

**The identification of ketotifen as a novel cardioprotective agent in patients
undergoing anthracyclines chemotherapy**

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Running title:

New indication of ketotifen as cardioprotective agent.

Highlights:

- Cancer is a foremost public health problem all over the world.
- Optimum administration of anthracyclines 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 the deferoxamine.
- Ketotifen is suggested to have a beneficial effect in the prevention of iron overload-associated disorders such as COVID-19.
- Can ketotifen act as a perfect oral iron chelator in preventing cardiotoxicity?

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. One hundred eleven eligible patients with breast cancer (age range, 30-60 year) were scheduled to receive anthracyclines 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 were 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; Mitochondria

There are no studies about the use of ketotifen in reducing expected cardio-toxicities from anthracyclines administration until the submission of this article.

This study adds a novel use of ketotifen as a cardioprotective agent.

1. 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 disease 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 anthracyclines-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 anthracyclines 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 anthracyclines 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]. Anthracyclines can also cause cardiomyopathy during chronic use and sometimes late-onset severe arrhythmia and ventricular dysfunction have occurred^[9]. It has been detected that the rate of survival with anthracyclines-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 toxicity⁹. Currently, echocardiography is used to monitor doxorubicin-induced cardiotoxicity, which is considered the principal assessment test^[11]. Commonly, chemotherapeutic drug-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 the patients are usually administering different treatment protocols^[13, 14].

Two main hypotheses are suggested on the subject of anthracyclines-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 the central point of oxidative stress; ii) Metabolic hypotheses in which the C-13 alcoholic metabolite of anthracyclines 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 anthracyclines-induced cardiotoxicity. The first and the oldest one is the iron chelator, deferoxamine, which was first approved for clinical use in the 1960s. 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].

1.1. Rationale and aim of the work

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 an 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].

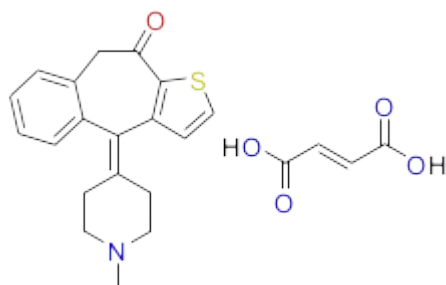


Figure I: Structural formula of ketotifen fumarate

Chelation-based therapy is one of the preferred medical treatment strategies for decreasing the toxic effects of metals like iron, calcium, 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]. Ketotifen has the qualifications of an efficient metal chelator as it has two atoms with more than one lone pair of electrons, sulfur, and oxygen. This criterion gives ketotifen the opportunity to bind with metal ions. Suggested forms of ketotifen chelates with iron, and calcium are presented in **Figure II**.

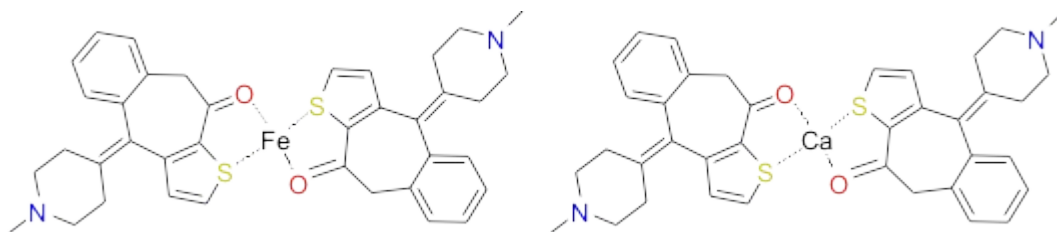


Figure II: Suggested ketotifen chelates with iron, and calcium

As the clinical use of anthracyclines is associated with the elevation of iron level, this iron overload is a major obstacle in the usage of doxorubicin and many other anthracyclines. In this study, we aimed to assess whether ketotifen can provide effective protection against the induced cardiotoxicity in patients treated with anthracyclines.

2. Materials and methods

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

Zaditen[®] is an oral dosage form which is manufactured by Novartis Pharma, Egypt. Each tablet contains 1 mg of ketotifen. 10 Tablets of zaditen[®] were soaked in 10 mL of distilled water for 12 hours and then filtered.

The filtrate titrated with 0.01 N solutions of both ferrous hydroxide ($Fe [OH]_2$) and calcium hydroxide ($Ca [OH]_2$) in three separated experiments using phenolphthalein (Ph. Ph.) as an indicator.

The same procedure was applied to the blank solution (distilled water).

Endpoints were recorded for all the above experiments.

The results are presented in **Table I**.

Table I: Results of complexmetric titration of ketotifen and blank samples against solutions of $Fe(OH)_2$ and $Ca(OH)_2$.

Experiment #	Endpoint of $Fe(OH)_2$	Endpoint of $Ca(OH)_2$
1.	5.6 mL	8.0 mL
2.	5.8 mL	8.2 mL
3.	5.7 mL	8.1 mL
Blank	0.1 mL	0.1 mL

Materials:

Calcium hydroxide ($Ca(OH)_2$) analytical grade; Merck Schuchardt, Germany

Ferrous hydroxide ($Fe(OH)_2$) analytical grade; Merck Schuchardt, Germany

Phenolphthalein (Ph. Ph.) analytical grade; Riedel-deHaen, Germany.

Equipment:

Clinical centrifuge (Minor 35, England).

Setting:

A total number of one hundred and eleven patients were recruited from the Oncology Department, Menoufia University Hospital, Egypt. The age range was 30-60 years in all groups. All were female patients with breast cancer. 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 is a randomized, prospective controlled trial. Eligible patients fulfilled the following criteria: female with breast cancer receiving anthracyclines chemotherapy in their protocol without any cardioprotective agent. They were initially have adequate baseline echocardiography. Patients with a history of any of the following pathological disorders were initially excluded: i) heart failure, ii) arrhythmia, iii) cardiac catheterizations, iv) angina, v) uncontrolled hypertension, vi) uncontrolled diabetes, and vii) impaired liver function. As well, patients who previously received anthracyclines-containing regimens were also not selected. Each patient was identified with a coded number to maintain privacy. The enrolled patients divided into two groups: **i) the control group**, 55 patients received their standard therapy (anthracyclines-containing chemotherapy without a cardioprotective agent) at a dose of 60 mg/m²/week over a period of 4 weeks in the induction phase and 3 more weeks in the re-induction phase (total 175 mg/m²); **ii) the ketotifen group**, 56 patients received anthracyclines-containing chemotherapy plus ketotifen. Ketotifen was given orally as one tablet (1 mg) three times daily for 5 months minimum. During cancer therapy, echocardiograms and blood samples were initially assessed and after 6 months of treatment for all patients to evaluate ejection fractions and different biomarkers. Regarding the therapeutic regimen in the control group, 14 patients received adriamycin with cyclophosphamide (AC). Eighteen 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. All patients in this group received three daily regular doses of 1 mg of ketotifen in addition to the specified chemotherapeutics.

Sample collection

Blood samples (5 ml) were collected from all patients before and after the treatment course. The serum supernatant was separated immediately from the blood by centrifugation for 15 minutes 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 appropriate 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. cTnI ELISA kits manufactured by Accu-Bind Company, USA used to assay Troponin-I. Total iron-binding capacity (TIBC) kits manufactured by BioSystem. S.A company, Spain was used for measurement of TIBC. Ferritin enzyme-linked immunosorbent assay (ELISA) kits manufactured by Accu-Bind company; USA was used for measurement of serum ferritin. Enzyme-linked immunosorbent assay (ELISA) kits (manufactured by Orgentec Diagnostika GmbH company, Germany used to assay anti-cardiolipin IgG. Iron-Chromazurol kits manufactured by BioSystem S.A company, Spain was used to assay iron. Enzyme-linked immunosorbent.

Statistical analysis

All data presented as mean \pm 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

From the above-tabulated results presented in **Table I**, it could be concluded that the ketotifen may chelate divalent cations (Fe^{++} and Ca^{++}), where the sulfur and oxygen

atoms of ketotifen are expected to bind with them, whereas, the blank (distilled water) did not.

A comparison between mean serum levels of all measured biomarkers and echocardiograms of patients in the control versus ketotifen group at baseline and after 6 months of treatment with anthracyclines is presented in **Table II**. It is obvious that there were no significant differences in the mean serum levels of all biomarkers measured in both groups at baseline. Whereas, after 6 months of treatment, patients in the ketotifen group, the mean of LDH level (U/L) was highly decreased compared with that in the control group (p -value ≤ 0.05). The results also exposed the statistically significant reduction in the mean of CK-MB level (ng/mL) in the ketotifen group (17.64 ± 2.81), versus that in the control group after 6 months of treatment with anthracyclines (32.99 ± 4.92 p -value ≤ 0.05). The mean level of troponin I in patients of the ketotifen group was significantly reduced at the end of the study compared with that of the control group (p -value ≤ 0.05). The tabulated data further presented that the mean ferritin level was dramatically dropped in patients who received ketotifen (64.6 ± 9.74 $\mu\text{g/L}$.) compared with that in the control group (269.31 ± 23.71 $\mu\text{g/L}$; p -value ≤ 0.002).

Table II: Measured biomarkers and echocardiograms of patients in control and ketotifen groups at baseline and after 6 months of treatment with anthracyclines.

Biomarkers	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 ± 16.72	255.87 ± 24.12	0.363	530.00 ± 26.06	227.53 ± 25.73	0.001*
CK-MB (ng/mL)	14.37 ± 2.49	14.46 ± 1.33	0.550	32.99 ± 4.92	17.64 ± 2.81	0.006*
Troponin I (ng/mL)	0.15 ± 0.09	0.16 ± 0.07	0.134	0.51 ± 0.09	0.15 ± 0.08	0.001*
ACL IgG (U/L)	6.91 ± 0.8	6.13 ± 0.20	0.048	14.38 ± 1.66	4.74 ± 0.62	0.002*
Iron (µg/dL)	89.06 ± 6.5	90.9 ± 8.73	0.464	178.25 ± 10.49	46.53 ± 6.34	0.008*
TIBC (µg/dL)	296.00 ± 26.33	298.07 ± 28.96	0.334	238.63 ± 17.62	320.13 ± 19.66	0.001*
Ferritin (µg/L)	196.63 ± 12.99	194 ± 17.84	0.786	269.31 ± 23.71	64.6 ± 9.74	0.001*
EF %	67% ± 4.00	68% ± 5.00	0.401	62% ± 3.00	68% ± 4.00	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

* *p*-value considered statistically significant if equal to or less than 0.05.

A comparison between mean serum levels of all measured biomarkers and echocardiograms of patients in both the control group and the ketotifen group at baseline and after 6 months of treatment with anthracyclines is presented in **Table III**. There were no statistically significant differences between the mean serum levels of measured biomarkers and the echocardiograms at the beginning of the study. As well, the *p*-values were above 0.05 in all patients as shown in **Table III**.

Table III: Mean serum levels of biomarkers and echocardiograms in control versus ketotifen group at baseline and after 6 months of treatment with anthracyclines.

Biomarkers	Control group n = 55	Control group n = 55	<i>p-value</i>	ketotifen group n = 56	Ketotifen group n = 56	<i>p-value</i>
	At baseline	After 6 months		At baseline	After 6 months	
LDH (U/L)	259.25 ± 16.72	530.00 ± 26.06	0.007*	255.87 ± 24.12	227.53 ± 25.73	0.297
CK-MB (ng/mL)	14.37 ± 2.49	32.99 ± 4.92	0.009*	14.46 ± 1.33	17.64 ± 2.81	0.102
Troponin I (ng/mL)	0.15 ± 0.09	0.51 ± 0.09	0.001*	0.16 ± 0.07	0.15 ± 0.08	0.562
ACL IgG (U/L)	6.91 ± 0.8	14.38 ± 1.66	0.003*	6.13 ± 0.20	4.74 ± 0.62	0.213
Iron (µg/dL)	89.06 ± 6.5	178.25 ± 10.49	0.001*	90.9 ± 8.73	46.53 ± 6.34	0.004*
TIBC (µg/dL)	296.00 ± 26.33	238.63 ± 17.62	0.005*	298.07 ± 28.96	320.13 ± 19.66	0.303
Ferritin (µg/l)	196.63 ± 12.99	269.31 ± 23.71	0.0139*	194 ± 17.84	64.6 ± 9.74	0.001*
EF %	67% ± 4.00	62% ± 3.00	0.080	68% ± 5.00	68% ± 4.00	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 (autoantibody); TIBC: Total Iron Binding Capacity; EF: ejection fraction.

A paired t-test is used through SPSS for statistical analysis.

* *p*-value is equal to less than 0.05 (statistically significant).

After 6 months of anthracyclines treatment, the differences in mean serum levels of biomarkers for all patients in the control group were found to be statistically significant. These results were used to determine the degree of cardiotoxicity as a result of anthracyclines treatment. The mean of lactate dehydrogenase (LDH) was dramatically elevated by more than 100% as a result of treatment with anthracyclines. Whereas, in the ketotifen group there was a statistically non-significant difference in the level of LDH. CK-MB, which is one of the heart toxicity markers. Patients in the control group were highly susceptible to the cardiotoxic effect of anthracyclines as indicated by the elevated level of the latter biomarker. Serum levels of CK-MB in the control group were elevated

by more than 2-folds. Whereas, there was a statistically non-significant difference in the mean serum levels of CK-MB in the ketotifen group (p -value = 0.102). The results also revealed highly statistically significant increases in all the mean serum levels of troponin I, ACL IgG, iron, TIBC, and ferritin in the control group patients (p -value ≤ 0.05). On the other hand, a statistically non-significant change in the percentage of Ejection Fraction (EF) was noticed as indicated by the obtained data in **Table III**.

A study of correlations between levels of LDH and iron with the ejection fraction in patients of the ketotifen group after 6 months of treatment with anthracyclines has conducted. This study helped us to rationalize the use of LDH level as an indicator to detect the degree of cardiotoxicity. The observed data showed that there is a direct positive correlation between mean blood levels of iron with that of LDH ($r = + 0.790$). In contrast, there is an indirect correlation between the mean levels of LDH with that of EF% ($r = - 0.697$). Accordingly, we have supposed that the serum levels of LDH could be employed as an indicator to detect the degree of cardiotoxicity. Graphical representation to compare the mean levels of LDH, Troponin, and ACL Ig in the groups under study before and after treatment with anthracyclines is presented in **Chart I**.

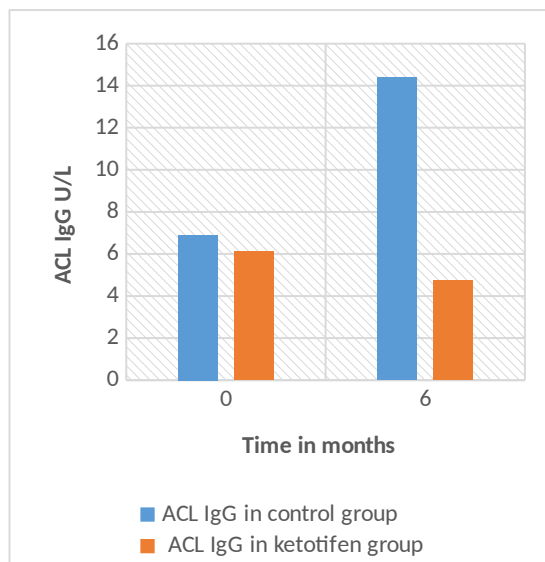
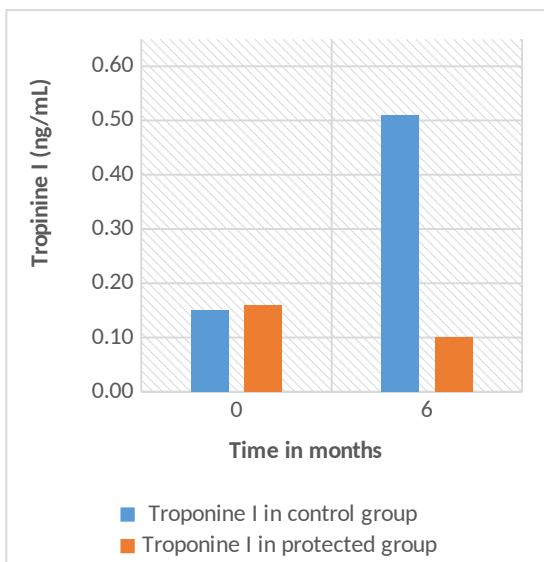
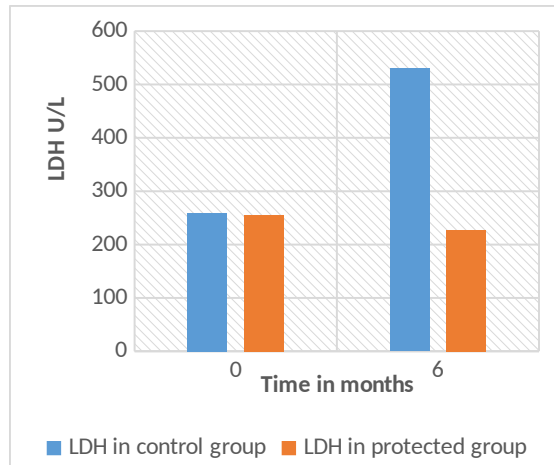


Chart 1: Changes in LDH U/L (upper row), Troponin I (lower left), and ACL Ig (lower right) in both groups before and after treatment with anthracyclines.

Control group: patients who received anthracyclines only without protection.

Protected group: patients who received anthracyclines plus 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]. To date, anthracyclines and taxanes remain the two main classes of drugs for breast cancer treatment. Among them, anthracyclines are the most ordinarily used and effective class. In the past few decades, they have become an important constituent of adjunctive and palliative therapy for breast malignancies.

Anthracyclines exhibit a range of toxic effects, including transient myelosuppression, mucositis, and hair loss. Cardiotoxicity remains a major concomitant risk with anthracyclines therapy because it may be permanent and progressive, leading to multimorbidity and strictly impacting the quality of life of cancer patients. 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 anthracyclines 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].

Results of the present *in vitro* chemical test revealed that ketotifen has an affinity to chelate with iron. Ketotifen is an FDA approved mast cell stabilizer and has fewer side effects compared with other commonly used iron-chelating agents. Therefore, it was suggested that concomitant administration of ketotifen with anthracyclines may present a beneficial impact as a cardioprotective agent.

Our results cast a new light on the significant cardioprotective effect of ketotifen in cancer patients receiving anthracyclines chemotherapy. This study recognized the findings of monitoring cardiotoxicity in patients on 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 who 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 compared with the ketotifen group. An additional novel finding is that ketotifen

maintains the function of mitochondria, as indicated by ACL IgG biomarkers, as its mean serum levels (U/L) were 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 compared with the ketotifen group. The mean serum level of ACL IgG (U/L) was 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 frequently reversible with the removal of systemic iron stores. Chronic therapy with subcutaneous deferoxamine (an iron chelator) has had a dramatic impact on survival in secondary iron overload from β -thalassemia^[32]. This study confirmed that the level of iron in the ketotifen group was highly decreased after 6 months (**Table II**). Simultaneously, a significant high fall in the level 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. Until now, there are no clinical studies on the effects of oral iron chelators as cardioprotective agents against anthracyclines cardiotoxicity.

It is crucial to investigate ways of protecting the heart following cancer chemotherapy. At present, the limited cardioprotective strategies of available cardioprotective agents such as dexrazoxane, ACE-inhibitors, ARB, and β -blockers are not in routine prophylactic use for anthracyclines cardiotoxicity. These results go beyond prior reports, showing a novel oral protecting agent that can be used for inhibition of cardiotoxicity induced by anthracyclines therapy. In order to prevent today's cancer patient from becoming tomorrow's cardiac patient, this finding may contribute to increasing the cancer survivor population.

Additionally, a recent study was reported by Liu and Li^[33] that suggested 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 released iron. Accordingly, we conclude that the ketotifen could have a potential

beneficial effect in managing diseases characterized by iron overload, including COVID-19.

Conclusions

In conclusion, this study indicates that firstly, ketotifen may have a novel cardioprotective effect in prevention of anthracyclines induced cardiotoxicity. Secondl, it has the ability to chelate iron overload, so suggested that it may have a beneficial effect in iron overload inducing diseases such as COVID-19. In the future, we will perform a further study to investigate the cardioprotective effect of ketotifen in pediatric patients with thalassemia-associated iron overload.

Conflicts of interest

Authors declare that they have no competing interests.

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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.

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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 anthracyclines-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 anthracyclines 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. *Anthracyclines 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 anthracyclines cardiotoxicity and improvement with heart failure therapy*. Circulation 2015; **131**: 1981-1988.
10. Lipshultz S E, Alvarez J A, Scully R E. *Anthracyclines 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 anthracyclines-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, Štěrbá M, Popelová O, Adamcová M, Hrdina R, Geršl V. *Anthracyclines-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 $\beta 3$ 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. Albin 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**.