Protective Effect of Benfotiamine against Doxorubicin-Induced Cardiotoxicity in Rabbits.

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Abstract :

The protective effect of benfotiamine against doxorubicin-induced cardiotoxicity was evaluated in rabbits. Pretreatment of rabbits with 70mg/kg benfotiamine orally 7 days before induction of cardiotoxicity with I.V 15mg/kg doxorubicin. injection resulted in significant reduction of the activities of lactate dehydrogenase and creatine phosphokinase enzyme in the serum compared to doxorubicin treated animals; benfotiamine also improves the histological changes produced by doxorubicin in the cardiac muscle compared to control. In conclusion, benfotiamine when used concomitantly with doxorubicin protects the myocardium against the cardiotoxicity induced by this cytotoxic drug.

الخلاصة

أن هذه الدراسة قد تم تصميمها لتقويم التأثير الوقائي المحتمل لمادة البنفوتيامين ضد التلف الذي تسببه مادة الدوكسوروبيسين في عضلة القلب في الأرانب من خلال زيادة عوامل التاكسد وتحفيز الموت المبرمج للخلية ومن خلال عوامل اخرى . أظهرت النتائج أن إعطاء مادة البنفوتيامين وبجرعة (٧٠ملغ/كغم من خلال الفم) للأرانب ولمدة ٧ أيام قبل جرعة الدوكسوروبيسين (٥ملغ/كغم عن طريق الوريد) أدى إلى انخفاض معنوي بمستويات فعالية أنزيمي LDH و CPK مقارنة بمستوياتها عند الأرانب الذين استلموا جرعة الدوكسوروبيسين فقط. كذلك أسهم البنفوتيامين في تحسين التلف الحاصل في نسيج العضلة القلبية لدى مجموعة الأرانب التي استلمت جرعة البنفوتيامين قبل جرعة الدوكسوروبيسين مقارنة بتلك التي استلمت جرعة الدوكسوروبيسين فقط. وأخيرا, نستنتج أن استعمال مادة البنفوتيامين قبل الدوكسوروبيسين قد يوفر الحماية الكافية لنسيج عضلة القلب من التلف الذي يسببه عقار الدوكسوروبيسين.

Introduction :

The anthracycline antibiotic, doxorubicin, is an important antineoplastic agent that show high efficacy against various types of malignancies[(1)]. It produces cardiotoxicity as a specific adverse effect mostly attributed due to extensive formation of reactive oxygen species (ROS) that associated with many events related to nucleic acid metabolism and induction of the immune system [(1,2)]. Recently, apoptosis of cardiomyocytes has been suggested as a common mechanism of acute and chronic loss of myocytes [(3)], and doxorubicin was reported as one of the most potent inducers of apoptosis in many cell lines of many types [(4,5)]. It has been suggested also that phosphokinase C (PKC) is one of the intracellular signaling mediator for the action of TNF-α, which is responsible for cytotoxicity and apoptosis in many cell types [(6)]. Benfotiamine, the lipid soluble prodrug of thiamine, is converted after administration to the biologically active thiamine pyrophosphate (TPP) [(7)], and due to its relatively higher rate of oral absorption and greatest intracellular access[(8, 9)]; it can replace the conventional thiamine wherever indicted. It inhibits diacylglycerol-protein kinase C pathway and $NF_k\beta$ activity through activating the transketolase, one of the pentose phosphate pathway enzymes [(10)]. This work was designed to evaluate the protective effect of orally administered benfotiamine against doxorubicin-induced cardiotoxicity in rabbits.

Materials and Methods :

Adult rabbits (locally bred white strain weighing 1-1.5kg) of both sexes, maintained in the animal house of the Collage of Pharmacy, University of Baghdad, were used for this study. Animals were allowed for a standard pellet diet and tap water *ad libitum*. The animals were allocated into three groups and treated as follow: Group 1 includes six rabbits treated with normal saline only, served as negative controls. Group 2, includes six rabbits given normal saline orally for seven days before induction of cardiotoxicity with i.v. injection of 15mg/kg doxorubicin, and served as positive controls. Group 3 includes six rabbits, pretreated with benfotiamine (70mg/kg) orally for 7 days before induction of cardiotoxicity with i.v injection of 15mg/kg doxorubicin. The animals were sacrificed 48 hr after administration of doxorubicin by an overdose of thiopental (100mg/kg). Blood samples were collected for the preparation of serum and estimating serum enzymes activities of Lactate dehydrogenase (LDH) [(11)], creatine phosphokinase (CPK) [(12)] and glutamic oxaloacetic transaminase (GOT) [(13)]. Histological sections of the myocardial tissues were prepared for evaluating the histopathological changes with ordinary microscope after fixing hearts in 10% formalin, processed and embedded in paraffin. 3µm. thick sections were cut on glass slides and stained with hematoxylin and eosin (H&E). The data were presented as mean ± S.E. The significance of differences between mean values

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v was evaluated utilizing unpaired Student's *t*-test. *P* alues less than 0.05 were considered significantly different.

Results :

Figure (1) indicated that serum level of LDH activity was significantly increased in doxorubicin treated rabbits compared to controls (P<0.05), while pretreatment with benfotiamine significantly reduces serum LDH activity compared to doxorubicin treated group. In figure (2), treatment with doxorubicin significantly elevated serum level of CPK activity compared to controls, and pretreatment of rabbits with benfotiamine before administration of doxorubicin resulted in CPK activity value that was significantly lower compared to doxorubicin treated animals and comparable to that of controls. There are no significant changes in serum levels of GOT activity in doxorubicin treated group and that group which pretreated with benfotiamine before doxorubicin (figure 3). Histological examination of tissue section from the heart muscle clearly showed that mild focal edema, focal cellular injury, vaculation of cardiomyocytes and disoriented nuclei were observed in doxorubicin treated group (figure 5). Pretreatment with benfotiamine before doxorubicin administration was protects cardiac tissue against doxorubicin-induced damage that shows normal heart tissue with no significant degenerative changes when compared with normal heart tissue slide (figure 4 & 6).

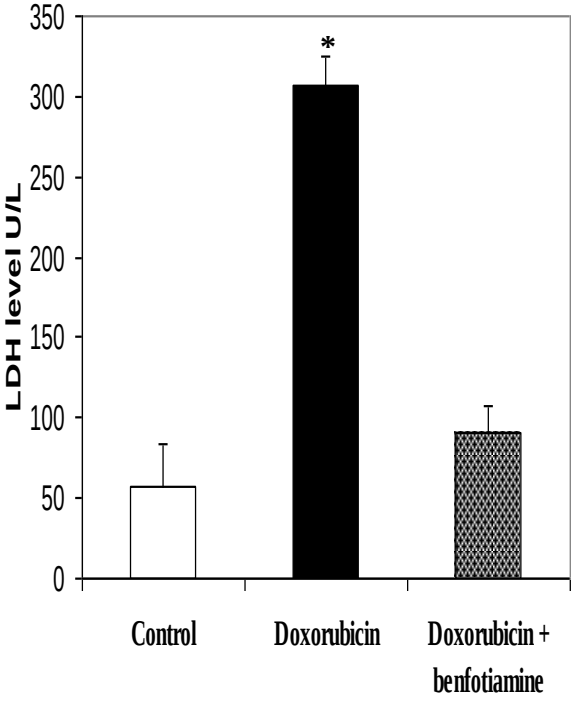

Figure (1) : Effect of pre-treatment with Benfotiamine on the serum level of Lactate dehydrogenase activity in rabbits with cardiac toxicity induced with doxorubicin. *P<0.05 compared with the control.

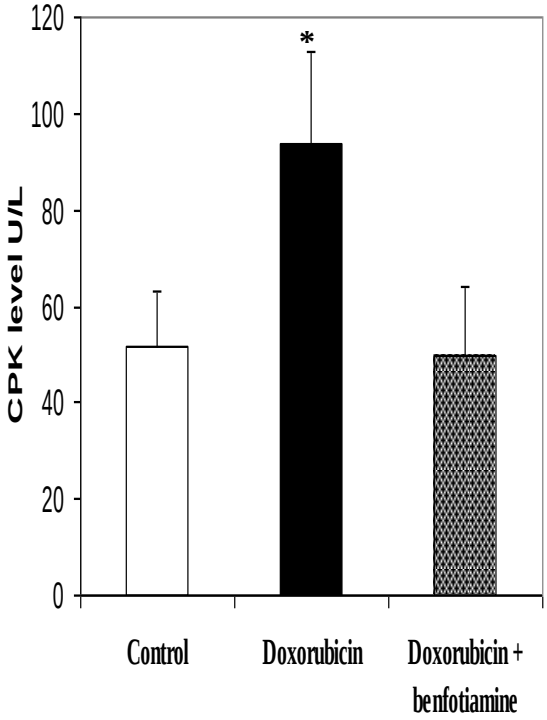

Figure (2) : Effect of pre-treatment with Benfotiamine on the serum level of creatine phosphate kinase (CPK) activity in rabbits with cardiac toxicity induced with doxorubicin.
*** *P*<0.05 compared with the control.**

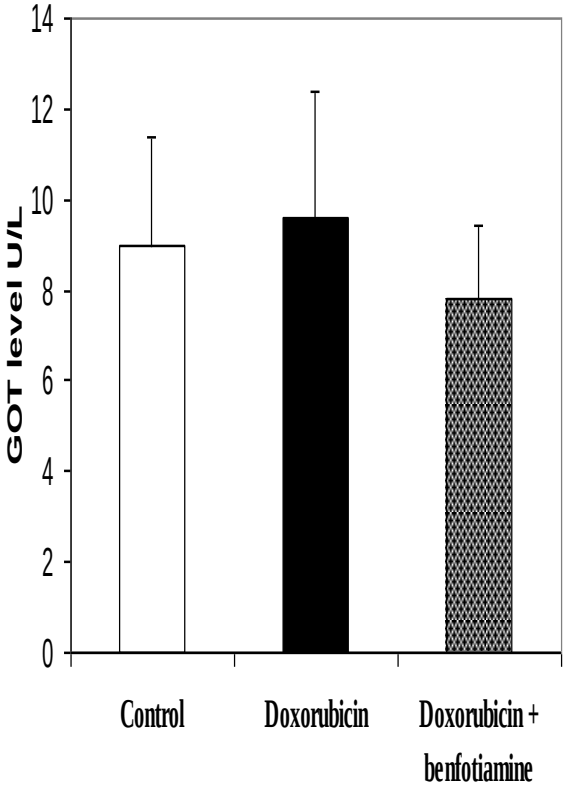

Figure (3) : Effect of pre-treatment with Benfotiamine on the serum level of Glutamate-Oxaloacetate aminotransferase (GOT) activity in rabbits with cardiac toxicity induced with doxorubicin.

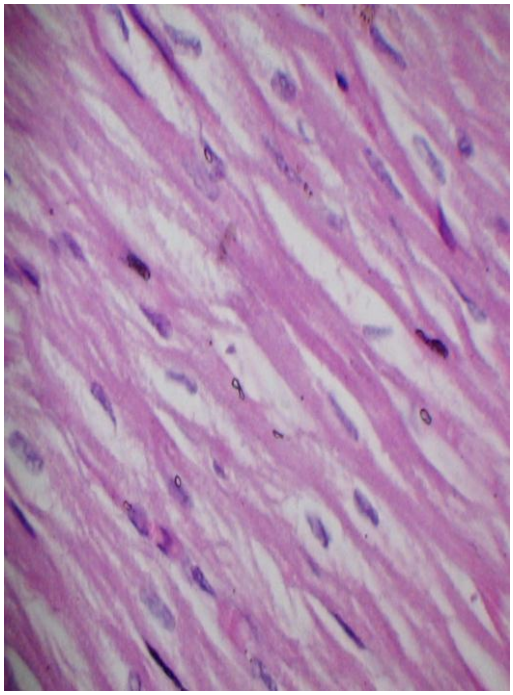

Figure (4) : Section showing normal rabbits myocardial tissue . Magnification : 20X, staining: haematoxylline & eosin.

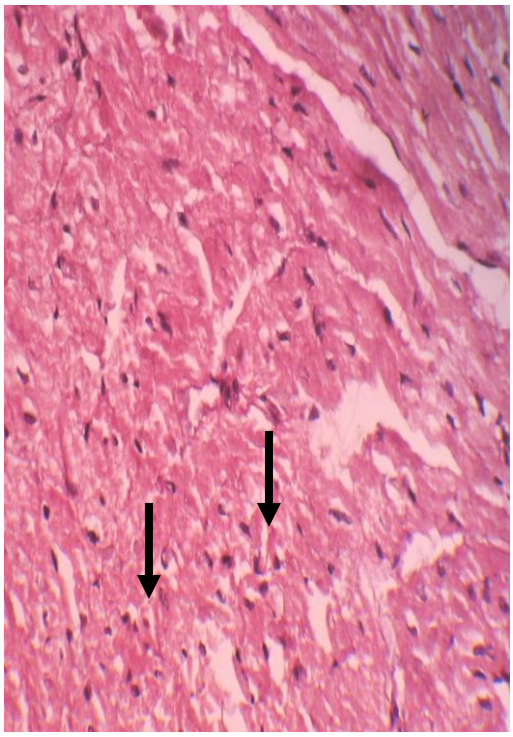

Figure (5) : Section showing morphological alteration of heart from doxorubicin-treated rabbits. Black arrow represents the vaculation of cardiomyocytes with focal edema. Magnification: 20X, staining: hematoxylene & eosin.

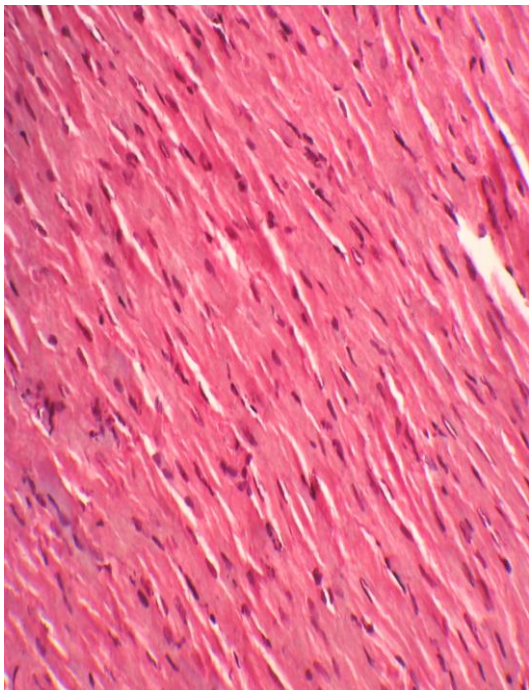

Figure (6) : Section showing the protective effect of Benfotiamine (70mg/kg) against cardiotoxic effect of doxorubicin. There are no significant changes. Magnification: 20X, staining hematoxylene & eosin.

Discussion :

The major adverse effect associated with doxorubicin use is the high incidence of cardiomyopathy and heart failure [14]. Several reports suggested that doxorubicin-induced apoptosis plays an important role in its cytotoxicity, which is linked to formation of reactive oxygen species (ROS) derived from redox activation of doxorubicin [15, 16]; and many other studies have focused on doxorubicin-induced apoptosis signaling mechanism [17, 16]. In the present study, the cardiotoxicity of doxorubicin was clearly demonstrated as a significant elevation in serum activity of LDH and CPK in doxorubicin-treated rabbits compared to control (figures 1and 2). While benfotiamine pretreated rabbits showed a significant reduction in LDH and CPK activity when compared to doxorubicin treated group. ($P < 0.05$). During the state of oxidative stress induced by doxorubicin treatment, cytotoxicity was mediated through the expression of different cytokines, especially TNF-α [18]; and benfotiamine, used in this study to protect the myocardium against toxicity by doxorubicin, was found to inhibit excessive release of TNF-α through a mechanism related to the inhibition of PKC enzyme that involved in the generation of $NF_k\beta$ the important signaling molecule in TNF-α release pathway [19, 20]. Modulation of TNF-α expression constitutes an attractive therapeutic choice in the alleviation of doxorubicin-induced apoptotic cardiotoxicity. Substances like benfotiamine,

which inhibits expression of TNF-α during the oxidative stress states, may have potential therapeutic value in this respect. Accordingly, the reported cardioprotective effect for benfotiamine in this study against doxorubicin-induced cardiotoxicity can be explained. Histological examination strongly supported this idea revealing the ability of benfotiamine in ameliorating the toxic effects of doxorubicin on the cardiomyocytes (figures 5 & 6). The histological features appeared in the heart of doxorubicin-treated rabbits clearly showed that the apoptotic cellular damage was more prevail than the necrotic one, mostly due to the absence of inflammatory cells and swelling of myocytes which consider the critical feature of necrosis (Figure 5). Therefore, benfotiamine may protect the heart against cytotoxic effect of doxorubicin by interfere with the process of toxicity through its ability to inhibit the apoptosis. In conclusion, benfotiamine protect the myocardium against doxorubicin-induced toxicity through a mechanism not related to antioxidant properties.

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