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Ameliorative Effect of Carvedilol and N-acetylcysteine on Doxorubicin-induced Cardiotoxicity in Rats, Possible Role of SIRT 1.

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ABSTRACT

Doxorubicin (DOX) is one of the most potent antitumor agents, but its use is limited by development of cardiotoxicity involving cardiomyocyte apoptosis and myocardial fibrosis. This study was aimed to evaluate the cardio- protective effects of carvedilol and N-acetylcysteine alone and in combination on doxorubicin-induced cardiotoxicity in rats. Male albino rats were classified into 7 equal groups; 1st group which is normal control group, 2nd and 3rd group involve normal rats receive carvedilol (10 mg/kg/day) and N-acetylcysteine (200 mg/kg/day) respectively. Cardiotoxicity was induced by I.P injection of 6 equal doses of doxorubicin (2.5 mg/kg) within two weeks. Cardio-toxic rats were divided into 4 groups; DOX untreated group, carvedilol treated group, N-acetylcysteine treated group and (carvedilol + N-acetylcysteine) treated group. The drugs were given for 14 day concomitantly with I.P doxorubicin administration. The current work revealed that treatment with either carvedilol or/and N-acetylcysteine resulted in a significant improvement of doxorubicin-induced cardiotoxicity; evident by a significant reduction of heart rate, ST segment elevation, serum creatinine phosphokinase (CPK-MB), troponin-I activity, serum interluken-6 (IL-6),cardiac malondialdehyde (MDA) , significant elevation of reduced glutathione (GSH) and serum SIRT 1 gene expression . Both drugs showed significant improvement of electrophysiological and biochemical parameters which were confirmed with histopathological examination with more significant effect of combination therapy over the effect of each drug alone.

Keywords: Doxorubicin, Cardiotoxicity, Carvedilol, N-Acetylcysteine, SIRT 1.

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INTRODUCTON

Doxorubicin (DOX), an anthracycline widely used chemotherapeutic, is one of the most potent antitumor agent, but its used is limited by development of cardiomyopathy involving cardiomyocytes apoptosis and myocardial fibrosis that may lead to congestive heart failure usually refractory to common medications **[1]**

Cardiomyopathy is the most severe form of chronic doxorubicin cardiotoxicity; its incidence is nearly 30% at cumulative doses **[2].**

Although doxorubicin- induced cardiotoxicity is dose dependent, some patients may develop cardiotoxicity at relatively low doses and some may receive high doses without developing cardiomyopathy **[3].**

Factors that enhance developing of cardiotoxicity at low doses include prior mediastinal or pericardial irritation, pre-existing heart disease, concomitant use of other cardio-toxic medications **[4].**

If the cardiac complication resulting from doxorubicin could be prevented or at least reduced, higher doses could be used and so increasing cancer cure rates can be reached **[5].**

Silent information regulator 1 (SIRT1) is a nicotinamide adenine dinucleotide (NAD+)-dependent class III histone deacetylase, which play an important role in regulation of several factors responsible for cellular defense mechanisms against oxidative stress and inflammation **[6]**

Carvedilol (CAR) is non selective beta blocker with α1-blocking activity decreasing heart rate, decreasing contractility, vasodilator and has antioxidant effect, Carvedilol has used in the treatment of congestive heart failure, hypertension, and also myocardial infarction**[7].**

N-Acetylcysteine (NAC) is a thiol-containing antioxidant. Its antioxidant action originates from its ability to stimulate glutathione (GSH) synthesis and scavenging reactive oxygen species (ROS), NAC has used as a chelator of some heavy metals such as chromium to protect against oxidative stress**[8].** NAC has been investigated for use as an antioxidant in treating an array of diseases including lived failure **[9].**

The aim of the present study was to evaluate the possible protective effect of carvedilol and N-acetylcysteine alone and in combination on doxorubicin-induced cardiotoxicity in rats and the possible mechanisms underlying this action.

MATERIAL AND METHODS

Animals

Adult male albino rats (n=70), each 150-200 g. at the beginning of the study. They were purchased from Faculty of Veterinary medicine, Benha University, Egypt. All animals were acclimatized for one week in controlled laboratory condition at 20 -25°C in a 12h light\dark cycle and had free access to standard diet and water. The study was approved by the ethical committee of Benha faculty of medicine, Benha University who adopts the guidelines for ethical conduct in the care and use of laboratory animals provided by National Research Center, Cairo, Egypt.

Drugs

Carvedilol (powder), N-Acetylcysteine (powder), other chemicals and reagents (Sigma- Aldich co., Cairo. Egypt.)

Experimental design

After acclimatization for one week, rats divided into 7 experimental groups, 10 rats each. **Group (1)**: Normal control group was fed with standard chow diet with no medication. **Group (2):** Normal Carvedilol treated group (CAR): normal rats of this group received carvedilol 10 mg/kg/day for 14 days orally**. Group (3):**

Normal N-acetylcysteine group (CAR), normal rats of this group received N-acetylcysteine 200 mg/kg/day for 14 days orally**.** The previous 3 groups received I.P injection of 6 equal doses of 2.5 ml/kg normal saline within two weeks at the same time of doxorubicin administration**. Group (4):** Doxorubicin untreated group (DOX), rats of this group received cumulative dose 15 mg/kg of doxorubicin through I.P injection of 6 equal doses of doxorubicin (2.5 mg/kg) within two weeks **[10]. Group (5):** DOX+CAR treated group, rats of this group received doxorubicin as group 4 with oral administration of carvedilol 10 mg/kg/day **[11] (**from the initial day of doxorubicin injection and for 14 days. **Group (6):** DOX+NAC treated group, rats of this group received doxorubicin as group 4 with I.P administration of N-acetylcysteine 200 mg/kg/day **[12]** from the initial day of doxorubicin injection and for 14 days. **Group (7):** DOX+ CAR+NAC treated group, rats of this group received doxorubicin as group 4 with oral administration of carvedilol 10 mg/kg/day and N-acetylcysteine 200 mg/kg/day from the initial day of doxorubicin injection and for 14 days.

At the end of the experiment, twenty four hours after last dose of doxorubicin, overnight fasted rats were anaesthetized by inhalation of ether and blood samples were collected from rat tail and processed for biochemical investigation. Then rats were sacrificed and heart of each rat was dissected immediately, washed with ice cold saline and divided into 2 parts. The first one was immediately frozen at -80°C and used for biochemical analysis of tissue MDA and GSH, this portion latterly was minced and homogenized .The crude homogenate was centrifuged at 7.700 for 30 minutes and the resultant supernatant was used for assay of hepatic MDA and GSH**[13].** The second part preserved in 4% formalin for histopathological and immune-histochemical examination.

Parameters measured

Electrophysiological parameter: Determination of

a- ST segment changes.
b- Heart rate (HR) by ECG.

Biochemical measurements

- Serum creatinine phosphokinase (CPK-MB) level **[14]**
- Troponin-I activity **[15].**
- Serum Interluken-6 (IL-6) **[16].**

Evaluation of cardiac malondialdehyde (MDA) level: [13].

Evaluation of cardiac reduced glutathione (GSH) level: [13].

Histopathologial examination

After.functional studies were completed, the heart was excised as a whole, put on cold (8° C) 30 mM KCl to achieve diastolic arrest. Both atria were excised; the ventricles were preserved in neutral formalin 10% and referred for histopathological examination. The hearts were cut into transverse sections from the ventricle and interventricular septum, each section was fixed with methanol and ethanol (1:1), processed with paraffin wax, sectioned at 5µm and stained with hematoxylin and eosin. The cardiac sections were examined under light microscopy for the presence of myocyte degenerative changes *[17].*

Immuno-histochemical examination

Hearts were fixed in formalin.and then put in paraffin according to the usual histological technique. Immunohistochemistry was used to determine the caspase-3 antigen. Samples were deparaffinized and rehydrated. Caspase-3 antibodies (Thermo Fisher Scientific, USA) were stored at 4°C overnight. The suitable horseradish peroxidase conjugated secondary antibodies were added and the samples were kept for 60 min at normal room temperature. Hematoxylin Myer was used as counter stain; the sections were examined by using light microscope.

RT- qPCR [18]

RNA was extracted from blood by using RNA Mini Kit (Qiagen, USA) .Sirt1, forward TGGACGAGCTG ACCCTTGA and reverse TCCTGCGGATGTGGAGATT [18].

Statistical analysis

The results were experienced as mean ± standard deviation of the mean (S.D). The overall significance was measured by One Way Analysis of Variance (ANOVA). The significance between individual groups was detected by t test. P value less than 0.05% was considered significant [19].

RESULTS

Heart rate (HR) and ST segment elevation changes

CAR and NAC treated normal rats showed non-significant effect on HR and ST segment elevation ($p > 0.05$) compared to control group. In DOX non-treated group there was statistically significant increase ($p<0.05$) in HR and ST segment elevation compared to control group. Treatment of DOX groups with CAR or/and NAC resulted in significant decrease ($p<0.05$) in HR and ST segment elevation compared to DOX non-treated group with more significant effect of combination therapy ($p<0.05$) if compared to each drug alone (Table 1) (Fig. 1&2).

Table (1): HR and ST segment elevation in different studied groups (Mean ± SD):

Parameters Groups	Heart rate (beat/min)	ST segment elevation (mm)
Normal control group	270.4 ± 8.5	0
Normal CAR treated group	272.6 ±9.2	0
Normal NAC treated group	274.5± 8.9	0
DOX untreated group	390.8 ± 35.4[a]	2.84 ± 0.32[a]
DOX+ CAR treated group	314.8 ± 31[a,b]	1.78 ± 0.23[a,b]
DOX+ NAC treated group.	350,7 ± 30[a,b,c]	2.32 ± 0.24[a,b]
DOX+ CAR+NAC treated group	280.8 ± 25[b,c,d]	1.05 ±0.18[a,b,c,d]

a: Significant difference versus Normal control group at $p<0.05$.
b: Significant difference versus DOX untreated group at $p<0.05$.
c: Significant difference versus DOX+CAR treated group at $p<0.05$.
d: Significant difference versus DOX+ NAC treated group at $p<0.05$.

Serum levels of CPK-MB, Troponin-I and IL-6

CAR and NAC treated normal rats showed non-significant effect on serum levels of CPK-MB, Troponin-I and IL-6 ($p > 0.05$) compared to control group. In DOX untreated group there was statistically significant increase ($p<0.05$) in these parameters compared to control group. Treatment of DOX groups with CAR or/and NAC resulted in significant improvement ($p<0.05$) in CPK-MB, Troponin-I and IL-6 serum levels compared to DOX non-treated group but it was still at significant higher level ($p<0.05$) if compared to control group with more significant effect of combination therapy ($p<0.05$) if compared to each drug alone (Table 2) (Fig. 3, 4 & 5).

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Table (2): Serum CK-MB& serum Troponin-I and IL6 in different studied groups (Mean ± SD)

Parameters Groups	CK-MB (ng / ml)	Troponin-I (ng/ml)	Serum (IL-6) nmol/L
Normal control group	5.4±0.23	0.37±0.03	0.224±0.021
Normal CAR treated group	5.37±0.25	0.38±0.03	0.232±0.025
Normal NAC treated group	5.42±0.31	0.37±0.02	0.226±0.023
DOX untreated group	96.56±7.2 [a]	3.53±0.28 [a]	1.38±0.12 [a]
DOX+ CAR treated group	48.4 ±4.8[a,b]	1.43±0.09 [a,b]	0.872±0.06 [a,b]
DOX+ NAC treated group.	67.8 ± 6.2[a,b,c]	2.34±0.18 [a,b,c]	0.735±0.07 [a,b]
DOX +CAR+NAC treated group	24.5±2.2[a,b,c,d] ↓55.3%	0.74±0.05[a,b,c,d] ↓77.8%	0.423±0.029[a.b.c,d]

a: Significant difference versus Normal control group at p<0.05.
b: Significant difference versus DOX untreated group at p<0.05.
c: Significant difference versus DOX+CAR treated group at p<0.05.
d: Significant difference versus DOX+ NAC treated group at p<0.05.

Cardiac Levels of (MDA) & (GSH) serum SIRT 1 expression in different studied groups

CAR and NAC treated normal rats showed non-significant effect on cardiac MDA &GSH levels and serum SIRT 1 expression. MDA was significantly (p<0.05) increased with significant (p<0.05) decrease of GSH and SIRT 1 expression in DOX u n treated group compared to control group, their levels significantly (p<0.05) improved in DOX+ CAR and DOX+ NAC treated groups, both groups showed insignificant difference between them, with more significant effect of combination therapy (p<0.05) if compared to each drug alone (Table 3) (Fig.6 &7& 8).

Table (3): Cardiac levels of (MDA) & (GSH) and serum SIRT 1 expression in different studied groups (Mean ± SD).

Groups	Cardiac (MDA) nmol/g	Cardiac (GSH) nmol/g	Serum SIRT 1 expression
Normal control group	35.2 ± 2. 6	0.282 ± 0.02	4.36±0.24
Normal CAR treated group	34.6 ± 2.4	0.278 ± 0.019	4.40±0.26
Normal NAC treated group	34.4 ± 2.3	0.291 ± 0.021	4.42±0.27
DOX untreated group	96.7 ± 8.2[a]	0.089 ± 0.005 [a]	0.82±0.05 [a]
DOX+ CAR treated group	60.58 ± 4.7 [a,b]	0.178 ± 0.013 [a,b]	3.18±0.22 [a,b]
DOX+ NAC treated group	58.3 ± 4.2 [a,b]	0.182 ± 0.016 [a,b]	3.12±0.21 [a,b]
DOX+ CAR+ NAC treated group	40.5 ± 3.8 [b,c,d]	0.252 ± 0.023 [b,c,d]	3.96±0.23 [b,c,d]

a: Significant difference versus Normal control group at p<0.05.
b: Significant difference versus DOX untreated group at p<0.05.
c: Significant difference versus DOX+CAR treated group at p<0.05.
d: Significant difference versus DOX+ NAC treated group at p<0.05.

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Histopathological examination

Histopathological examination of the heart for detection of signs of acute ischemia and inflammation was done at the end of the experiment in different groups:

Group (1) control normal group: showed normal cardiac muscle (Fig.8).with central elongated nuclei, eosinophilic cytoplasm and striations **Group (2)** Normal CAR treated group: showed normal cardiac muscle (Fig.9).**Group (3)** Normal NAC treated group: showed normal cardiac muscle (Fig. 10).**Group (4)** DOX untreated group: showing (A) Disarrangement of myocardial fibers with cytoplasmic vacuoles (B) cellular infiltration (C) Interstitial edema (Fig.11).**Group (5)** DOX+ CAR treated group: showing (A) Moderate Disarrangement of myocardial fibers with no cytoplasmic vacuoles (B) congested blood vessel. (C) Interstitial edema (Fig.12).**Group (6)** DOX+ NAC treated group: showing (A) moderate cardiac muscle necrosis (B) congested blood vessel. (Fig.13).**Group (7)** DOX+ CAR+ NAC treated group: showing normal myocardium, mild congested blood vessel (Fig.14).

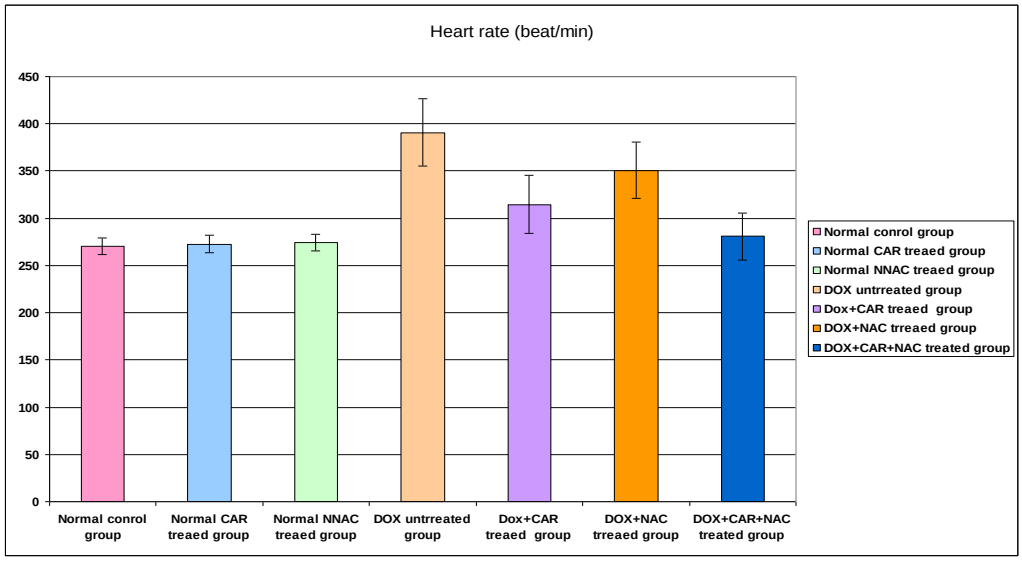

Fig.(1): Heart rate in different studied groups.

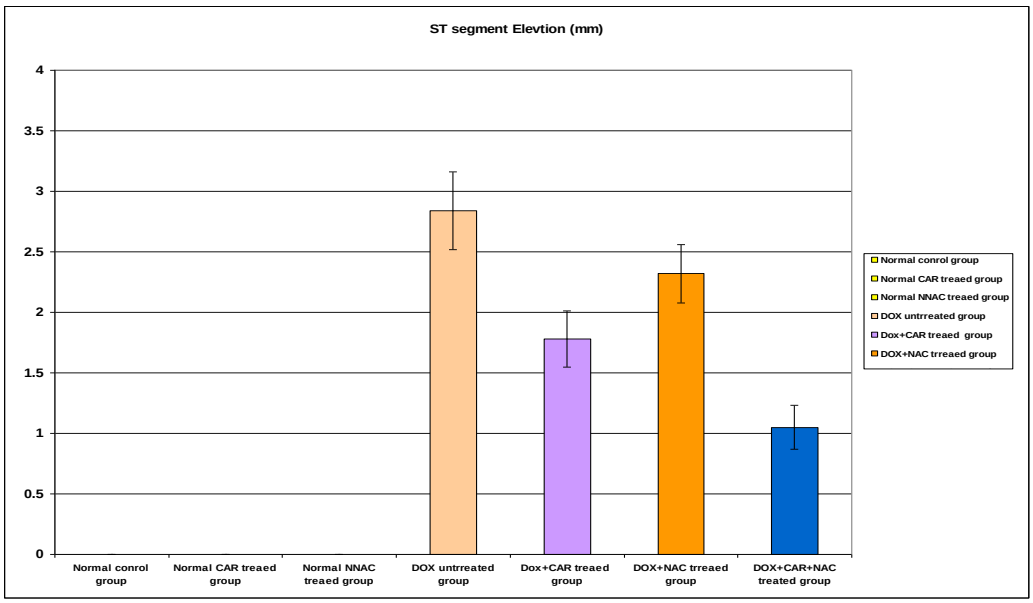

Fig. (2) ST segment elevation in different studied groups

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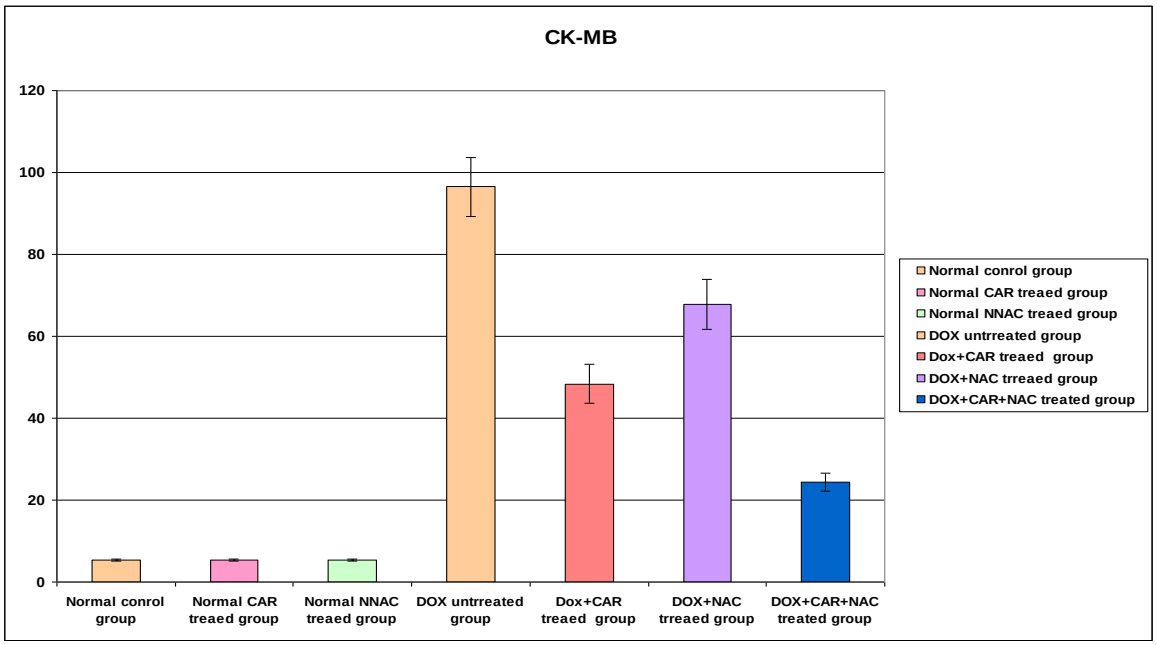

Fig. (3): Serum CK-MB in different studied groups.

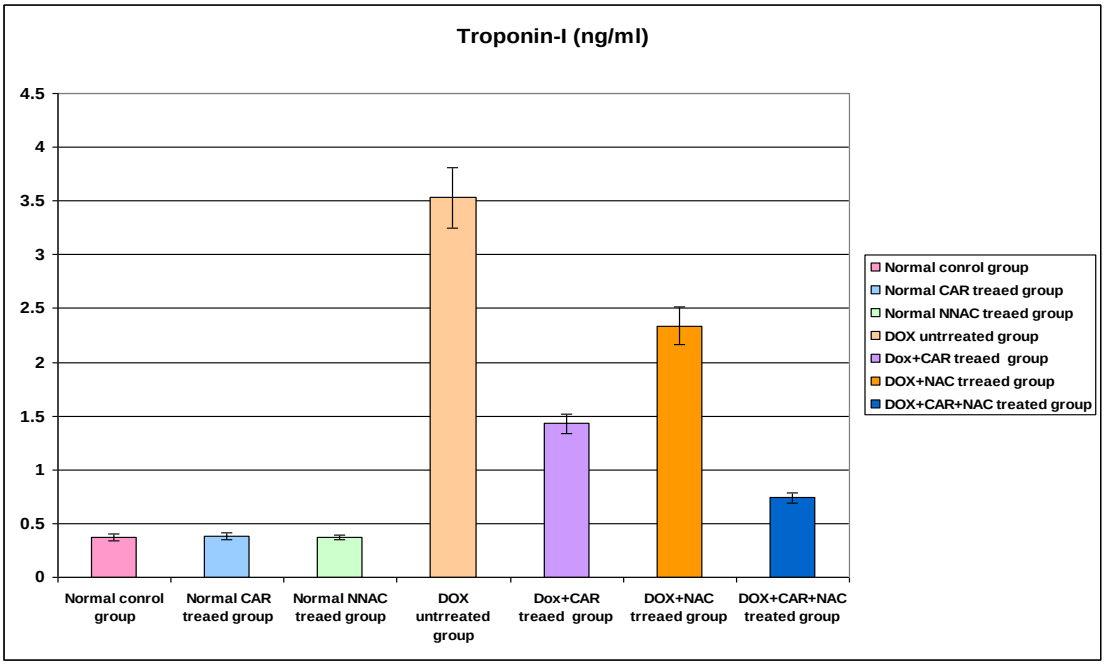

Fig. (4): Serum Troponin-I in different studied groups

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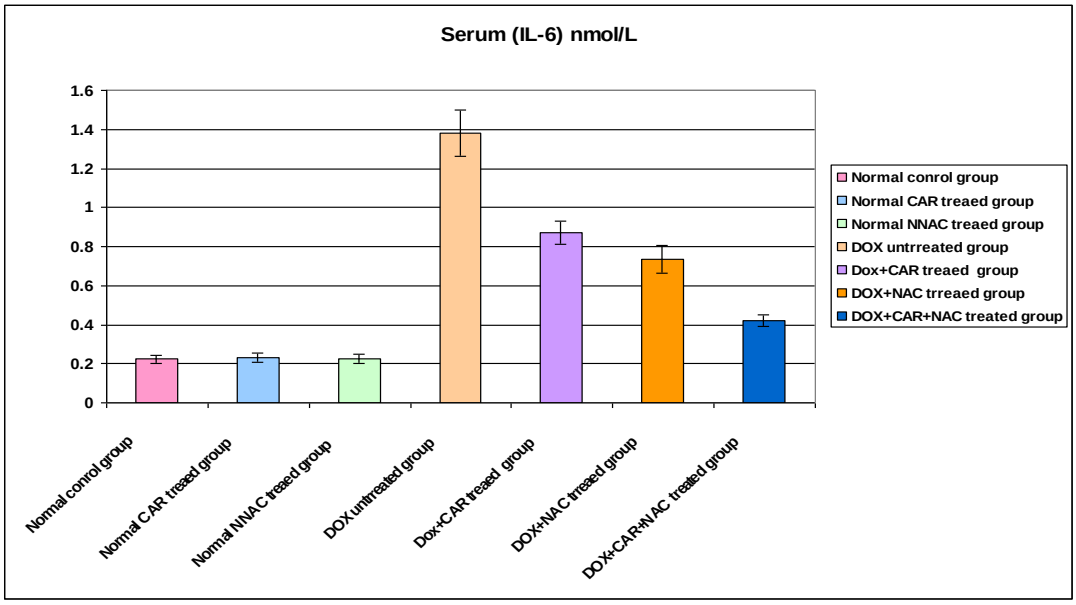

Fig. (5): Serum IL6 in different studied groups

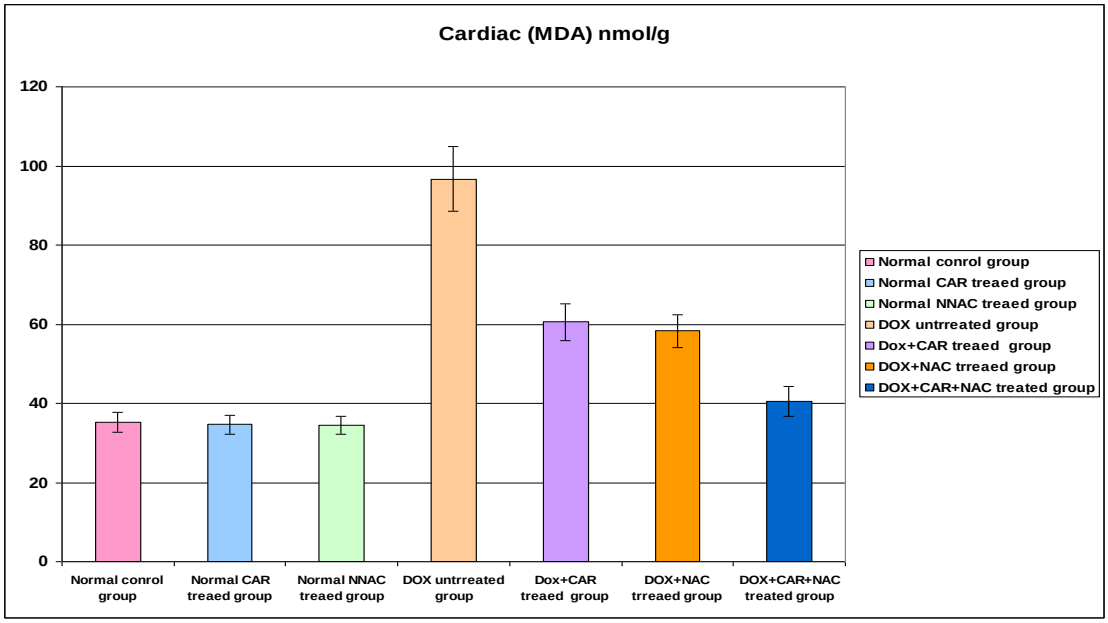

Fig. (6): Cardiac levels of (MDA) in different studied groups

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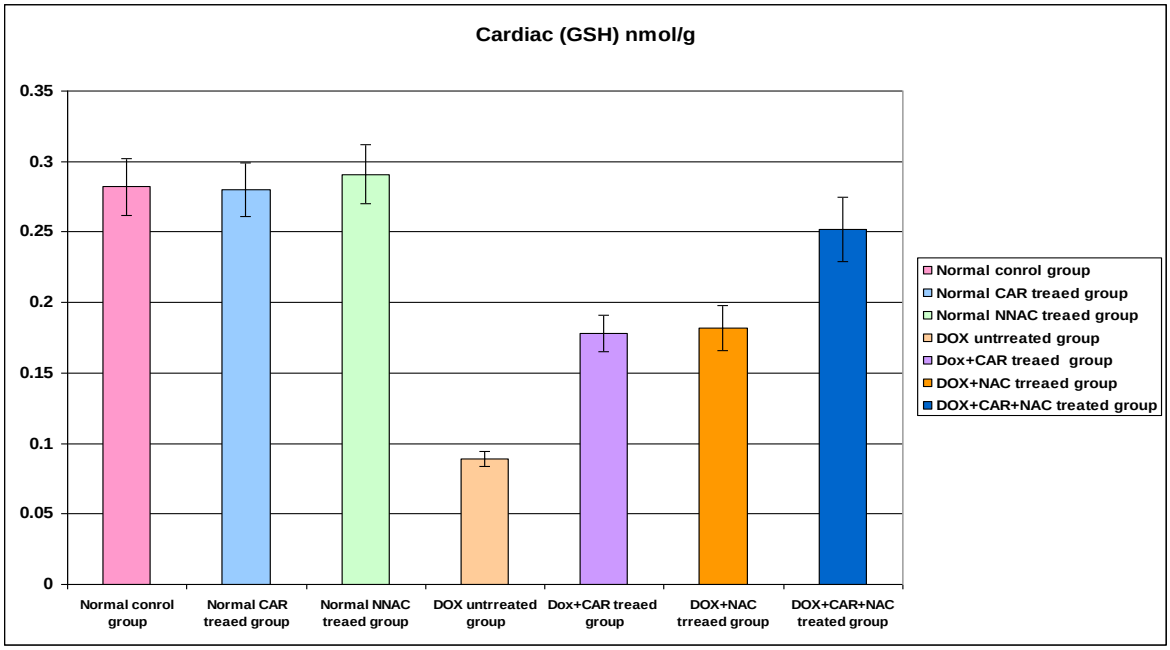

Fig. (7): Cardiac levels of (GSH) in different studied groups

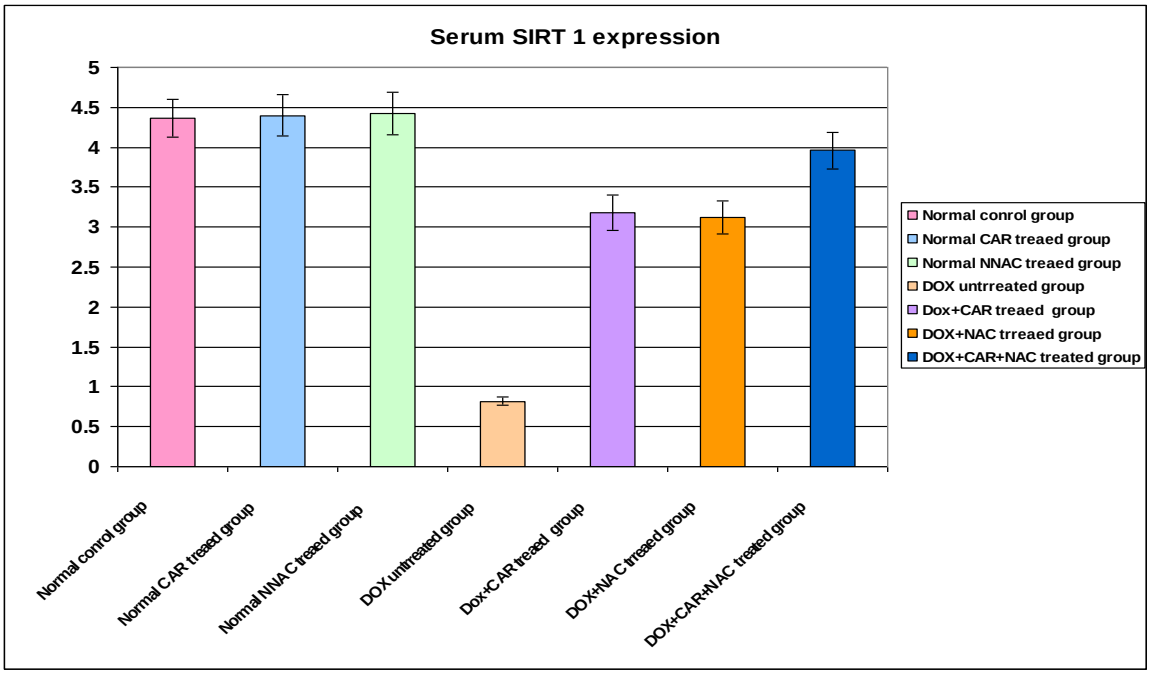

Fig. (8): Serum SIRT 1 expression in different studied groups (Mean ± SD).

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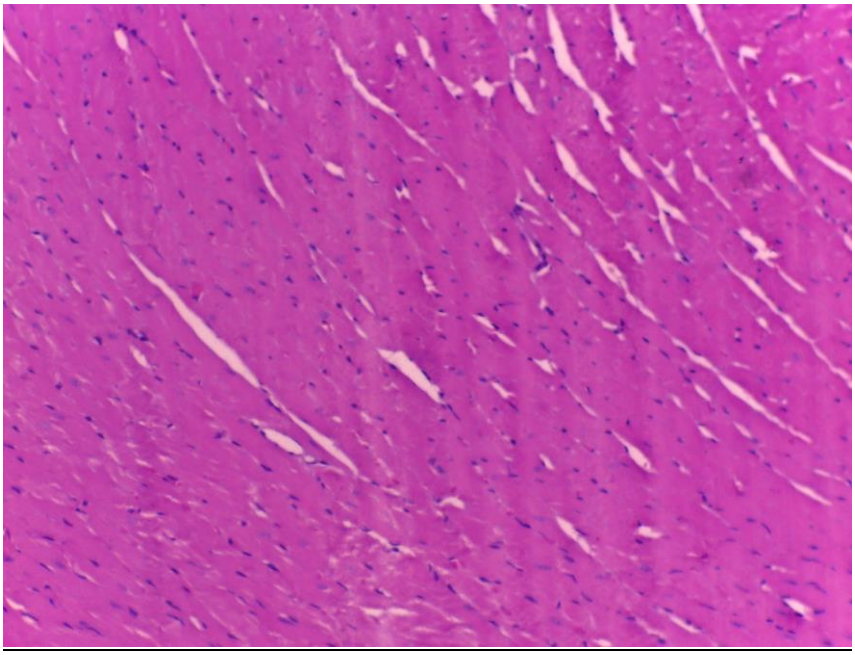

Fig. (9): Histopathology of normal control group (H & E x 200).

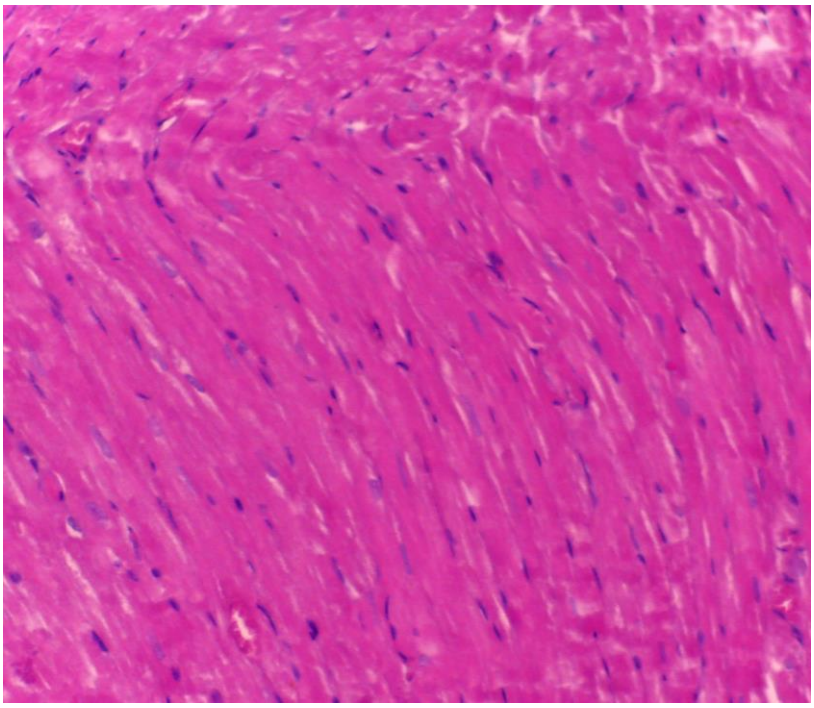

Fig. (10) Histopathology of normal CAR treated group: showed normal cardiac muscle (H & E x 200).

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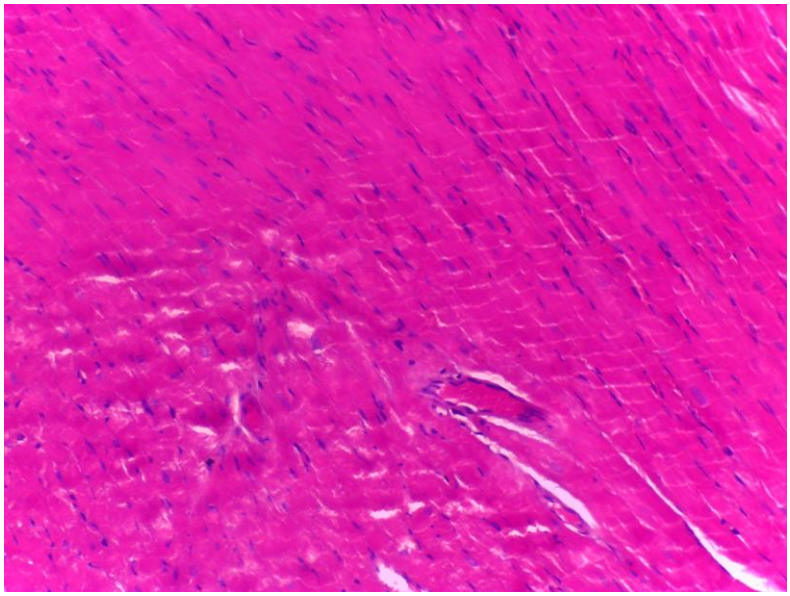

Fig. (11): Histopathology of **normal NAC treated group**: showed normal cardiac muscle (H & E x 200).

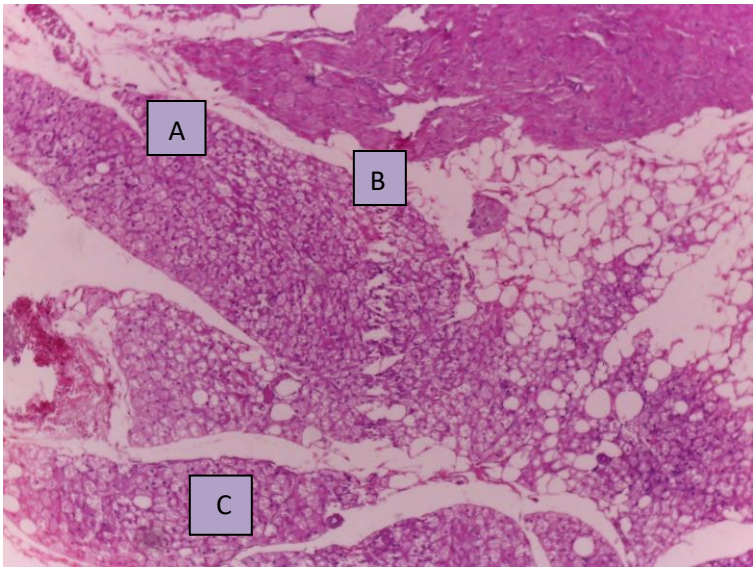

Fig. (12): Histopathology of DOX untreated rats (group 4) showing (A) Disarrangement of myocardial fibers with cytoplasmic vacuoles (B) cellular infiltration (C) Interstitial edema (H&Ex200).

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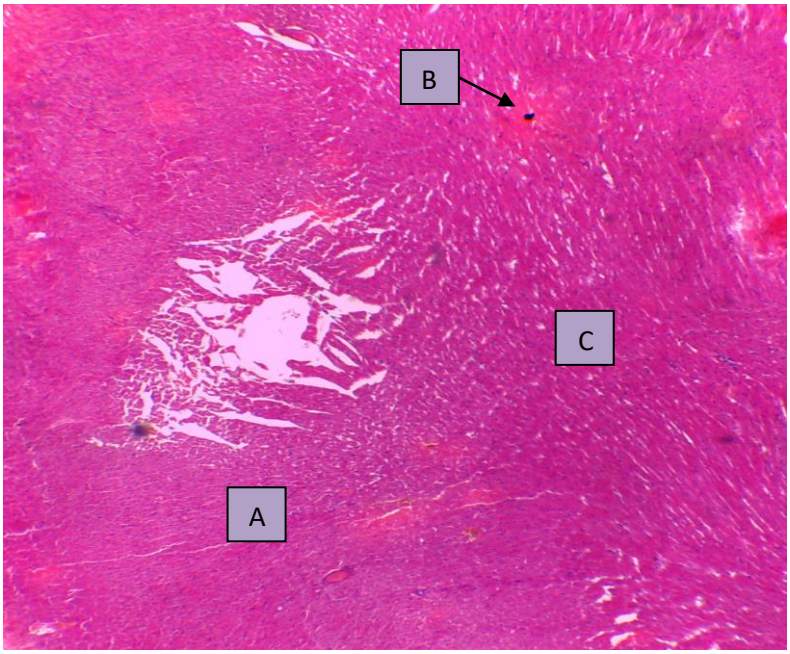

Fig. (13): Histopathology of DOX+ CAR treated rat (group 5) showing (A) Moderate Disarrangement of myocardial fibers with no cytoplasmic vacuoles (B) congested blood vessel. (C) Interstitial edema (H&Ex100).

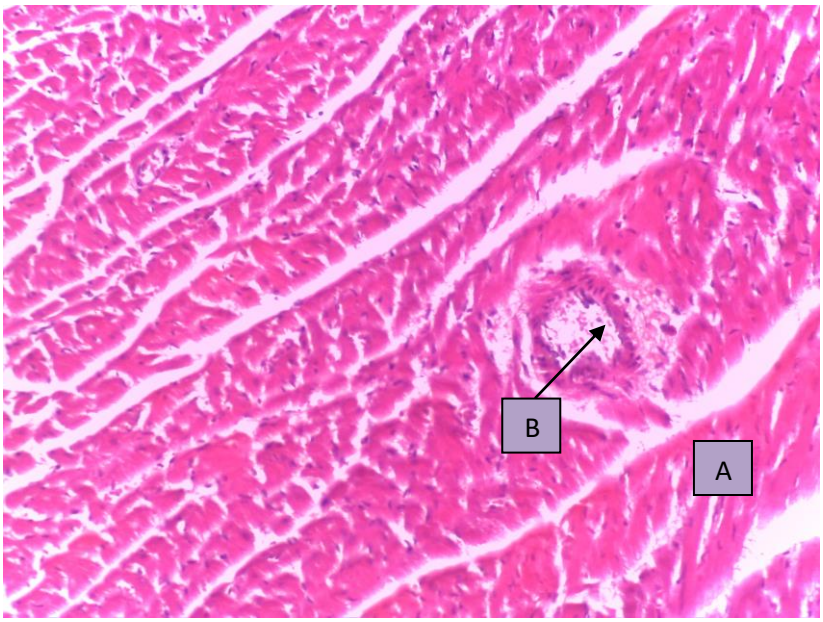

Fig. (14): Histopathology of DOX+ NAC treated rat (group 6) showing (A) moderate cardiac muscle necrosis.(B) congested blood vessel. (H&Ex200).

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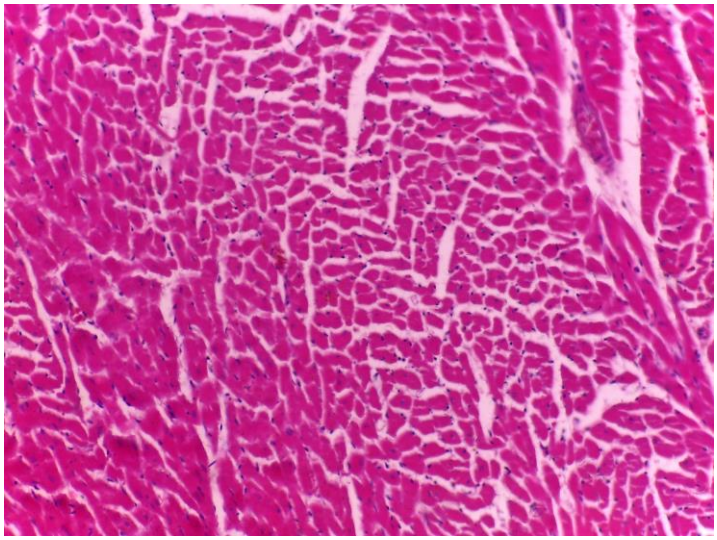

Fig. (15): Histopathology of DOX+ CAR+ NAC treated rat (group 7) showing normal myocardium, no hemorrhage and no vacuolated fibers (H & E x 200).

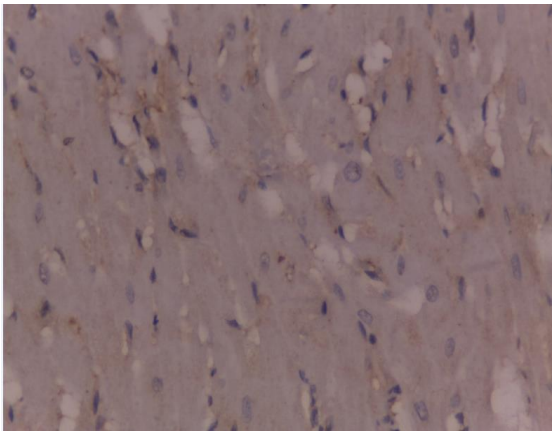

Fig. (16): A photomicrograph of normal control rats(group 1) showing minimal expression of caspase 3.

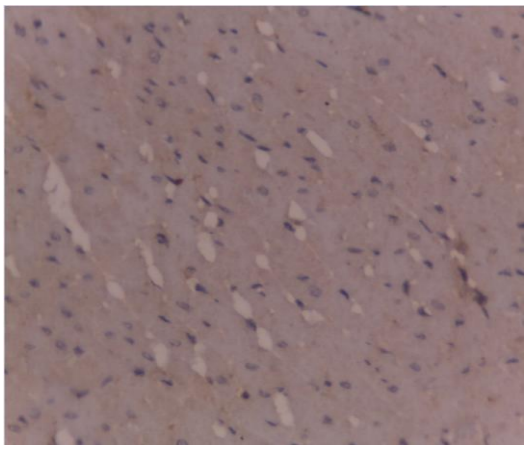

Fig. (17): A photomicrograph of normal CAR treated rats(group 2) showing minimal expression of caspase 3.

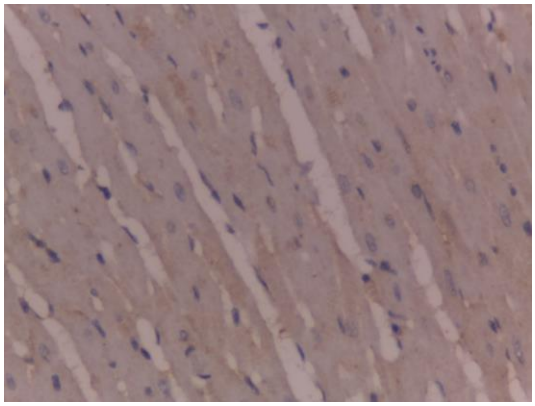

Fig. (18): A photomicrograph of normal NAC treated rats (group 3) showing minimal expression of caspase 3.

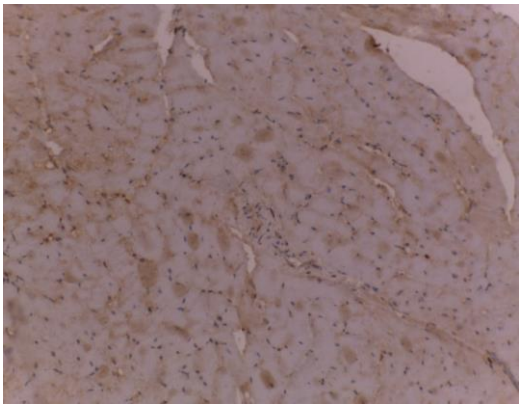

Fig. (19): A photomicrograph of DOX untreated rat (group 4) showing marked expression of caspase 3.

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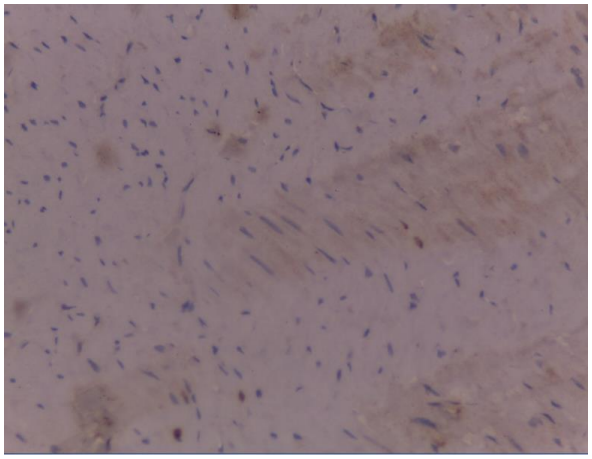

Fig. (20): A photomicrograph of DOX+ NAC treated rats(group 5) showing moderate expression of caspase 3.

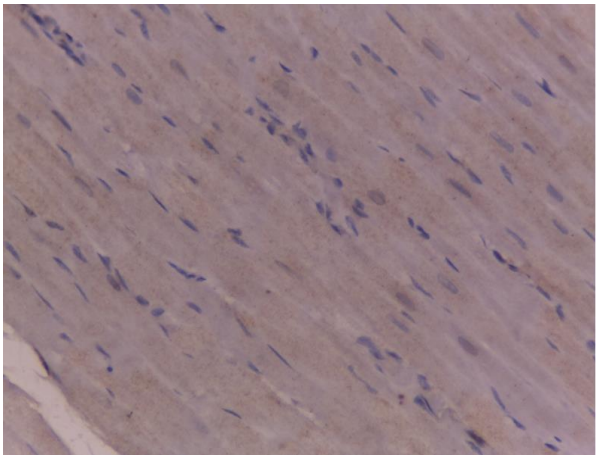

Fig. (21): A photomicrograph of DOX+ NAC treated rats(group 6) showing moderate expression of caspase 3.

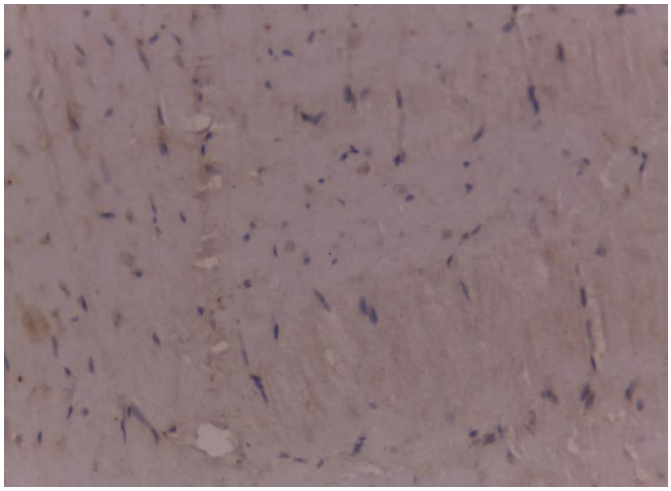

Fig. (22): A photomicrograph of DOX+ CAR+ NAC treated rats (group 7) showing minimal expression of caspase 3

DISCUSSION

Doxorubicin is one of the most widely used cytotoxic drugs for the treatment of a variety of cancers including leukemia, lymphomas and solid tumors [1]. The usage of doxorubicin is limited by development of cardiomyopathy involving cardiomyocyte apoptosis and myocardial fibrosis that may lead to congestive heart failure usually refractory to common medications [20].

Many previous studies demonstrated that the cardiopretective effect of SIRT1 through suppression of NF-KB and regulation of p53 signal responsible for cardiomyocyte inflammatory responses and apoptosis [21&22]. In addition, SIRT1 up regulate the activity of antioxidants such as superoxide dismutase (SOD) and catalase suggesting that overexpression of SIRT1 associated with increase cell defenses and promote the cell survival in response to Dox-induced cardiotoxicity[23].

Silent information regulator 1 (Sirt1), a NAD+-dependent class III histone deacetylase, has the ability to deacetylate important metabolic players such as peroxisome coactivator 1 alpha, which is a key regulator of oxidative metabolism responsible for oxidative stress protection systems including manganese superoxide dismutase and catalase [21].

Sirt1 can increase survival rate and cell resistance from stress to protect against Dox-induced oxidative damage and cell death. Overexpression of Sirt1-through up-regulating the antioxidants actions- can protect the heart from oxidative stress [21&23].

Furthermore, previous studies have demonstrated that Sirt1 can suppress NF-κB and adjust P53 signal, responsible for regulation of inflammation and apoptosis [22&23].

So, Sirt1 plays critical roles in regulating inflammation, oxidative stress, and apoptosis.

This work was designed to evaluate the possible protective effect of carvedilol and N-acetylcysteine alone and in combination on doxorubicin-induced cardiotoxicity in rats and the possible mechanisms underlying this action.

The obtained data in the present work revealed that I.P injection of a cumulative dose 15 mg/kg of doxorubicin through 6 equal doses (2.5 mg/kg) within two weeks resulted in significant alteration in heart rate, ST segment elevation, serum creatinine phosphokinase (CPK-MB), troponin-I activity, serum interluken-6 (IL-6), cardiac MDA & GSH and SIRT 1 expression.

These data are in agreement with, **[24] Maryam et al., (2010)** who demonstrated ST segment elevation in doxorubicin treated rats.

These ECG changes could be explained by **[25]** who reported that ECG changes may be due to disturbance of calcium movement across the cell membrane or may be due to loss of cell membrane integrity as reported by **[26].**

Also these results are in agreement with **[27] (Rashikh et al., (2011)** who detected elevations in creatinine phosphokinase and Troponin-I activity in rats after a cumulative dose of doxorubicin.

These elevations in cardiac enzymes could be explained by **[28]Hadi et al., (2012)** who determined that the myocardium contain a lot of diagnostics marker enzymes in rats for myocardial infarction which released as metabolic damage occurred, Hence, the serum levels of these marker enzymes reflect the membrane disturbance in integrity and permeability.

Our results also, in agreement with **[26] Zhou et al., (2008**) who demonstrated elevation in lipid peroxidation and decrease in GSH in doxorubicin induced cardio-toxic rats.

The present study revealed significant histopathological changes in the form of degeneration, vacuolization, inflammation and interstitial hemorrhage in doxorubicin treated rats which confirm the cardio-toxic effect of doxorubicin, these finding were in agreement with**[29](Shao et al., 2007).**

Several mechanisms were involved in doxorubicin induced cardiotoxicity including mitochondrial damage that may cause respiratory chain defects which allow production of free radicals and release of cytochrome c lead to induction of apoptosis **[21].** In addition, doxorubicin may produce changes in vascular endothelium-derived vasoactive mediators (endothelin-1 and cardiac nitric oxide) **[30].**

Also cellular damage may be due to increase intracellular iron accumulation producing free radicals that immediately cleaving DNA preventing its repair and replication **[24]**. Also, doxorubicin alters cardiac specific gene expression including structural, metabolic and enzyme activities **[31]** and doxorubicin activates mitogen-activated protein kinases, p38 and JNK.

The data of the present work showed that, administration of carvedilol 10 mg /kg / day orally as monotherapy concomitantly with doxorubicin injection and for 14 days resulted in significant reduction of heart rate, ST segment elevation, serum CPK-MB level, serum troponin-1, serum IL-6 level and cardiac MDA with significant elevation cardiac of GSH and SIRT 1 expression compared to doxorubicin cardio-toxic non treated rats.

These results were in agreement with**[32] Spallarossa et al.,(2004)** and**[33]Oliveira et al., (2004)**

who suggested that carvedilol is potentially protective against doxorubicin cardiotoxicity by decreasing free radical release and apoptosis in cardiomyocytes.

Mousa et al., (2018) [34]reported that carvedilol markedly improves malondialdehyde , superoxide dismutase , insulin-like growth factor , vascular endothelial growth factor levels and histological and immunohistochemical structure of cardiac muscle and improve cardiac function in doxorubicin-induced cardiotoxicity in rats.

Cardio-protective effect of carvedilol in this study can be explained by **[33]Oliveira et al., (2004)** who concluded that the cardiopretective effects of "carvedilol against DOX-induced mitochondrial cardiotoxicity are due to its inherent antioxidant activity and not due to its beta-adrenergic receptor blocking effect as oxidative stress, mitochondrial dysfunction, and histopathological lesions in the cardiac tissue induced by DOX , all of which are inhibited by carvedilol and not by atenolol which is other beta-adrenergic receptor antagonist lacking antioxidant properties".

Also **Mousa et al., (2018) [34]** suggested possible role of insulin-like growth factor-1 as a mechanism of cardiopretective effect of carvedilol in doxorubicin- induced cardiotoxicity.

In addition **Zhang et al., (2019) [35]**mentioned that carvedilol has protective effect against doxorubicin cardiotoxicity by augmenting the expression and activities of the anti-oxidative enzymes as well as suppress the inflammatory response, proved by the reduction of pro-inflammatory cytokines (COX2, TNF-α, IL-6, IL-1β and IL-18), which was associated with the inactivation of nuclear factor κB. Also CAR attenuates DOX-induced apoptosis and autophagy through down-regulating cleaved caspase-3.

The present study showed that The data of the present work showed that, administration of NAC 200 mg /kg / day orally as monotherapy concomitantly with doxorubicin injection and for 14 days resulted in significant reduction of heart rate, ST segment elevation, serum CPK-MB level, serum troponin-1, serum IL-6 level and cardiac MDA with significant elevation cardiac of GSH compared to doxorubicin cardiotoxic non treated rats.. These data are supported by previous **Arica et al., (2013)[12]** studies which revealed that NAC improve myocardial functions and improve histopathological picture of the heart in rats exposed to DOX-induced cardiotoxicity as it improve biochemical parameters(thiobarbituric acid reactive substance TBARS, lactate dehydrogenase , aspartate transaminase, nitric oxide NO and creatine kinase levels) as well as it preserve general architecture .

Also our results were in agreement with **[21]Wang (2012**) who reported that pretreatment with (NAC) attenuated intracellular ROS accumulation, cytotoxicity in DOX-induced cardiotoxicity.

These results can be explained**[36]Goyal et al., (2016)** who demonstrated that NAC improved the DOX- induced cardiotoxicity in a murine model of chemotherapy-induced cardiac dysfunction by decreasing oxidative stress and apoptosis.

Also **[37] Yoshida (2009)** reported that NAC attenuated doxorubicin induced oxidative stress, DNA damage, ATM activation, and p53 induction in cultured cardiac myocytes.

Many previous studies suggested the anti-oxidant effect as a cardiopretective mechanism of NAC, **[38] Mansour et al., (2015)** revealed that NAC attenuates cyclophosphamide-induced cardiotoxicity in rats by inhibiting oxidative stress and improving the antioxidant enzymes activity.

(Zhao et, al.2018) [39] demonstrated that antioxidant N-acetylcysteine (NAC) attenuated SIRT1 repression increased SIRT1 expression and decreased SIRT1 protein breakdown in antimony treated cells.

(Yang et, al. 2018) [40] suggested that NAC exerted a protective effect against PM2.5-induced respiratory oxidative stress by regulating the SIRT1 expression .

Several previous studies demonstrated the beneficial cardio-protective effect of combination therapy between CAR and other statins **as** rosuvastatin **[11)** and carnosic acid (CAA) **[35],** but -up to our knowledge – no study demonstrate the combination between CAR and NAC.

According to the present study, co-administration of CAR + NAC concomitantly with doxorubicin injection and for 14 days resulted in significant reduction of heart rate, ST segment elevation, serum CPK-MB level, serum troponin-1, serum IL-6 level and cardiac MDA with significant elevation cardiac of GSH compared to doxorubicin cardio-toxic non treated rats and compared to monotherapy with either each drug alone, these results were confirmed with histopathological examination, this greater cardio-protective effect may be due to synergistic effects of both drugs .

In conclusion, these findings suggest that Carvedilol and N-acetylcysteine may have cardio-protective effect mainly by their antioxidant mechanism which could be mediated through upregulating the expression of SIRT1 providing a good combination to ameliorate doxorubicin-induced cardiotoxicity.

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