OPEN ACCESS

Haya: The Saudi Journal of Life Sciences
Abbreviated Key Title: Haya Saudi J Life Sci
ISSN 2415-623X (Print) | ISSN 2415-6221 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

Side Effects of Anthracycline Chemotherapy on the Heart of Laboratory Animals

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DOI: 10.36348/sjls.2024.v09i07.006 | **Received:** 06.06.2024 | **Accepted:** 10.07.2024 | **Published:** 15.07.2024

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Abstract

Anthracycline is one of the chemical drugs commonly used worldwide for the treatment of various types of cancer. The high doses of it cause unwanted toxic side effects on the tissues. Our study designed to demonstrated the physiological and histopathological effect of the drug on the heart organ of experimental animals. For this purpose, twenty adults male rats were used in this study and were divided into four main groups. Control group, Azoxymethane treated group, Azoxymethane qrand anthracycline treated group, and anthracycline treated group only. Blood of the experimented animals was collected in order to measure biochemical parameters such as glutathione, lactate dehydrogenase, malondialdehyde, peroxynitrite, Creatinine Kinase, C-reactive protein Creatine kinase –myocardial band, and Myoglobin concentrations. In this study a significant increase and decrease was observed in biochemical parameters. Histological examination on the heart shown that rats treated with AOM have been revealed only hypertrophy of cardiac muscles, while other groups were observed hypertrophy of cardiac muscles furthermore. Aorta in AOM group have been revealed Adverse histological changes and transformation in the thickness aortic wall layers. The tissue sections of the groups treated with Anthracyclin showed a negative effect on the aortic wall layers, the endothelial layer and the middle layer, as well as the outer layer in the sections prepared from these groups.

Keywords: Cancer, chemotherapy, anthracycline, azoxymethane.

INTRODUCTION

Cancer is one of the most dangerous and widespread diseases in countries of the world, as the uncontrolled growth of normal cells as a result of changing the genetic material leads to the formation of cancer cells that invade peripheral tissues and then spread to the rest of the organs, causing the destruction of cells in various organs of the body (Kumar *et al*., 2003). When a normal cell is exposed to carcinogenic factors, these substances cause changes in the genetic material, and the primary genes in the normal cell are transformed into a mutant cell, which then acquires the character of a tumor cell in the initiation process (Butel., 2000). Then it suffers from division, forming a cancer cell, then it grows, forming a distinct tumor through the process of promotion (Steen, 2000), and then these cells begin to multiply through the process progression, it spreads at the expense of normal cells and migrates through the bloodstream to other sites in the body in process metastasis (Nevidjon & Sowers, 2000). One of the side effects of cancer cells is an increase in body fat and its deposition in the walls of blood vessels and in the heart muscle, causing myocardial infarction and the emergence of heart and arterial diseases, as well as the production of large quantities of free radicals that cause damage to tissues and organs, numerous of studies have demonstrated that abnormal levels of lipids are intimately related to carcinogenesis and cancer metastasis. Malignant transformation and accelerated cancer cell proliferation are in high demand for energy, which induces alterations in lipid metabolism to allow the survival of cancer cells (Zhu *et al*., 2016).

Anthracycline is widely used and is one of the effective anti-tumor treatments derive from the Streptomyces bacterium. Despite its effectiveness against a group of solid and malignant blood tumors, its

clinical use causes toxicity in healthy tissues due to increased formation of free radicals, causing side effects on tissues, especially cardiac tissue (Panal *et al*., 2023).

Anthracyclines are toxic to both tumor cells and other rapidly dividing cells, such as those in the bone marrow and the heart. This is why anthracyclines can have serious side effects, such as bone marrow suppression and cardiotoxicity, that may result in cardiac contractile dysfunction, cardiomyopathy, ventricular dysfunction, pericarditis-myocarditis syndrome, arrhythmias and heart failure (Shinlapawittayatorn *et al.*, 2022). Aldoxorubicin is a doxorubicin derivative containing a carboxylic hydrazone and serves as a prodrug of doxorubicin. It covalently binds to albumin in the blood until reaching the acidic tumor environment, which dissolves the hydrazone linker, thus releasing doxorubicin into the tissue, it is one of the chemotherapy that has side effects on tissues (Esha *et al*., 2017), therefore the current study was conducted to find out the harmful effects of this drug on the body's tissues, especially the heart organ.

MATERIAL AND METHODS

Animals experimental design: This study was carried out on twenty adult male mices, aged (3-4 months) and (25-27gm) body weight in the animal house of college of science, university of Basrah. Animals were divided into four main groups, each of five mices. control group injected with normal saline, second and third group injected with carcinogen (azoxymethane) (15 mg/kg body weight) once a week for two weeks in intraperitoneally cavity for cancer induction (Suaeyun *et al*., 1997), the third group injected in addition to the carcinogen the anthracycline drug (Aldoxorubicin) (25 mg/kg body weight) intraperitoneal once a week for six months, while fourth group were injected with anthracycline drug only (25 mg/kg body weight) once a week for six months (Zhao *et al*., 2012).

Sample collection and biochemical tests: About 3 ml of blood was taken and placed in tubes without anticoagulant and placed in a centrifuge to obtain blood serum for biochemical tests of glutathione(GSH) (Tietz,1999), malondialdehyde(MDA) (Guidet & Shah, 1989), peroxynitrite ($ONOO^-$) (Vanuffelen *et al*., 1998), lactate dehydrogenase(LDH,LDH2) (Tietz,1999), Creatinine Kinase(CK) concentrations, creatine kinase – myocardial band (CK-MB) , C- reactive protein (CRP), myoglobin (Myo).

The histological study: After two weeks of carcinogen injection, rats were explained to detect cancer occurrence. The other groups were explained a week after the last dose, and parts of heart were taken and fixed in 10% formalin for the purpose of the histological study (Drury *et al.*,1967).

Statistical analysis: The statistical analysis of the results of the experiment was done using the statistical package for the 2008(Social Science, version 20(SPSS)) program to study the effect of the materials used in the experiment and to determine the significant differences between the average of the probability level ($p \leq 0.05$) (Duncan,1955).

RESULTS AND DISCUSSION

The obtained result in Table (1) revealed significant increase ($p \leq 0.01$) in the CK, LDH, LDH2 in AOM group compared with control group The reason for this increase is due to the fact that the free radicals resulting from AOM work to break down and remove nitric oxide, NO, which has antioxidant properties induction, in addition to the damage that causes the lining of the arteries, which stimulates phagocytes to produce large amounts of free radicals that oxidize fats, especially LDL-C. His concentration rises. Also the reason is due to the high level of lipid peroxidation and its products (MDA), which stimulate the oxidation process of fats, especially LDL.C and phospholipids (Mostafa *et al*., 2014; Mostafa *et al*., 2015). Thus, it leads to the formation of epithelial damage areas in the blood vessels and an increase in the permeability of the vessels and their lack of elasticity due to atherosclerosis.

The results also showed a significant increase ($P \leq 0.01$) in the concentration of CK, LDH, LDH2 in serum of animals exposed to Anthracycline compared with control group, This increase in enzyme concentrations is attributed to an increase in lipid peroxidation and produce large amount of free redicals, its products (MDA), which stimulates the oxidation process of fats, especially LDL-C, and thus leads to the formation of epithelial damage areas of blood vessels, an increase in vessel permeability and a lack of elasticity, which leads to atherosclerosis. (Panel *et al.*,2023).

Studies also indicated that high levels of fats in the blood due to treatment with anthracycline for a long period lead to the accumulation of fats in the epithelial lining of the arteries, especially the coronary arteries forming Atherosclerotic plaques that thicken and harden the artery wall and when these plaques rupture, it causes a thrombotic blockage that prevents the heart muscle from being supplied with blood, causes Myocardial infarction, Coronary heart diseases and Angina pectoris.

The study also showed a significant increase ($P \leq 0.01$) in CK, LDH, LDH2 concentrations of the AOM and Anthracycline group compared to all groups, and the increase in heart enzymes causes induced antioxidant stress lead to damage heart tissues as a result of damage to the myocardial cells leading to the secretion of these enzymes from the muscle into the blood (Sonia *et al*., 2021).

The results of the current study showed that the treatment with AOM led to a significant increase ($p \leq 0.01$) in the concentration of MDA in the blood serum of animals table (1) compared with control group. and the reason for this increase is due to oxidative stress

resulting from the effect of hydrogen peroxide, which causes an increase in free radicals that play a role in the reactions of fat oxidation of the cellular membranes, partially damaging them and losing their flexibility by the process of fat peroxidation, which leads to an increase in the production of MDA. Also, an increase in free radicals causes a decrease in antioxidants and causes damage in different body tissues as pancreatic beta cells, they affect the process of insulin production and increase blood sugar, which causes an increase in the activity of the fatty a cycle COA oxidase which stimulated the β-oxidation process of fatty acids and the formation of H_2O_2, causing an increase in lipid peroxidation and production MDA (Sonia *et al*., 2021). The study also showed a significant increase in the concentration of MDA in the drug group and AOM and drug group, which is attributed to the production of free redicals from drug metabolism, the most important of which is OH^- (Kim *et al*., 2008). It is also due to the cellular damage caused by the drug, and this results from the formation of an ion complex, which generates free radicals, which in turn cause severe damage to the cellular membranes and interference in the structure of the cellular structure (Billingham *et al*.,1978), These results are consistent with the researchers result (Lands *et al*.,1999).

Free iron also plays an important role in the peroxidation of fat resulting from the drug anthracycline. Therefore, high doses of the drug cause an imbalance between the active oxygen species ROS, enzymes and antioxidants, and thus tissue damage results as a result of the effect of the new drug (Essick and Sam, 2010).

The results also showed a highly significant decrease in the concentration of GSH in all groups compared to the control group (Table 1) as a result of increased oxidative stress and increased free radicals, which causes increased consumption of GSH, which is considered a non-enzymatic antioxidant that removes free radicals and thus protects cell membranes from oxidative damage (Abd-alwahab, 2014).

The study showed a significant increase in the concentration of $ONOO^-$ and CRP in all groups compared to the control group (Table 1). The reason is due to the increase in free radicals, especially negative superoxide O^-, Therefore, it remains free and active inside the body to search for a single electrolyte to bind with it. Therefore, it reacts with nitric oxide NO^- to produce $ONOO^-$(Yagmurea *et al*.,2007; Mohammad *et al*.,2011).

Table 1: Creatinine Kinase (CK), Malondialdehyde (MDA), Glutathione (GSH), Peroxynitrite (ONOO), Lactate dehydrogenase (LDH, LDH2), C-reactive protein (CRP), concentrations in the treated Groups Compared with the Control Group

Tests	Groups			
	Control group	AOM Group	Anthracyclin Group	AOM+ Anthracyclin
CK(U/L)	339.8±7.9 d	725.4±4.1 c	939.4±4.4 b	970.4.±5.4 a
LDH(U/L)	73.0±2.62 d	122.0±3.63 c	145.0±4.22 b	185.4±5.64 a
LDH2(U/L)	69.4±6.768 d	90.4±4.722 c	120.4.±4.00 b	186.6±0.89 a
GSH(µmol/L)	±1.724 *a*11.92	± 1.141 b10.78	± 0.998 b10.70	± 1.169 b10.82
MDA(µmol/L)	± 0.767 c5.06	± 0.924 a8.84	± 0.288 a8.46	± 1.238 b6.78
ONOO (µmol/L)	± 8.64 d74.32	± 4.86 b143.50	± 2.73 c109.46	± 4.95 b143.60
CRP(mg/l)	5±0.65d	13±0.99c	15±1.2b	19±1.35a

showed a significant differences at level p≤ 0.01. Different letters

Histological microscopic examination of a cross-section of the aorta for a group of AOM (Figure 2) compared with normal tissue (Figure 1) showed the occurrence of histological changes in the thickness of the layers of the aortic wall due to the production of hydrogen peroxide, forming active oxygen species that cause the peroxidation of lipids in cell membranes and their MDA products, which stimulate the oxidation process of lipids, especially LDL-C and phospholipids, and thus lead to the formation of Areas of epithelial damage in the blood vessels, an increase in the permeability of the vessels and a decrease in their elasticity, causing their destruction (Sundus *et al*.,2020).

The histological section of the drug only and AOM and drug (Figure 2,3) also showed a negative effect on the layers of the aortic wall, the inner, middle, and outer layer, compared to the control group (Figure1). The reason is attributed to the toxicity of the drug and AOM on the vascular wall of the aorta (Joel *et al*., 2014), which leads to the production of free radicals and oxidative stress, causing an increase in the process of catabolism and catabolism of fats for the purpose of using them in producing energy inside. The cell as well as the appearance of apoptosis as a result of using the drug (Wolfram *et al*., 2023; Mowei *et al*., 2023). The drug has negative effects on all tissues, and this is consistent with what the researcher found (Sundus & Majid, 2021) Histological examination of the heart for the AOM group (Figure 6) compared with control group (Figure 5) showed the occurrence of harmful tissue changes, such as hypertrophy of some heart muscles and amyloid deposition, the result of oxidative stress, which increases the peroxidation of fats in the tissues, depletes tissue glutathione, and changes in the antioxidant enzymatic system. An increase in active oxygen species is toxic to cells and tissues, causing cell breakdown and death (Sundus & Majid, 2021).

It was also observed in the picture of the combination of the drug and AOM (Figure 7,8), the enlargement of some heart muscles and the deposition of amyloid as a result of metabolic disturbances as a result of the toxicity of the drug and an increase in active oxygen species as a result of increased peroxidation of fats and unsaturated fatty acids and free radicals attacking DNA and accelerating the process of apoptosis of heart cells (Varghese *et al*., 2021). Abnormal Ca^{2+} homeostasis is especially important, for the nature of the contractile activity of the heart, which is strictly dependent on the cation. Anthracyclines dysregulate expression and function of Ca^{2+} channel and dysregulating mitochondrial calcium fluxes, impairing cardiac mitochondrial calcium homeostasis and disrupting cation regulation: this leads to cardiac intracellular Ca^{2+} overload, increased diastolic calcium concentration, a decrease in Ca^{2+} transients amplitude and, overall, to the impairment of calcium contractile function (Bauckneht *et al*., 2019) Further, anthracyclines increase ROS production and oxidative stress: this, on the one hand leads by itself to impaired cardiac contractile function . Many studies indicate that the cardiac mitochondrial Ca^{2+} overload and dysfunction could represent the main cause of cardiac contractile dysfunction. Dox induces the release of cytochrome c from mitochondria, and impairs cardiac mitochondrial respiration, leading to loss of ATP production, apoptosis and cell death (McGowan *et al.*;,2017; Henriksen *et al*.,2018; Bhagat *et al*., 2020). Therefore, it has been proven that the drug anthracycline has side effects on all tissues.

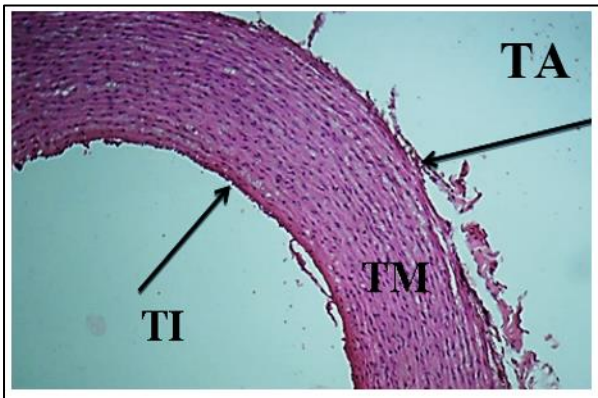

Figure (1): Aorta of Control group showed tunica intima (TI), tunica media (TM) and tunica adventitia (TA) (H&E X100).

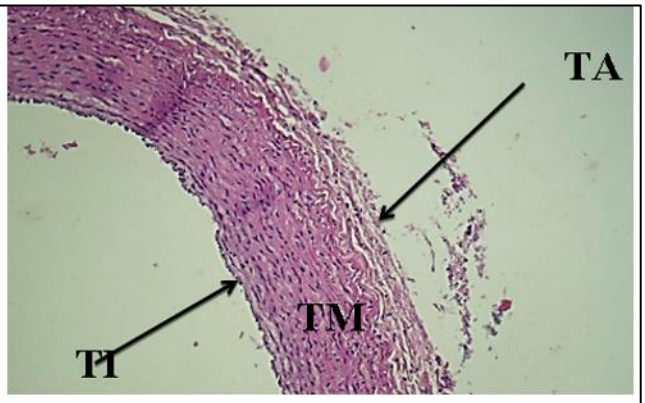

Figure (2): Aorta of mice administrated with AOM showed tunica intima (TI), tunica media (TM) and tunica adventitia (TA) (H&E X100).

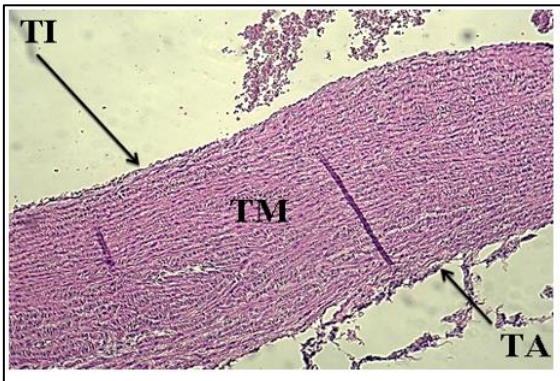

Figure (3): Aorta of mice administrated with Anthracyclin+AOM showed tunica intima (TI), tunica media (TM) and tunica adventitia (TA) (H&E X100).

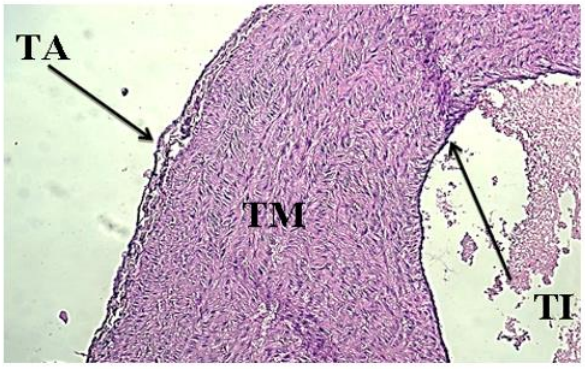

Figure (4): Aorta of mice administrated with Anthracyclin showed tunica intima (TI), tunica media (TM) and tunica adventitia (TA) (H&E X100).

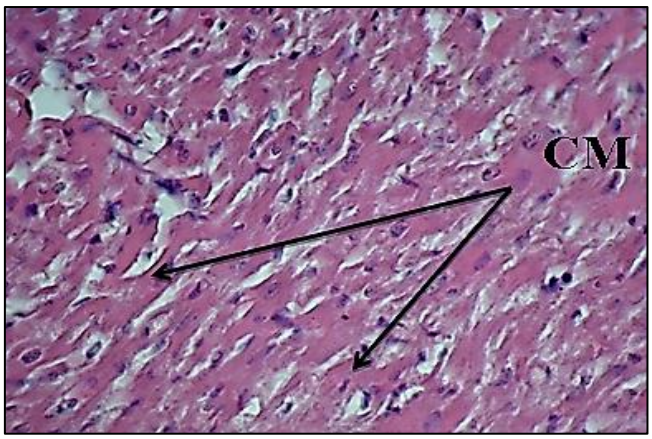

Figure 5: Heart of Control group showed normal cardiac muscles (CM) (H&E- X400)

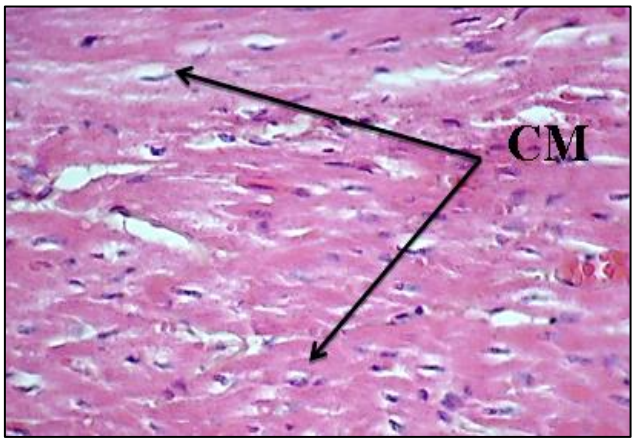

Figure 6: Heart of mice administrated with AOM showed hypertrophy of cardiac muscles (CM) (H&E X400). H&E 400X

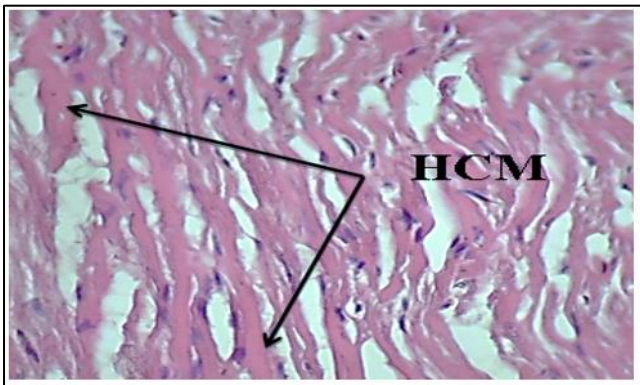

Figure (7): Heart of mice administrated with Anthracyclin showed hypertrophy some of cardiac muscles (HCM) (H&E X400).

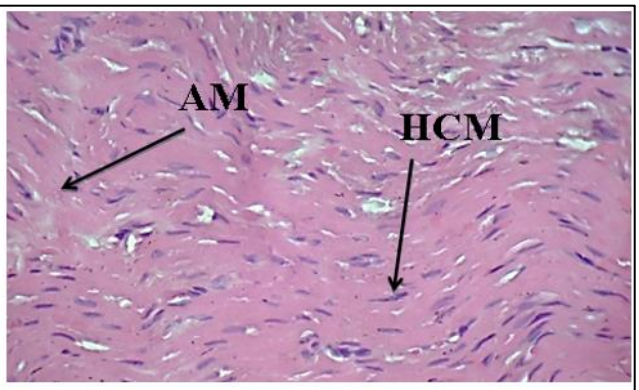

Figure (8): Heart of mice administrated with AOM+Anthracyclin showed hypertrophy of cardiac muscles (HCM) with amyloid (AM) (H&E X400).

CONCLUSION

We conclude from the current study:

1. The study showed the effect of carcinogen azoxymethane (AOM)) on the biochemical tests.
2. Effects carcinogen upon the heart tissue.
3. Side effects for anthracycline drug on heart tissue and biochemical tests.
4. Side effects for AOM and Anthracycline drug on heart tissue and biochemical tests.

REFERENCES

- Butel, J. S. (2000). Viral carcinogenesis: revelation of molecular mechanisms and etiology of human disease. *Carcinogenesis*, *21*(3), 405-426.

- Kumar, V.; Cotran, R.S. and Robbins, S.L. (2003). Robbins basic pathology, (7th ed.), W.B. Saunders Co., Pennsylvania, USA, pp: 165-210.
- Nevidjon, B. M., & Sowers, K. W. (2000). A nurse's guide to cancer care. *(No Title)*.
- Steen, H. B. (2000). The origin of oncogenic mutations: where is the primary damage?. *Carcinogenesis*, *21*(10), 1773-1776.
- Zhu, Y., Aupperlee, M. D., Zhao, Y., Tan, Y. S., Kirk, E. L., Sun, X., ... & Haslam, S. Z. (2016). Pubertal and adult windows of susceptibility to a high animal fat diet in Trp53-null mammary tumorigenesis. *Oncotarget*, *7*(50), 83409.
- Mattioli, R., Ilari, A., Colotti, B., Mosca, L., Fazi, F., & Colotti, G. (2023). Doxorubicin and other anthracyclines in cancers: Activity, chemoresistance and its overcoming. *Molecular Aspects of Medicine*, *93*, 101205.
- Shinlapawittayatorn, K., Chattipakorn, S. C., & Chattipakorn, N. (2022). The effects of doxorubicin on cardiac calcium homeostasis and contractile function. *Journal of cardiology*, *80*(2), 125-132.
- Sachdev, E., Sachdev, D., & Mita, M. (2017). Aldoxorubicin for the treatment of soft tissue sarcoma. *Expert opinion on investigational drugs*, *26*(10), 1175-1179.
- Suaeyun, R., Kinouchi, T., Arimochi, H., Vinitketkumnuen, U., & Ohnishi, Y. (1997). Inhibitory effects of lemon grass (Cymbopogon citratus Stapf) on formation of azoxymethane-induced DNA adducts and aberrant crypt foci in the rat colon. *Carcinogenesis*, *18*(5), 949-955.
- Zhao, X., Zhang, J., Tong, N., Chen, Y., & Luo, Y. (2012). Protective effects of berberine on doxorubicin-induced hepatotoxicity in mice. *Biological and Pharmaceutical Bulletin*, *35*(5), 796-800.
- Burtis, C. A., & Ashwood, E. R. (1999). Tietz textbook of clinical chemistry. *Philadelphia*, *1999*, 1654-5.
- Guidet, B. & shah, S. V. (1989). *Am J. Physiol*; 257(26):440. cited by Muslih, R. K., Al-Nimer, M. S & Al-Zamely, O. Y. (2002) . The level of Malondialdehyde after activation with H_2O_2 and $CuSO_4$ and inhibition by deferoxamine and Molsidomine in the serum of patient with acute Myocardial infarction. *National journal of chemistry*; 5: 139-148.
- Al-Zamely, O. Y., Al-Nimer, M. S., & Al-Muslih, R. K. (2001). Detection the level of peroxynitrite and related antioxidant status in the serum of patients with acute myocardial infraction. *Nation. J. Chem*, *4*(1), 625-637.
- Carleton, H. M., Drury, R. A. B., & Wallington, E. A. (1967). *Histological technique* (Vol. 3). London:: Oxford University Press.
- Duncan, D. B. (1955). Multiple range and multiple F tests. *biometrics*, *11*(1), 1-42.
- Waly, M. I., Al-Rawahi, A. S., Al Riyami, M., Al-Kindi, M. A., Al-Issaei, H. K., Farooq, S. A., ... & Rahman, M. S. (2014). Amelioration of azoxymethane induced-carcinogenesis by reducing oxidative stress in rat colon by natural extracts. *BMC complementary and alternative medicine*, *14*, 1-10.
- Waly, M. I., Al-Ghafri, B. R., Guizani, N., & Rahman, M. S. (2015). Phytonutrient effects of date pit extract against azoxymethane-induced oxidative stress in the rat colon. *Asian Pacific Journal of Cancer Prevention*, *16*(8), 3473-3477.
- Mattioli, R., Ilari, A., Colotti, B., Mosca, L., Fazi, F., & Colotti, G. (2023). Doxorubicin and other anthracyclines in cancers: Activity, chemoresistance and its overcoming. *Molecular Aspects of Medicine*, *93*, 101205.
- Ramos, S., Goya, L., & Martín, M. A. (2021). Antioxidative stress actions of cocoa in colonic cancer: Revisited. In *Cancer* (pp. 337-348). Academic Press.
- Alabdullah, S. W., & Humoud, M. N. (2021). Physiological and Histological Study of Doxorubicin Effect on Kidney Tissue in Cancer Induced Rats. *Indian Journal of Forensic Medicine & Toxicology*, *15*(2), 4302-4308.
- Kim, K. H., Oudit, G. Y., & Backx, P. H. (2008). Erythropoietin protects against doxorubicin-induced cardiomyopathy via a phosphatidylinositol 3-kinase-dependent pathway. *Journal of Pharmacology and Experimental Therapeutics*, *324*(1), 160-169.
- Lands, L. C., Grey, V. L., & Smountas, A. A. (1999). Effect of supplementation with a cysteine donor on muscular performance. *Journal of Applied Physiology*, *87*(4), 1381-1385.
- Billingham, M. E., Mason, J. W., Bristow, M. R., & Daniels, J. R. (1978). Anthracycline Cardiomyopathy Monitored by Morphologic Changes [1]. *Cancer treatment reports*, *62*(6), 865-872.
- Essick, E. E., & Sam, F. (2010). Oxidative stress and autophagy in cardiac disease, neurological disorders, aging and cancer. *Oxidative medicine and cellular longevity*, *3*(3), 168-177.
- Alabdullah, S. W., Alsamir, S. A., & AlRufaei, I. A. (2020). Effect of Thymoquinone on some biochemical and hormonal indices and their protective effect on the genital organs of rats after cancer induction in Laboratory. *EurAsian J. BioSci*, *14*, 567-573.
- Salazar-Mendiguchía, J., González-Costello, J., Roca, J., Ariza-Solé, A., Manito, N., & Cequier, Á. (2014). Anthracycline-mediated cardiomyopathy: basic molecular knowledge for the cardiologist. *Archivos de cardiología de México*, *84*(3), 218-223.
- Kong, M., Pan, Q., Cheng, X., Li, J., Gao, Y., & Tian, X. (2023). Anthracycline-induced delayed-onset cardiac toxicity: A case report and

literature review. *Experimental and Therapeutic Medicine*, *26*(5), 1-7.

- Bhagat, A., & Kleinerman, E. S. (2020). Anthracycline-induced cardiotoxicity: causes, mechanisms, and prevention. *Current Advances in Osteosarcoma: Clinical Perspectives: Past, Present and Future*, 181-192.
- Henriksen, P. A. (2018). Anthracycline cardiotoxicity: an update on mechanisms, monitoring and prevention. *Heart*, *104*(12), 971-977.
- McGowan, J. V., Chung, R., Maulik, A., Piotrowska, I., Walker, J. M., & Yellon, D. M. (2017). Anthracycline chemotherapy and cardiotoxicity. *Cardiovascular drugs and therapy*, *31*, 63-75.
- Varghese, S. S., Eekhoudt, C. R., & Jassal, D. S. (2021). Mechanisms of anthracycline-mediated cardiotoxicity and preventative strategies in women with breast cancer. *Molecular and Cellular Biochemistry*, *476*(8), 3099-3109.
- Bauckneht, M., Pastorino, F., Castellani, P., Cossu, V., Orengo, A. M., Piccioli, P., ... & Marini, C. (2020). Increased myocardial 18F-FDG uptake as a marker of Doxorubicin-induced oxidative stress. *Journal of Nuclear Cardiology*, *27*(6), 2183-2194.
- Dempke, W. C., Zielinski, R., Winkler, C., Silberman, S., Reuther, S., & Priebe, W. (2023). Anthracycline-induced cardiotoxicity—are we about to clear this hurdle?. *European Journal of Cancer*, *185*, 94-104.