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Cardiac and Renal Protective Roles of Aliskiren in Daunorubicin Induced Acute Toxicity in Rats

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Abstract **Original Research Article**

Daunorubicin (DNR) is the potent broad-spectrum antineoplastic drugs widely used in treatment of cancers including acute myeloid leukemia and acute lymphocytic leukemia. But therapeutic use of DNR is limited due to cardiomyopathy. The renin-angiotensin system (RAS) plays crucial role in the development of cardiomyopathy. Changes of heart and increase duration of long-term survival with cardiac hypertrophy by inhibition of the RAS cascade and most effectively involve in the remodelling of the myocardium. Aliskiren (ALK) a recent drug of a direct inhibitor of the renin enzyme and keep safe cardiomyopathy in anthracycline - induced toxicity. Also the inhibition of the renin activity by aliskiren may effective and good approach in the safety of Anthracycline induced toxicity. The present study was focused to find the possible outcome of Aliskiren against DNR-induced cardiotoxicity. The acute model dose of 1.25 mg/kg DNR intraperitoneally in sixteen equal increasing doses induced cardiomyopathy in rats. DNR treatment remarkably increased the activities of serum creatine kinase (CK-MB), lactate dehydrogenase (LDH), cardiac cell caspase -3 catalase. ALK 100 mg/kg treatment safe the animal heart cell remarkably from rise in CK-MB, LDH and CAT. This study state that ALK save rats from DNR-induced cardiomyopathy.

Keywords: Daunorubicin, Cardiomyopathy, Aliskiren, Telmisartan and serum creatine kinase.

INTRODUCTION

Daunorubicin (DNR) is one of the potent broad-spectrum antineoplastic anthracycline antibiotics, mainly used to treat a variety of malignancy including acute myeloid leukemia, acute lymphocytic leukemia, chronic myelogenous leukemia, Kaposi's sarcoma and neuroblastoma [1, 2]. Daunorubicin has been used with more chemotherapeutic agents to management of the blastic phase of chronic myelogenous leukemia [3]. However, the therapeutic application of DNR has limited by its undesirable toxicity especially in the heart such as cardiac heart failure, pericarditis-myocarditis, left ventricular dysfunction, sinus tachycardia, supraventricular tachycardia, heart block, and ventricular arrhythmias have been reported [4]. The mechanism of cardiac toxicity is not known although the widely accepted mechanism for that are free radical formation and interference with the mitochondrial electron transport chain. The cardiac cell is rich in mitochondria and has a relatively poor ability to rid itself of free radicals due to its dependency on the glutathione peroxidase cycle consequently especially susceptible to free radical target [5].

The novel concept of cardio toxicity has broader intimation especially with regard to myocardial function because loss of cardiac cells could exacerbate heart failure. The pathway propose that the drug-sensitive cells clinically relevant concentrations of DNR trigger then generation of ROS that leads to a process and makes the beginning ROS blowing up the rate limiting step in DNR-induced apoptosis mark for the primary apoptotic initiation step [6].

Several types of agents have been used to manage the anthracycline induced cardiomyopathy such as ω-3 polyunsaturated fatty acids, beta-blockers (carvedilol and nebivolol), Talmisartan and spironolactone [7-10]. But most these drugs have clinical disadvantages including a markably decrease in HDL levels and inability to prevent cardiomyopathy mortality and weight loss. Various reported studies encountered that angiotensin-converting enzyme

inhibitors and angiotensin receptor blockers (ARBs) have role as a save and protective effects against anthracycline cardiotoxicity [11].

The manifestation from prior studies recommends and proved vital role of renin-angiotensin system (RAS) in the development of cardiomyopathy. RAS cascade inhibition and the changes of heart muscle cell increase duration of long-term survival in animal and humans with cardiac hypertrophy as well as failure and reperfusion injury. The most effective system in the remodelling of the myocardium is RAS facilitates cardiac oxidative injury and it has been evaluated in various studies that the remodelling process after damage could be reduced via blockade of RAS cascade system [10, 9].

Antihypetensive agents prevent apoptosis in the cell independently to its antihypertensive efficacy and significantly decreases lipid peroxidation by exhibiting an antioxidant role in hypertensive patients. It is also found that safety effects of spironolactone against oxidative stress in the cardiac cells of uraemic rats. Uraemic rats showed a clear cut enhance in superoxide production and decrease expression of NADP oxidase subunits in the left ventricle as a beneficial effects of antihypertensive drugs in cardiac oxidative stress [12]. Aliskiren a recent drug of a direct inhibitor of the renin enzyme and Protect cardiomyopathy in anthracycline - induced toxicity. Furthermore the inhibition of the renin activity by aliskiren could be effective and authentic approach in the protection of Anthracycline induced toxicity [13]. The aim of the present study was to investigate the possible effects of Aliskiren against DNR-induced cardiotoxicity in rats using biochemical markers of oxidative stress and cellular injury.

MATERIALS AND METHODS

Experimental animals

The study synopsis was approved by the Institutional Animal Ethics Committee (IAEC) of Jaipur National University, Jaipur Rajasthan. Albino rats of Wistar strain, with body weight 160–200 g were procured from Central Animal House Facility of Jaipur National University Jaipur and process under standard laboratory procedure conditions at 20–25 8C. The animals were kept in propylene cages under controlled conditions of illumination and had a free access to commercial pellet diet and water ad libitum.

Drugs and chemicals

Daunorubicin (Jubilant Life sciences limited Bhartiagram Gajraula, Distt. Amroha UP), aliskiren (Dabur India Ltd., Sahibabad, UP, India) and Telmisartan were gratefully received by Glenmark Pharmaceutical Ltd., Kisanpura, and Himachal Pradesh for the study. Caspase-3 inhibitor assay kits by BioVision USA, LDH and CK-MB assay kits were by Reckon Diagnostics Ltd. Delhi were purchased. All the other chemicals used were received of analytical and HPLC grade for all biochemical assays.

Experimental schedule

After set new conditions for study all the animals were randomly distributed into six groups of eight animals each and treated as follows:

- Group I received physiological saline of 0.5 mL/kg i.p. and similar chedule as group II and served as control
- group II received Daunorubicin four times a week (alternate day) in sixteen equal doses for a time of four weeks in a cumulative dose of 1.25 mg/kg, i.p.
- Groups III, IV, and V received 30 mg/kg ALK, 100 mg/kg ALK, 10 mg/kg Telmisartan respectively every day by per oral for 42 days with dose of DNR 1.25 mg/kg, i.p. similar to the schedule as group II
- Group VI received only 100 mg/kg aliskiren through gavage for 42 days.

The rats were anesthetized with ether for collection of blood samples after 24h of last dose of DNR from the tail vein then enzymatic parameters such as CK-MB, LDH and caspase-3 activity were determined in blood.

Biochemical estimation in serum

Creatine kinase myocardial band isoenzyme (CK-MB) [14], lactate dehydrogenase (LDH) [15] was estimated in serum by enzymatic kits using an UV-Visible spectrophotometer.

Caspase-3 activity in myocardium

The activity of caspase-3 was determined by the detection of chromophore p-nitroanilide after cleavage from the labeled substrate DEVD-p-nitroanilide [16]. In brief, 50 mL supernatant from homogenized tissue with cooled lysis buffer was used from each sample and 50 mL of Reaction Buffer was added to each sample. Then, 5 mL of the 4 mM DEVD-pNA substrate (200 mM) was added and incubated at 37 8C for 30 min to permit a dissociation of p-nitroanilide (pNA) from the conjugate DEVDpNA. The activity was read by Elisa at 405 nm using 96 well plates.

Proteins in cardiac tissues

The protein content was determined by the method of Lowry [17], using bovine serum albumin as a standard.

Data and statistical analysis

The results were expressed as in standard error of mean (S.E.M.). The group of data were compared with the analysis of variance (Anova) followed by Dunnett's t test to identify significance among groups. The significance of values was considered statistically t at $P < 0.05$.

RESULTS

Effect of ALK on Creatine Kinase, Lactate dehydrogenase, Angiotensin I Total protein, albumin Creatinine, Blood urea nitrogen and *Caspase-3* in DNR induced experimental cardiomyopathy in rats

Creatine Kinase (CK-MB) activity

The Creatine Kinase, Lactate dehydrogenase, Angiotensin I Total protein, albumin Creatinine, Blood urea nitrogen and *Caspase-3* were significantly (P< 0.01) increased. Treatment of rats with DNR (15 mg/kg, intraperitoneally) in single acute dose caused a significant (P<0.01) increase in serum CK-MB enzyme activity (202.66%) as compared to normal control group. ALK 30 pretreated group did not show protection (i.e non-significant; P>0.05) from elevated levels of CK-MB as compared to DNR control group. However, pretreatment with ALK 50, ALK 100 and TEL 10 caused significant reduction in serum CK-MB activity (22.71%, 43.5% and 24.16% respectively) whenever comared to DNR control group.

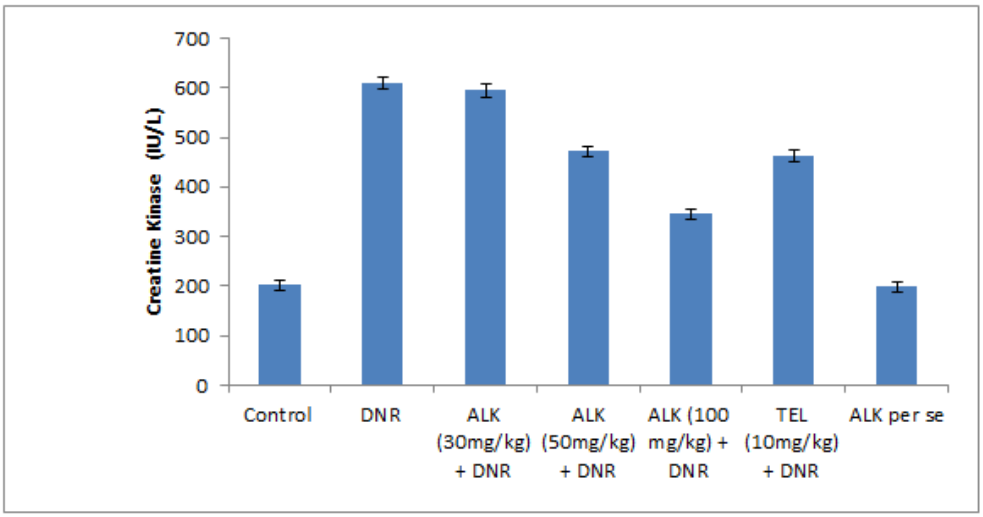

Fig-1: Creatine kinase (CK-MB) activity in serum

Lactate dehydrogenase (LDH) activity

Treatment of rats with DNR (15 mg/kg, i.p.) in single acute dose caused a significant (P<0.01) increase in serum LDH enzyme activity (175.2%) as compared to normal control group. ALK 30 pretreatment group did not show protection (i.e non-significant; P> 0.05) from increased levels of LDH as contrast to DNR control group. However, pretreatment with ALK 50, ALK 100 and TEL 10 caused significant reduction in serum LDH activity (20.8%, 36.98% and 27.3% respectively) when compared to DNR control group.

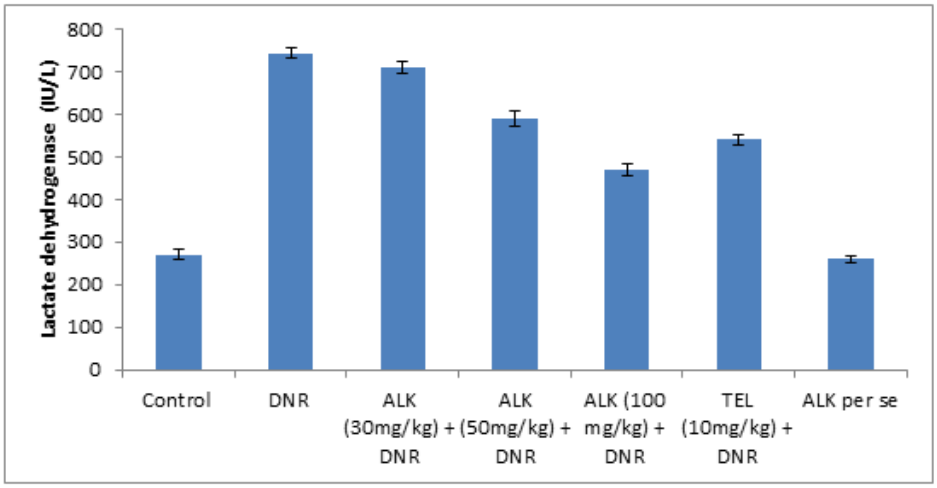

Fig-2: Lactate dehydrogenase (LDH) activity in serum.

Angiotensin I level in plasma

As shown in below figure, significantly ($p<0.01$) increased level of angiotensin I (317.36%) was found in DNR control group as compared to normal control group. However, ALK 50 and ALK 100 pretreated groups showed significant ($p<0.01$) reduction in angiotensin I level (by 25.3% and 60.24% respectively) as compared to DNR control group. Although, angiotensin I was found to be supplementarily increased in TEL 10 + DNR group by 7.53 % as compared to DNR control group.

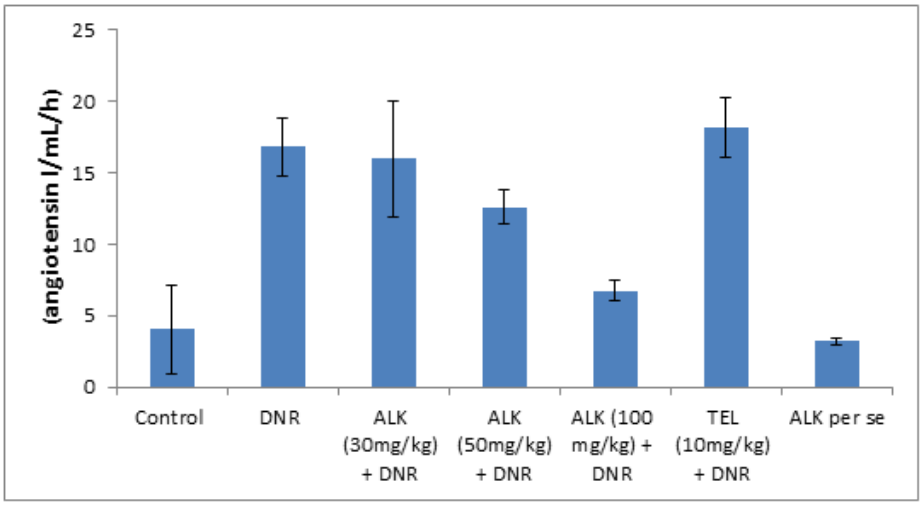

Fig-3: Angiotensin I level in plasma

Total protein in plasma

A decreased concentration of plasma total protein was found in DNR control group by 33.3% as compared to normal control group. Pretreatment with ALK 50, ALK 100 and TEL significantly restored the reduced levels of total protein by 18.96%, 37.77% and 24.5% respectively in plasma whenever compared to DNR control group.

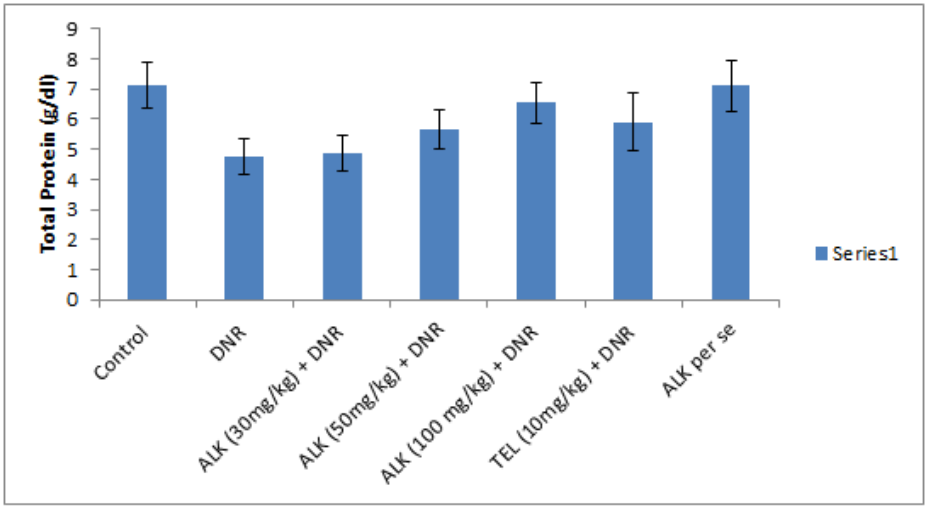

Fig-4: Total protein in plasma

Albumin in Plasma

A decreased concentration of albumin was found in DNR control group by 30.0% as compared to normal control group. Pretreatment with ALK 50, ALK 100 and TEL significantly restored the reduced levels of albumin (16.82%, 33.68% and 20.2% respectively in plasma when compared to DNR control group.

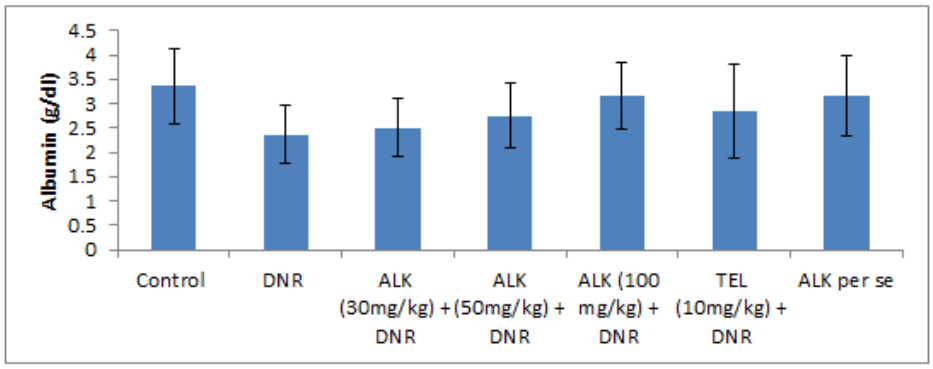

Fig-5: Albumin in Plasma

Creatinine in plasma

An increased concentration of creatinine (76.9%) was found in DNR control group as compared to normal control group. However, Pretreatment with ALK 50 (30.4%), ALK 100 (43.5%) and TEL 10 (30.4%) significantly reduced the increase levels of creatinine in plasma.

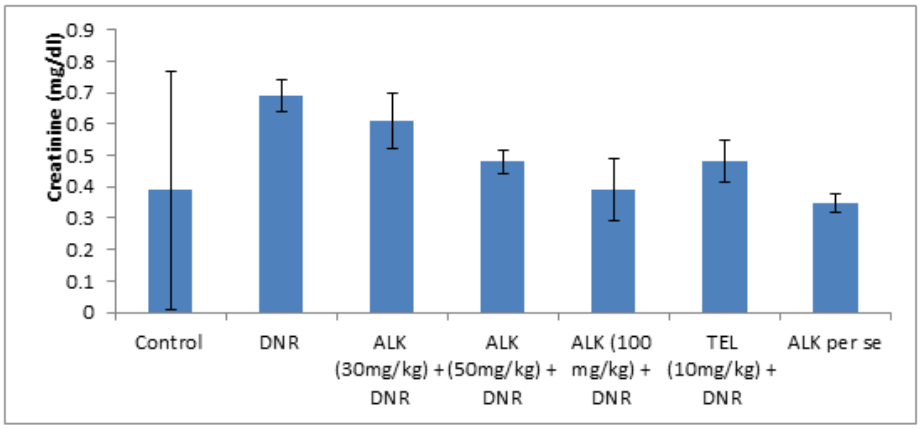

Fig-6: Creatinine in plasma

Blood urea nitrogen in plasma

An increased concentration of creatinine urea (140.5%) was found in DNR control group as compared to normal control group. However, Pretreatment with ALK 50 (25.9%) ALK 100 (50.2%) and TEL 10 (40.1%) significantly reduced the increase levels of urea in plasma.

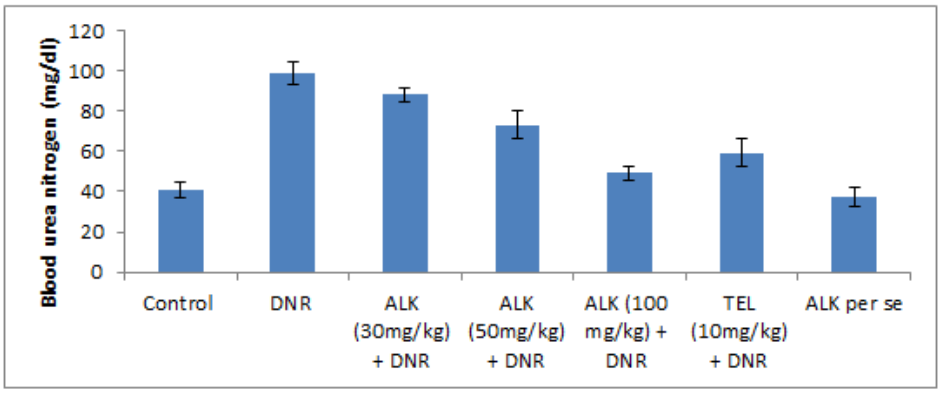

Fig-7: Blood urea nitrogen in plasma

Caspase-3 activity in cardiac tissue

DNR control group showed a 4.6 folds increase of caspase-3 activity in myocytes as compared to normal control group. However, ALK 50, ALK 100 and TEL 10 pretreatment showed significant ($P<0.01$) attenuation in increased levels of caspase-3 activity in myocytes by 36.53%, 61.81% and 32.5% respectively than DNR control group.

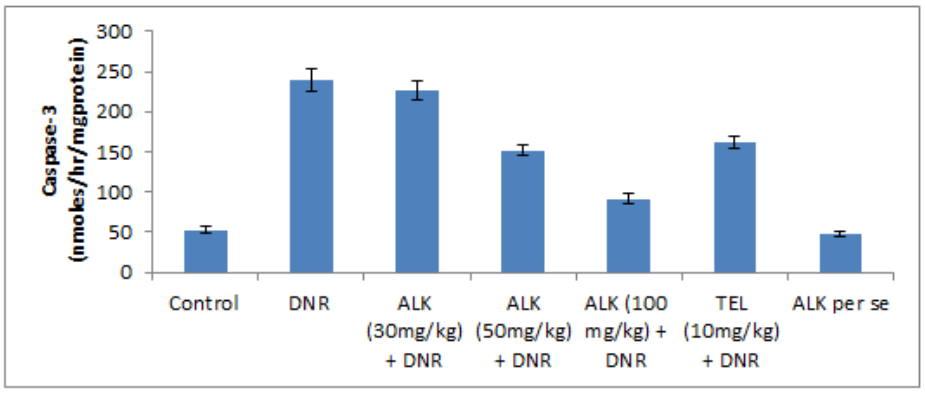

Fig-8: Caspase-3 activity in myocyte

DISCUSSION

DNR is one of the main anthracycline for the treatment of neoplasm. Effective anticancer therapy with DNR is severely limited by side effects such as cardiomyopathy and congestive heart failure [18, 19]. The mechanism of DNR-induced cardiomyopathy has not yet been fully understood but present study provides a good focus into pathology and clearly marks the inclusion of myocardial apoptosis and oxidative stress. We focused on studying the modifications in various apoptotic factor in terms of caspase-3, cardiac enzyme activity, oxidative stress markers, and anatomical structure of myocytes. The results of this study have proved that sixteen equal cumulative doses of DNR 1.25 mg/kg, i.p. DNR induces chronic cardiomyopathy in rats which is consistent with the previous studies reported by other investigators [20, 21].

The programmed cell death phenomenon or Apoptosis is a dynamic process which is evaluated through the caspase-3 activity. It is induced by a variety of factors and plays a important role in a many of cardiovascular diseases. The anthracycline antibiotics (DNR) induce myocyte apoptosis as many recent reported studies have shown [6, 22]. Ang II stimulation induces the apoptosis of ventricular myocytes in different animal models [23, 24], whereas angiotensin converting enzyme inhibitors [25] and an ARB [26] have been shown to lower myocyte apoptosis in DNR-induced cardiomyopathy. Present paper is reporting that treatment by 100 mg/kg of ALK significantly lower DNR-induced myocyte apoptosis as observed by the lower in caspase-3 activity. Hence, it could be proposed to be ALK as an anti-apoptotic agent. The Oxidative stress indicator serum CK-MB and LDH are principal myocardial enzymes in the evaluation of myocardial injury and congestive heart failure

CONCLUSION

On the basis of our investigation and finding it can be suggested that daunorubicin induced cardiomyopathy has some component of renin-angiotensin system. Our results shows that Aliskiren pretreatment could be an adjunct to reduce daunorubicin -induced cardiac adverse effects. Furthermore studies are required to validate and established the the association between Daunorubicin induced cardiomyopathy and renin-angiotensin system.

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