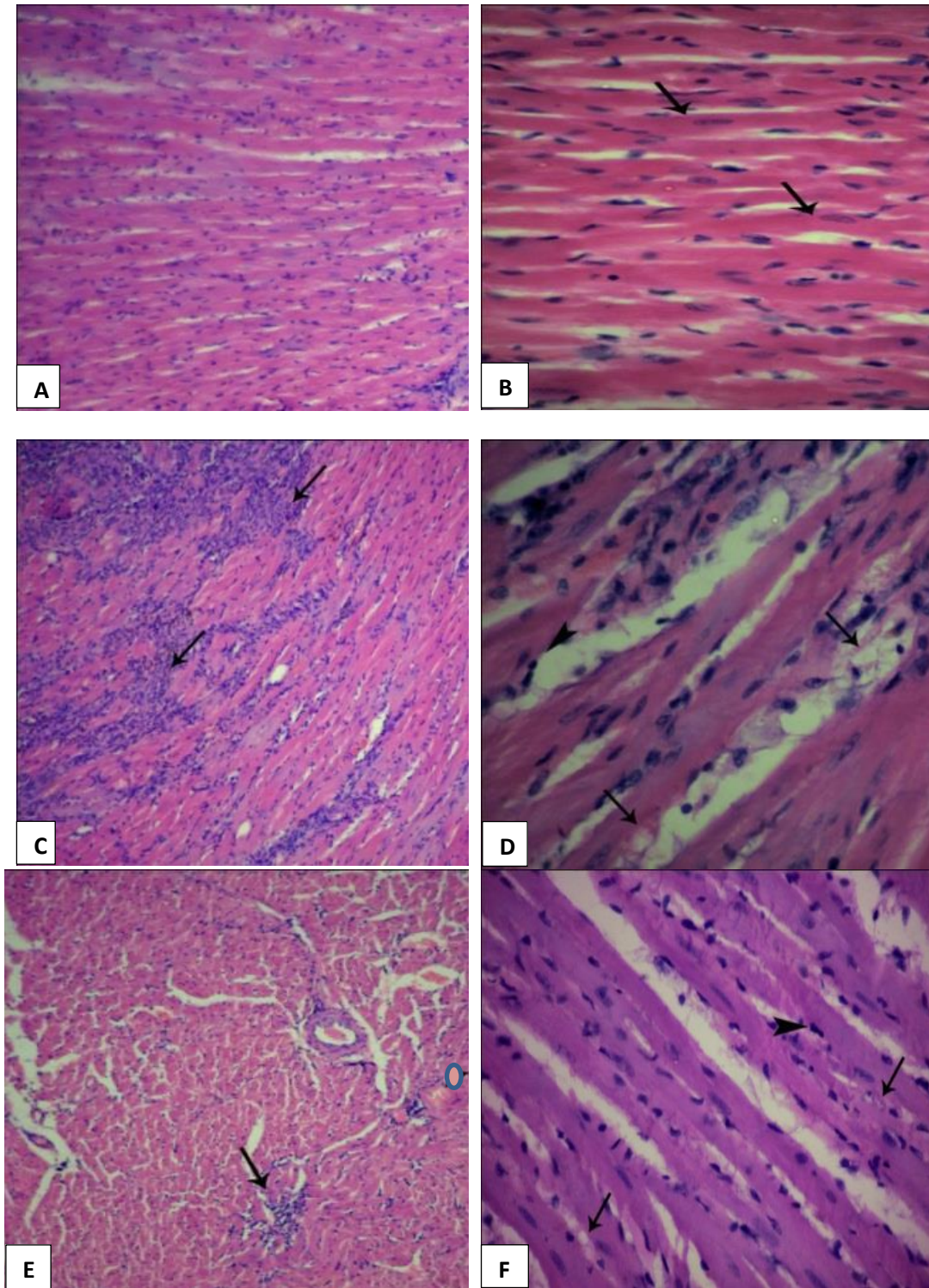


Fig. (2): Photomicrographs of sections from rats' hearts of all groups showing:

A: Normal myocardial tissue of the control group with branching and anastomosing cardiac muscle fibers with uniform diameters (H&E 125 X).

B: Normal myocardial tissue of the control group with longitudinal cut muscle fibers with acidophilic cytoplasm and central vesicular nuclei (↑). Notice the diameter of the cardiomyocytes (H&E 250X)

C: Large areas of mononuclear cellular infiltration (↑) of the doxorubicin group (H&E 125x)

D: Longitudinal muscle fibers of the doxorubicin group with marked vacuolation of the sarcoplasm (↑) and the nuclei appear deeply stained small and pyknotic (arrowhead). Notice the apparent increase in the cardiomyocyte diameter in relation to the control group (H&E 250 X).

E: Small areas of mononuclear cellular infiltration (↑) of Doxorubicin+ Exogenous NADPH group (H&E 125X).

F: Longitudinal muscle fibers of Doxorubicin+ Exogenous NADPH group with little vacuolation of the sarcoplasm (↑) and some nuclei appear deeply stained small and pyknotic (arrowhead).

Notice the apparent decrease in the cardiomyocyte diameter in relation to the doxorubicin group (H&E 250X).

DISCUSSION

The pathogenesis of Doxorubicine-induced cardiotoxicity is multifactorial and includes several mechanisms (**Abdel-Daim et al., 2107**). Many detrimental effects have been related to DOX-induced cardiotoxicity, including oxidative stress, lipid peroxidation, DNA/RNA damage, autophagy suppression, endoplasmic reticulum-mediated apoptosis, Superoxide anions and hydroxyl radicals generated during the metabolism of DOX lead to cellular membrane injury (**Wu et al., 2018**).

The present study was designed to evaluate the cardioprotective effect of exogenous reduced NADPH on DOX-induced cardiotoxicity in rats and the possible underlying mechanisms of this protection.

The current work has demonstrated acute cardiotoxicity with a single dose of DOX administration in experimental albino rats. We observed that DOX has significantly altered ECG waves in the form of tall T wave /prolonged PR interval /depressed ST segment /prolonged QT and normal QRS complex amplitude. The findings of all these ECG changes in the present study were supported by previously reported

studies done by **Aygun and Gul., (2019)**.

An altered membrane function due to DOX-induced lipid peroxidation might be responsible for most of the ECG changes (**Emeka and Al-Ahmed., 2017**).

We observed that the administration of exogenous reduced NADPH has a significant protective effect on these acute changes in ECG.

The same results were obtained by **Ma et al., (2019); and Fatma et al., (2021)** who reported that the cardiotoxicity induced by DOX was evidenced by ECG changes in which prolonged QT interval occurred.

In contrast to our results, the studies done by **Kumar et al., (2012) and Jensen et al., (1984)** have documented that DOX produce nonspecific ST changes and the Detection of the ST segment in rat ECG was stated to be difficult, as the T wave always increases in continuity with the S wave.

In agreement with the present study, **Reyes et al., (2015)** found that NADPH was markedly depleted in isolated rats' hearts subjected to myocardial ischemia-reperfusion (I/R). Also, **Zhu et al., (2020)** reported that

administration of exogenous NADPH has protective effects against myocardial I/R injury and ameliorates acute changes in ECG.

In the present study, DOX treatment showed a significant rise in the level of CK-MB, LDH and cardiac troponin (cTnI) if compared to control groups, however, the pretreatment of DOX-treated rats with exogenous reduced NADPH showed a significant decrease in the level of these cardiac injury markers. Moreover, treatment with DOX showed also a significant increase in the MDA level and a significant reduction in the GSH level if compared to control groups. While pretreatment of DOX-treated rats with exogenous reduced NADPH showed a significant decrease in the level of MDA and a significant increase in GSH level if compared to DOX treated rats' group.

Cardiac enzyme biomarkers such as cardiac troponin (cTnI), creatinine kinase (CK), and lactate dehydrogenase (LDH) are released into the circulation when myocardial infarction or damage to myocytes occurs increasing their levels in the bloodstream and thus used as prognostic indicators for major cardiovascular disorders. CK adds a phosphate group to creatinine to produce high-energy phosphocreatine, where its increase could be indicative of cardiac muscle damage (Wang et al., 2019). The activity of (LDH), an enzyme widely expressed in tissues increases after about 18 hours post myocardial infarction and remains elevated for about one week (El-Bakry and Abd Elrahman., 2017).

Cardiac troponin I (cTnI) is released from cardiomyocytes into the bloodstream in case of myocardial damage, so it is a specific biomarker for cardiac injury. It is the gold standard non-invasive diagnosis of myocardial injury, as it increases significantly 4 hours after myocardial

infarction and remains high for seven days (Hammadi et al., 2015)

Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) that detoxify free radicals, are produced under oxidative stress disorders induced by DOX (Nadia et al., 2020). As the heart lacks the antioxidant enzymes for scavenging free radicals, thus free radicals may accumulate and cause peroxidation of lipids and cardiomyocytes apoptosis (Zare et al., 2019), thus malondialdehyde (MDA) as a marker of lipid peroxidation increases and antioxidant enzymes, SOD and GSH decrease (Nadia et al., 2020).

The result of the present study was in accordance with Zhu et al., (2020) who reported that NADPH administration decreased circulating LDH levels in cardiac ischemia-reperfusion injury (I/R/I) in rats. This result may be due to the potent effect of NADPH on protecting the mitochondria and preserving energy production (Xia et al., 2009).

As regards the effect of pretreatment with NADPH on DOX-treated rats on (cTnI) level our result is in line with Nagah et al., (2022) who reported that co-administration of NADPH with aluminum phosphide-induced cardiotoxicity in a rat model caused a highly significant decrease in (cTnI). NADPH also can improve the cellular defense against oxidative stress by regeneration of the antioxidant enzymes, thus decreasing cardiomyocyte injury. (Zhu et al., 2020)

Xiao et al., (2018) showed that NADPH treatment reduced oxidative stress in renal (I/R/I), which was reflected by changes in GSH and MDA levels in kidney tissues. One possible explanation for these findings is that NADPH may scavenge ROS by

preserving GSH levels (Ghosh et al., 2014).

The studies were done by Corso et al., (2018) and Huai-Qiang et al., (2020) are also in agreement with our results where they documented that NADPH plays a key role in cellular antioxidation systems by providing reducing equivalents to generate reduced forms of antioxidant molecules, where GSH reductase converts GSSG to GSH using NADPH as an important cofactor, then GSH acts as a co-substrate for GSH peroxidase that reduces hydrogen peroxide (H₂O₂) and other peroxides to H₂O or alcohol to deactivated ROS. In addition, in some cell types, NADPH binds to the important H₂O₂-disposing enzyme, catalase and reactivates it when it has been inactivated by H₂O₂ (Huai-Qiang et al., 2020).

Our data showed that doxorubicin significantly increased TNF α , IL-1 β and Caspase-3 compared with the controls. On the other hand, pretreatment of DOX-treated rats with NADPH shows a significant decrease in these inflammatory and apoptotic markers.

This is in agreement with the study done by Hongdang et al., (2015) who showed that myocardial cell apoptosis-associated protein of Caspase-3 and Cyt c expressions were increased when treated with 1 μ mol DOX. Also, Shama et al., (2022) documented that DOX administration elevated the expression of apoptotic proteins, Bax, and caspase-3, while it lowered the expression of Bcl-2.

Nagah et al., (2022) documented that exogenous NADPH administration showed a marked increase in cell viability and showed a marked decrease in the late apoptotic cells when they use flow cytometry analysis of cardiac

myocytes: in aluminum phosphide-induced cardiotoxicity in rats.

Similar results were obtained from Xiao et al., (2018) who proved that exogenous NADPH administration may defend renal tissues from potential ischemia/reperfusion damage by inhibiting apoptotic pathways, as evidenced by the decreased levels of Bax and elevated Caspase-3

Zhao et al., (2016) reported that the administration of NADPH inhibited the increased levels of pro-inflammatory target genes of NF- κ B, such as Cox2. NADPH reduces inflammation during renal (I/R/I) by inhibiting NF- κ B signaling.

This also coincides with Li et al., (2016) who documented that NADPH has neuroprotective effects on cerebral ischemic injury through antioxidative and anti-inflammatory effects.

DOX administration caused severe histopathological lesions, which were characterized by severe inflammatory cell infiltration, loss of myofibrils, and vacuolation of the sarcoplasm. These results are in agreement with Argun et al., (2015). Additionally, in this study, NADPH has an anti-inflammatory effect as it improved histopathological features of DOX-induced cardiotoxicity and decreased inflammation, degeneration, and necrosis of myocardium. These results are in agreement with Li et al., (2016)

Conclusion

We found that pretreatment with exogenous reduced NADPH protects DOX-induced oxidative stress, decreases cardiac apoptosis, and tempers DOX-induced cardiotoxicity. These results suggest that exogenous

reduced NADPH could be used as a potential therapeutic tool aiming to prevent DOX-induced cardiotoxicity. Our study opens the perspective to clinical studies for consideration of exogenous reduced NADPH as a potential chemoprotection in the combination chemotherapy with DOX to limit its cardiotoxicity.

RECOMMENDATIONS

Further studies are needed to investigate cardiotoxicity and other system toxicities in rats intoxicated with DOX and to investigate the positive effect of exogenous NADPH in the treatment of DOX in humans.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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REFERENCES

- Abdel-Daim, M.M, Khalifa, H.A, and Ahmed, A.A. (2017):** Allicin ameliorates doxorubicin-induced cardiotoxicity in rats via suppression of oxidative stress, inflammation and apoptosis. *Cancer Chemother Pharmacol*; 80(4): 745-753.
- Agledal, L, Niere, M, and Ziegler, M. (2010).** ‘The phosphate makes a difference: cellular functions of NADP’, *Redox Rep.*, 15(1), p. 2–10.
- Ahmed, A. Z, Satyam, S. M., Shetty, P, et al. (2021):** Methyl Gallate Attenuates Doxorubicin-Induced Cardiotoxicity in Rats by Suppressing Oxidative Stress. *Hindawi Scientifica*, 2021:1-12
- Argun, M, Üzümlü, K, Sönmez, M.F., et al. (2015):** Cardioprotective effect of metformin against doxorubicin cardiotoxicity in rats. *Anatol. J. Cardiol.*, pp 15
- Aygun, H., & Gul, S. S. (2019).** Cardioprotective effect of melatonin and agomelatine on doxorubicin-induced cardiotoxicity in a rat model: an electrocardiographic, scintigraphic and biochemical study. *Bratislavske lekarske listy*, 20(4), 249–255.
- Beutler, E., Duron, O., Kelly, B., et al. (1963):** Improved method for the determination of blood glutathione. *J Lab. Clin. Med.*, 61:882-888.
- Chan, S.W, Ziegel, S, and Callard, I.P (1987):** [Plasma progesterone in snakes](#). *Comp Biochem Physiol A Comp Physiol*. 44(2): 631-637.
- Chattopadhyay, A., Biswas, S., Bandyopadhyay, D., et al. (2003):** Effect of isoproterenol on lipid peroxidation and antioxidant enzymes of myocardial tissue of mice and protection by quinidine. *Molec. Cell. Biochem.*, 245: 43-49.
- Corso, C. R. & Acco, A. (2018):** Glutathione system in an animal model of solid tumors: From regulation to therapeutic target. *Crit. Rev. Oncol./Hematol.* 128, 43–57

- Ding, D., Su, D., Li, X., et al. (2015):** Serum levels of monocyte chemoattractant protein-1 and all-cause and cardiovascular mortality among patients with coronary artery disease. *PLoS One.*; 10 (3): e0120633
- El-Bakry, S.T., and Abdel Rahman, Y.H. (2017):** Synergistic effect of Galantamine carried on a novel nano-drug delivery system on induced heart disorders in Alzheimer's disease. *Ejbps.*; 4(10): 45-55.
- Emeka, P. M. and Al-Ahmed, A., (2017):** "Effect of metformin on ECG, HR and BP of rats administered with cardiotoxic agent doxorubicin," *International Journal of Basic & Clinical Pharmacology*, vol. 6, no. 5, pp. 1054–1059
- Fatma, E. R., Moshira, M. A., Mahmoud, H. E., et al. (2021):** Possible protective effects of quercetin on doxorubicin-induced cardiotoxicity in rats Year: Volume: 34 | Issue: 1 | Page: 333-339 *monufia medical journal*
- Ghosh, D., Levault, K.R., and Brewer, G.J. (2014):** Relative importance of redox buffers GSH and NAD (P)H in age-related neurodegeneration and Alzheimer disease-like mouse neurons. *Aging Cell*, 13, 631–640
- Hajra, S., Patra, A. R., Basu, A., et al. (2018):** "Prevention of doxorubicin (DOX)-induced genotoxicity and cardiotoxicity: effect of plant-derived small molecule indole-3- carbinol (I3C) on oxidative stress and inflammation," *Biomedicine & Pharmacotherapy*, 101(4): 228–243
- Hammadi, M.H., labiy, N.M., and Hamoodi, S.R. (2015):** 'Study the predictive value of Troponin –I and histopathological changes during acute and chronic myocardial infarction in adult male rats', *MJS*, 26(2), pp. 13-17.
- Henriksen, P.A. (2018):** Anthracycline cardiotoxicity: an update on mechanisms, monitoring and prevention *Heart*, 104 (12) pp. 971-977
- Hongdang, X.u, Han, Y.u, Zhang, M. et al. (2015):** Protective role of Osthole on myocardial cell apoptosis induced by doxorubicin in rats *Int J Clin Exp Pathol*;8(9):10816-10823 www.ijcep.com /ISSN:1936-2625/IJCEP0009448
- Huai-Qiang, J.u, Jin-Fei, L., Tian, T. et al. (2020):** NADPH homeostasis in cancer: functions, mechanisms and therapeutic implications *Signal Transduction and Targeted Therapy* 5:231
- Jensen, R. A., Acton, E. M. and Peters, J. H. (1984):** "Doxorubicin cardiotoxicity in the rat: comparison of electrocardiogram, transmembrane potential and structural effects," *Journal of Cardiovascular Pharmacology*, vol. 6, no. 1, pp. 186–200.
- Kumar, S. R., Marfatia, S., Tannenbaum, C., et al. (2012):** "Doxorubicin-induced cardiomyopathy 17 years after chemotherapy," *Texas Heart Institute Journal*, vol. 39, no. 3, p. 424,
- Lamberg, S. and Rothstein, R. (1978):** *Laboratory Manual of Histology and Cytology*. 2nd ed.,

A.V.I. Publishing Company, West Port Connecticut, 137-140.

- Lewis, C. A., Parker, S. J., Fiske, B. P., et al. (2014).** ‘Tracing compartmentalized NADPH metabolism in the cytosol and mitochondria of mammalian cells’, *Mol Cell*, 55(2), pp. 253–263.
- Li, M., Zhou, Z.P, Sun, M., et al. (2016):** Reduced nicotinamide adenine dinucleotide phosphate, a pentose phosphate pathway product, might be a novel drug candidate for ischemic stroke. *Stroke*; 47:187–95. doi: 10.1161/STROKEAHA.115.009687.
- Lisa, M.; Kopp, R.B.; Womer, C. L., et al. (2019):** Effects of dexrazoxane on doxorubicin-related cardiotoxicity and second malignant neoplasms in children with osteosarcoma: a report from the Children’s Oncology Group: volume 5, Article number: 153294
Accesses
- Ma, T., Kandhare, A.D, Mukherjee-Kandhare, A.A, et al. (2019):** Fisetin, a plant flavonoid ameliorates doxorubicin-induced cardiotoxicity in experimental rats: the decisive role of caspase-3, COX-II, cTn-I, iNOs and TNF- α . *Mol Biol Rep*; 46:105–118.
- McGowan, R. Chung, A. Maulik, I. Piotrowska, J. M. Walker, and Yellon, D. M. (2017):** “Anthracycline chemotherapy and cardiotoxicity,” *Cardiovascular Drugs and therapy*, vol. 31, no. 1, pp. 63–75,
- Nadia, N.A., Yasser, H. A, and Asmaa, M. (2020):** Cardioprotective Effect of *Moringa Oleifera* against Doxorubicin Cardiotoxicity in Leukemia Rat Model *International Journal of Pharmaceutical and Psychopharmacological Research (eIJPPR) | | Volume 10| Issue 2| Page 148-161*
- Nagah, A., Wafaa, E., Islam, M., et al. (2022):** Ameliorative Effects of Erdosteine and Exogenous Reduced Nicotinamide Adenine Dinucleotide Phosphate on Aluminium Phosphide Induced Cardiotoxicity in Adult Albino Rats., *Mnsoura journal of forensic medicine and clinical toxicology*, vol 30.
- Ping, Z., Aiqun, M., Jiwu, L., Liang, S. (2017):** TNF Receptor 1/2 Predict Heart Failure Risk in Type 2 Diabetes Mellitus Patients. *Int Heart J.*; 58 (2): 245 – 249.
- Qi, W., Boliang, T., Xiaoxi, F. et al. (2020):** “Cardamonin protects against doxorubicin-induced cardiotoxicity in mice by restraining oxidative stress and inflammation associated with Nrf2 signaling,” *Biomedicine & Pharmacotherapy*, vol. 122, Article ID 109547.
- Renu, K., Abilash, V.G., Tirupathi, P.B., et al. (2018):** Molecular mechanism of doxorubicin-induced cardiomyopathy - an update *Eur. J. Pharmacol.*, 818 pp. 241-253
- Reyes, L.A, Boslett, J., Varadharaj, S., et al. (2015):** Depletion of NADP (H) due to CD38 activation triggers endothelial dysfunction in the post-ischemic heart. *Proc Natl Acad Sci U S A.*; 112: 11648–53. Doi: 10.1073/pnas.1505556112

- Saleh, Y.; Abdelkarim, K.; Herzallah, et al. (2020):** “Abela GS Anthracycline-induced cardiotoxicity: mechanisms of action, incidence, risk factors, prevention, and treatment,” *Heart Fail Reviews*, vol. 25
- Shama, M., Muhammad, U., Qurat, u., et al. (2022):** Isorhamnetin: a flavonoid, attenuated doxorubicin-induced testicular injury via r regulation of steroidogenic enzymes and apoptotic signaling gene expression in male rats: *Toxicology Research*, 1–11
- Wang, J., Jing, j., Mengjie, X., et al. (2021):** Molecular mechanisms of doxorubicin-induced cardiotoxicity: novel roles of sirtuin 1-mediated signaling pathways. *Cellular and Molecular Life Sciences* volume 78, pages 3105–3125
- Wang, W., Ren, D., Wang, C.S., et al. (2019):** High sensitivity C-reactive protein to prealbumin ratio measurement as a marker of the prognosis in acute coronary syndrome. *Sci Rep.*; 9 (1):11583.
- Weng, X., Li, S.d, Song, Q.a, et al. (2018):** Protective Effect of Nicotinamide Adenine Dinucleotide Phosphate on Renal Ischemia-Reperfusion Injury *Kidney Blood Press Res*; 43: 651–663
- Wu, K.H, Ho, C.T, Chen, Z.F, et al. (2018):** The apple polyphenol phloretin inhibits breast cancer cell migration and proliferation via inhibition of signals by type 2 glucose transporter. *J Food Drug Anal.* : 26(1): 221-23
- Xia, W., Wang, Z., Wang, Q., et al. (2009):** Roles of NAD (+)/NADH and NADP (+)/NADPH in cell death. *Curr Pharm Des.*; 15: 12–19
- Xiao, W., Wang, R. S., Handy, D. E., et al. (2018).** ‘NAD (H) and NADP (H) Redox Couples and Cellular Energy Metabolism’, *Antioxid Redox Signal*, 28(3), pp. 251–272.
- Ying, W. (2008):** NAD⁺/NADH and NADP⁺/NADPH in cellular functions and cell death: regulation and biological consequences. *Antioxid Redox Signal.*; 10: 179–206. Doi: 10.1089/ars.2007.1672.
- Zare, M.F.R., Rakhshan, K., Aboutaleb, N., et al. (2019):** Apigenin attenuates doxorubicin-induced cardiotoxicity via reducing oxidative stress and apoptosis in male rats. *Life Sci.*; 116623.
- Zhao L, X.u, Tao, X., Han, X., et al. (2016):** Protective Effect of the Total Flavonoids from *Rosa laevigata* Michx Fruit on Renal Ischemia-Reperfusion Injury through Suppression of Oxidative Stress and Inflammation. *Molecules*; DOI: 10.3390/molecules21070952
- Zhao, X.; and Tong, N. (2012):** Protective effects of berberine on doxorubicin-induced nephrotoxicity in mice. *J Transl Med.*; 10:120–8. Doi: 10.1186/1479-5876-10-120.
- Zhu, J., Wang, Y., Chai, X., et al. (2020):** Exogenous NADPH ameliorates myocardial ischemia-reperfusion injury in rats through activating AMPK/mTOR pathway *Apr*; 41(4): 535–545.

الملخص العربي

التأثير الوقائي لنيكوتيناميد ادينين ثنائي نوكليويتيد الفوسفات المختزل على سمية القلب الناتجة عن دكسوروبسين في الجرذان البيضاء البالغة

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١ - قسم الفارماكولوجى بكلية الطب البشرى جامعة بنها.

٢. قسم الطب الشرعي والسموم الإكلينيكية بكلية الطب البشرى جامعة بنها

الخلفية العلمية: يعتبر عقار دكسوروبسين واسع الاستخدام فى علاج العديد من انواع السرطانات ولكنه يؤدى الى احداث سمية بالقلب بالجرعات العالية.

الهدف من الدراسة: معرفة التأثير الوقائي لنيكوتيناميد ادينين ثنائى نوكليويتيد الفوسفات المختزل على سمية القلب الناتجة عن دكسوروبسين فى الجرذان البيضاء البالغة

المواد العلمية ووسائل البحث: تم استخدام ست وثلاثون جرذا مقسمة على خمس مجموعات كالاتى: المجموعة الضابطة السلبية , مجموعة المادة المذيبة والتي قسمت الى مجموعتين فرعيتين , مجموعة نيكوتيناميد ادينين ثنائى نوكليويتيد الفوسفات المختزل (٨مجم/كجم/اليوم بالحقن الوريدي لمدة ٧ ايام) , مجموعة دكسوروبسين (٢٥مجم/كجم/جرعة واحدة تعطى فى اليوم السابع) , مجموعة عقارى نيكوتيناميد ادينين ثنائى نوكليويتيد الفوسفات المختزل و دكسوروبسين. تم عمل رسم للقلب فى اليوم الثامن واخذ عينات الدم لقياس مستوى التربونين القلبي , كرياتين كينيز , اللكتات ديهيدروجينيز , المالوندهايد (احد مؤشرات اجهاد التاكسد) والجلوتاثيون (احد مضادات الاكسدة). وقياس مستوى انترليوكن , معامل هدم الاورام وكاسبس ٣ فى انسجة القلب. كما تم اخذ عينات من نسيج القلب للمجموعات المختلفة لفحصها تحت الميكروسكوب الضوئى

النتائج: قد نتج عن هذه الدراسة زيادة ذو دلالة احصائية فى مستوى التربونين القلبي , كرياتين كينيز , اللكتات ديهيدروجينيز , المالوندهايد , انترليوكن , معامل تكسير الاورام وكاسبس ٣ ونقص ذو دلالة احصائية فى مستوى الجلوتاثيون فى مجموعة دكسوروبسين مقارنة بالمجموعة الضابطة. ونقص ذو دلالة احصائية فى مستوى التربونين القلبي , كرياتين كينيز , اللكتات ديهيدروجينيز , المالوندهايد , انترليوكن , معامل تكسير الاورام وكاسبس ٣ وزيادة ذو دلالة احصائية فى مستوى الجلوتاثيون فى مجموعة عقارى نيكوتيناميد ادينين ثنائى نوكليويتيد الفوسفات المختزل و دكسوروبسين مقارنة بمجموعة دكسوروبسين. وتتوافق هذه التغيرات البيوكيميائية مع التغيرات الباثولوجية فى انسجة القلب للمجموعات

الخلاصة: يمكن القول ان نيكوتيناميد ادينين ثنائى نوكليويتيد الفوسفات المختزل يحمي من سمية القلب الناجمة عن عقار دكسوروبسين بعدة طرق وهى انه مضاد للاكسدة ومضاد للالتهاب.