Iranian Journal of Pharmaceutical Sciences
Spring 2005: 1(2): 85-90

Original Article

Protective Effects of Lycopene and Tomato Extract Against Doxorubicin-Induced Cardiotoxicity

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Abstract

The protective effect of tomato extract and lycopene on acute doxorubicin-induced myocardial toxicity was evaluated in mice. Doxorubicin toxicity, induced by a single intraperitoneal injection (15 mg/kg), was revealed by elevated serum CPK_{MB} and histopathological observations. Tomato extract (1.2 and 2.4 g/kg, i.p.) and lycopene (1.7 and 3.5 mg/kg, i.p.), prevented the rise in serum CPK_{MB} and ameliorated cardiac cell injury. These results suggest that tomato extract and lycopene inhibit doxorubicin-induced cardiotoxicity and might serve as a combination chemotherapeutic agent with doxorubicin to limit its cardiotoxic effects.

Keywords: Doxorubicin; Cardiotoxicity; Tomato; Lycopene.
Received: January 3, 2004; *Accepted:* April 24, 2005

1. Introduction

Anthracycline antibiotics, including doxorubicin are among the most important antitumor agents. Doxorubicin alone or in combination with other drugs, has a broad activity against hematological malignancies and solid tumors. However, its therapeutic utility is limited by its toxic effects including cardiotoxicity, which can seriously endanger the patient's condition [1, 2]. The exact mechanism by which doxorubicin causes alterations in myocardial structure and function is still unknown. Among several hypotheses postulated to explain its cardiotoxicity, the most plausible seems to be the induction of free radical production, which induces lipid peroxidation and causes oxidative damage to the heart [3, 4].

Tomato (*Lycopersicon esculentum*) continues to be a popular and highly consumed fruit in many countries. Lycopene is the most prominent carotenoid in tomatoes, and it is relatively resistant to heat in the processing of tomatoes. Processed tomato products are better sources of lycopene than fresh tomatoes, and they are more bioavailable as well [5]. Carotenoids exert antioxidant activity, and lycopene exhibits the highest overall single oxygen-quenching carotenoid, twice as that of b-carotene [6]. Tomatoes also contain modest amounts of vitamin A, vitamin E, vitamin C and the flavonoid quercetin [7]. Therefore, the aim of this study was to evaluate the

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efficacy of tomato decoction and lycopene in the prevention of acute doxorubicin-induced cardiotoxicity in mice.

2. Materials and methods

2.1. Chemicals

Doxorubicin was purchased from Pharmacia and Upjohn Company. CPK_{MB} kits were obtained from BETA Company.

2.2. Animals

Male albino Balb/c mice, weighing 25-35 g housed in ventilated rooms at a temperature of 24±2 ºC, with 12 h light/dark cycle and 60±5% humidity. They were provided with food and water ad libitum.

2.3. Preparation of tomato extract

Ripe tomatoes were purchased from the local market of Mashhad, Iran. The fruits were homogenized in a blender, and were extracted using decoction method in boiling water for 30 min. The extract was then filtered and concentrated under reduced pressure to the desired volume. The residual water was evaporated to dryness at 40 ºC on a water bath. The yield (w/w) of the extract was 10%.

2.4. Isolation of lycopene

Lycopene was isolated from the tomato extract using a modified method previously explained by Lin and Chen [8]. Briefly, 8 g of dried tomato extract was placed in a 60 ml vial and mixed with 0.2 g calcium carbonate and 40 ml acetone-benzene (1:1, v/v). The mixture was shaken in a shaker at 140 rotation/min for 30 min. The upper phase was collected and poured into a 500 ml flask. The lower phase was extracted again with the same solvent (32 ml), and was shaken for 30 min. The upper phase was also collected and poured into the same flask. The organic layer was washed three times with distilled water, and was dried under reduced pressure. The remaining residue was lycopene.

2.5. Experimental procedure

Doxorubicin was given intraperitoneally to the mice, at a dose of 15 mg/kg. The tomato extract (1.2 and 2.4 g/kg) or lycopene (1.7 and 3.5 mg/kg) was given i.p. for 3 consecutive days starting the day before the administration of doxorubicin. In the control groups, normal saline was administered for the same days without doxorubicin treatment. Three days after doxorubicin administration, mice were sacrificed by decapitation under light ether anesthesia. The protocols used conformed to guidelines of the conduct of animal experiments issued by Faculty of Pharmacy and were approved by the committee on the ethics of animal experiments in Mashhad University of Medical Sciences. After bleeding, the heart was rapidly removed and fixed in 10% neutral buffered formalin for at least 48 h. Tissues were processed for microscopic examination using a standard protocol, and paraffin sections were stained with haematoxylin and eosin. Tissue damage was evaluated by scoring the light microscopic observations as follows: +1, mild necrosis; +2, moderate necrosis; +3, severe necrosis. Serum creatine kinase-MB CPK_{MB} was assayed as described by Oliver [9]. A CPK_{MB} kit based on this method was obtained from BETA Company and the provided instructions were followed.

2.6. Statistical analysis

The results are expressed as mean±SEM. Data were analyzed by one-way ANOVA. Sequential differences among the means were calculated at the level of $p<0.05$, using tukey contrast analysis as needed.

3. Results

Doxorubicin injection (15 mg/kg) resulted in a significant elevation of serum CPK_{MB} levels, measured on the third day after the injection of doxorubicin (Table 1). Administration of the tomato extract or

Table 1. Protective effect of tomato extract and lycopene on doxorubicin-induced elevation of serum CPK_{MB}.

Treatment	CPK_{MB} (IU/lit serum)
Saline	192.3 ± 12.7
Doxorubicin (15 mg/kg)	1502.6 ± 190.7*
+ Tomato extract (1.2 g/kg)	818.2 ± 58.6**
+ Tomato extract (2.4 g/kg)	492.4 ± 46.1**
+ Lycopene (1.7 mg/kg)	892.6 ± 63.9**
+ Lycopene (3.5 mg/kg)	468.5 ± 49.8**

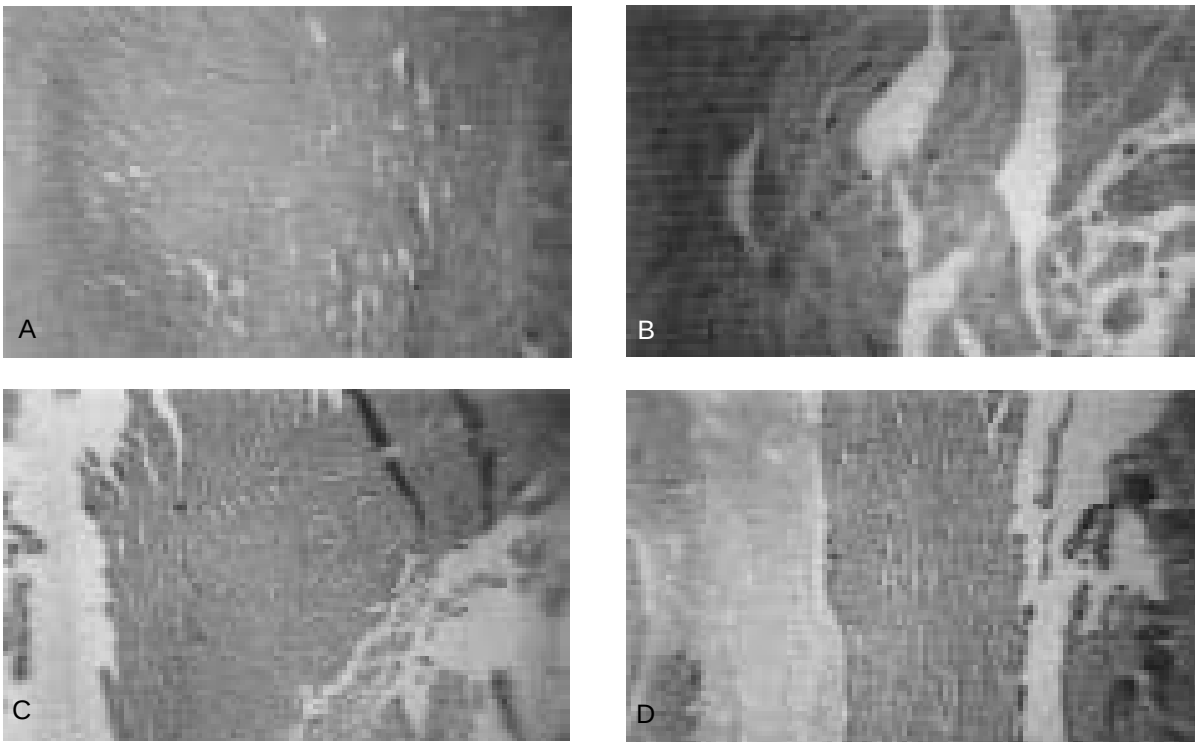
Doxorubicin was injected i.p. to the mice and the animals were sacrificed 3 days later and serum CPK_{MB} was measured. The tomato extract or lycopene was injected i.p. for three consecutive days started one day before doxorubicin administration. n = 6; mean±SEM; *Significantly different from control $p<0.001$; **Significantly different from doxorubicin-treated animals.

lycopene with both doses used significantly prevented the elevation of serum CPK_{MB} levels (Table 1). Higher doses of both the tomato extract and lycopene showed stronger protective effect against doxorubicin-induced cardiac damage. In histopathological studies, the hearts of doxorubicin-treated animals showed moderate to sever focal myocardial degeneration accompanied by mononuclear cell infiltration (Figure 1). Treatment with the tomato extract or lycopene significantly prevented doxorubicin-induced cardiac damages (Figure 1C and 1D). However, slight myocardial inflammation and mild cell injury were observed in the heart tissue of the animals treated with 2.4 g/kg of the tomato extract or 3.5 mg/kg of lycopene.

4. Discussion

Weak antioxidant capacity, particularly low catalase activity in the heart, may be a factor which makes the heart prone to oxidative damage [10]. Cardiotoxicity has been recognized as a complicating factor in

Figure 1: Micrographs of myocardium of mice 3 days after treatment with (A) normal saline; (B) doxorubicin 15 mg/kg; (C) doxorubicin 15 mg/kg plus tomato extract 2.4 g/kg; and (D) doxorubicin 15 mg/kg plus lycopene 3.5 mg.kg. Treatment (i.p.) with tomato extract or lycopene started one day before doxorubicin injection and continued for 3 days.



using doxorubicin in cancer chemotherapy. Previous studies have shown that doxorubicin toxicity is associated with oxidative stress [11]. Since therapeutic strategies are aimed to limit free radical-mediated cardiac injury by doxorubicin, we hypothesized that tomato extract or lycopene treatment would alter cardiotoxicity induced by doxorubicin.

It is reported that lycopene supplementation decreased LDL oxidation, suggesting a decreased risk for coronary heart disease [12]. Lycopene demonstrated a protective effect against myocardial infarction, confirming its beneficial effects on the heart [13]. Our results clearly indicate that tomato extract and lycopene treatment protect against acute doxorubicin cardiotoxicity, as assessed by CPK_{MB} activity and histopathological studies.

The mechanisms by which tomato extract and lycopene ameliorate doxorubicin cardiotoxicity remains to be elucidated. They possibly inhibit lipid peroxidation by scavenging free radicals produced by doxorubicin, therefore, blocks the free radical chain reaction and lipid peroxidation. Also, they may have a membrane stabilizing effect which is supported by reducing the serum CPK_{MB} activity and mild cell abnormality under light microscopic examination. Further studies such as measurement of catalase and glutathione in the heart and chemical interaction between lycopene and doxorubicin are needed to determine the exact mechanism of lycopene cardioprotection.

In conclusion, the present findings demonstrate that the tomato extract protects against acute doxorubicin-induced cardiotoxicity and may be considered as a potential useful candidate in the combination chemotherapy with doxorubicin.

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