Biochemical Study on Attenuating the Cardiotoxic Effect of Doxorubicin through Transforming into Mesoporous Nanoparticles Form

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A B S T R A C T

The aim of this study was transforming doxorubin by encapsulating it in mesoporous silica compared to free doxorubicin. In vivo study, free doxorubicin, mesoporous silica preparations were assessed by different measurements like particles size, zeta potential and TEM. Several parameters have been measured to assess the cardiotoxicity (Albumin, lactate dehydrogenase (LDH), Aspartate Aminotransferase (AST), Anticardiolipin IgG, Creatine kinase-*MB (*CK MB), C-reactive protein (CRP), Tumor necrosis factor (TNF α)). The experimental animals were divided into seven groups as follow: group (1) Control. (2) Group B: received doxorubicin. (3) Group C: mesoporous doxorubicn. Rats injected with free doxorubicin caused significantly elevated levels of all parameters measured compared to control group (albumin 4.435 ± 0.085 vs 2.910 ± 0.10, anticardiolipin 9.4 ± 0.256 vs 19.31 ± 0.4391, AST 64.60 ± 1.79 vs 244.8 ± 9.86, CK 14.04± 1.794 vs 73.25± 1.240, CRP 4.904±0.45 vs 23.96 ± 0.95, LDH 212±10.1 vs 754±16.4, TNF α 34.62±1.05 vs 126.6±6.2°). In addition there was a significant variation of mesoporous silica results compared to free doxorubicin. Conclusion: There was a marked cardiotoxicity of doxorubicin could be decreased by transforming it into mesoporous form.

Keywords: cadiotoxicity, myocardial infarction, doxorubicin, mesoporous, nanoparticles.

Abbreviations: (LDH) lactate dehydrogenase, (AST) Aspartate Aminotransferase, *(*CK MB) Creatine kinase-*MB,* (CRP) C-reactive protein, (TNF α) Tumor necrosis factor, dox. doxorubicin, (gp) group, (EDTA) Ethylene diamine tetra acetic acid, (CVD) cardio vascular diseases, (CAD) coronary artery disease . (TEM) Transmission electron microscopy. ECG. Electrocardiography, MPEPC methoxy poly (ethylene glycol)-poly (ε-caprolactone)

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1. INTRODUCTION:

Myocardial infarction is a major cause of death and disability worldwide may be the first manifestation of coronary artery disease (CAD) or it may occur, repeatedly, in patients with established disease. *(Qanitha et al., 2017).* The term myocardial infarction reflects cell death of cardiac myocytes caused by ischaemia, which is the result of a perfusion imbalance between supply and demand *(Marina et al., 2016).* Ischaemia in a clinical setting most often can be identified from the patient's history and from the ECG. *(Marta et al., 2017).* Doxorubicin cardiotoxicity can be acute, occurring during and within 2–3 days of its administration. The incidence of acute cardiotoxicity is approximately 11%. *(Irawati et al., 2017).* Many mechanisms implicated in cardiotoxicity

of doxorubicin by activation of reactive oxygen species and oxidative stress that play major role in several disorders including cancer, significant upregulation of the expression of death receptors (TNFR1, Fas, DR4 and DR5) at both protein and mRNA levels, Mitochondrial dysfunction and calcium overloading *(Arivalagan et al., 2018).* Nano-formulated drugs along with Dox were studied for their anticancer effect like liposomal oleonalic acid which attenuates the cardiotoxic effects of Dox and created a synergistic anticancer effect. Synergistic anti-glioma effect of honokiol, combination with MPEPC nanoparticles, honokiol and Dox – poly (D,L-lactide-coglycolide) are different forms of doxorubicin nanoparticles (*Yangyang et al., 2018).*

Nanotechnology can be defined as the science and engineering involved in the design, synthesis, characterization, and application of materials and devices whose smallest functional organization *(Lina et al., 2017).*

The emergence of nanoscience and nanotechnology, which is creation and utilization of materials and tools at the nanometer scale, has been a great influence on a number of industries and particularly the pharmaceutical industry *(Challa 2010).* As recombinant technology and biotechnology changed the landscape of pharmaceutical industry, nanotechnology is poised to lift the pharmaceutical industry that is at cross roads today, to new levels. *(Anna et al., 2018).* However, unlike the biotechnology industry which influenced primarily the pharmaceutical industry, nanotechnology has broader applications and therefore, the nanotechnology tools and materials developed for other industries also have potential opportunities in the pharmaceutical industry as well. *(Ilhwan et al., 2017).* However, what is clearly lacking is a model for sorting out the plethora of nanotechnology tools that exists and strategically correlating with potential opportunities into different segments of pharmaceutical R&D. In addition, there is going to be a paradigm shift in the pharmaceutical industry towards personalized medicine as a new standard of care integrating therapeutics with diagnostics. It is, therefore, important to develop a more scientific approach for strategic implementation of nanotechnology tools in the pharmaceutical industry *(Karthik et al., 2018).*

Mesoporous silica materials was discovered in 1992 by the Mobile Oil Corporation have received considerable attention due to their superior textual properties such as high surface area, large pore volume, tunable pore diameter, and narrow pore size distribution *(Chachchaya et al., 2017).* The surface functionalization is generally needed to load proper type of drug molecules, specific actions can also have a natural quality or characteristics by the functionalization through chemical links with other materials such as stimuli-responsive, luminescent or capping materials, leading to smart, and multifunctional properties *(Ilaria et al., 2017).* Porous silica based materials are among the most beneficial compounds which can provide more opportunities for treatment of cancer therapy and provide a pathway toward the treatment of challenging diseases *(Daniel et al., 2017).* MSNs have been used in controllable drug delivery, gene transport, gene expression, biomarking, biosignal probing, imaging agent, detecting agent, drug delivery vehicles, and other important biological applications ***(Nowald et al., 2017).*** The present study aimed to investigate the effect of doxorubicin and mesoporous doxorubicin on myocardial infracted rat model.

2. MATERIAL AND METHODS:

2.1. Materials

Doxorubicin hydrochloride provided from *(Eimic company, cairo,egypt).* CTAB, TEOS

(SiO2>28.0%) and sodium hydroxide (>96%) were purchased from Sigma-Aldrich,

2.2. Animals:

I. Source: The male albino rats were provided by the animal house unit of The National Organization for Drug Control and Research (NODCAR), Giza, Egypt. Their weight was from (150-180 grams), their age was about thirteen to fourteen weeks.

II. Diet: The standard diet was a mixture composed of (72.2% carbohydrate,

3.4% fats, 19.8% proteins, 3.6% cellulose, 0.5% vitamins and minerals and 0.5% salts), obtained from Kahira Company for Poultries, Cairo, Egypt.

III. Adaptation: The animals were housed in the animal house unit of NODCAR, and allowed free access to standard diet and water under standardized conditions (12.00h light: 12.00h dark, 25±2°C) and away from stressful stimuli.

2.3. Collection of blood samples:

Blood sample collected after 48 hours from the last dose, centrifuged, serum collected and divided into seven Epindorf tubes, stored at -20°C until analysis *(Melissa et al., 2009).*

This study was performed on thirty adult albino rats were divided as follow:

1. Control group (group A): Ten albino rats received saline.

2. Group B: Ten albino rats received a cumulative doses of doxorubicin (15 mg/kg) *(Wuqiang et al., 2008)* given as a dose of (5 mg/kg) every other two days, rats slaughtered and plasma collected after 48 hours from the last dose.

3. Group C: Ten albino rats received cumulative doses of doxorubicin (20 mg/kg) encapsulated on mesoporous silica given as a dose of (5 mg/kg) taken every other two days, rats slaughtered and plasma collected after 48 hours from the last dose.

2.4. Preparation of mesoporous nanoparticles

2.4.1. Method using CTAB as surfactant

The mixture of CTAB (1.5 g), 2.0 M NaOH (1.75ml) and water (120 ml) is heated to 80°C for 30 mins to reach pH 12.3. To this clear solution, add TEOS (2.335 g) is rapidly added via injection (stirring at 550 rpm), a white precipitate is observed after 3 mins of constant stirring. The products are then isolated by hot filtration, washed with copious amount of water and methanol. An acid extraction is performed using mixture of methanol (100 ml), conc. HCl (1 ml) and previously prepared sample (1.0 g) for 6 hours using hot plate. *(lodha et al ., 2012).*

2.4.2. Loading procedure

The passive method was used to load MSN with doxorubicin. Deionized Water as a solvent was used for loading, depending on the polarity index. 100 of MSN was added to 100 mg of doxorubicin. Afterward the suspensions were brought to equilibrium under gentle stirring for 24 hours. The loading procedure was carried out by using successively water as solvent. Apart from the effect of solvent, the influence of temperature and time on the loading procedure was investigated *(Meysam et al., 2015).*

2.5. Nanoparticles Characterization.

2.5.1. Transmission Electron Microscopy.

Transmission Electron Microscopy is a procedure that is used to characterize the morphology of NPs, operates on the same basic principles as the light microscope but uses electrons instead of light. *(Yuri et al., 2015)*

2.5.2. Dynamic Light Scattering

Dynamic light scattering is a non-invasive technique for measuring the size of particles and

molecules in suspension. The technique of DLS measures the speed of particles which undergoing Brownian motion (Brownian motion is the random movement of particles due to collisions caused by bombardment of the solvent molecules that surround them).

The speed of the Brownian motion is influenced by particle size, sample viscosity and temperature. *(Angelina et al., 2016)*

2.5.3. Zeta potential measurements.

Zeta potential has often been used for characterizing colloidal drug delivery systems. It is a measure of the surface electrical charge of the particles. The magnitude of zeta potential gives an indication of the potential stability of a colloidal system. If all the particles have relatively large negative or positive zeta potentials, they will repel each other and create dispersion stability. If the particles have low zeta potential values, there is no force to prevent the particles from agglomerating and there is dispersion instability *(Paolino et al., 2006).*

2.5. Measuring serum cardiac marker enzymes

Cardiac dysfunction was evaluated by measuring the elevation in serum activities of LDH *(Pankajet al., 2017),* AST *(Sameer et al., 2017),* and CKMB *(Matthew et al., 2017)* (spectrophotometrically) using commercially available kits. The results were expressed in u/l.

2.6. Measuring serum cardiac function proteins .

Cardiac dysfunction was assessed by measuring the alteration in plasma levels of albumin *(Ismail and Bulent, 2017)* (spectrophotometrically) and elevation in the concentration of anticardiolipion *(Androniki et al., 2000)* (ELISA), CRP (spectrophotometrically) *(Endre and Walker, 2017)* , TNFα (ELISA) *(Ahmad and Sharma, 2012)* using commercially available kits.

2.7. Statistical analysis

Data are expressed as mean±SEM. The level of statistical significance was taken at $P< 0.05$ using one way analysis of variance (ANOVA), followed by Tukey–Kramer multiple comparison test to judge the difference between various groups. All analysis and graphics were performed using Instant and graph pad Prism computer program.

3. RESULTS:

The serum albumin concentration was significantly decreased in group B (dox free) 2.9g/ dl and C (Mes. Dox. Gp) 4.081 g/ dl when compared to control group 4.44 g/ dl, also the study showed significant increase in the activities of group B measured enzymes (LDH, CK MB and AST) (754, 244.8 and 73.25) U/L respectively and the concentration of proteins (Anticardiolipin Ig G, CRP, TNFα) (19.31, 23.96 and 126.6) respectively of group B (dox free) compared to group C (LDH, CK MB and AST) (332, 23.51and 83.8) respectively and (Anticardiolipin Ig G, CRP and TNFα) (10.69, 5.46 and 45.08) respectively. (All parameters concentrations and activities of group B and group C were significantly increased from control group except the concentrations of (CRP and TNFα) in group C which were not significant from control. Moreover, group C was significantly decreased from group B in all parameters measured.

Effect of free dox. and meso. Dox on some measured parameters in rats

Table 1. Data below represents the comparison between control group, group B (dox.free) and group C (meso. dox.) in different parameters (Albumin, AST, Anticardiolipin IgG, CK MB, CRP, LDH, TNF α respectively)

Figures

Gp / Mean ±SEM	Albumin	AST	Anti-Cardiolipin	CK MB	CRP	LDH	TNF α
Control gp	4.435±0.085	64.60± 1.8	9.4±0.26	14.04±1.8	4.904±0.45	212±10.1	34.62±1.05
Group B	2.910±0.10	244.8±9.8586	19.31±0.44	73.25±1.24	23.96±0.95	754±16.4	126.6±6.2°
Group C	4.081± 0.06	83.8±1.0	10.69± 0.24	23.51±0.69	5.46±0.29	332±8.51	45.08±0.88

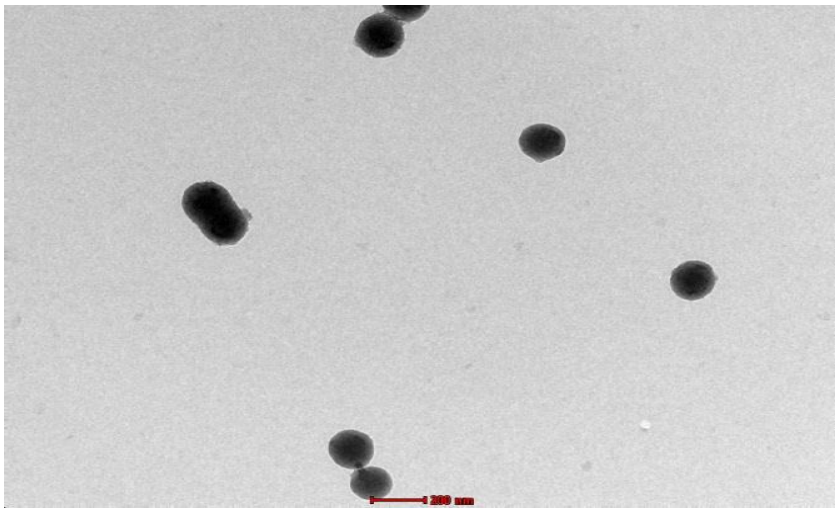

Fig.1. shows the mesoporous shape and vesicle size of average 230 nm in size measured

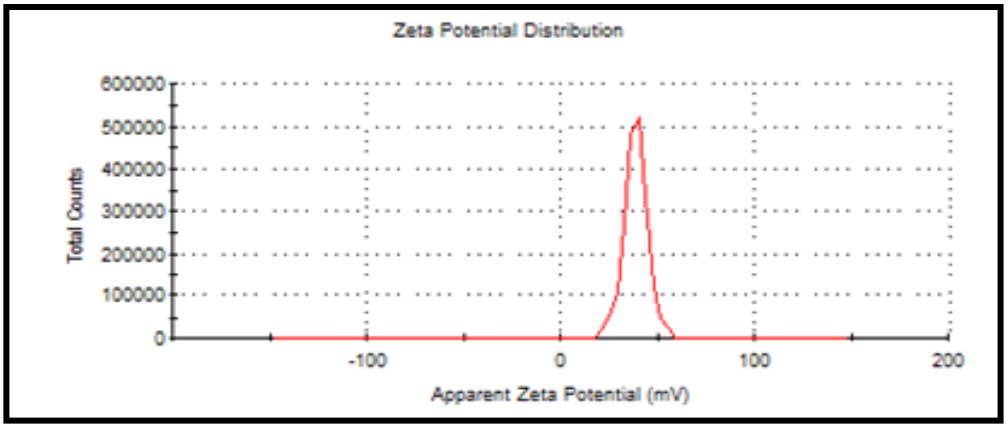

Fig.2. measurement of Zeta potential mesoporous silica loaded with doxorubicin shows apparent zeta potential of average 38.3 (mv) .

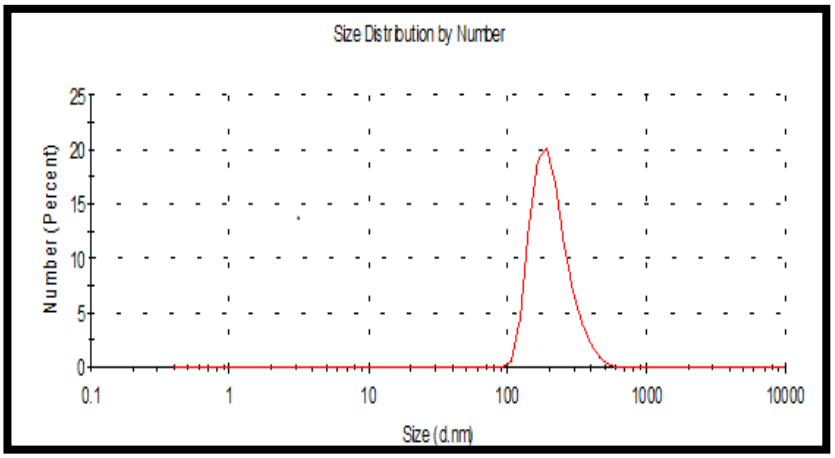

Fig.3. The size distribution of loaded mesoporous silica vesicles shows particles size average of 230 nm

4. DISCUSSION:

According to the preceding view about the cardiotoxicity induced by doxorubicin in group B, and it was clear the toxicity resulted from the administration of doxorubicin (15mg/kg) in the light of measuring different parameters which is matched by the work of *(Kaviyarasi et al., 2018),* they stated that doxorubicin is a highly effective anticancer agent but causes cardiotoxicity in many patients. Many substances could be used to decrease the cardiotoxicity in general or the cardiotoxicity induced by doxorubicin. In our study there was a clear evidence of toxicity induced by doxorubicin which has been chosen as a model of cardiotoxic drugs, the toxicity indicated by increase in AST, CKMB, LDH enzymes concentration and also in increase in inflammatory mediators like CRP, TNFα, also in thrombosis related proteins like anticardiolipin and decrease in albumin plasma concentration. AST exists in human tissues as two distinct isoenzymes (c-AST), and (m-AST). In particular, m-AST is infrequently enhanced after myocardial injury, increases later and apparently provides

different biological information compared to c-AST. LDH and its isoenzyme LDH-1 increase in blood 5–10 hours after AMI, reaches the maximum value in blood in 60–144. CK-MB subforms provide a reliable and specific diagnosis with high accuracy in the first hours of onset of cardiac symptoms *(Elisa and Montagnana, 2016).* These three enzymes activities were chosen to evaluate the cardiotoxicity which was appeared in a significant increase in their activities in free dox. treated group compared to control group and a significant decrease of their activities observed in the meso. Dox. treated group compared to free dox. treated group. CRP as an inflammatory marker, has a pro-inflammatory effect causing expression of adhesion and an inflammatory molecules, increased in patients with unstable angina (*De Servi et al., 2004)*. Death receptors including TNF receptor 1 (TNFR1), Fas, DR4 and DR5, are crucial mediators of apoptosis under physiological conditions, produced by various types of cells (e.g., T cells, Natural Killer cells) and are normally present in blood and tissue microenvironment. Their mechanism involved in triggering activation of a caspase cascade, cleavage of cellular proteins, and ultimately apoptosis of target cells (*Twomey et al., 2015).* Antiphospholipid antibodies (aPL) are associated with the recurrent pregnancy loss and thrombosis that characterizes the antiphospholipid antibody syndrome (APS), there is evidence that indicates the involvement of both genetic and environmental factors. The ability of aPL to induce a procoagulant phenotype plays a central role in the development of arterial and venous thrombotic manifestations. Inflammation serves as a necessary link between this procoagulant phenotype and actual thrombus development *(Rohan and Silvia, 2011).* These parameters were used as indicators of cardiotoxicity. The inflammatory potentiation in CRP, thrombus initiation in anticardiolipin and the apoptotic implication in TNF α reflect various mechanisms of cardiotoxicity and the development of infarction. These parameters significantly increased in the free doxorubicin group with significant improvement observed in the mesoporous doxorubicin treated group which denotes the role of the modification of mesoporous on its cardiotoxicity mechanisms. Both of groups (B) and (C) (albumin, anticardiolipin, CRP) concentrations and the activities of three enzymes were significantly elevated compared to control group except TNF α and CRP concentration in group C that was not significant compared to control group. Also there was a significant improvement observed in group C by an obvious decrease in activities and concentrations of all parameters measured. These findings indicate that modification of substances by transforming into nanopraticles form or loading on a substance like PEG, silver or mesoporous is a way to modify the pharmacokinetics, bioavailability of the drug or it could overcome the serious side effects of the drug. .

One of the main causes of doxorubicin toxicity induced by initiation of ROS, as was stated by *(Asensio et al., 2017).* In this regard, doxorubicin is a potent and exogenous ROS generator that may eventually be involved in delayed cardiomyopathy when used in cancer chemotherapy. *(De and Borm, 2008)* stated that the whole nanoparticles system leads to a special function related to treating, preventing or diagnosing diseases. It could be seen that loading mesoporous doxorubicin may act as the masking formula for doxorubicin toxicity; it was obvious from the parameters chosen for the comparison

between two forms (the free one and the mesoporous one) and the assessment of the toxicity of the two forms, the influence of loading doxorubicin and encapsulation in the mesoporous uniform silicated vesicles. This novel method could be a solution to overcome the hazard of administrating doxorubicin parenterally and also the use of this method of using mesoporous nanoparticles may be a solution to lessen the toxicity of many drugs with poor therapeutic index.

5. CONCLUSION:

Mesoporous nanoparticles of doxorubicin remarkably decrease the cardiotoxicity induced by doxorubicin. This novel preparation could be used as an alternative to free doxorubicin due to its relative safety.

6. REFERENCES:

Ahmad, M.I.; and Sharma, N.; 2012: Biomarkers in Acute Myocardial Infarction. *J Clin Exp Cardiolog 3:22*

Androniki, B.; Arthu, J. M.; Charles, W. F.; Wojciech, Z.; and Luc, F.; 2000: For the Thrombogenic Factors, Recurrent Coronary Events Investigators *Circulation.;102:1258-63*

Angelina, D.; Schoenen, B. l.; Angela, S.; Vera, M.; Melanie, K.; Jess, G.; and Katharina , W.; 2016: Macrophage Polarization by Titanium Dioxide (TiO_2) Particles: Size Matters. *ACS Biomater. Sci. Eng.*2(6): 908–19

Anna, A. P.; Daniel, M.; Ravindra, P.; Ilona, W.; Ute, S.; Pavel, A. L.; 2018: Fish-Microarray: A Miniaturized Platform for Single-Embryo High-Throughput Screenings advanced functional materials. 28,(3)

Arivalagan, P.; Thomas, N. J.; Immanuel, E.; Bharath, K.; Joe, A. J.; Indira, K.; 2018: Toxicity of Doxorubicin (Dox) to different experimental organ systems. *Life Sciences ;200: 26-30*

Asensio, L., Soler, F., Pascual, D., Fernández, F., Lax, A., 2017: Doxorubicin-induced oxidative stress: The protective effect of nicorandil on HL-1 cardiomyocytes. *PLoS One.*;12:1–21.

Chachchaya, T., Apanee, L., Thanyalak, C., Nuwong, C., Shih, Y., and Yuji, Y., 2017: Synthesis and characterization of Zr incorporation into highly ordered mesostructured SBA-15 material and its performance for CO_2 adsorption. *Microporous and Mesoporous Materials ;253:18-28*

Challa, S. S.; 2010: Nanotechnology tools in pharmaceutical R&D. *Materialstoday ;12(1) 24-30*

Daniel, A. R., Antoine M., and Vijay C., 2017: Antibody fragments as nanoparticle targeting ligands: a step in the right direction *Chem. Sci.*;8:63-77

De, J. W. H., and Borm, P. J., 2008: a. Drug delivery and nanoparticles:applications and hazards. *Int. J. Nanomedicine*.;3:133–49.

De, S. S.; Mariani, M.; Mariani, G.; Mazzone, A.; 2005*:* C-reactive protein increase in unstable coronary disease cause or effect? *J Am Coll Cardiol. 46(8):1496-502.*

Elisa, D.; and Martina, M.; 2016: An historical approach to the diagnostic biomarkers of acute coronary syndrome. *Ann Transl Med.; 4(10): 194.*

Endre, Z. H .; Walker, R.J.; 2017: Biomarkers of Kidney Disease (Second edition) pp, 485-511

Ilaria, R.; Monica, T.; and Luca, D. S.; (2017): Synthetic vs Natural: Diatoms Bioderived Porous

Materials for the Next Generation of Healthcare Nanodevices. *Adv. Healthcare Mater.6(3):11-25* .

Ilhwan H.; Chin-Y. K.; and Jun-B. P.; 2017: Advances in hot-melt extrusion technology toward pharmaceutical objectives. *J Ph Invest. 47, (2) 123–132*

Irawati, K., Katherine, J. M., Lauren, E. C., Anthony, G. M. B., Brian, M. H., Andrew, P. M., and Evan, R. T., (2017): Discovery of the Antitumor Effects of a Porphyrazine Diol (Pz 285) in MDA-MB-231 Breast Tumor Xenograft Models in Mice. *ACS Med. Chem. Lett; 8* (7):705–709

Ismail O.; and Bulent O.; 2017: Ischemia-modified albumin as a marker of acute coronary syndrome: the case for revising the concept of "n-terminal modification" to "fatty acid occupation of albumin. *Dis Markers.;(2):1-8*

Kaviyarasi, R., Abilash, V.G., Tirupathi, B., and Sankarganesh, A., 2018: Molecular mechanism of doxorubicin-induced cardiomyopathy – An update. *European Journal of Pharmacology 818: 241-253*

Karthik, S.; Balu, K. S.; Suriyaprabha, R.; and Manivasakan, P.; 2018: Screening the UV-blocking and antimicrobial properties of herbal nanoparticles prepared from Aloe vera leaves for textile applications. *IET Nanobiotechnology ;12(4) 459 –65*

Lina, A.A., Mohammed, A.A., Farkaad, A.K., Najihah, M.H., Nurhidayatullaili, M.J., Wageeh, A.Y., 2017: Graphene– gold based nanocomposites applications in cancer diseases; Efficient detection and therapeutic tools *European Journal of Medicinal Chemistry;139: 349-366*

Lodha, A., Lodha, M., Patel, A., Chaudhuri, J., Dalal, J., Edwards, M., and Douroumis, D., 2012: Synthesis of mesoporous silica nanoparticles and drug loading of poorly water soluble drug cyclosporin A. *J Pharm Bioallied Sci.;4(1): 92-4.*

Marina, p., Alessandro, B., Fabrizio, M., Antonio, P., Mario, M., Veronica, D., Giacomo, M., Gabriele, F., Alberto, Z. and Giovanni, L. E., (2016): Outcome of cardiac surgery in patients with low preoperative ejection fraction BMC *16:97*

Marta, G, F., Xiaoran, L,, Vasanti, S. M., Qi, S., Walter, C.W., Jo, A. E.M., : Nut Consumption and Risk of Cardiovascular Disease. *Journal of the American College of Cardiology;70 (20):2519-32*

Melissa, K. T.; Daniel, W. C.; David C.; Andrew K. G.; William E. G.; Karl E. K.; William R.; Martin S.; Lynn S.; 2009: Standard operating procedures for serum and plasma collection: early detection research network consensus statement standard operating procedure integration *J Proteome Res.; 8(1): 113–117.*

Matthew, D. A.; Allan, S. J.; Roy, C. Z.; and Jeffrey C. T.; 2017: Eliminating creatine kinase–myocardial band testing in suspected acute coronary syndromea value-based quality improvement. *JAMA Intern Med.;177(10):1508-1512*

Meysam, M., Kambiz, G., Zohreh, B., Noushin, B., and Seyed, A., 2015: Preparation and In-vitro Evaluation of Rifampin-loaded Mesoporous Silica Nanoaggregates by an Experimental Design Mortazavia. *Iranian Journal of Pharmaceutical Research 14 (2): 359-371*

Nowald, C., Käsdorf, B.T., and Lieleg, O., 2017: Controlled nanoparticle release from a hydrogel

by DNA-mediated particle disaggregation. *Journal of Controlled Release;246(28):71-78*

Paolino, D., Fresta, M., Sinha, P., Ferrari, M., 2006: Drug delivery systems. In: Webster JG (ed) Encyclopedia of medical devices and instrumentation, 2nd edn. Wiley, New York, pp 437–495.

Pankaj, G.; Paul, M.; Asma, L. F.; Sethumadhavan, V.; Balazs, D.; Amardeep, G. D.; Sven, P.; Christian, M.; and Philip, H.; 2017: Cardiac biomarkers of acute coronary syndrome: from history to high-sensitivity cardiac troponin. Intern Emerg Med.; 12(2): 147–55.

Qanitha, A., de Mol B., Burgner, D. P., Kabo, P., Pabittei, D. R., and Yusuf, I., 2017. Pregnancy-related conditions and premature coronary heart disease in adult offspring. *Heart*.;9:90–5.

Rohan, W.; and Silvia, S. P.; 2011: Pathophysiology of the antiphospholipid antibody syndrome. *Auto Immun Highlights. ; 2(2): 35–52.*

Sameer, A.; Melissa, C. C.; Jacob, A. M.; Wesley, M. J.; Adam, C. F.; Sidney, C. S.; and Prashant, K.; 2017: Abstract 15577: Elevated serum aspartate transaminase as a predictor of early mortality in patients with non-ST-segment elevation myocardial infarction. *Circulation; 136(1)55-77.*

Twomey J. D.; Kim S. R.; Zhao L.; Bozza W. P.; Zhang B.; 2015: Spatial dynamics of TRAIL death receptors in cancer cells. *Drug Resist Updat.;19:13-21.*

Wuqiang, Z.; Weinian, S. R.; Mark, P.; Randall, C.; and Loren J. F.;. (2008): A mouse model for juvenile doxorubicin-induced cardiac dysfunction. *Pediatr Res.; 64(5): 488–494.*

Yangyang, C., Qiumian, Y., Tian, G., Jianyuan, K., 2018: Preparation of Doxorubicin-loading Sodium Alginate Nanoparticles and its Anticancer Activity in HepG2 cells. *Lawarence press;8(05)79-83*

Yuri, T., Mari, K., Shiori, Y., Atsushi, W., and Takashi, M., 2015: Comparison of optical data from flow cytometry and microscopy of leukocytes after exposure to specific reagents *Microscopy (Oxf) 64 (5): 305-10.*