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Impact of Doxorubicin Chemotherapy on Oxidative Stress Status in Heart and Liver: An experimental Study on Rats

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Abstract

The purpose of the current study was to assess how doxorubicin treatment affected the heart and liver oxidative stress indicators in female Wistar rats. For in-vivo study, ten female albino Wistar rats were divided into two groups (n=5), control group and doxorubicin treated group. GSH, MDA levels and SOD, GST activities in heart and liver were measured in order to evaluate various oxidative stress markers. The tissue histology of the heart was examined. Results of the in-vivo rats study show that doxorubicin induces oxidative stress by decreasing the level of GSH level and increasing MDA level, SOD and GST activities in the DOX group compared to the control group. Furthermore, in comparison to the control group, DOX caused histological changes in the heart cells in the DOX group. This study concludes that doxorubicin treatment causes oxidative stress in the heart and liver of rats, which is detrimental to those organs.

Keywords: Doxorubicin; Oxidative Stress; Heart; Liver; Rats

Abbreviations

GST: Glutathione S Transferase; SOD: Superoxide Dismutase; NADPH: Nicotinamide Adenine Dinucleotide Phosphate; GSH: Reduced Glutathione; H2O2: Hydrogen Peroxide, HO•: Hydroxyl Free Radical; ROS: Reactive Oxygen Species; O2-: Superoxide Radical

Introduction

Uncontrolled cell proliferation is a common feature of cancer illness, and it may result in metastasis, or the migration and spread of tumor cells to other organs [1]. Globally, cancer is a significant public health issue [2]. After cardiovascular disorders, cancer is the second most common cause of mortality [3]. In an effort to defeat cancer, numerous therapeutic approaches are being researched [4]. There are other cancer therapies available, but chemotherapy is among the most important because it is thought to be the most successful and widely used technique for the majority of cancer types [5]. Chemotherapy is the medical use of chemicals to stop cancerous cells or disease-causing microorganisms without significantly harming healthy cells. Chemotherapy, like doxorubicin chemotherapy, is

accompanied with adverse effects that inevitably lead to treatment failure [6]. Doxorubicin (Adriamycin) is considered as one of the most effective among the available drugs of cancer [7]. An antitumor antibiotic known as an anthracycline glycoside, doxorubicin, is first-line therapy for a variety of cancer types when combined with other chemotherapy medications [8]. Unfortunately, doxorubicin's use and effectiveness can be compromised by its toxic and adverse effects on a variety of organs. Doxorubicin induced cardiotoxicity and hepatotoxicity [9]. The therapeutic application of anthracycline antibiotics is limited by side-effects mainly, chronic cardiotoxicity and hepatotoxicity [10]. In the current study, the aim was to evaluate the impact of doxorubicin chemotherapy on oxidative stress markers in heart and liver of female Wistar rats.

Materials and Methods

Animal Care and Experimental Design

In this investigation, ten female Wistar rats weighing 184.84 ± 8.48 weeks were used from the Pasteur Institute of Algiers. The rats at the Echahid University Hamma Lakhdar-El-Oued pet store are bred at the Faculty of Natural and Life Sciences. The animals were kept in identical conditions; a photoperiod (12 hours of light and 12 hours of darkness) at room temperature; during their adaption period. The rats live in plastic cages with free access to food and water, following a standard diet [11]. Over a period of four weeks, the experiment was carried out.

Raw materials	Quantity (g / kg)	Percentage (%)
Maize	326	32.6
Sucrose	326	32.6
Protein	168	16.8
Oil	80	8
Cellulose	40	4
Minerals	40	4
Vitamin	20	2

Table 1: Standard Diet Composition.

Following a time of adaption, the animals had been divided into two experimental groups, each consisting of five individuals:

Group 1 (Control): Normal rats received weekly injections of 1.5milliliters per kilogram of physiological saline for four weeks.

Group 2 (DOX): Normal rats received weekly injections of 1.5milliliters per kilogram of doxorubicin for four weeks.

Doxorubicin was injected intraperitoneally (one dose per week at a rate of 1.5milligrams per kilogram) to cause heart and liver damage [2].

Sacrifice and Tissues Collection

Following a 16-hour fast, the animals were slaughtered while inhaling a small amount of chloroform (94%) to induce slight anesthesia. After carefully removing the heart and liver, they were cleaned in normal saline (NaCl), and kept at -20°C to do the oxidative damage analyses.

Tissue Samples Preparation and Tissular Oxidative Stress Parameters

Homogenates were prepared following the method done by Boulaares I, et al. [2]. GSH, MDA, SOD and GSTs level was measured according to the method described by Weckbecker G, et al. [12], Draper HH, et al. [13], Beauchamp C, et al. [14] and Habig WH, et al. [15], respectively.

Histopathological Study

Upon sacrificing the rats, the heart tissues were removed and kept in a 10% formaldehyde fixative solution until slide preparation was ready. Afterwards, the tissues received a series of ethanol dehydration, toluene cleaning, paraffin immersion, and hematoxylin and eosin staining applications. Camera-equipped microscopes were used to examine the resultant slides, and computer screens were used to display the photos that the camera had taken.

Statistical Analysis

In order to express the results as either an average ± ES (standard deviation), the study used the student's t-test for independent samples. Minitab 13.0 software was used to analyse all the data, and a P-value of less than 0.05 was used to assess statistical significance.

Results

Oxidative Stress Parameters

Our data showed a highly significant decrease in GSH level in heat and liver ($P<0.01$), as well as a significant increase in MDA level, SOD and GST activities in heart and liver in control as compared with DOX group (Figure 1).

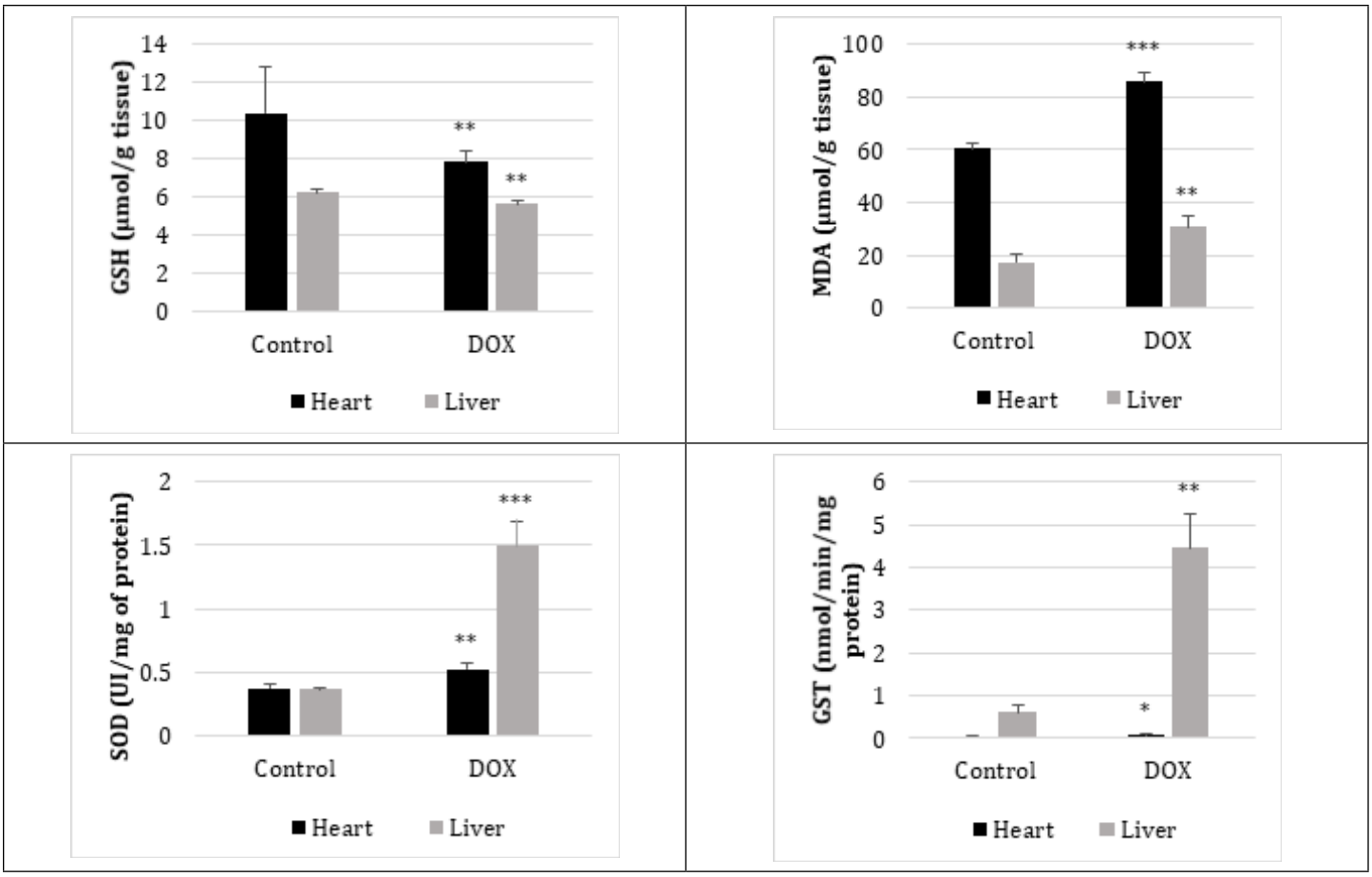

Figure 1: Oxidative stress parameters levels in heart and liver in control and DOX groups.

Histopathological Study

Photomicrograph of the heart tissues of control rats appeared normal myofibrillar structure and normal cells. Heart tissues of DOX-treated rats showed deformations of muscle fibers with hemorrhage, inflammation and vacuolization of cardiomyocytes (Figure 2).

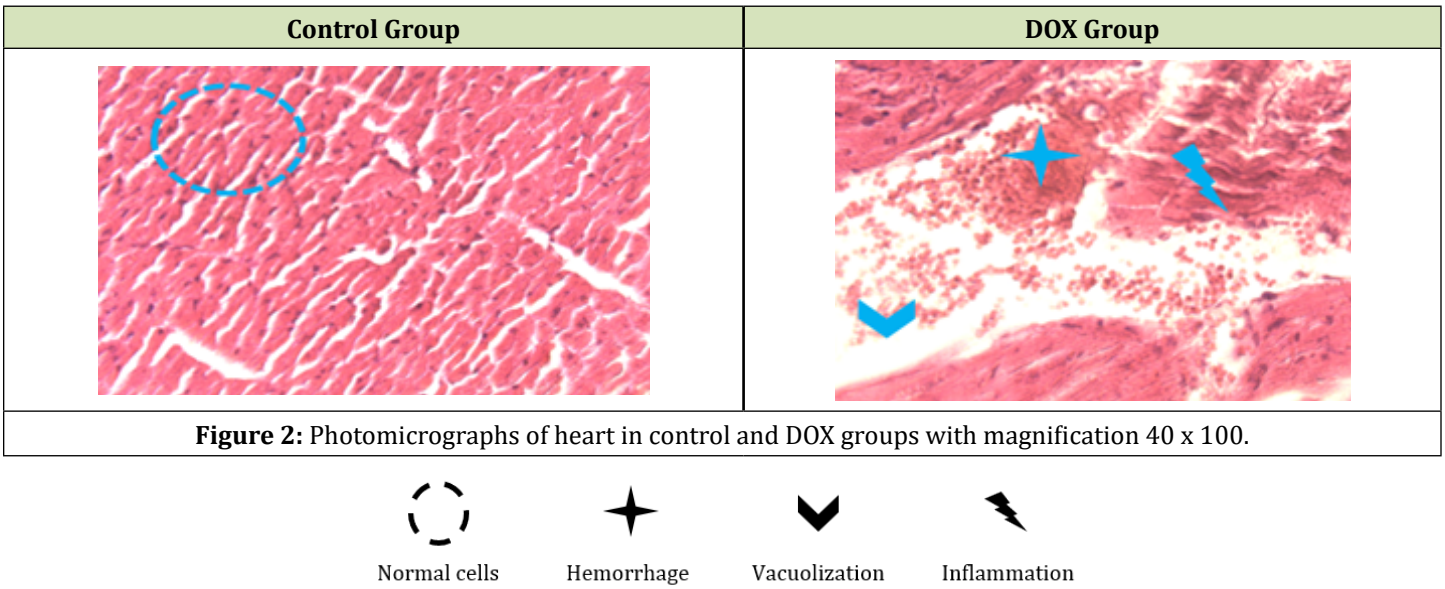

Figure 2: Photomicrographs of heart in control and DOX groups with magnification 40 x 100.

Groups	Control group	DOX group
Hemorrhage	- - - -	+ + + +
Inflammation	- - - -	+ + + +
Vacuolization	- - - -	+ + + +

Table 2: Photomicrographs of Heart in Control and DOX Groups.

Discussion

Chronic cardiotoxicity and hepatotoxicity are the main side effects that restrict the therapeutic use of anthracycline medicines [10]. Numerous investigations have demonstrated a connection between doxorubicin-induced cardiotoxicity and elevated oxidative stress and reactive oxygen species (ROS) generation [16]. Superoxide radical (O2-), hydrogen peroxide (H2O2), and hydroxyl free radical (HO•) are the three main forms of ROS that cause cardiotoxicity [17]. In our investigation, rats administered DOX noticed a significant decrease in their liver and heart levels of reduced glutathione (GSH) as compared to the control group. These results are consistent with Khan et al.'s stated that doxorubicin causes the heart to produce free radicals and reduces its capacity to detoxify ROS [18]. Furthermore, our findings show that the DOX group's liver and heart had higher MDA levels. The fundamental mechanism is that doxorubicin increases malonaldehyde levels by reduction of one-electron reduction of nicotinamide adenine dinucleotide phosphate (NADPH) cytochrome P-450 reductase enzyme [19]. The level of lipid peroxidation was evaluated by measuring MDA, which is the final product of lipid peroxidation [20]. Elevation of oxidized lipids (MDA) confirms doxo-induced oxidative stress [21]. A non-enzymatic mechanism that uses iron and an enzymatic pathway that uses the mitochondrial respiratory chain are the two pathways by which the toxicity is first caused by an imbalance in the creation of free radicals [22]. The first method involves the production of a semiquinone free radical by the activity of several NADPH-dependent reductases, which result in the reduction of the Doxo to the equivalent Doxo semiquinone by one electron. Superoxide radicals are produced when quinone-semiquinone generated from Doxo conducts redox cycling in the presence of oxygen. The second process for the formation of doxorubicin free radicals is a non-enzymatic pathway involving interactions with iron. Doxorubicin not only makes the tissue produce more free radicals, but it also makes it less able to detoxify reactive oxygen species [21]. Doxorubicin is probably harmful to the liver [23]. By phospholipid activation and lipid peroxidation, which raise intracellular Ca2+ and cause the production of ALT and eventually result in apoptotic cell death, these free radicals could damage the hepatic membranes [24]. Superoxide dismutase (SOD) and glutathione S transferase (GST) are examples of enzymatic antioxidants. Our study found that these enzymes had high activity levels because the body was trying to repair itself by increasing the activity of these enzymes due to an increase in free radicals, which are the substrate of SOD and GST. Compared to other organs, the heart has a higher number of mitochondria and comparatively lower amounts of antioxidant enzymes, making it more susceptible to the damaging effects of free radicals and doxorubicin-induced cardiotoxicity [25]. In this regard, it's possible that mitochondria not only directly experience doxorubicin toxicity, but also cause oxidative stress, which contributes to overall cell damage [26].

After administering DOX injections to rats for four weeks, the animals developed severe histopathological abnormalities that included myocardial swelling, hemorrhage, inflammation and vacuolization in the tissue. These anatomical and cellular alterations align with additional findings on DOX induced cardiomyopathy in rats. Increased inflammatory responses within the myocardium are one of the many potential molecular pathways associated with doxorubicin-induced structural alteration [27].

Doxorubicin causes an important increase in the concentrations of particular inflammatory chemokines and cytokines, such as TNF-α, IL-1β, IL-6, COX-2, and CCL2/MCP-1 [28]. Important mediator proteins, cytokines are necessary for the immune system's networking and communication. Monocytes or lymphocytes can create cytokines with pro- and anti-inflammatory properties. Chemokines are defined as cytokines possessing chemotactic activity. It is believed that a key factor in immune response homeostasis and inflammation, which underlie many diseases, is the balance between pro-inflammatory cytokines (IL-1β, IL-2, TNFα, Il-6, IL-8, IFN-γ...) and anti-inflammatory cytokines (IL-10, IL-4, TGFβ) [29]. Another early occurrence in cardiac stress is inflammation. Affected cardiac tissues show increased production and release of inflammatory cytokines and chemokines, as well as elevated levels of endothelial adhesion molecules. The main line of defense for the heart against infections and tissue damage is the innate immune system [30]. Prospective COX-2 inhibitors may potentially be suitable as cancer chemotherapy preventive medications due to the evident correlation between inflammation and carcinogenesis [31]. Apoptosis is induced by doxorubicin, and this leads to cardiotoxicity. Doxorubicin induces oxidative stress and increases the production of ROS [17]; this apoptosis may be caused vacuolization in the tissue.

Several clinical studies have indicated that a number of chemotherapies result in direct vascular injury; however, the mechanism and characteristics of this toxicity are still unknown [32]. Hemorrhage is the loss of blood from a blood vessel and can be caused by free radicals or vascular toxicity carried on by doxorubicin.

Conclusion

In conclusion, this study demonstrates the harmful effects of doxorubicin chemotherapy on the liver and heart in rats by inducing oxidative stress, which may also demonstrate the harmful effects of DOX treatment on cancer patients.

References

1. Boulaares I, Guemari IY, Derouiche S (2020) Analysis of Some Hematological and Biochemical Markers in Women Cancer Patients Receiving Doxorubicine Chemotherapy. J Pharm Biosci 8(1): 29-34.

2. Boulaares I, Derouiche S, Guemari IY (2024) Protective effect of ObE against Doxorubicin-Induced immunosuppression and Cardiotoxicity in Rats. Res J Pharm Technol 17(4): 1839-1843.

3. Lodi M, Priksz D, Fulop GA, Bodi B, Gyongyosi A, et al. (2019) Advantages of prophylactic versus conventionally scheduled heart failure therapy in an experimental model of doxorubicin-induced cardiomyopathy. J Transl Med 17(1): 229.

4. Boulaares I, Derouiche S, Niemann J (2024) Can manganese oxide nanoparticles synthesized by green route be used in Tumor Therapy, A Narrative Review. Arch Razi Inst.

5. Abbas Z, Rehman S (2018) An overview of cancer treatment modalities. Neoplasm 1: 139-157.

6. Lei J, Wang H, Zhu D, Wan Y, Yin L (2019) Combined effects of avasimibe immunotherapy, doxorubicin chemotherapy, and metal–organic frameworks nanoparticles on breast cancer. J Cell Physiol 235(5): 4814-4823.

7. Wakharde A, Awad A, Bhagat A, Karuppayil S (2018) Synergistic activation of doxorubicin against cancer: A review. Am J Clin Microbiol Antimicrob 1(2): 1-6.

8. Chetehouna S, Derouiche S, Atoussi O (2024) Identification of New Alkaloids from Algerian Purslane by HPLC-QTOF-MS and Beneficial Effect of Purslane Enriched with Zinc on Experimental Alzheimer Disease in Rats. Curr Trends Biotech Pharm 18(1): 1595-1607.

9. Salouege I, Ali RB, Said DB, Elkadri N, Kourda N, et al. (2014) Means of evaluation and protection from doxorubicin-induced cardiotoxicity and hepatotoxicity in rats. J Cancer Res Ther 10(2): 274-278.

10. Damodar G, Smitha T, Gopinath S, Vijayakumar S, Rao Y (2014) An evaluation of hepatotoxicity in breast cancer patients receiving injection Doxorubicin. Ann Med Health Sci Res 4(1): 74-79.

11. Southon S, Gee JM, Johnson I (1984) Hexose transport and mucosal morphology in the small intestine of the zinc-deficient rat. Br J Nutr 52(2): 371-380.

12. Weckbecker G, Cory JG (1988) Ribonucleotide reductase activity and growth of glutathione-depleted mouse leukemia L1210 cells in vitro. Cancer Lett 40(3): 257-264.

13. Draper HH, Hadley M (1990) Malondialdehyde determination as index of lipid Peroxidation. Methods in enzymology: Elsevier 186: 421-431.

14. Beauchamp C, Fridovich I (1971) Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem 44(1): 276-287.

15. William HH, Michael JP, William BJ (1974) Glutathione S-transferases. J Biol Chem 249(22): 7130-7139.

16. Gasser A, Chen Y-W, Audebrand A, Daglayan A, Charavin M, et al. (2019) Prokineticin receptor-1 signaling inhibits dose-and time-dependent anthracycline-induced cardiovascular toxicity via myocardial and vascular protection. Cardio Oncology 1(1): 84-102.

17. Zhu H (2018) Doxorubicin-Induced Cardiotoxicity. Cardiotoxicity pp: 47-66.

18. Khan G, Haque SE, Anwer T, Ahsan MN, Safhi MM, et al. (2014) Cardioprotective effect of green tea extract on doxorubicin-induced cardiotoxicity in rats. Acta Pol Pharm 71(5): 861-868.

19. Anber Z (2018) Effect of doxorubicin and cyclophosphamide regimen versus taxane on liver enzymes in Iraqi women with breast cancer. Biomed Res 29(21): 3869-3873.

20. Chetehouna S, Derouiche S, Reggami Y, Boulaares I (2024) Sonchus maritimus Extract-Loaded Niosomes Bioconjugated by Linoleic Acid in Hepatic Encephalopathy Induced by High-Fructose Diet in Albino Wistar Rats. Arch Razi Inst 79(1): 189-200.

21. Dogan M, Firinci F, Balci YI, Gurses D, Polat A, et al. (2014) Cardioprotective effect of clarithromycin on doxorubicin-

induced cardiac toxicity in rats. International Journal of Hematology and Oncology 24: 30-35.

22. Chetehouna S, Derouiche S, Boulaares I, Reggamie Y (2024) Phytochemical profile, anti-inflammatory analysis and cytotoxic activity of SmE-SeNPs against breast (MCF-7) cancer cells. Biocat Agricult Biotech 57: 103122.

23. Bengaied D, Ribeiro A, Amri M, Scherman D, Arnaud P (2017) Reduction of hepatotoxicity induced by doxorubicin. J Integr Oncol 6(3): 1-12.

24. Sasu A, Herman H, Folk A, Balta C, Rosu M, et al. (2016) Protective effects of silymarin on epirubicin-induced hepatotoxicity in mice. Studia Universitatis "Vasile Goldis" Arad. Seria Stiintele Vietii (Life Sciences Series) 26(3): 305-316.

25. Li J, Wan W, Chen T, Tong S, Jiang X, et al. (2019) miR-451 Silencing Inhibited Doxorubicin Exposure-Induced Cardiotoxicity in Mice. Biomed Res Int 1: 1-11.

26. Adamcova M, Skarkova V, Seifertova J, Rudolf E (2019) Cardiac troponins are among targets of doxorubicin-induced cardiotoxicity in hiPCS-CMs. Int J Mol Sci 20(11): 2638.

27. Hijazi MA, Jambi HA, Aljehany BM, Althaiban MA (2019) Potential Protective Effect of Achillea fragrantissima against Adriamycin-Induced Cardiotoxicity in Rats via an Antioxidant and Anti-Inflammatory Pathway. Biomed Res Int 1: 1-10.

28. Guo RM, Xu WM, Lin JC, Mo LQ, Hua XX, et al. (2013) Activation of the p38 MAPK/NF-κB pathway contributes to doxorubicin-induced inflammation and cytotoxicity in H9c2 cardiac cells. Mol Med Rep 8(2): 603-608.

29. Chetehouna S, Derouiche S, Reggami Y, Boulaares I, Frahtia A (2024) Gas Chromatography Analysis, Mineral Contents and Anti-inflammatory Activity of *Sonchus maritimus*. Trop J Nat Prod Res 8(4): 6787-6798.

30. Chen L, Deng H, Cui H, Fang J, Zuo Z, et al. (2018) Inflammatory responses and inflammation-associated diseases in organs. Oncotarget 9(6): 7204-7218.

31. Boulaares I, Derouiche S, Niemann J (2024) *Ocimum basilicum* L.: A Systematic Review on Pharmacological Actions and Molecular Docking Studies for Anticancer Properties. J Biochem Technol 15(1): 12-18.

32. Joseph H, Stemmer SM, Tsarfaty I, Shalgi R, Aharon I (2015) Chemotherapy-induced vascular toxicity-real-time in vivo imaging of vessel impairment. J Vis Exp 95: e51650.