Novel use of Ketotifen as a cardio-protective agent in patients undergoing anthracycline chemotherapy

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Running title:

New indication of ketotifen as cardioprotective agent.

Highlights:

- Cancer is a foremost public health problem in the United States and many other parts of the world
- Optimum administration of anthracycline is limited due to its cardiotoxicity
- Oxidative stress results in augmented calcium concentration in the interior of myocardial fiber and damages the cell.
- The oldest iron chelator is deferoxamine, which was first approved for use in the prevention of cardiotoxicity still seems to be insufficient.
- Ketotifen suggested a beneficial effect in iron overload inducing diseases such as COVID-19
- Can ketotifen act as a perfect oral iron chelator in preventing cardiotoxicities?

Abstract

Objective: The present study aimed to investigate the possible cardioprotective effects of ketotifen and to assess its activity as an iron-chelating agent in patients receiving anthracyclines for the treatment of breast cancer.

Patients & Methods: This was a randomized, prospective, controlled clinical trial. 111 eligible patients with breast cancer (age range, 30-60 year) were scheduled to receive anthracycline chemotherapy. The patients divided into two groups: Patients (n=56) assigned to The ketotifen group received ketotifen 1 mg three times daily for six consecutive cycles of treatment, and patients assigned to The control group (n= 55) without ketotifen treatment.

The echocardiogram for each patient was recorded two times at baseline and at the end of the study. As well, blood samples were collected from all patients.

Results: The findings showed a statistically significant reduction in the mean serum levels of common cardiotoxicity accompanied biomarkers in The ketotifen group compared with The control group ($P \leq 0.05$). The mean serum levels of total iron-binding capacity was significantly elevated in The ketotifen group ($P \leq 0.001$). There was a direct correlation between the mean serum levels of iron and that of lactate dehydrogenase (LDH) ($r = + 0.79$). On the other hand, there were indirect correlations between mean serum levels of LDH and both the percentage of ejection fraction and the total iron-binding capacity ($r = - 0.69$ and -0.697, respectively).

Conclusion: Oral administration of ketotifen appears to be efficient and safe as a novel cardioprotective agent for the prevention of anthracyclines induced cardiotoxicity. Additionally, ketotifen suggested a beneficial effect in iron overload inducing diseases such as COVID-19.

Keywords: Cardiotoxicity; Ketotifen; Anthracyclines; Cancer chemotherapy; Mitochondria

There are no studies about the use of ketotifen in reducing expected cardio-toxicities from anthracycline administration until submission of this article.
This study adds novel use of ketotifen as a cardioprotective agent.

Introduction:

Cancer is the foremost public health problem in the United States and many other parts of the world [1]. Cancer chemotherapy or radiotherapy can cause short- and long-standing cardiovascular complications[2]. In the US National Health and Nutrition Examination survey of 1,807 cancer survivors followed for 7 years, 33% died as a result of heart diseases consequences [3]. To date, The US Food and Drug Administration (FDA) has approved more than 150 anticancer drugs, with anthracyclines being the most widely used. Considering the support of therapy for several decades, conventional anthracycline-containing regimens have proven benefits in terms of response rate, time to disease progression, and overall survival[4-6]. Doxorubicin (adriamycin) and daunorubicin (daunomycin) are two members of the anthracycline group. Doxorubicin is known to induce serious cardiotoxicity, which is believed to be mediated by oxidative stress and complex interactions with iron[7]. These two drugs are acquired from actinobacteria (*Streptomyces peucetius*)[8]. The clinical use of anthracycline is still limited because of its cardiotoxicity, which may be acute and results in arrhythmia, myocarditis, pericarditis, or acute left ventricular failure. These symptoms decrease immediately after pulling out of the treatment, but limit the further use of the drug [9]. Anthracycline can also cause cardiomyopathy during chronic use and sometime late-onset severe arrhythmia and ventricular dysfunction have occurred[9]. It has been detected that the rate of survival with anthracycline-associated cardiotoxicity is much lower than that with ischemic or dilated cardiomyopathy[10]. Doxorubicin-induced cardiotoxicity is dose-dependent, so controlled monitoring of dose is the best possible way to control toxicity[9]. Currently, echocardiography is used to monitor doxorubicin-induced cardiotoxicity, which is considered the principal assessment test [11]. Commonly, chemotherapeutic drugs-induced cardiotoxicity is associated with myocardial cell loss, apoptosis, or necrosis, which may be directly or indirectly mediated by oxidative stress[12]. In practice, the determination of the precise mechanism of doxorubicin-induced cardiotoxicity is not clear because most of patients are usually administrating different treatment protocols[13, 14].

Two main hypotheses are suggested on the subject of anthracycline-induced cardiotoxicity: i) Iron and free radical theory, in which the incidence of high oxidative stress and depletion of endogenous antioxidants is observed. This hypothesis suggested

that the myocardial mitochondria are central point of oxidative stress; ii) Metabolic hypotheses in which the C-13 alcoholic metabolite of anthracycline acts on the myocardium and obstruct the myocardial energy pathway and intracellular calcium concentrations. Unifying the hypothesis in the C-13 alcoholic metabolite is further acted by oxidative stress, which results in augmented calcium concentration at the interior of myocardial fiber and damages the cell. This may additionally enhance lipid peroxidation and loss of selective membrane permeability[15]. The role of free radicals occupies the central position. It has been hypothesized that oxidative stress not only causes myocardial death but also directly affects the excitation-contraction properties of cardiac muscles[5, 16]. Free radicals, mainly nitrite-free radicals, are the major culprit of oxidative stress[17]. There are many common cardioprotective agents for the prevention of anthracycline-induced cardiotoxicity. The first and the oldest one is the iron chelator, deferoxamine, which was first approved for the clinical use in the 1960_s. However, the prevention of cardiotoxicity still seems to be insufficient[3, 18].

Ketotifen is an oral anti-allergic drug established in 1970 by Sandoz Pharmaceuticals, Switzerland. It is a benzocycloheptathiophene derivative and was initially marketed as an inhibitor of anaphylaxis[19]. Ketotifen was initially developed as a drug that would prevent the release of vasoactive substances from mast cells. It is an oral alternative to cromoglicate, but its actions in asthma are possibly attributable to its antihistaminic effect, which occurs within minutes after oral administration and lasts for up to 12 hours. Ketotifen also alleviates mast cells, prevents histamine release, inhibits eosinophil accumulation in the lungs of animals exposed to platelet-activating factor, and reverses β-adrenoceptor tachyphylaxis[20]. Numerous pharmacodynamic properties are belonging to ketotifen, because they inhibit the release and/or the activity of mast cells and basophil mediators, such as histamine, neutrophil, eosinophil chemotactic factors, arachidonic acid metabolites, prostaglandins, and leukotrienes[21]. Extra possible modes of action of ketotifen include its ability to reverse β_2-agonist-induced reductions in β-adrenoreceptor density and to alter the attraction of these receptors and increase intracellular concentrations of cyclic adenosine monophosphate[22].

Ketotifen has a similar chemical structure to some first-generation antihistamines, such as cyproheptadine[21]. The chemical structure of ketotifen fumarate (zaditen®) is illustrated in **Figure I**. Ketotifen is freely absorbable from the gastrointestinal tract after oral administration and achieves the peak plasma concentration within 2-4 hours. Ketotifen fumarate is referred to as a mast cell stabilizer because it inhibits normal mast cell degranulation by avoiding the intracellular calcium influx associated with this phenomenon.[21, 23, 24]. Chelation-based therapy is one of the preferred medical treatment strategies for decreasing toxic effects of metals like iron and others[25]. Metal chelators are capable of binding to the toxic forms of metal ions producing complex structures that are easily eliminated from the body. Metal chelators are chemical compounds whose structures permit them to simultaneously attach two or more electron donor atoms to a metal ion giving one or more complex ring structures[26].

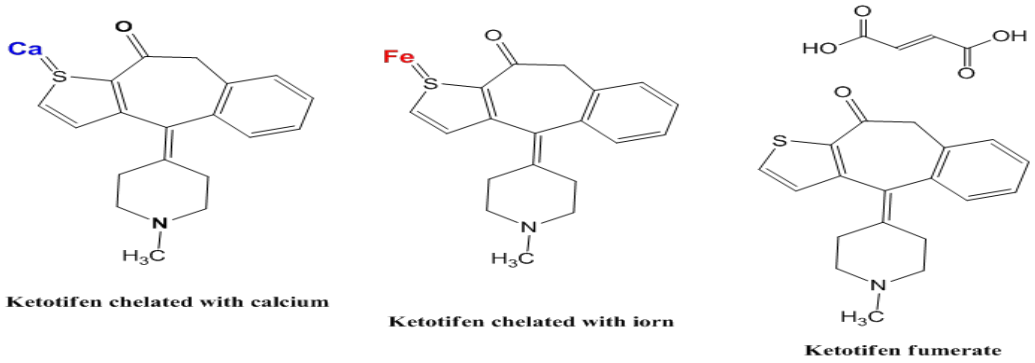

Figure I: Structural formula of ketotifen fumarate, ketotifen chelated with iron, and

As the clinical use of anthracycline is hampered due to its cardiotoxicity. In this study we aimed to assess whether ketotifen can provide effective protection against anthracycline-induced cardiotoxicity in patients with breast cancer.

Drug, Patients, and Methods

Ketotifen has a molecular weight of 309.4 Dalton, and its chemical formula is $C_{19}H_{19}NOS$. To measure the chelating efficiency of ketotifen[27], we made a simple chemical test (complexmetry) as follows:

1-10 tables of zaditen ^{R} 1 mg (ketotifen) were soaked in 10 ml of distilled water for 12 hrs. and then filtered. zaditen ^{R} 1 mg tablet is manufactured by Novartis Pharma, Egypt, and was purchased from a community pharmacy.

2-The filtrate `titrated with N/100 calcium hydroxide (Ca [OH] $_2$) and, by N/100 ferrous hydroxide (Fe [OH] $_2$) other times using phenolphthalein (Ph. Ph.) as an indicator.

3- Changing the color of the filtered solution from colorless to pink indicates the endpoint.

4-This procedure was repeated three times when the filtrate was titrated with Ca ([OH] $_2$) and with Fe ([OH] $_2$).

5-The same procedures were applied to the blank solution (distilled water).

6-The results are illustrated in Table I

Table I: Results of complexmetry test when ketotifen was titrated by calcium hydroxide, and ferrous hydroxide versus blank (distilled water).

Number of procedure	Endpoint of $Ca(OH)_2$	Endpoint of $Fe(OH)_2$
1	8 ml	5.6 ml
2	8.2 ml	5.8 ml
3	7.8 ml	5.4 ml
Distilled water (blank)	0.2 ml	0.2 ml

The results proved that that ketotifen may chelate divalent cations (Ca^{++} and Fe^{++}). The sulfur atom in ketotifen binds with them, whereas the blank (distilled water) did not.

Materials:

1- Calcium hydroxide (Ca [OH] $_2$) analytical grade; Merck Schuchardt, Germany
2- Ferrous hydroxide (Fe [OH] $_2$) analytical grade; Merck Schuchardt, Germany
3- Phenolphthalein (Ph. Ph.) analytical grade; Ridedel-deHaen, Germany.

Equipment:

Clinical centrifuge (Minor 35, England).

Setting:

The patients were recruited from the Oncology Department, Menoufia University Hospital, Egypt. The study was designed and conducted in compliance with the ethical principles of Good Clinical Practice Guidelines and the Declaration of Helsinki[28]. The

study protocol was approved by the National Research Ethics Committee [Menoufia University, Research Ethics Committee]. The study was registered in ClinicalTrials.gov under Identifier: NCT04435028. Informed written consent was obtained from all patients.

Study design:

The current study was a randomized, prospective controlled trial. The patients were identified by coded numbers to maintain privacy. The age ranged to 30-60 years in all groups. All were female patients with breast cancer. Fifty-five were enrolled in The control group and 56 women in The ketotifen group respectively. Regarding the therapeutic regimen in The control group, 14 patients received adriamycin with cyclophosphamide (AC). 18 patients received 5-fluorouracil + adriamycin +cyclophosphamide (FAC), and 23 patients received epirubicin + cyclophosphamide. In The ketotifen group, 19 patients received adriamycin with cyclophosphamide (AC). 17 patients received 5-fluorouracil + adriamycin +cyclophosphamide (FAC), and 20 patients received epirubicin + cyclophosphamide.

Eligible patients fulfilled the following criteria: female patients with breast cancer receiving anthracycline chemotherapy in their protocol without any cardioprotective agent. They have an adequate baseline echocardiography. The excluded patients from the study were patients with i) a history of heart failure, ii) arrhythmia, iii) cardiac catheterizations, iv) angina, v) uncontrolled hypertension, vi) uncontrolled diabetes, vii) patients with impaired liver functions tests, and viii) patients who previously received anthracycline-containing regimens.

The enrolled patients divided into two groups:

Control Group: 55 patients received their standard therapy (anthracycline-containing chemotherapy without a cardioprotective agent) at a dose of 60 mg/m^2/week/4 times in the induction phase and 3 times in the re-induction phase (total 175 mg/m^2).

***Ketotifen Group*:** 56 patients received anthracycline-containing chemotherapy plus ketotifen as a cardioprotective agent. Ketotifen will be given orally as one tablet (1 mg/tablet) 3 times daily (zaditen ^{R} 1mg), before and during the chemotherapeutic cycles for 6 cycles of treatment (at least 5 months).

All patients were exposed to complete history and clinical examination, and all patients were enrolled to be evaluated every month during their cancer therapy (for 6 cycles of treatment) using echocardiograms and blood samples.

Sample collection

Intravenous blood sample (5 ml) was obtained from all patients before and after the treatment course. The serum supernatant was separated immediately from the blood by centrifugation for 15 min at 3000 rpm, and the serum was coded and stored at −80 °C for biochemical analysis.

The serum levels of biomarkers as LDH, CK-MB, troponin I, TIBC, ferritin, anti-cardiolipin IgG and, iron were measured using the following kits:

LDH kits manufactured by BioSystem S.A company, Spain used to assay LDH spectrophotometrically.

CK-MB was assayed using CK-MB kits manufactured by BioSystem S.A company, Spain.

Iron-Chromazurol kits manufactured by BioSystem S.A company, Spain was used to assay iron. Enzyme-linked immunosorbent assay (ELISA) kits (manufactured by Orgentec Diagnostika GmbH company, Germany used to assay anti-cardiolipin IgG. Enzyme-linked immunosorbent. Assay (ELISA) kits manufactured by Accu-Bind Company, USA used to assay Troponin-I (cTnI). Ferritin enzyme-linked immunosorbent assay (ELISA) kits manufactured by Accu-Bind company, USA was used for measurement of serum ferritin.

Total iron binding capacity (TIBC) kits manufactured by BioSystem. S.A company, Spain was used for measurement of TIBC.

Statistical analysis

All data presented as mean ± SD, Unpaired t-test, and ANOVA test were used. A Chi-square test was used for statistical analysis of nominal data. Correlation between variables was evaluated by Pearson's correlations. The statistical analysis was done using IBM SPSS statistical package version 22.0 (SPSS Inc; USA, 2013). The level of

significance was set at $p \leq 0.05$. Data were also presented as figures using Microsoft Excel 2016 software.

Results:

The results of the present study conclude that ketotifen chelates with divalent cations (Ca^{++}, and Fe^{++}), where the sulfur atom in ketotifen binds with them, whereas, the blank (distilled water) did not as shown in Table I.

There were no differences in the mean serum levels of all parameters measured at the beginning of the study as well echocardiograms, the *p*-value is more than 0.05 as shown in Table II.

Table II Mean serum levels of parameters and echocardiograms in control and ketotifen groups at baseline and after 6 months.

	Control group n=55	Control group n=55	*p-value*	ketotifen group n=56	Ketotifen group n=56	*p*-value
	At baseline	After 6 months		At baseline	After 6 months	
LDH (U/L)	259.25 ± 95.71	530.00 ± 86.99	0.007*	255.87 ± 86.99	227.53 ± 69.49	0.297
CK-MB (ng/mL)	14.37 ± 4.00	32.99 ± 11.15	0.009*	14.46 ± 3.58	17.64 ± 6.91	0.102
Troponin I (ng/ml)	0.15 ± 0.10	0.51± 0.14	0.001*	0.16 ± 0.10	0.15± 0.08	0.562
ACL IgG (U/L)	6.91 ± 1.24	14.38 ± 2.99	0.003*	6.13 ± 0.2	4.74 ± 4.18	0.213
Iron (mcg/dL)	89.06 ±15.41	178.25± 19.90	0.001*	90.9 ±15.34	46.53± 16.30	0.004*
TIBC (µg/dL)	296.00 ± 72.12	238.63 ± 55.18	0.005*	298.07 ± 57.31	320.13 ± 68.15	0.303
Ferritin (µg/l)	196.63 ± 87.63	269.31 ± 70.53	0.0139*	194 ± 35.56	64.6 ± 27.82	0.000*
EF %	67% ± 4	62% ± 3	0.08	68% ± 5	68% ± 4	0.924

All data are representing mean ±SD
SD: standard deviation
LDH: lactate dehydrogenase enzyme
CK-MB: creatinine kinase-MB iso-enzyme.
Troponin I: it is a part of the troponin protein complex in the myocardium.
ACL IgG: anti-cardiolipin antibody (autoantibodies)
TIBC: Total Iron Binding Capacity
EF: ejection fraction
A paired t-test is used through SPSS for statistical analysis
When the *p*-value is less than 0.05, it will be considered clinically significant.

The changes in the means serum levels of biomarkers of all patients in The control group, after 6 months of anthracycline treatment are statistically significant. These results were used to determine the degree of cardiotoxicity as a result of anthracycline treatment. The mean of lactate dehydrogenase (LDH) is dramatically increased by more than 204 % after 6 months' treatment by anthracycline. Whereas, in The ketotifen group there is a non-statistically significant difference in LDH. Patients in The control group are highly affected by anthracycline treatment particularly toxicity of the heart as there is more than a 2.29-fold increase in serum levels of CK-MB after 6 months of treatment by anthracycline. Whereas, patients in THE ketotifen group, there is a statistically non-significant difference (p-value = 0.102) in the mean serum levels of CK-MB after 6 months of treatment by anthracycline. The results showed a highly statistically significant (p-value $\leq$ 0.05) increase in all mean serum levels of troponin I, ACL IgG, iron, TIBC, and ferritin in patients in The control group. As well, a statistically non-significant change in EF% was noticed as seen in Table II.

Table III shows a statistically significant difference between mean serum levels of all measured parameters, like troponin I, ACL IgG, and ferritin (p-value $\leq$ 0.05) in The control group at baseline versus 6 months' treatment of anthracycline.

There are no statistically significant differences between measured EF% in patients in The control group at baseline versus after anthracycline treatment. (p = 0.08).

The results, as shown in Table IV, indicate that there were no significant differences changes in the means serum levels of all biomarkers measured of both groups at baseline. But after 6 months of treatment, the patients in The ketotifen group, the mean of LDH (U/L) was highly decreased compared with that in The control group (p-value $\leq$ 0.05). The results showed a statistically significant difference reduction in the mean of CK-MB (ng/mL) in the ketotifen group (17.64 ± 6.91), versus that in the control group after 6 months with anthracycline treatment (32.99 ± 11.15 p-value $\leq$ 0.05). Patients in the ketotifen group, the mean of troponin I (ng/ml) was a statistically significant decline when compared with that in the control group after 6 months of treatment (p-value $\leq$ 0.05). Table IV showed that the mean ferritin was dramatically declined in patients received

ketotifen (64.6 ± 27.82 µg/L.), when compared with that in the control group (269.31 ± 70.53 µg/L.). (*p*-value ≤ 0.002).

From Table V the results showed that a direct positive correlation between mean blood levels of iron in the ketotifen group with the mean of LDH (r = + 0.79). At the same time, the mean of LDH has an indirect correlation with the mean of EF% after 6 months of anthracycline treatment. (r = - 0.697). So, we can use serum levels of LDH as an indicator for the degree of cardiotoxicity.

Table III Measured biomarkers and echocardiograms of patients in the control group at baseline and after 6 months

Measured Parameter	Control group at baseline n=55	Control group after 6 months n=55	*p*-value
LDH (U/L)	259.25 ± 95.71	530.00 ± 86.99	0.007*
CK-MB (ng/mL)	14.37 ± 4.00	32.99 ± 11.15	0.009*
Troponin I (ng/ml)	0.15 ± 0.10	0.51± 0.14	0.001*
ACL IgG (U/L)	6.91 ± 1.24	14.38 ± 2.99	0.000*
Iron (mcg/dL)	89.06 ±15.41	178.25± 19.90	0.000*
TIBC (µg/dL)	296.00 ± 72.12	238.63 ± 55.18	0.005*
Ferritin (µg/l)	196.63 ± 87.63	269.31 ± 70.53	0.0139*
EF %	67% ± 4	62% ± 3	0.08

All data are representing mean ±SD
SD: Standard Deviation
LDH: lactate Dehydrogenase enzyme
CK-MB: Creatine kinase-MB iso-enzyme.
Troponin I: It is a part of the troponin protein complex in the myocardium.
ACL IgG: Anti-cardiolipin antibody (autoantibodies)
TIBC: Total Iron Binding Capacity
EF: Ejection Fraction
A paired t-test is used through SPSS for statistical analysis
When the *p*-value is less than 0.05, it will be considered clinically significant.
*: statistically highly significant.
Control group: patients received anthracycline without the use of ketotifen.

Table IV Measured biomarkers, and echocardiograms in the patients in control and, ketotifen groups at baseline, and after 6 months' treatment by anthracycline.

	Control group N=55	Ketotifen group N=56	p-value	Control group N=55	Ketotifen group N=56	*p*-value
	At baseline			After 6 months		
LDH (U/L)	259.25 ± 95.71	255.87 ± 86.99	0.363	530.00 ± 86.99	227.53 ± 69.49	0.001*
CK-MB (NG/ML)	14.37 ± 4.00	14.46 ± 3.58	0.550	32.99 ± 11.15	17.64 ± 6.91	0.006*
Troponin I (ng/mL)	0.15 ± 0.10	0.16 ± 0.10	0.134	0.51± 0.14	0.15± 0.08	0.001*
ACL IGg (U/L)	6.91 ± 1.24	6.13 ± 0.20	0.048	14.38 ± 2.99	4.25 ± 1.16	0.002*
Iron (mcg/dL)	89.06 ±15.41	90.90 ± 15.34	0.464	178.25± 19.90	46.53± 16.30	0.008*
TIBC (µg/dL)	296.00 ± 72.12	298.07± 57.32	0.334	238.63 ± 55.18	320.13 ± 68.15	0.001*
FERRITIN (µg/L)	196.63 ± 87.63	194.00 ± 35.56	0.786	269.31 ± 70.53	64.6 ± 27.82	0.001*
EF %	67% ± 4	68% ± 5	0.401	62% ± 3	68% ± 4	0.009*

All data are representing mean ±SD
SD: Standard deviation
LDH: lactate Dehydrogenase enzyme
CK-MB: Creatine kinase-MB iso-enzyme.
Troponin I: it is a part of the troponin protein complex in the myocardium.
ACL IgG: Anti-cardiolipin antibody (autoantibodies)
TIBC: total iron-binding capacity
EF: ejection fraction
A paired t-test is used through SPSS for statistical analysis
*When *p*-value is less than 0.05, it will be considered clinically significant.

Table V The correlations between some biomarkers in the ketotifen group after 6 months' treatment by anthracycline.

	LDH levels in The ketotifen group after 6 months	TIBC levels in The ketotifen group after 6 months
EF % for The ketotifen group after 6 months	r = - 0.697	
LDH levels in The ketotifen group after 6 months		r = - 0.597
Iron levels in The ketotifen group after 6 months	r = + 0.79	

EF: Ejection fraction
r: Correlation coefficient.
LDH: lactate dehydrogenase

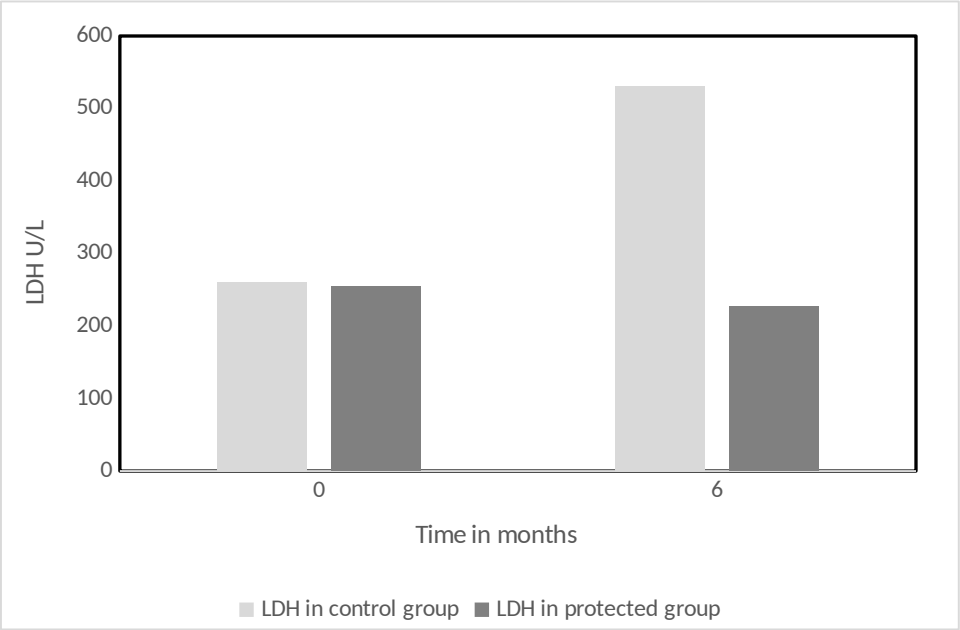

Figure (2): Represents the changes in serum lactate dehydrogenase (LDH U/ L) in both groups before and after treatment by anthracycline.

Control group: patients who received anthracycline only without protection.

Protected group: patients who received anthracycline plus protection by ketotifen

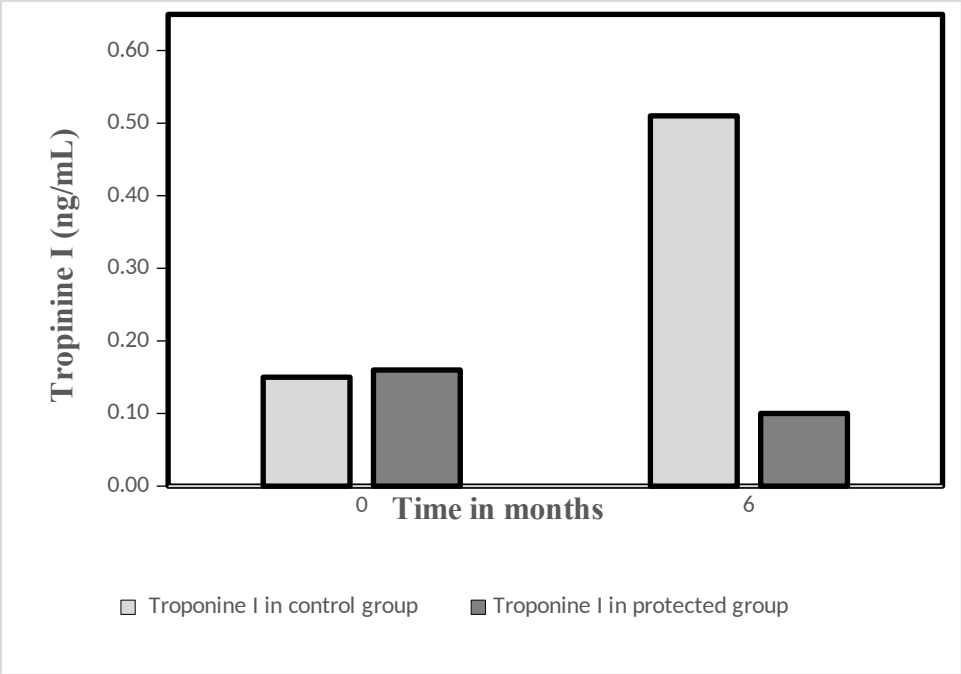

Figure (3): Represents the changes of serum Troponin I in both groups before and after treatment by anthracycline.

Control group: patients received anthracycline only without protection.
Ketotifen group: patients received anthracycline with protection by ketotifen

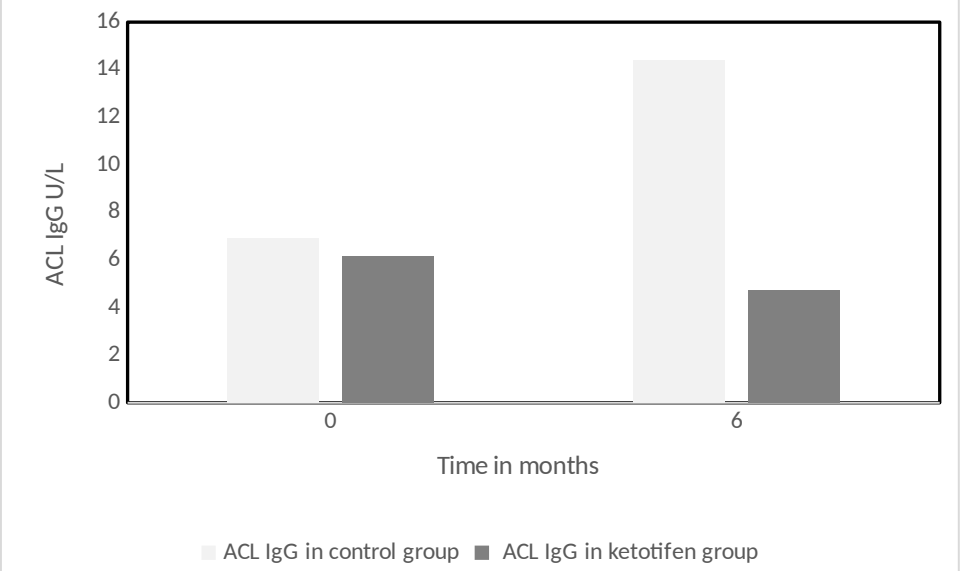

Figure (4): Represents the changes in mean serum levels of ACL Ig in both groups before and after treatment by anthracycline.

ACL Ig: anti-cardiolipin antibody (autoantibodies)
Control group: patients received anthracycline only without protection.
Ketotifen group: patients received anthracycline with protection by ketotifen

Discussion

As mentioned in the introduction section, breast cancer is one of the greatest collective malignant tumors that dramatically affect the health of women[29]. At present, anthracyclines and taxanes are the two main classes of drugs for breast cancer treatment. Anthracyclines are amongst the most ordinarily used and effective drugs in breast cancer treatment. In the past 30 years, they have become an important constituent of adjunctive and palliative therapy for breast cancer.

Anthracyclines belong to a class of antineoplastic antibiotics, which interfere with cell replication by acting on the DNA at several levels, showing an effect in every phase of the cell cycle. Doxorubicin and epirubicin are commonly used in clinical practice. The administration is only via an intravenous infusion; metabolism is mainly hepatic and excretion via the bile route, while urinary elimination accounts for approximately 1/6 of the total amount. Anthracyclines exhibit a range of toxic effects, including transient myelosuppression, mucositis, and hair loss. Cardiotoxicity remains a major risk because it may be permanent and progressive, leading to multimorbidity and strictly impacting the quality of life in patients with breast cancer. Acute cardiotoxicities, as well as the potential effect of cumulative doses, increase the risk of congestive heart failure[30]. Iron chelating agents such as dexrazoxane (DEX), a most promising cardioprotective agent, is effective in reducing both acute and chronic cardiotoxicity induced by anthracycline therapy. It has been widely used in the United States and Europe for various clinical applications. Dexrazoxane is mainly used to reduce the incidence and severity of cardiomyopathy caused by doxorubicin in patients with advanced breast cancer[31].

The results of the *vitro* chemical test revealed that ketotifen has the affinity to chelate with iron. Ketotifen is registered as a drug by the FDA and has fewer side effects compared with other commonly used iron-chelating agents. Therefore, association with chemotherapy may be of value as a cardioprotective agent.

Our results cast a new light on the significant cardioprotective effect of ketotifen in patients with breast cancer receiving anthracycline chemotherapy. Overall, these findings are by findings reported by Gurusher S. et al. 2007[7]. A higher body iron stores in patients who have received multiple blood transfusions, prolonged iron

supplementation, or those with unpredicted iron regulatory gene mutations may be predisposed to anthracyclines cardiotoxicity.

The present study established the findings of monitoring cardiotoxicity in patients receiving anthracyclines, which include monitoring EF% that was gutted from an echocardiogram. The study proved that EF% was highly decreased in patients who did not receive ketotifen. However, in the patient received ketotifen with an anthracycline, their EF% was not affected. As well as cardiotoxicity biomarkers: serum levels of troponin I were highly elevated in the control group when compared with the ketotifen group after 6 months treatment of anthracyclines. An additional novel finding is that ketotifen maintains the function of mitochondria, which is monitored by ACL IgG, as its mean serum levels (U/L) was highly elevated after 6 months of anthracyclines treatment in the control group. This finding is indicating a loss of mitochondria function in the control group when compared with the ketotifen group. The mean serum levels of ACL IgG (U/L) was very close to its mean at baseline Table III in the ketotifen group.

Together, the current findings approve that the iron profile was improved in The ketotifen group as a chelating agent for iron. Since 1979, myocyte injury, as measured clinically, is often reversible with removal of systemic iron stores. Chronic therapy with subcutaneous deferoxamine (chelating agent for iron) has had a dramatic impact on survival in secondary iron overload from β-thalassemia[32]. The present study confirmed the findings of the mean serum levels of iron in The ketotifen group was highly decreased after 6 months (Table II). At the same time, a significant high decline of the mean serum levels of ferritin in The ketotifen group, compared with that in The control group. So, this finding introduces a new role of ketotifen in reducing iron overload. The results demonstrate two things. First, a significant reduction in serum iron overload. Second, improvement in mitochondrial function. Up till now, there are no clinical studies on the effects of oral iron chelation as a cardioprotective agent against anthracyclines cardiotoxicity.

We must continue to investigate ways of protecting the heart following cancer chemotherapy, at present the limited cardioprotective strategies available –dexrazoxane, ACE-inhibitors, ARB, and beta-blockers – are not in routine prophylactic use. These results go beyond prior reports, showing a novel oral protecting agent. That can be used

for inhibition of cardiotoxicity induced by anthracyclines therapy. Which may lead to increasing the cancer survivor population, and, representing a strong motivator to explore larger randomized controlled trials in cancer cardioprotection. In order to prevent today's cancer patient from becoming tomorrow's cardiac patient.

On this basis, we conclude that, ketotifen could have a potential beneficial effects in managing diseases characterized by iron overload including the coronavirus disease 2019. A study by Wenzhong and Hualan has demonstrated that the ORF8 and surface glycoprotein could bind to the porphyrin. At the same time, orf1ab, ORF10, and ORF3a proteins could coordinate attack the heme on the 1-β-chain of hemoglobin to dissociate the iron to form the porphyrin. The attack will cause less and lose hemoglobin that can carry oxygen and carbon dioxide. The lung cells have extremely intense poisoning and inflammatory due to the inability to exchange carbon dioxide and oxygen frequently, which eventually results in ground-glass-like lung images[33]. The previous study confirmed that the pathogenesis of COVID-19 is due to its invasion of hemoglobin, releasing iron from hemoglobin resulting in iron overload. Our study proved that ketotifen can chelate with the released iron furthermore may have potential beneficial effects in diseases such as COVID-19 management.

Conclusions

In conclusion, this study indicates that firstly, ketotifen may have a novel cardioprotective effects in prevention of anthracyclines induced cardiotoxicity. Secondly, ketotifen appears to be efficient, safe and taken orally. Lastly, it has the ability to chelate iron overload, so suggested a beneficial effect in iron overload inducing diseases such as COVID-19.

Conflicts of interest

The authors have declared that no competing interests exist.

Funding statement

The writers provided no financial support for this article's study, authorship, and/or publishing.

All authors share the data underlying the findings of their manuscripts. Data sharing allows researchers to verify the results of an article, replicate the analysis, and conduct secondary analyses.

Acknowledgment:

I would like to express my sincere gratitude to Dr. Rezik Ayyad, a professor in the Medicinal Chemistry Department, Faculty of Pharmacy, Azhar University, Cairo, Egypt for supporting us in this study.

References

1. Siegel RL, Jemal A, Wender RC, Gansler T, Ma J, Brawley O.W. *An assessment of progress in cancer control.* CA: a cancer journal for clinicians 2018; 68: 329-339.
2. Vejpongsa P, Yeh ET. *Prevention of anthracycline-induced cardiotoxicity: challenges and opportunities.* Journal of the American College of Cardiology 2014; 64: 938-945.
3. Zhang J, Cui X, Yan Y, Li M, Yang Y, Wang J, Zhang J. *Research progress of cardioprotective agents for prevention of anthracycline cardiotoxicity.* American journal of translational research 2016; 8: 2862-2872.
4. Carvalho C, Santos RX, Cardoso S, Correia S, Oliveira PJ, Santos MS, Moreira PI. *Doxorubicin: the good, the bad and the ugly effect.* Current medicinal chemistry 2009: 16: 3267-3285.
5. McGowan JV, Chung R, Maulik A, Piotrowska I, Walker J M, Yellon D M. *Anthracycline chemotherapy and cardiotoxicity.* Cardiovascular drugs and therapy, 2017; **31**: 63-75.
6. Cagel M, Grotz E, Bernabeu E, Moretton M A, Chiappetta D A. *Doxorubicin: nanotechnological overviews from bench to bedside.* Drug discovery today 2017; **22**: 270-281.
7. Panjrath, G.S., V. Patel, Valdiviezo C I, N. Narula, Narula J, Jain x. *Potentiation of doxorubicin cardiotoxicity by iron loading in a rodent model.* Journal of the American College of Cardiology 2007; **49**: 2457-2464.
8. Manivasagan P, Kang K H, Sivakumar K, Li-Chan, Kim S K. *Marine actinobacteria: an important source of bioactive natural products.* Environmental Toxicology and Pharmacology 2014; **38**: 172-188.
9. Cardinale D, Colombo A, Bacchiani G, Tedeschi I, Meroni C A, Veglia F, Civelli M, Lamantia G, Colombo N, Curigliano G. *Early detection of anthracycline cardiotoxicity and improvement with heart failure therapy.* Circulation 2015; **131**: 1981-1988.
10. Lipshultz S E, Alvarez J A, Scully R E. *Anthracycline associated cardiotoxicity in survivors of childhood cancer.* Heart 2008; **94**: 525-533.
11. Wang L, Tan T C, Halpern E F, Neilan T G, Francis S A, Picard M H, Hochberg J S, Abramson A E. *Major cardiac events and the value of echocardiographic evaluation in patients receiving anthracycline-based chemotherapy.* The American journal of cardiology 2015; **116**: 442-446.
12. Angsutararux P S, Luanpitpong S, Issaragrisil. *Chemotherapy-induced cardiotoxicity: overview of the roles of oxidative stress.* Oxidative medicine, cellular longevity 2015.

13. Popat R, Oakervee H E, Hallam S, Curry N, Odeh L, Foot N, Esseltine D L, Drake M, Morris C, Cavenagh J D. *Bortezomib, doxorubicin, dexamethasone (PAD) front–line treatment of multiple myeloma: updated results after long–term follow–up.* British journal of haematology 2008; **141**: 512-516.
14. Ichikawa Y, Ghanefar M, Bayeva M, Wu R, Khechaduri A, Prasad S V N, Mutharasan R K, Naik T J, Ardehali H. *Cardiotoxicity of doxorubicin is mediated through mitochondrial iron accumulation.* The Journal of clinical investigation 2014; **124**: 617-630.
15. Outomuro D, Grana D R, Azzato F, Milei J. *Adriamycin-induced myocardial toxicity: new solutions for an old problem?* International journal of cardiology 2007; **117**: 6-15.
16. Münzel T, Gori T, Keaney J F, Maack C, Daiber A. *Pathophysiological role of oxidative stress in systolic and diastolic heart failure and its therapeutic implications.* European heart journal 2015; **36**: 2555-2564.
17. Šimůnek T, Štěrba M, Popelová O, Adamcová M, Hrdina R, Geršl V. *Anthracycline-induced cardiotoxicity: overview of studies examining the roles of oxidative stress and free cellular iron.* Pharmacological reports 2009; **61**: 154-171.
18. Wouters K.A, Kremer L C, Miller T L, Herman E H, Lipshultz S E. *Protecting against anthracycline–induced myocardial damage: a review of the most promising strategies.* British journal of haematology 2005; **131**: 561-578.
19. Kamide R, Niimura M, Ueda H, Imamura S, Yamamoto S, Yoshida H, Kukita A. *Clinical evaluation of ketotifen for chronic urticaria: multicenter double-blind comparative study with clemastine.* Annals of allergy 1989; **62**: 322-325.
20. D'Arcy P F. *Meyler's side effects of drugs, twelfth edition: M.N.G. Dukes (Ed.) Elsevier Science Publishers BV, Amsterdam, 1992. ISBN: 0-444-98524-8. Price US$297.00 Dfl.475.00.* International Journal of Pharmaceutics 1993; **94**: 241.
21. St-Pierre J, Kobric M, Rackham A. *Effect of ketotifen treatment on cold-induced urticaria.* Annals of allergy 1985; **55**: 840.
22. Grant S M, Goa K L, Fitton A, Sorkin E M. *Ketotifen.* Drugs 1990; **40**: 412-448.
23. Gallant–Behm CL, Hildebrand K A, Hart D A. *The mast cell stabilizer ketotifen prevents development of excessive skin wound contraction and fibrosis in red Duroc pigs.* Wound Repair and Regeneration 2008; **16**: 226-233.
24. Hildebrand K A, Sutherland C, Zhang M. *Rabbit knee model of post–traumatic joint contractures: the long–term natural history of motion loss and myofibroblasts.* Journal of orthopaedic research 2004; **22**: 313-320.
25. Poprac P, Jomova K, Simunkova M, Kollar V, Rhodes C J, Valko M. *Targeting free radicals in oxidative stress-related human diseases.* Trends in pharmacological sciences 2017; **38**: 592-607.
26. Flora S J, Pachauri V. *Chelation in metal intoxication.* International journal of environmental research and public health 2010; **7**: 2745-2788.
27. Seeger C, Christopeit T, Fuchs K, Grote K, Sieghart W, Danielson U H. *Histaminergic pharmacology of homo-oligomeric β3 γ-aminobutyric acid type A receptors characterized by surface plasmon resonance biosensor technology.* Biochemical pharmacology. 2012; **84**: 341-351.
28. Association W M. *Declaration of Helsinki. Ethical principles for medical research involving human subjects.* http://www. wma. net/e/policy/b3. htm, 2008.
29. Albini A, Pennesi G, Donatelli F, Cammarota R, De Flora S, Noonan D M. *Cardiotoxicity of anticancer drugs: the need for cardio-oncology and cardio-oncological prevention.* Journal of the National Cancer Institute 2010; **102**: 14-25.

30. Zhang S, Liu X, Bawa-Khalfe T, Lu L S, Lyu Y L, Liu L F, Yeh E T. *Identification of the molecular basis of doxorubicin-induced cardiotoxicity* Nature medicine 2012; **18**: 1639-1642.
31. Minotti G, Menna P, Salvatorelli E, Cairo G, Gianni L. *Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity*. Pharmacological reviews 2004; **56**: 185-229.
32. Wolfe L, Olivieri N, Sallan D, Colan S, Rose V, Propper R, Freedman M H, Nathan D G. *Prevention of cardiac disease by subcutaneous deferoxamine in patients with thalassemia major*. New England Journal of Medicine 1985; **312**: 1600-1603.
33. Liu W, Li H. *COVID-19: attacks the 1-beta chain of hemoglobin and captures the porphyrin to inhibit human heme metabolism*. Preprint revised on 2020; **10**.