AJPS, 2018, Vol. 18, No.2 **Date of acceptance: 24-9-2018**

Histopathological Study on The Effect of Neuregulin-1 Against Cardiotoxicity Induced By Trastuzumab in Adult Mice

yousuf gays majeed*, mustafa ghazi al-abbassi*, ghaith ali jasim al-zubaidy*

Department of Pharmacology and Toxicology, College of Pharmacy, Al-Mustansiriyah University.

pharm.mustafa-alabbassi@uomustansiriyah.edu.iq

DOI: https://doi.org/10.32947/ajps.18.02.0376

Abstract:

Objective: This work carried out to investigate the potential therapeutic effect of Neuregulin-1 against cardiotoxicity induced by trastuzumab in male mice.

Materials and Methods: Forty five adult male and female mice (weighing 35-50 gm) were divided into 3 groups randomly, each group contains 15 animals, group A which received distilled water as control by using intraperitoneal injection (IP) route for 10 days group B, received Trastuzumab as 6 mg/kg/day by using intraperitoneal injection route for 10 days while group C, received Neuregulin-1 (2.5 mg/day/animal) just before the administration of Trastuzumab (6mg/kg/day) given by IP route for 10 days. The significance of difference were tested by using ANOVA followed by Tukey test. Statistical significance depends on the $P<0.05$.

Results: The histological score of cardiac tissue changes showed that a highly increase in mice administrated with trastuzumab when compared to control group while pretreatment with Neuregulin-1 , made a decrease in histological changing score when compared with trastuzumab group. The percentage of immunohistochemical score of stain uptake for TNF-α and IL-1β expression in cardiac tissue shows a highly increase in mice administrated when compared with control group, furthermore, pretreated with neuregulin-1 showed a decrease in the percentage of staining when compared with trastuzumab group.

Conclusion: Neuregulin-1 can be used as a cardioprotective in cardiac complications of cancer treatment by its ability to attenuate the cardiotoxic effect of trastuzumab by decreasing in histological changing score and decrease in the percentage of staining when compared with trastuzumab group.

Key words: Neuregulin-1, Cardiotoxicity, Trastuzumab, TNF-α, IL-1β.

التأثير المحتمل للنيورجيلين-1 على سمية عضلة القلب المستحثة من قبل عقار التراستوزوماب في الفئران البالغة

يوسف قيس مجيد*

مصطفى غازي العباسي*

غيث علي جاسم*

فرع الادويه والسموم / كلية الصيدلة-الجامعه المستنصرية

الخلاصة:

الهدف:

للتحقيق في التأثيرات العلاجية المحتملة لل نيوروغيلين 1-ضد التسمم القلبي المستحدث بواسطة عقار التراستوزوماب في الفئران.

المواد وطرق العمل: أستخدمت في هذه التجربه خمس واربعون من ذكورواناث الفئران البيضاء من سلالة وستر ذات الاوزان التي35 غرام. الحيوانات وزعت على ثلاث مجاميع بصورة عشوائية تحتوي كل مجموعة على13 فأر.

الجموعة الاولى: أعطيت محلول الماء المقطرعن طريق الحقن البريتوني لمدة 15 ايام.

الجموعة الثانية: أعطيت عقار التراستوزوماب) 6ملغ/كلغ/اليوم(عن طريق الحقن البريتوني لمدة 15 ايام.

المجموعه الثالثه: أعطيت النيروجيلين 2.5) 1-ملغ/للحيوان الواحد/اليوم(عن طريق الحقن البريتوني مباشرة

قبل اعطاء عقار التراستوزوماب 6) ملغ/كلغ/اليوم(لمدة 15 ايام.
النتائج:أظهرت نتائج الدراسة الحالية أن تركيز التروبونين القلبي الأول كان معنويا في مجموعة التراستوزماب عند مقارنته مع مجموعة التحكم بينما كان التركيز أقل معنويا في المجموعة C عند مقارنته مع مجموعة التراستوزوماب. بينما أظهرت نتائج مضادات الأكسدة الكلية، قيم أعلى بكثير في المجموعة B والمجموعةC بالمقارنة مع مجموعة التحكم. في حين سجلت البيانات انخفاضا في المجموعة C مقارنة بالمجموعة B مع عدم وجود نتائج هامة .) P = 0.822 (كما أظهرت التغييرات النسيجية لعضلة القلب أن هناك زيادة كبيرة في صبغة لانسجة الفئران التي اعطيت عقار التراستوزوماب بالمقارنة مع مجموعة التحكم أثناء المعالجة مع نيوروجيلين1- ، والتي جعلت الانخفاض في تغيير النسيجي بالمقارنة مع مجموعة تراستوزوماب.
الاستنتاج:يمكن أستخدام النيروجيلين- 1 كواقي لعضلة القلب من علاج السرطان من خلال قدرته على تخفيف تأثير السمية لعقار التراستوزوماب عن طريق تخفيف تأثير مؤشرات الالتهاب الناتج من تأثيرعقار التراستوزوماب على عضلة القلب.
الكلمات المفتاحية : نيوروجيلين-1، تسمم عضلة القلب، التراستوزوماب، عامل نخر الورم الفا، انترلوكين-1بيتا

Introduction:

Cardiotoxicity is a direct effect of the drug on the heart and indirect effect due to enhancement of haemodynamic flow alterations or due to thrombotic events [1]. The improvement in cancer management and cardiotoxicity that have a relationship which lead to improve survival and the adverse effects of treatments that have significant effect on patient outcome [2–4]. Most of anticancer drugs may cause severe cardiac dysfunctions by the dose dependent toxicity in those patients in treatment period and for health care professionals in multiple periods of antineoplastic agents [5, 6]. Cardiotoxic events may comprise some blood pressure changes, arrhythmias and cardiomyopathy [7]. The intensity of side effects can be either acute, subacute, or as chronic progress [8]. Trastuzumab is considered as humanized monoclonal antibody against the extracellular domain of human epidermal growth factor receptor 2 (HER2) that improves survival with those patients of metastatic breast cancer and prolongs disease free survival and overall survival in patients with early-stage breast cancer (EBC) treated trastuzumab as adjuvant therapy [9-12]. Cardiotoxicity that related to Trastuzumab therapy is classified as TypeII which is mechanistically and clinically distinct, is not dose-dependent, reversible, and is not related to ultrastructural changes [13, 14]. The HER2 signalling pathway has an important role in the cardiac development and function and some studies showed that mice models of lacking HER2 may involve multiple properties of dilated cardiomyopathy, including wall thinning, chamber dilation, and decreased contractility [15, 16]. The most acceptable mechanism of drug-induced cardiac toxicity is due to oxidative stress and the generation of reactive oxygen species (ROS) [17, 18].

Cytokines are highly potent, pleiotropic, endogenous peptides produced by a variety of cell types. Tumor necrosis factor-alpha (TNF-α), interleukin-1α (IL-1 α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) have been classified as proinflammatory cytokines. Cytokine secretion is stimulated in response to myocardial ischemia or infarction and has reflective effects on myocardium that provokes several pathological changes in cardiac myocyte that contribute to the phenotype reprogramming or remodelling: progressive myocyte apoptosis, myocyte hypertrophy, defects in contractility, and inflammatory signal transduction [19].

Interleukin-1 (IL-1) has a role in mediating acute inflammation during ischemic injury in the heart, which leads to both necrosis and apoptosis of cardiomyocyte [20]. Interleukine-1β, the essential form of circulating IL-1, is primarily synthesized as a precursor (proIL-1β) that becomes activated by caspase-1 cleavage in the setting of a macromolecular structure known as the inflammasome [21]. It has a central and important role in the development and progression of a variety of cardiovascular conditions, most notably

coronary atherosclerosis and congestive heart failure. IL-1 binds the transmembrane IL-1 type I receptor (IL-1RI) and initiates down-stream signals [22].
Tumor necrosis factor-α is a product of activated macrophages, are increased in the serum of patients with severe congestive heart failure. Local cellular effects of TNF-α may cause immune activation and coagulation, TNF-α appears to play an important role in regulation of nitric oxide metabolism in leukocytes, vascular endothelial cells, and vascular smooth muscle cells [23].
Despite of the effective treatment of chemotherapy, a cardiotoxicity has been occurred and for that reason many strategies required at the starting of treatment CVD risk factors, preventing cardiac side effects just before or during chemotherapy and also many protocols used for the management of cardiac side effects either sub-clinically or clinically. Thus, many of cardio-protective agents are used with early identification and induction of therapy to get the best results of getting rid from cardiac side effects. Angiotensin-converting enzyme (ACE) inhibitors are considered as the best agent of systolic heart failure therapy as many studies showing a free mortality benefit [24]. Several studies showed the protective role for ACE inhibitors in cancer-induced cardiotoxicity and the significant reduction in left ventricular ejection fraction (LVEF) [25]. Beta blockers have an important role in decreasing the developing risk of cancer therapy related cardiomyopathy and the important effects of this class showed with agents like carvedilol and nebivolol [26]. Statins are lipid lowering drugs can reduce morbidity and mortality due to their actions as anti-inflammatory and antioxidant properties [27,28]. Sildenafil, a phosphodiesterase-5 inhibitor which can limit the ischemic and myocardial injury and provide the protection from doxorubicin-induced myocardial toxicity by its effect on nitric oxide pathway and on the opening of ATP-sensitive potassium channels in mitochondria [29,30].
Neuregulin-1 (NRG-1) is belongs to family of neuregulin growth factor, it is responsible for many processes in cell it is could be either via paracrine or juxtacrine signalling mediated by tyrosine kinase receptor called “erythroblastic leukemia viral oncogene homologs (ErbBs)” of epidermal growth factor receptors [31]. So that’s mean, NRG-1 is going to reduce in the pathologic changes in those studied animals with heart failure accompanied with other cardiovascular diseases through one or of these mechanisms mention previously and thus lead to the improvement in cardiac muscle integrity and performance and also enhance the function of left ventricles and decreased mortality [32].
This work was carried out to investigate the potential therapeutic effect of neuregulin-1 against cardiotoxicty induced by trastuzumab in mice.

Materials and Methods

Animals and Study Design:

Forty-five adult male and female mice (weighing 35-50 gm) were have been used in the experiment after getting approval from ethical committee in collage of pharmacy/Al- Mustansiriyah University. Animals were divided into 3 groups randomly each group contains 15 animals, group A (control group) which received Distilled water as control by using intraperitoneal injection route for 10 days group B (TRZ group) received Trastuzumab (TRZ) as 6 mg/kg/day by using intraperitoneal injection route for 10 days while group C (NRG-1+TRZ group) received Neuregulin-1 (2.5 mg/day/animal) just before the administration of Trastuzumab 6mg/kg/day given by IP route for 10 days.

Treatment Administration:

Each day of the experiment a calculated dose of Trastuzumab and Neuregulin-1 for each mouse (in both group B and C) with equal volume of distilled water (in Group

A) was drawn in an insulin syringe then delivered to the animal via intraperitoneal injection of a conscious mouse. Using adequate manual restraint, the animal was turned over, so abdomen is exposed and injection should be in the lower right or left quadrant of abdomen trying to avoid hitting bladder, liver, or other internal organs. As the animal injected, a gentle aspiration to check if the needle hit an internal organ or not, if no blood incoming with the aspiration that's mean no damage had been occurred and complete the injection.

Animal Sacrifice:

At the end of 10 days, mice euthanized by decapitation and after opening the abdominothoracic cavity the heart was easily identified and transferred to the fixative after washing with normal saline after carefully cutting thoracic bone [33].

Tissue Preparation and Staining:

After fixation with 10% buffered formalin tissue processing will be started according to Bancroft and Stevens the tissues were processed as follows, Fixation, dehydration, clearing, impregnation, embedding, sectioning, using semi enclose Benchtop tissue processor Leica TP1020, heat paraffin embedding module Leica EG1150H, cold Plate for modular tissue embedding system Leica EG1150C, after programing according to type of staining (33).

Assessment of Histological Analysis of the Heart Tissue:

1- A digital microscope system with Leica DM4000 B LED was used to capture five zones of a slide which randomly at X 20, X40 magnification were selected.

2- The myocardial damage was determined by scoring method depending on the severity as follows: [34]

•Score 1 = normal myocardial cells;

•Score 2 = scant number of myocardial cells (< 5%) showing necrosis, partial absence of myocardial basement membrane, a few apoptotic cells (one or two cells/high magnification field in an affected area);

•Score 3 =5–15% of myocardial cells showing necrosis, partial absence of myocardial basement membrane, no more than four apoptotic cells per field;

•Score 4 = multiple foci of necrotic myocardial cells (16–25%), partial absence of myocardial basement membrane, up to five apoptotic cells/field;

•Score 5 = myocardial cells (26–35%) showing necrosis in confluent areas, marked loss of myocardial basement membrane, up to six apoptotic cells/field;

•Score 6 = more than 35% of myocardial cells showing necrosis in multiple massive or coalescent areas (which also show hyper contraction bands and myofibrillar loss), complete absence of myocardial basement membrane, seven or more apoptotic cells/field).

Immunohistochemistry Staining:

A biotin free-horseradish peroxidase conjugate,3-3'diaminobenzidine

HRP/DAB system is intended for use with primary antibody from rabbit or mouse for the qualitative identification of antigens by light microscopy and immunohisto-chemistry in formalin fixed and paraffin embedded tissues. After that the primary antibody will bind to specific antigens that already existed within the specimen, followed by sequential incubation with a horseradish peroxidase conjugate to the primary anti body and unbound enzyme is removed by washing. Staining is completed, and the brown end product is formed after incubation with the substrate-chromogen (3-3'diaminobenzidine) (DAB) and it is oxidized when it donates electrons to activate the HRP/H2O2 reaction [33].

Immunohistochemical Analysis of Heart Tissue: [35]

Immunohistochemical was utilized for analyses of IL-1β and TNF-α as color of stain uptake, and depend on the type of color to be either as positive or negative;

- Blue color = Negative (No inflammation or necrosis, nearly normal)

- Brown color = Positive (Inflammation or necrosis).

Results

Histopathological Changes:

At sacrifice, microscopic findings of control group of mouse heart show a branched appearance, normal myofibrillar structure with striations and continuity with adjacent myofibrils (Figure 1-1) occurs mainly in score 1. TRZ-induce mouse revealed marked interstitial edema and myofibrillar degeneration with infiltration of neutrophil granulocytes (Figures 1-2) the characterizations of score 3. The tissue sections from treatment groups (Figures 1-3) showing that, apparently normal myocardial muscle fiber, no myocyte damage or inflammation, interstitial edema & discontinuity with adjacent myofibrils then the morphology of cardiac muscle fibers had been relatively well preserved with no evidence of focal necrosis when compares with TRZ-induced group, which is the main features of score 1.

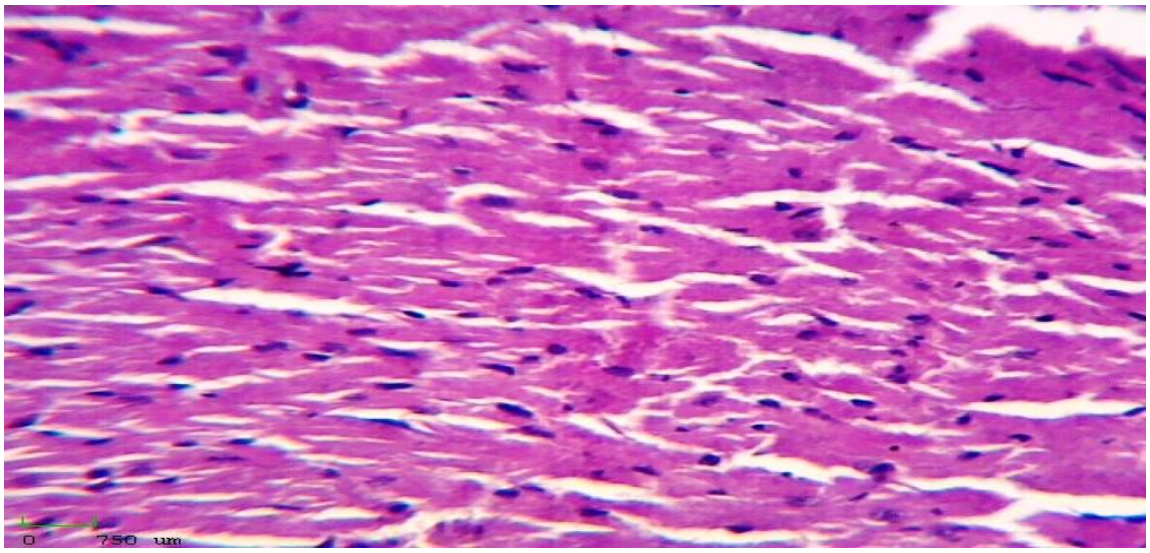

Figure (1-1): Light microscopic section of mouse cardiac tissue of H&E stain of control group (A) which received IP distilled water for 10 days showing normal myocardial muscle fiber, no myocyte damage or inflammation.(40X)

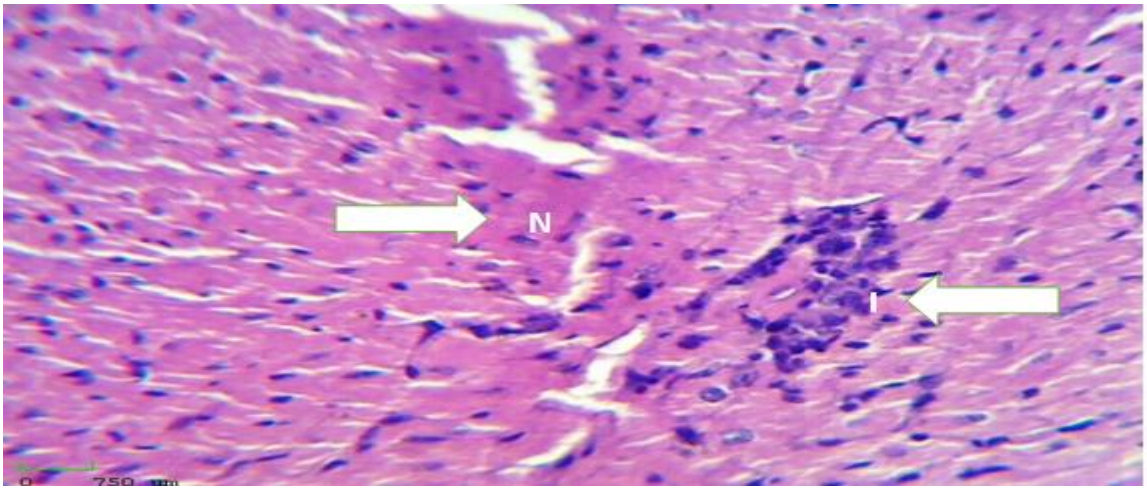

Figure (1-2): Light microscopic section of mouse cardiac tissue of H&E stain of group (B) which received Trastuzumab as 6 mg/kg/day by using IP injection route for 10 days, the myocardial cells showing necrosis, partial absence of myocardial BM, no more than four apoptotic cells per field(40X).(N necrosis , I inflammation)

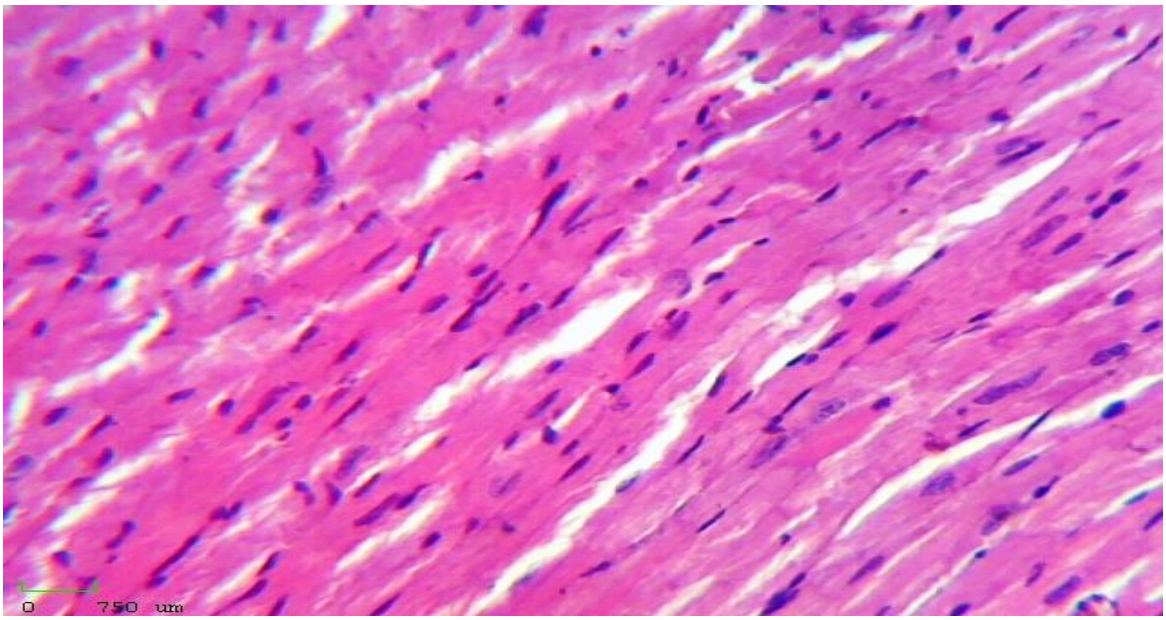

Figure (1-3): Light microscopic section of mouse cardiac tissue of H&E stain of group (C) which received Neuregulin-1 (2.5mg/day/animal) just before the administration of Trastuzumab 6 mg/kg/day given by IP route for 10 days. Showing normal myocardial muscle fiber, no myocyte damage or inflammation. (40X)

Changes in Immunohistochemistry of Inflammatory Markers:

Interliukine-1beta (IL-1β):

The percentage of stain up take in the figure (1-4) showed:

The stain in control group as shown in the figure (1-5) which represent 100% negative immunohistochemically, while TRZ group was positively stained as shown in the figure (1-6) expressing high percentage of 23%. The NRG-1 pretreated group with negative immunohistochemical stain uptake as shown in the figure (1-7).

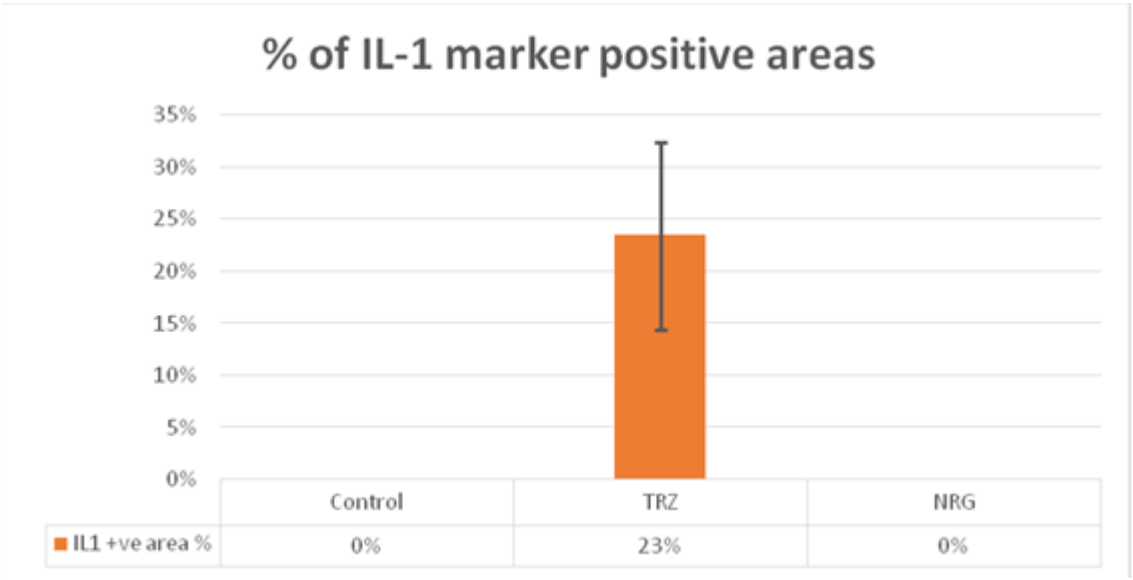

Figure (1-4): Percentage of IL-1β stain uptake among study groups.

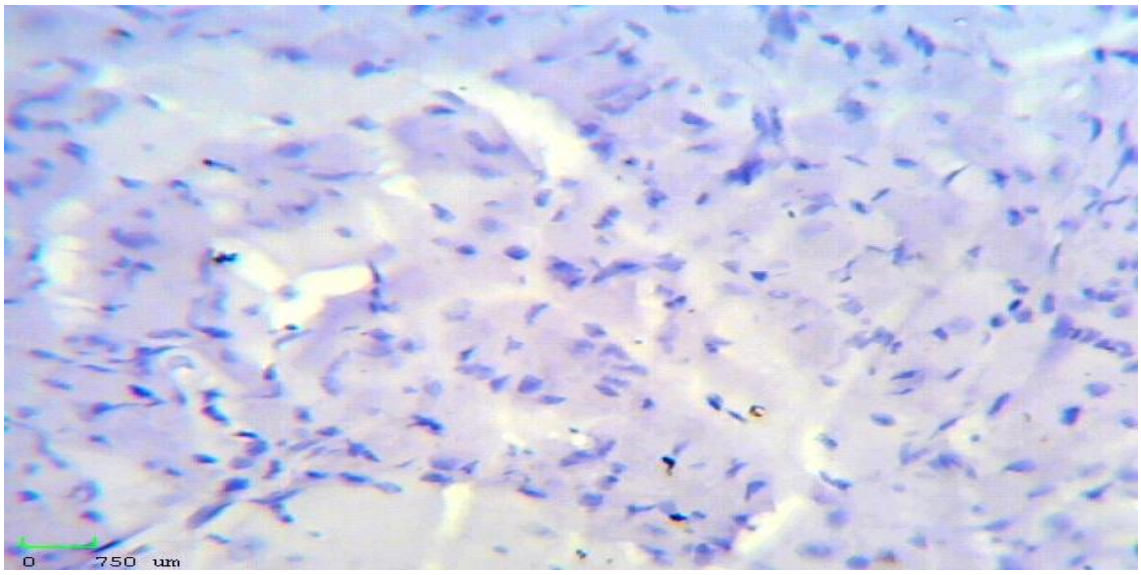

Figure (1-5): Microphotograph of immunohistochemical (IHC) staining of mouse cardiac tissue of control group (A) of IL-1Beta showing negative cytoplasmic immunohistochemical stain. (40X).

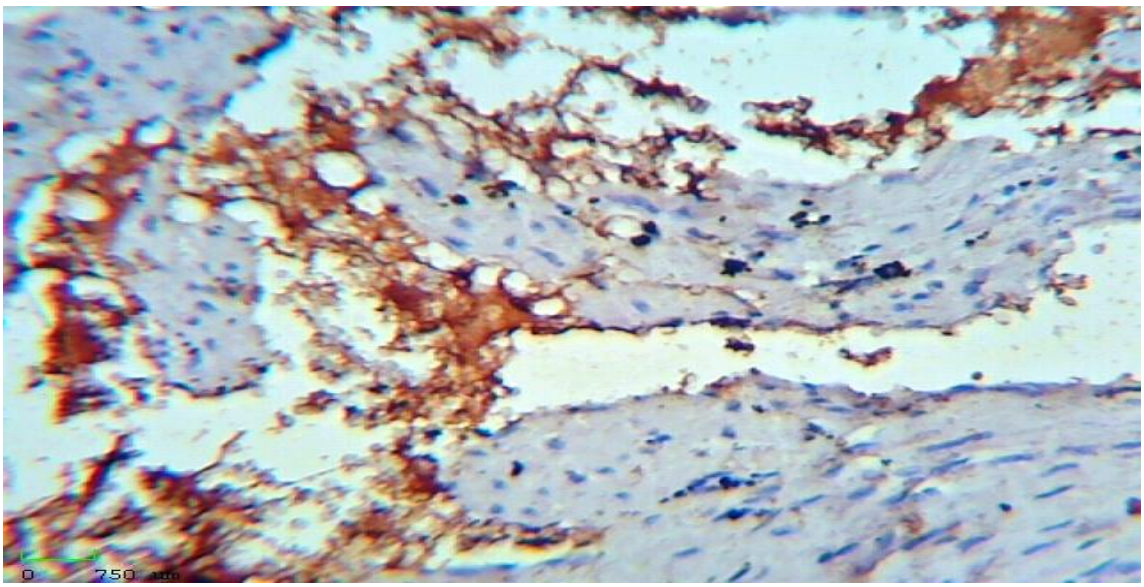

Figure (1-6): Microphotograph of immunohistochemical (IHC) staining of mouse cardiac tissue of group (B) which received Trastuzumab as 6 mg/kg/day by using IP injection route for 10 days of IL-1Beta showing +ve brown cytoplasmic immunohistochemical stain. (40x).

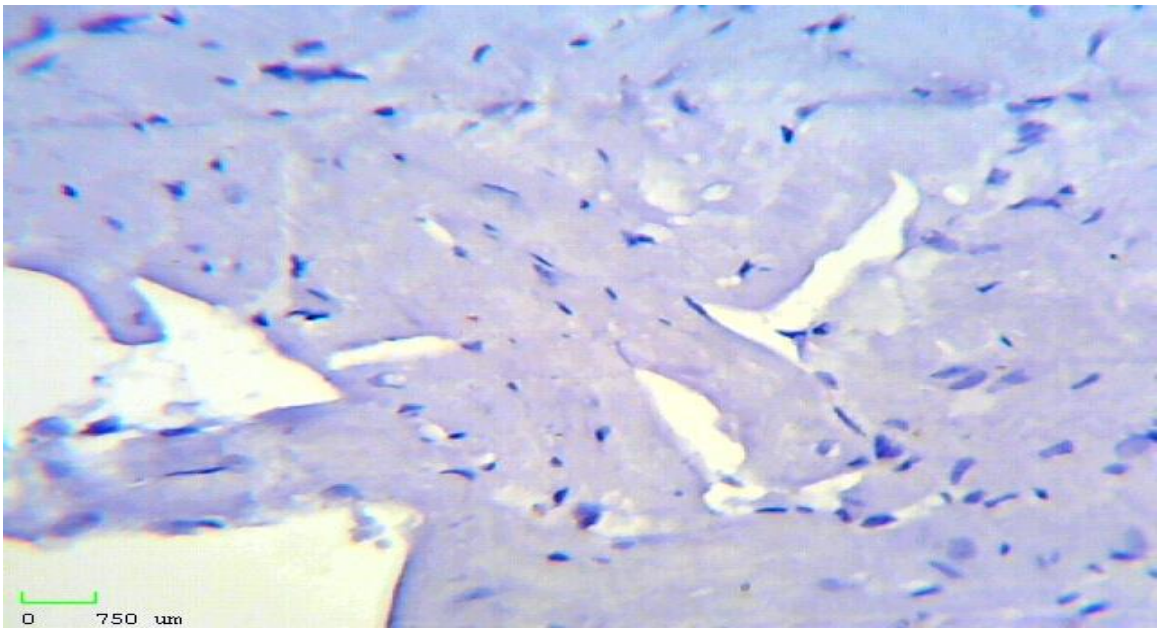

Figure (1-7): Microphotograph of immunohistochemical (IHC) staining of mouse cardiac tissue of group (C) which received Neuregulin-1 (2.5 mg/day/animal) just before the administration of Trastuzumab 6 mg/kg/day given by IP route for 10 days of IL-1Beta showing negative cytoplasmic immunohistochemical stain. (40x).

Tumor Necrosis Factor-Alpha (TNF-α):

The percentage of stain up take in the figure (1-8) showed:

The weak up take of stain in control group as shown in the figure (1-9) which represent 100% of negative immunohistochemical staining, while TRZ group observed a strong uptake of stain expressing extremely high percentage 42% as shown in the figure (1-10) The NRG-1 pretreated group with negative immunohistochemical stain uptake as shown in the figure (1-11).

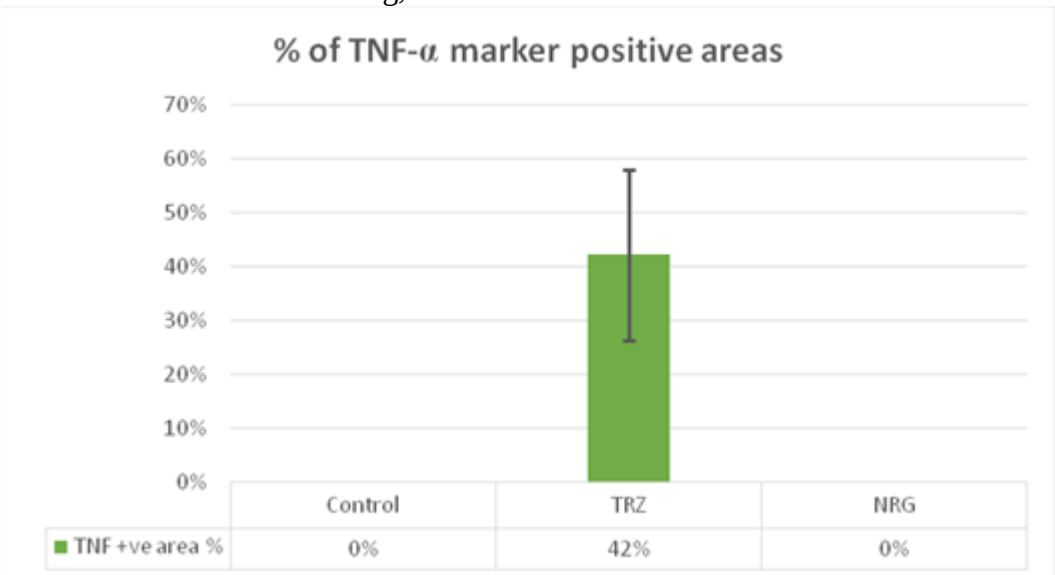

Figure (1-8): Percentage of TNF-α stain uptake among study groups.

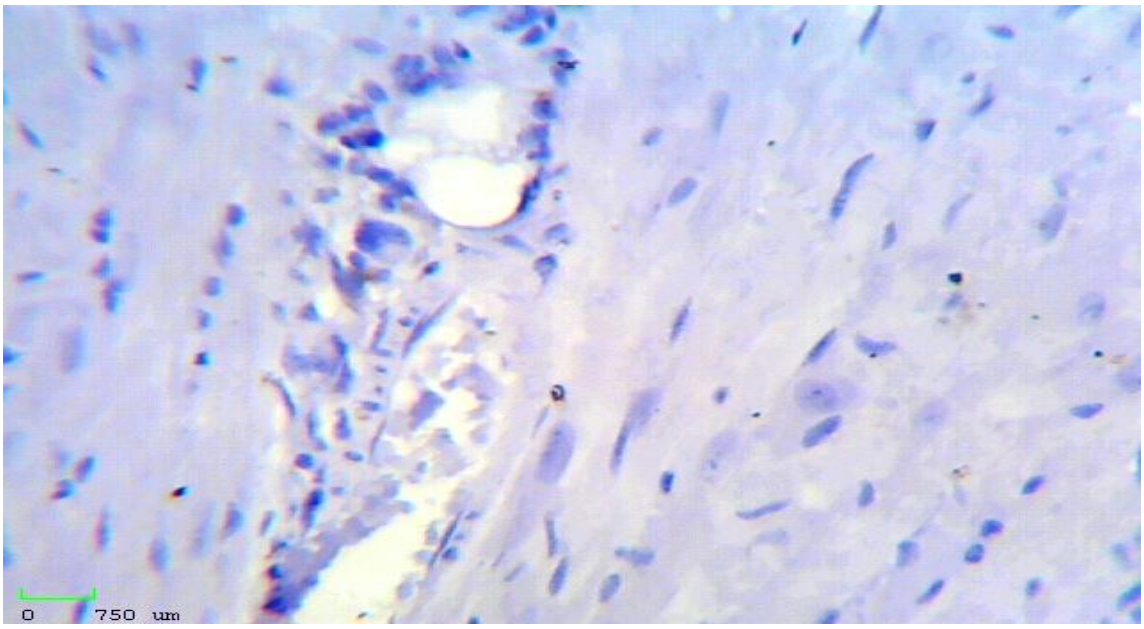

Figure (1-9): Microphotograph of immunohistochemical (IHC) staining of mouse cardiac tissue of control group (A) of TNF-alpha showing negative cytoplasmic immunohistochemical stain. (40X).

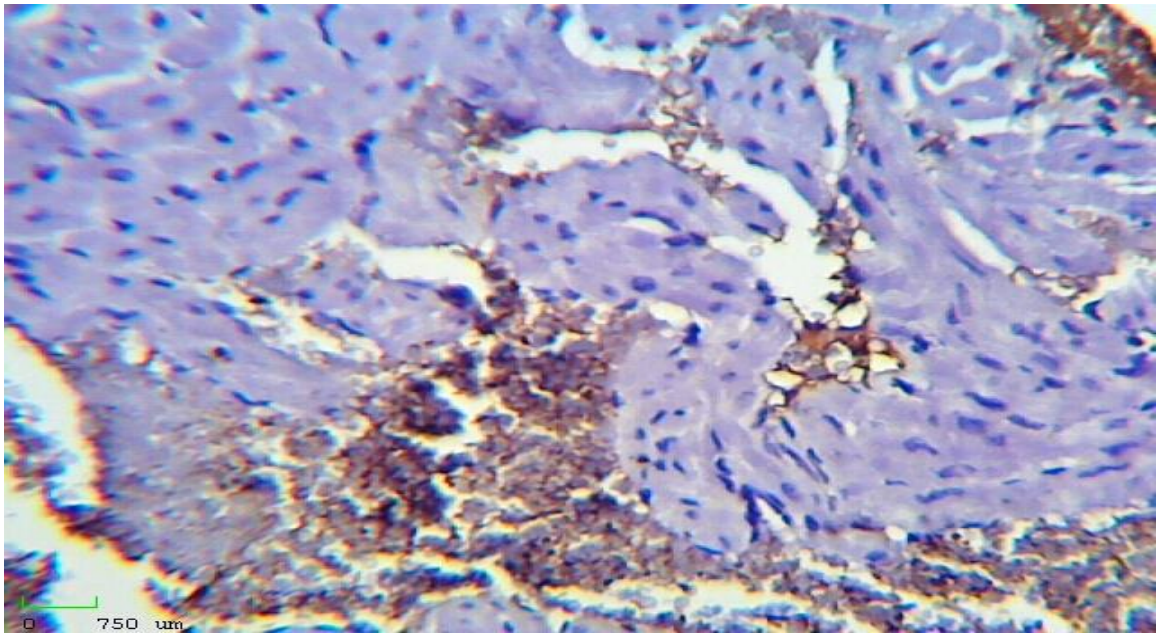

Figure (1-10): Microphotograph of immunohistochemical (IHC) staining of mouse cardiac tissue of group (B) which received Trastuzumab as 6 mg/kg/day by using IP injection route for 10 days of TNF-alpha showing +ve brown cytoplasmic immunohistochemical stain. (40X).

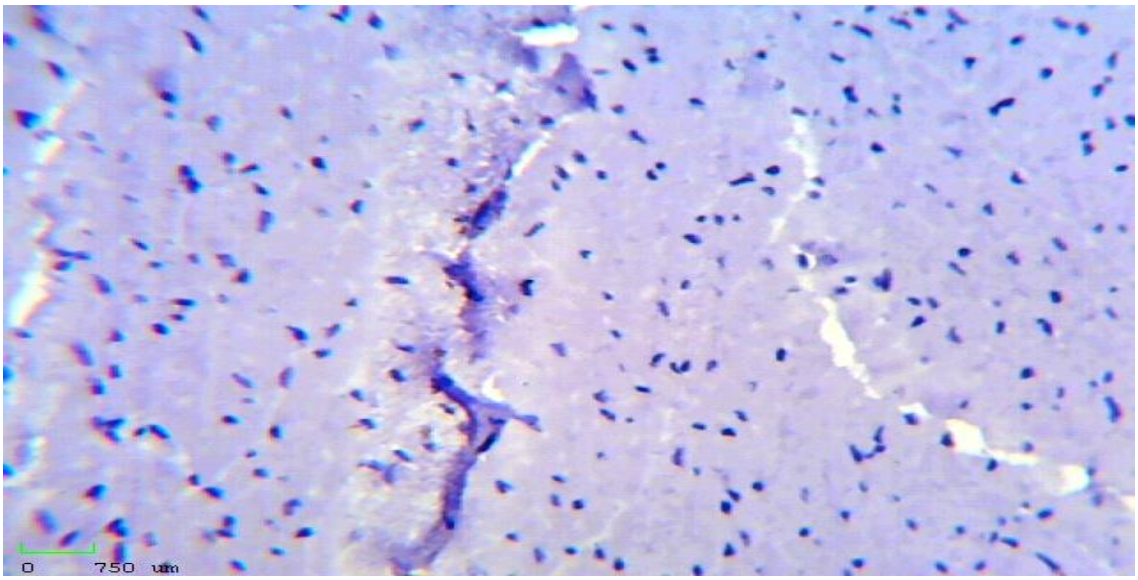

Figure (1-11): Microphotograph of immunohistochemical (IHC) staining of mouse cardiac tissue of group (C) which received Neuregulin-1 (2.5 mg/day/animal) just before the administration of Trastuzumab 6 mg/kg/day given by IP route for 10 days of TNF-alpha showing negative cytoplasmic immunohistochemical stain. (40X).

DISSCUSSION

Drug related cardiotoxicity may affect health and drug development. There are several indicators of cardiotoxicity which significantly changed only after severe heart injuries occurrence. In this study the investigation of most sensitive and reliable indicators to evaluate and predict the cardiac dysfunction. Thus, mice cardiotoxicity models had been created by Trastuzumab (6 mg/kg/day), followed by different statistical and integration tests and after selecting some of biomarkers that evaluate the occurrence of cardiotoxicity like biochemical and histopathological assessment.

In this study control group show no histopathological changes (normal appearance myocardial muscle fiber) no myocyte damage or inflammation. While, in trastuzumab treated group diffused myocyte necrosis with marked inflammation. Trastuzumab in high doses induces morphological and functional variations in the heart leading to myocardial ischemia, hypoxia, necrosis, and fibroblastic hyperplasia with decreased myocardial compliance and inhibition of diastolic and systolic function, which nearly resembles local myocardial infarction-like pathological changes seen in human myocardial infarction [36].

In the present study, treatment of mice with exogenous NRG-1 may had gave a protection for cardiac muscle and thus attenuate TRZ-induced cardiotoxicity and improve myocardial function. An assessment of the structural effects after the treatment with NRG-1 on the heart in TRZ-induced cardiotoxicity in mice and the results justified that there was a protective role for NRG-1 in reduction of cytotoxic effect of trastuzumab. These findings came to agreement with a previous study that concluded, NRG-1/ErbB signaling has an important role in cardiac dysfunction and that NRG-1 treatment result in the improvement of cardiopulmonary function and structure. The NRG-1 has the ability to reduce the remodeling of arteries, endothelial function improvement, and restores right ventricular function [37].

Histological results totally support biological markers assessed for cardioprotective effect for NRG-1 in the present study.

Interleukin-1 (IL-1) has a role in mediating acute inflammation during ischemic injury in the heart, which leads to both necrosis and apoptosis of

cardiomyocyte [20]. Interleukine-1β, the essential form of circulating IL-1, is primarily synthesized as a precursor (proIL-1β) that becomes activated by caspase-1 cleavage in the setting of a macromolecular structure known as the inflammasome [21]. It has a central and important role in the development and progression of a variety of cardiovascular conditions, most notably coronary atherosclerosis and congestive heart failure. IL-1 binds the transmembrane IL-1 type I receptor (IL-1RI) and initiates down-stream signals [22].

In the present study, the stain in control group showed in the figure (1-5) which represent 100% negative immunohisto-chemically, while TRZ group was positively stained as shown in the figure (1-6) expressing high percentage of 23%.

The first effect of IL-1 on the heart by directly suppressing the contractile force of cardiac muscle and this will weaken the heart, also IL-1-mediated inflammation in the heart attracts IL-1-producing cells from the bone marrow that lead to production of IL-1 and other inflammatory mediators contributing to cell death of cardiac muscle. In a previous study where plasma from patients with moderate to severe heart failure contains biologically active IL-1 and injected into healthy mice results in suppression of the contractile force of the heart in mice [38].

Conditions like congestive heart failure has a continued poor prognosis although of the available therapeutic options leading to the importance of looking for the observations that have important role for an immunomodulatory approach to decompensated cardiac failure. Cytokines like IL-1 may play an important role in the pathogenesis of myocardial dysfunction. IL-1 along with other pro-inflammatory molecules and their effectors may contribute to myocardial injury, the belief in continued investigations of cytokines increase in the understanding of influence of pro-inflammatory molecules in cardiac function and the modulation effect of such factors that improve the myocardial response to injury. There are specific observations that showing the importance role for IL-1 in this process when IL-1 is increases in several cardiac disease. IL-1 is presented by myocardial cells in response to injury, gene expression alterations seen in response to IL-1 resembling the phenotype of the heart failure, and also IL-1 response to the negative transcriptional regulators making them of potential targets for therapeutic manipulation [39].

The NRG-1 pretreated group with negative immunohistochemical stain uptake as shown in the figure (1-7). In the present study, treatment with NRG-1, suppressed the severity of cardiac dysfunction and reduced the percentage of IL-1β positive myocardial cells compared with which observed immunohistochemistry. Accordingly, the beneficial effects of NRG-1 may be partly due to the suppression of inflammatory cytokines in which these findings are agree with other previous studies [40,41].

Neuregulin-1 (NRG1) is released from microvascular endothelial cells. It acts via ErbB4/ErbB4 homodimers and ErbB4/ErbB2 heterodimers to stimulate protective pathways. The ErbB2 pathway is required by cell to survive and work (PI3/AKT and MEK/ERK pathways) and is initiated as myocardium be under stress or adverse hemodynamics. The cell cycle re-entry is going to be induced through the activation of NRG1/ErbB pathway in those differentiated cardiomyocytes and promote the cardiomyocyte proliferation and repair. The NRG/ ErbB is considered as an important target of therapy for cancer growth and cardiac diseases. Blocking this receptor by TRZ may inhibits tumor growth and improve the prognosis [42].

Several data showed those patients treated with TRZ having a high incidence of causing cardiac dysfunction, limiting the clinical potential of TRZ. This erbB signaling pathway has an important protective effect on cardiac survival and homeostasis. Several studies showed that the neuregulin derived from endothelium has a protective effect on cardiomyocyte

and the vascular smooth muscle cells. The endothelial cells have erbB receptors, which represented as targets for signaling by this pathway, a mediator of importance in vascular preservation and endothelial angiogenic response [43].

Similar results have been observed in rats in vivo, wherein administration of NRG-1 has rescued mitochondrial function, reduced oxidative stress, and inhibited cytochrome-c release in coronary ligation-induced heart failure [44].

The protective effect of neuregulin-1 was also associated with a suppression of interleukin-1b mRNA levels. These data suggest that neuregulin-1 protects cells from delayed, ischemia-induced apoptotic cell death by inhibiting pro-inflammatory responses. These results demonstrated that NRG-1 can regulate inflammatory and stress gene expression and may give new insight to the molecular mechanisms involved in the cardioprotective role of neuregulins in trastuzumab induced cardio toxicity [45, 46].

Tumor necrosis factor-α is a product of activated macrophages, are increased in the serum of patients with severe congestive heart failure. Local cellular effects of TNF-α may cause immune activation and coagulation, TNF-α appears to play an important role in regulation of nitric oxide metabolism in leukocytes, vascular endothelial cells, and vascular smooth muscle cells. The weak up take of stain in control group showed in the figure (1-9) which represent 100% of negative immunohistochemical staining, while TRZ group observed a strong uptake of stain showed in the figure (1-10) expressing extremely high percentage 42%. Expression of TNF-α is improved in various pathophysiological states associated with increased oxidative stress, which in turn helps the induction of various other cytotoxic substances on endothelial cells and myocytes. This has intense pathological significance, in terms of development of cardiomyopathy, myocyte apoptosis, transmural myocarditis and biventricular fibrosis as well as overall functional deterioration of heart [23].

The NRG-1 pretreated group with negative immunohistochemical stain uptake showed in the figure (1-11). In this study, the percentage of inflammatory markers up-regulated during ischemic episode induced by trastuzumab, while they were down regulated in NRG-1 pre-treated groups, validating their protective effect, which is in line with a previous study that stated that TNF-α and IL-6 were increased in the TRZ group, indicating a systemic inflammation, were attenuated by rhNRG-1 treatment. The increase circulating TNF-alpha stimulates intracellular adhesion molecule-1 (ICAM-1) expression on cardiac myocyte, which in turn promotes adhesive interaction between transmigrated neutrophils and cardiac myocytes of the re-perfused myocardium. The end result is the release of harmful substances, such as oxygen free radicals, leukotrienes and cytokines from these neutrophils, so the immune histopathological marked decrease in percentage of staining intensity of pretreated group is approved this concept [47].

References

1- Albini A, Pene´is G, Donatelli F et al. Cardiotoxicity of anticancer drugs: the need for cardio-oncology and cardio-oncological prevention. J Natl Cancer Inst 2010; 102: 14–25.
2- Oeffinger KC, Mertens AC, Sklar CA et al. Chronic health condition in adult survivors of childhood cancer. N Engl J Med 2006; 355: 1572–1582.
3- Kerkela R, Grazette L, Yacobi R et al. Cardiotoxicity of the cancer therapeutic agent imatinib mesylate. Nat Med 2006; 12: 908–916.
4- Yeh ET, and Bickford CL. Cardiovascular complications of cancer therapy: incidence,patho-genesis, diagnosis and management. J Am Coll Cardiol. 2009; 53: 2231–2247.
5- T. H. Connor, G. DeBord, J. R. Pretty et al. Evaluation of antineoplastic drug exposure of health care workers at

three university-based US cancer centers. Journal of Occupational and Environmental Medicine. 2010; 52(10): 1019–1027.

6- M. Pieri, L. Castiglia, P. Basilicata, N. Sannolo, A. Acampora, and N. Miraglia. Biological monitoring of nurses exposed to doxorubicin and epirubicin by a validated liquid chromatography/fluorescence detection method. The Annals of Occupational Hygiene. 2010; 54(4): 368–376.

7- K. J. Schimmel, D. J. Richel, R. B. van den Brink, and H. J. Guchelaar. Cardiotoxicity of cytotoxic drugs. Cancer Treatment Reviews. 2004; 30(2): 181–191.

8- H. Broder, R. A. Gottlieb, and N. E. Lepor. Chemotherapy and cardiotoxicity. Reviews in Cardiovascular Medicine. 2008; 9(2): 75–83.

9- Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med. 2001; 344: 783-792.

10- Piccart-Gebhart MJ, Procter M, Leyland- Jones B, et al. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. N Engl J Med. 2005; 353:1659-1672.

11- Romond EH, Perez EA, Bryant J, et al. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. N Engl J Med. 2005; 353: 1673-1684.

12- Slamon D, Eiermann W, Robert N, et al. Adjuvant trastuzumab in HER2-positive breast cancer. N Engl J Med. 2011; 365:1273-1283.

13- Ewer MS, and Lippman SM. Type II chemotherapy-related cardiac dysfunction: time to recognize a new entity. J Clin Oncol. 2005; 23(13): 2900–2.

14- Ewer MS, Vooletich MT, Durand JB, Woods ML, Davis JR, Valero V, et al. Reversibility of trastuzumab-related cardiotoxicity: new insights based on clinical course and response to medical treatment. J Clin Oncol. 2005; 23(31): 7820–6.

15- Kuramochi Y, Guo X, and Sawyer DB. Neuregulin activates erbB2-dependent src/FAK signaling and cytoskeletal remodeling in isolated adult rat cardiac myocytes. J Mol Cell Cardiol. 2006; 41(2): 228–35.

16- Crone SA, Zhao YY, Fan L, Gu Y, Minamisawa S, Liu Y, et al. ErbB2 is essen- tial in the prevention of dilated cardiomyopathy. Nat Med. 2002; 8(5): 459–65.

17- Wouters KA, Kremer LC, Miller TL, Herman EH, and Lipshultz SE. Protecting against anthracycline-induced myocardial damage: a review of the most promising strategies. Br J Haematol. 2005; 131:561–578.

18- Olson RD, and Mushlin PS. Doxorubicin cardiotoxicity: analysis of prevailing hypotheses. FASEB J. 1990; 4: 3076–3086.

19- Nian M, Lee P, Khaper N, Liu P. Inflammatory cytokines and postmyocardial infarction remodeling. Circ Res. 2004 Jun 25;94(12):1543–53.

20- Suzuki K, Murtuza B, Smolenski RT, Sammut IA, Suzuki N, Kaneda Y, et al. Overexpression of Interleukin-1 Receptor Antagonist Provides Cardioprotection Against Ischemia-Reperfusion Injury Associated With Reduction in Apoptosis. Circulation. 2001 Sep 18;104(suppl 1):I – 308 – I – 313.

21- Dinarello CA. Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. Blood. 2011 Apr 7;117(14):3720–32.

22- Zhu J, Zhang J, Zhang L, Du R, Xiang D, Wu M, Zhang R, Han W. Interleukin-1 signaling mediates acute doxorubicin-induced cardiotoxicity. Biomedicine & Pharmacotherapy. 2011 Oct 31;65(7):481-5.

23- Mukherjee S, Banerjee SK, Maulik M, Dinda AK, Talwar KK, Maulik SK.

Protection against acute adriamycin-induced cardiotoxicity by garlic: Role of endogenous antioxidants and inhibition of TNF-α expression. BMC Pharmacol. 2003 Dec 20; 3:16.

24- Yancy CW, Jessup M, Bozkurt B et al. 2013 ACCF/AHA Guideline for the management of heart failure. A report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. J. Am. Coll. Cardiol. 2013; 62(16): 147–239.

25- Cardinale D, Colombo A, Sandri MT et al. Prevention of high-dose chemotherapy induced cardiotoxicity in high-risk patients by angiotensin-converting enzyme inhibition. Circulation. 2006; 114(23): 2474–2481.

26- Kaya MG, Ozkan M, Gunebakmaz O et al. Protective effects of nebivolol against anthracycline-induced cardiomyopathy: a randomized control study. Int. J. Cardiol. 2013; 167(5): 2306–2310.

27- Damrot J, Nubel T, Epe B, Roos WP, Kaina B, and Fritz G. Lovastatin protects human endothelial cells from the genotoxic and cytotoxic effects of the anticancer drugs doxorubicin and etoposide. Br. J. Pharmacol. 2006; 149(8): 988–997.

28- Huelsenbeck J, Henninger C, Schad A, Lackner KJ, Kaina B, and Fritz G. Inhibition of Rac1 signaling by lovastatin protects against anthracycline-induced cardiac toxicity. Cell Death Dis. 2011; 2(8): e190.

29- Mahmood AM, Al-Abbassi MG, Al-Obeidy MM. Effect of Sildenafil Citrate against Cardiotoxicity Induced by Isoproterenol in Male Rats, Int. J. Pharm. Sci. Rev. Res., 41(1), November - December 2016; Article No. 27, Pages: 138-144

30- Ibrahim H.Nashmee, Mustafa Al-Abassi,Mustafa Al-Obedy. Biochemical and Immunohi-stochemical Study Of Sildenafil on Various Tissues of Male Rats. The University of al-Mustansiriyah/ College of Pharmacy. 2016.

31- Falls D. Neuregulins: functions, forms, and signaling strategies. Exp Cell Res. 2003; 284(1): 14–30.

32- Liu, X. et al. Neuregulin-1/erbB-activation improves cardiac function and survival in models of ischemic, dilated, and viral cardiomyopathy. J. Am. Coll. Cardiol. 2006; 48: 1438–1447

33- Bancroft JD, and Gamble M. Theory and Practice of Histological Techniques. Elsevier Health Sciences. 2008; 742.

34- Zhang J, et al. Isoproterenol-induced cardiotoxicity in sprague-dawley rats: correlation of reversible and irreversible myocardial injury with release of cardiac troponin T and roles of iNOS in myocardial injury. Toxicologic pathology. 2008; 36(2):277-8.

35- Maryla Krajewska. Image Analysis Algorithms for Immunohistochemical Assessment of Cell Death Events and Fibrosis in Tissue Sections. J.Histo. cyto. 2009

36- Li H, Xie YH, Yang Q, Wang SW, Zhang BL, Wang JB, Cao W, Bi LL, Sun JY, Miao S, Hu J. Cardioprotective effect of paeonol and danshensu combination on isoproterenol-induced myocardial - injury in rats. PloS one. 2012 Nov 6;7(11):e48872.

37- Mendes-Ferreira P, Maia-Rocha C, Adão R, Mendes MJ, Santos-Ribeiro D, Alves BS, Cerqueira RJ, Castro-Chaves P, Lourenço AP, De Keulenaer GW, Leite-Moreira AF. Neuregulin-1 improves right ventricular function and attenuates experimental pulmonary arterial hypertension. Cardiovascular research. 2015 Oct 26;109(1):44-54.

38- Van Tassell BW, et al. Enhanced interleukin-1 activity contributes to exercise intolerance in patients with

systolic heart failure. PLoS ONE. 2012; 7:e33438.

39- Long CS. The role of interleukin-1 in the failing heart. InThe Role of Inflammatory Mediators in the Failing Heart. Springer US. 2001 (pp. 13-25).

40- El-Shitany NA, El-Desoky K, El-Shitany NA, El-Desoky K. Protective Effects of Carvedilol and Vitamin C against Azithromycin-Induced Cardiotoxicity in Rats via Decreasing ROS, IL-1Beta and TNF-alpha production and Inhibiting NF- and Caspase-3 Expression. Oxidative Med Cell Longev. 2016 May 5;2016, 2016:e1874762.

41- Ibrahim H.Nashmee, Mustafa Al-Abassi ,Mustafa Al-Obedy. Biochemical and Immunohist-ochemical Study Of Sildenafil on Various Tissues of Male Rats. The University of al-Mustansiriyah/ College of Pharmacy. 2016.

42- Madonna R, Cadeddu C, Deidda M, et al. Improving the preclinical models for the study of chemotherapy-induced cardiotoxicity: aPosition Paper of the Italian Working Group on Drug Cardiotoxicity and Cardioprotection. Heart failure reviews. 2015 Sep 1;20(5):621-31.

43- Hedhli N, Kalinowski A, S Russell K. Cardiovascular effects of neuregulin-1/ErbB signaling: role in vascular signaling and angiogenesis. Current pharmaceutical design. 2014 Sep 1;20(30):4899-905.

44- Rupert CE, Coulombe KL. The roles of neuregulin-1 in cardiac developpment, homeostasis, and disease. Biomarker insights. 2015;10(Suppl 1):1.

45- Xu Z, Jiang J, Ford G, Ford BD. Neuregulin-1 is neuroprotective and attenuates inflammatory responses induced by ischemic stroke. Biochemical and biophysical research communications.2004Sep17;322(2):440-6.

46- Xu Z, Ford GD, Croslan DR, Jiang J, Gates A, Allen R, Ford BD. Neuroprotection by neuregulin-1 following focal stroke is associated with the attenuation of ischemia-induced pro-inflammatory and stress gene expression. Neurobiology of disease. 2005 Aug 1;19(3):461-70.

47- Gurevitch J, Frolkis I, Yuhas Y, Lifschitz-Mercer B, Berger E, Paz Y, et al. Anti–Tumor Necrosis Factor-Alpha Improves Myocardial Recovery After Ischemia and Reperfusion. J Am Coll Cardiol.1997Nov 15;30(6):1554–61.