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REDUCING THE HEART BIOCHEMICAL AND HISTOLOGICAL EFFECT OF DOXORUBICIN BY ARTEMISININ COMPOUND

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Abstract

The current study included a statement doxorubicin effect on the heart muscle and cardiotoxicity enzymes (cTnI, CK-MB, Myoglobin and LDH), in addition, reduction effect of it by artemisinin compound. Tn-I, CK-MB, and LDH showed high significant values effected by doxorubicin compared with other compounds, while the myoglobin showed high significant value effected by ARD compound. The interaction effect of periods and compounds factor showed high significant effect on Tn-I, CK-MB, and LDH by doxorubicin at the third week, but myoglobin show high significant effect by ARD.The period factor of compounds treatment showed no significantly effect on Tn-I, CK-MB, and LDH in contrast with mayoglobin.
Key words: cardiotoxicity enzyme, reducing, artemisinin, doxorubicin

Introduction

Chemotherapy treatment of cancer began at 20th century with attempts to narrow the universe of chemicals that might affect the disease (DeVita *et al.,* 2008).One of these drugs is doxorubicin (DOX) which is a potent anthracycline antibiotic, (Ibsen *et al.,* 2010; Hemeida *et al.,* 2008). There are many researcher tried to reduce the side effect of doxorubicin (Homady *et al.,* 2018).

Chemical treatments have negative effects on the body, Hair loss, weight loss, as well as liver tissue damage caused by toxic effects of chemical and respiratory disorders, etc. (Remesh, 2017). Alternative medicine has been used to reduce the toxicity and side effects of chemotherapy leading to positive and acceptable results in many breast, colon and skin cancers (Sak, 2012). Artemisia herba-alba was known for its therapeutic and various it ,artemisinin compounds have been isolated from Artemisia. herba-alba, possibly the most important being the compound which has medicinal properties (Ali *et el.,* 2017).

Material and Methods

The Experimental Design for Animals

120 animals were taken from laboratory mice and were divided into three main groups (one, two, three weeks) at different time intervals. Each group was then divided into four groups. The first was the control group given to Normal Saline and the other three groups, Artemisinin at a concentration 60 mg and the second gave the doxorubicin component with a concentration 5mg/kg b.wt. and valence gave a mixture of ART and DOX called ARD concentration 60, 5mg/kg b.wt.) respectively and after Period of time the animal was sacrificed and fixed in formalin (10%) for histopathological study and 0.2 mm thickness fixed in the qluteraldehyd for Transmission electron microscope study

Blood Collection

0.75-1 ml of blood was collected from the heart of each mouse directly using disposable syringe. The blood sample placed in serum gel tube, left for 30 minutes. The serum was prepared via centrifugation at 3000 rpm for 10 minutes and kept frozen at -20ºC (unless immediately analyzed) and then used.

Ultra-structural Study by the Electron Microscope

The Specimen Preparation

The electronic microscope has been used to modulate the method as described below in the next source (AL-Zahid, 2014; Ghadially, 2017).

Fixation by using (2.5% gluteraldyhayde for 3 days as chemical treatment to remove water and preserve the tissue as much as possible in its original state, washing by PBS three times for (1 h) the last one stay overnight in the refrigerator, fixation and oxidation by osmium tetroxide for 90min,washing by distilled water (2time) for (5min) each time, dehydration by (30, 50% ethanol alcohol for 20 min and in 70% overnight ,complete the dehydration by ethanol (80, 90, 95%) 20 min for each concentration, (100%) ethanol for 15 min 2 time, Acetone (100%), min for tow time, prepare equal volume of acetone and araldite (1:1) and put it in the shaker for (75min), pure araldite day and over night, embedding with araldite in plastic capsule and labeled.

Grid staining

Filtration the uranyl stain drop like on grid for (90 min), washing with alcohol (70%), the grid put in lead stain for 45 min, washing with D.W, dry by filter paper, Put in the capsule and then examined

Result

Ultra-Structural of Heart Muscle

The doxorubicin showed ultra structure changes for cardiac muscle as arrangement of microfilament and losing attachment, which was the cause of the breakdown of cardiac fibers and their disintegration and spacing, so began to lose these fibers function and flexibility, lead to muscle dysfunction. While the ARD and ART were not affect and remain like a control. Picture (1, 2, 3, 4).

The current study evaluated the effects of DOX, ART, and ARD compounds on cardiac enzymes function, mice serum cardiac troponin I (cTnI), CK-BM, LDH and myoglobin during treatment schedule (3 weeks).

Tn-I, CK-MB, and LDH showed high significant values effected by doxorubicin compared with other compounds, table (1,2,4), while the myoglobin showed high significant value effected by ARD compound, Table (3). The interaction effect of periods and compounds factor showed high significant effect on Tn-I, CK-MB, and LDH by doxorubicin at the third week, table (1, 2, 4), but myoglobin show high significant effect by ARD, Table (3). The period factor of compounds treatment showed no significantly effect on Tn-I, CK-MB, and LDH in contrast with mayoglobin. Table (1, 2, 3, 4)

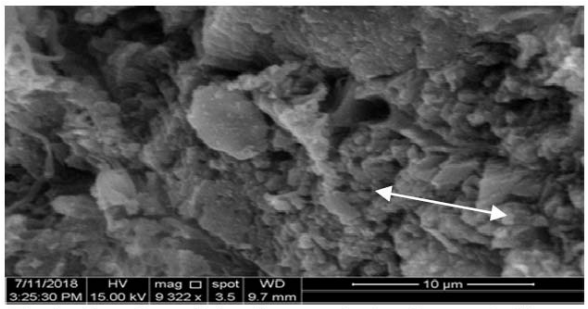

Fig. 1 : Show doxorubicin effect on animal cardiac muscle fiber

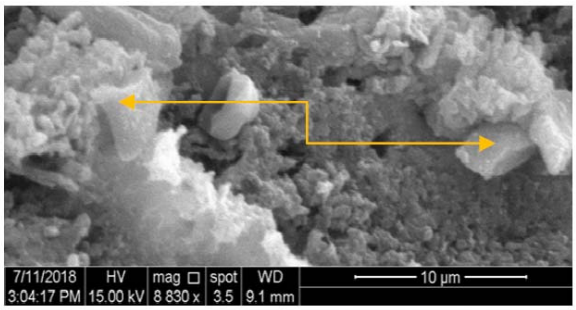

Fig. 2 : Show normal fibers of control animals cardiac muscle

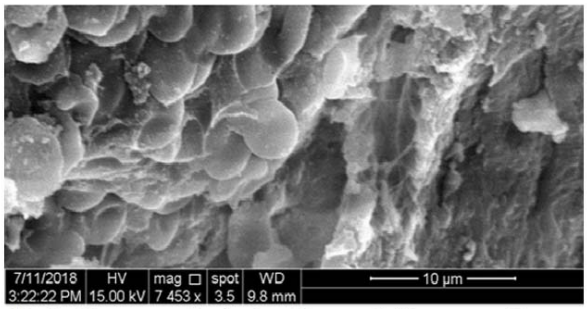

Fig. 3 : Shows normal animal cardiac muscle fibers, treated by artemisinin ART

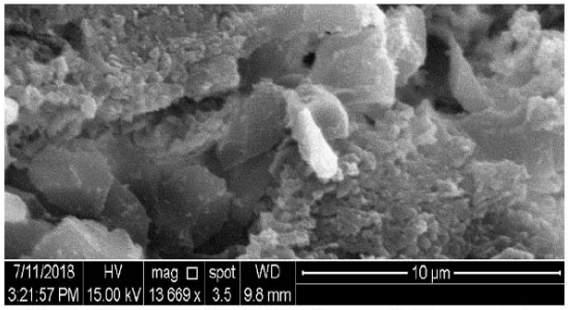

Fig. 4 : Shows normal animal cardiac muscle fibers, treated by ARD

Table 1 : Showing the averages of compounds effect on mice for three weeks, Tn-I.

Tn-I	Period (week)			Average b
Groups	W1	W2	W3	
Control	0.11±0.002	0.09±0.0019	0.1±0.002	0.1
ART	0.067±0.004	0.051±0.0007	0.024±0.001	0.04
DOX	0.21±0.01	0.37±0.0134	0.55±0.012	0.37
RAD	0.09±0.079	0.12±0.01	0.15±0.013	0.12
Average a	0.11925	0.15775	0.206	
LSD, P‧ 0.001	a=0.2362	ab=0.4090	b=0.2322	

Table 2 : Showing the averages of compounds effect on mice for three weeks, CK-MB.

CK-MB	Period (week)			Average b
Groups	W1	W2	W3	
Control	3±0.021	2.9±0.024	3.1±0.02	3.0
DOX	2.8±0.05	2.4±0.053	1.9±0.073	2.36
ART	4.1±0.07	4.7±0.13	5.8±0.079	4.86
RAD	3.5±0.1	3.8±0.132	4.1±0.053	3.8
Average a	3.35	3.45	3.725	
LSD, P‧ 0.001	a=0.821	ab=1.422	b=0.858	

Table 3 : Showing the averages of compounds effect on mice for three weeks, myoglobin.

Myoglobin	Period (week)			Average b
Groups	W1	W2	W3	
Control	24±0.24	26±0.23	25±0.26	25.0
ART	32±0.53	35.8±1.27	39.8±1.01	35.8
DOX	24±0.53	31±0.26	33±0.79	29.3
RAD	34±1.06	40±1.32	46±1.04	40.0
Average a	28.5	33.2	35.95	
LSD, P‧ 0.001	a=2.677	ab=4.637	b=2.565	

Table 4 : Showing the averages of compounds effect on mice for three weeks, LDH

LDH	Period (week)			Average b
Groups	W1	W2	W3	
Control	720±2.4	700±2.99	710±2.65	710
ART	619±10.37	810±15.9	1059±28.9	829
DOX	1481±20.47	1570±18.61	1770±21.27	1607
RAD	960±15.9	1420±13.2	1654±14.3	1344
Average a	945	1125	1298.25	
LSD, P‧ 0.001	a=365.4	ab=632.8	b=354.1	

Discussion

The increased risk of cardiac dysfunction by doxorubicin can manifest acutely during treatment or chronically third weeks after treatment has ceased as show in picture (3.1).

Doxorubicin induced damage causing myocyte death and the injured regions would be abnormal as not a normal tissue, lead to dyssynchrony and deformation. In both human and animal studies two-dimensional strain echocardiography appeared more sensitive than standard echocardiography protocols providing additional doxorubicin induced cardiac injury (Ho *et al*., 2010; Migrino *et al.,* 2008).

Doxorubicin accumulates in the nucleus and the mitochondria (Das *et al*., 2016). Similar to the nucleus, the mitochondria contains topoisomerase 2β, a specific target of doxorubicin. The functional role of mitochondrial topoisomerase II remains unclear, however it has been suggested to participate in deactivating newly synthesized mtDNA circles wears give fibers irregular and disassembled (Zhang *et al*., 2014). Our study agree with this study (Kavazis *et al.,* 2017).

The cardiac injury increase terponine in circulate blood (GS Panjrath *et al.,* 2006; Aziz *et al.,* 2017) and CK-MB (Aziz *et al*., 2017). Cardiac toxicity is a major dose-limiting factor for application of some medication like DOX which well known as cardiac toxic compound (GS Panjrath *et al.,* 2006) and closer this analysis with (Mitry *et al.,* 2016).

There are many researcher tried to reduce the side effect of doxorubicin (Beak *et al*., 2017).

The ART have not effect on cardiac muscle and that result positive to use treatment and some modification in future make more compounds safety as anticancer with less cardio toxicity and this agree with (Homady *et al*., 2018; Hakacova *et al*., 2013). ARD decrease toxicity effect on myocardium and agree with study (Wu *et al.,* 2013).

It is widely accepted that the DOX induces cardiac injury via several mechanisms, including activation of ubiquitin-proteasome system, sarcomere reorganization, induction of pro-inflammatory cytokines, free radical generation and apoptotic cell death that are the typical changes in DOX-induced heart failure (Octavia *et al*., 2012). and associated with a decrease in antioxidants and an increase in oxygen free radicals leading to an increase in oxidative stress, followed by the development of a variety of subcellular cells Myocardial, typical of heart injury caused by DOX (Devaraj *et al*., 2015).

DOX has been pumps. Moreover, doxorubicin abolished the calcium loading activity of cardiac sarcoplasmic reticulum vesicle (Liu *et al*., 2008).

But ART was result level less from normal limit because less streets on endocardium which is stimulate this enzymes form less and Cause toxicity result from DOX on tissue heart which coming closer result search (Aziz *et al*., 2017). Decided experimental ARD to decrease effect and coming result.

Conclusion

1. Doxorubicin has sever effect on cardiomyocyte which lead to heart dysfunction.
2. Doxorubicin toxicity were less when combination with artemisinin and this is the result of antagonistic action of mixture from during measurement parameter cardiac (ART).
3. Artemisinin don't find any effect toxicity on cardiac muscle.

Reference

DeVita, V.T. and Chu, E. (2008). A history of cancer chemotherapy. Cancerres, 68(21): 8643-8653.

Ibsen, S.; Zahavy, E.; Wrasdilo, W.; Berns, M.; Chan, M. and Esener, S. (2010). A novel doxorubicin prodrug with controllable photolysis activation for cancer chemotherapy. Pharm. Res., 27(9): 1848–1860.

Hemeida, R.A. and Mohafez, O.M. (2008). Curcumin attenuates methotraxate-induced hepatic oxidative damage in rats. Journal of the Egyptian National Cancer Institute, 20(2):141-148.

Homady, M.H.; Kadhim, H.A.; Al-Kelaby, K.K.A.; Aziz, D.Z. and Kadhim, N.J. (2018). Cytotoxic activity of compounded anthracycline against

rhabdomyosarcoma cancer cell line. Plant Archives, 18(1): 941-946.

Remesh, A. (2017). Toxicities of anticancer drugs and its management. International Journal of Basic & Clinical Pharmacology, 1(1): 2-12.

Sak, K. (2012). Chemotherapy and dietary phytochemical agents. Chemotherapy research and practice.

Ali, M.; Abbasi, B.H.; Ahmad, N.; Khan, H. and Ali, G.S. (2017). Strategies to enhance biologically active-secondary metabolites in cell cultures of Artemisia–current trends. Critical reviews in biotechnology, 37(7): 833-851

AL-Zahid, (2014). Histological and Immunohistochemical Studies of Artemisia herba alba on mouse bearing adenocarcinoma.

Ghadially, F.N. (2017). *Diagnostic electron microscopy of tumours*. Butterworth-Heinemann.

Ho, E.; Brown, A.; Barrett, P.; Morgan, R.B.; King, G.; Kennedy, M.J. and Murphy, R.T. (2010). Subclinical anthracycline-and trastuzumab-induced cardiotoxicity in the long-term follow-up of asymptomatic breast cancer survivors: a speckle tracking echocardiographic study. Heart, 96(9): 701-707.

Migrino, R.Q.; Aggarwal, D.; Konorev, E.; Brahmbhatt, T.; Bright, M. and Kalyanaraman, B. (2008). Early detection of doxorubicin cardiomyopathy using two-dimensional strain echocardiography. Ultrasound in medicine & biology, 34(2): 208-214.

Das, S.; Filippone, S.M.; Williams, D.S.; Das, A. and Kukreja, R.C. (2016). Beet root juice protects against doxorubicin toxicity in cardiomyocytes while enhancing apoptosis in breast cancer cells. *Molecular and cellular biochemistry*, 421(1-2): 89-101.

Zhang, H.; Zhang, Y.W.; Yasukawa, T.; Dalla, I.; Khiati, S. and Pommier, Y. (2014). Increased negative supercoiling of mtDNA in TOP1mt knockout mice and presence of topoisomerases IIα and IIβ in vertebrate mitochondria. Nucleic acids research, 42(11): 7259-7267.

Kavazis, A.N.; Morton, A.B.; Hall, S.E. and Smuder, A.J. (2017). Effects of doxorubicin on cardiac muscle subsarcolemmal and intermyofibrillar mitochondria. Mitochondrion, 34: 9-19.

Panjrath, G.S. and Jain, D. (2006). Monitoring The cardiotoxicity it causes Chemotherapy: the role of nuclear imaging J. Cardiol., 13 : 415-426

Beak, J.Y.; Huang, W.; Parker, J.S.; Hicks, S.T.; Patterson, C.; Simpson, P.C. and Jensen, B.C. (2017). An oral selective alpha-1A adrenergic receptor agonist prevents doxorubicin cardiotoxicity. JACC: Basic to Translational Science, 2(1): 39-53.

Mitry, M.A. and Edwards, J.G. (2016). Doxorubicin induced heart failure: Phenotype and molecular mechanisms. IJC heart & vasculature, 10: 17-24

Hakacova, N.; Klingel, K.; Kandolf, R.; Engdahl, E.; Fogdell-Hahn, A. and Higgins, T. (2013). The first therapeutic use of artesunate in the treatment of human herpes virus 6B myocarditis in the child. Journal of Clinical Virology, 57 (2), 157-160.)

Wu, G. S., Lu, J. J., Guo, J. J., Huang, M. Q., Gan, L., Chen, X. P., & Wang, Y. T. (2013). Synergistic anti-cancer activity of the combination of dihydroartemisinin and doxorubicin in breast cancer cells. Pharmacological Reports, 65(2): 453-459

Octavia, Y.; Tocchetti, C.G.; Gabrielson, K.L.; Janssens, S.; Crijns, H.J. and Moens, A.L. (2012). Doxorubicin-induced cardiomyopathy: from molecular mechanisms to therapeutic strategies. Journal of molecular and cellular cardiology, 52(6): 1213-1225.

Singh, G.; Singh, A.T.; Abraham, A.; Bhat, B.; Mukherjee, A.; Verma, R. and Burman, A.C. (2008). Protective effects of Terminalia arjuna against Doxorubicin-induced cardiotoxicity. Journal of Ethnopharmacology, 117(1): 123-129.]

Liu, L.; Shi, R.; Shi, Q.; Cheng, Y. and Huo, Y. (2008). Protective effect of saponins from Panax notoginseng against doxorubicin-induced cardiotoxicity in mice. Planta medica, 74(03): 203-209.

Aziz, D.Z.; Homady, M.H.; Kadim, H.A. and Al-Kelaby, K.K.A. (2017). Assessment of Compounded Doxorubicin in Cardiac Tissue of Experimental Animals. Pak. J. Biotechnol. Vol, 14(4): 811-816.