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Silymarin prevents adriamycin-induced cardiotoxicity and nephrotoxicity in rats

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ABSTRACT

Adriamycin is a potent anticancer agent, its clinical use is limited for its marked cardiotoxicity and nephrotoxicity. The present study aimed to investigate the possible protective role of the natural antioxidant silymarin on ADR-induced heart and kidney toxicity. Studies were performed on four groups of rats. 1 – control group, 2 – silymarin group (50 mg/kg), 3 – adriamycin group (10 mg/kg), 4 – adriamycin + silymarin group. On the third day after ADR injection, plasma was separated for determination of LDH, CPK, cholesterol and total lipids. 30 days after ADR injection, plasma was separated for determination of creatinine and urea levels. Frozen heart specimens (72 h) and frozen kidney specimens (30 days) were used for estimation of lipid peroxides and GSH contents. Histopathological examinations of heart and kidney sections were also done. Pretreatment of ADR-treated rats with silymarin resulted in a significant decrease in the plasma CPK, LDH, creatinine and urea. On the other hand silymarin pretreatment did not change ADR-induced hyperlipidemia. Silymarin pretreatment significantly decreased the myocardial MDA contents. In addition, silymarin pretreatment normalized renal tissue contents of MDA and GSH. Histopathological examination of heart and kidney sections revealed that ADR caused only mild myocardial injury in silymarin pretreated rats. Also, silymarin pretreatment inhibited ADR-induced renal tubular damage in rats. These results have suggested that, silymarin ameliorated ADR-induced cardiotoxicity and protected against ADR-induced nephrotoxicity in male albino rats. The mechanisms of silymarin induced protection against ADR-induced toxicities were proved to be due to inhibition of lipid peroxidation and protection against GSH depletion.

1. Introduction

Adriamycin (ADR) is an anthracycline chemotherapeutic agent that has been commonly used in treatment of a wide range of cancers (Blum and carter, 1974). Unfortunately, the clinical use of ADR is associated with sever cytotoxic side effects including cardiotoxicity and nephrotoxicity (O'Donnell et al., 1985; Milner et al., 1991; Koima et al., 1993; Itoh et al., 2004). The mechanism of adriamycin-induced cardiotoxicity and nephrotoxicity is most likely mediated by the formation of an iron–anthracycline complex that generates free radicals, which in turn, causes diverse oxidative damage on critical cellular components and membrane lipids in the plasma membranes and mitochondria (Billingham et al., 1978; Doroshow and Davies, 1986; Mimnaugh et al., 1986; Dorr et al., 1996; Shan et al., 1996; Fadillioglu et al., 2004; Deman et al., 2001). Previously, it has been also reported that hyperlipidemia appears to contribute to adriamycin-induced cardiotoxicity (Kunitomo et al., 1985; Washio et al., 1994; Iliskovic and Singal, 1997).

The hypothesis was proposed that, if ADR cardiotoxicity and nephrotoxicity is related to free radicals formation, lipid peroxidation and hyperlipidemia so antioxidants that lower serum lipids may protects against ADR-induced toxicities in hearts and kidneys (Siveski-Iliskovic et al., 1995).

Silymarin, an antioxidant flavonoid complex isolated from the seed of *Silybum marinum* (milk thistle) possesses a powerful free radical scavenging properties (De Groot and Raven, 1998; Kren and Walterova, 2005). In addition, as antioxidant, silymarin regulates the intracellular contents of the reduced glutathione (GSH) and chelates metal ions (iron and copper) (Hikino and Kiso, 1988; Muzes et al., 1990; Borsari et al., 2001). Recently, silymarin received attention due to its alternative beneficial activities as hypocholesterolemic and cardioprotective agent (Kren and Walterova, 2005).

Therefore, this study aimed to investigate the protective effect of silymarin against ADR-induced cardiotoxicity and nephrotoxicity.

Abbreviations: ADR, adriamycin; GSH, reduced glutathione; i.p., intraperitoneally; LDH, lactate dehydrogenase; CPK, creatine phosphokinase; MDA, malondialdehyde.

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In addition, the influence of silymarin on oxidant–antioxidant status and serum lipids has been investigated.

2. Materials and methods

2.1. Chemicals

Adriablastina (10 mg adriamycin hydrochloride), Pharmacia Italia S.P.A. Italy was used in this study. Silymarin was purchased from Sigma, USA. All other chemicals used were of analytical grade.

2.2. Animals and treatments

Adult male albino rats weighing 120–150 g were obtained from National Researches Center (Cairo, Egypt). They were housed under controlled environmental conditions (12:12-h light/dark cycle) and had free access to standard pellet chow and water. Animals were fasted 12 h before experiments.

The rats were divided into four groups of 12 animals each. Control group was intraperitoneally (i.p.) injected with sodium hydroxide pH less than 7.8 for 30 days. ADR group was i.p. injected with a single dose of 10 mg/kg ADR, which is well documented to induce cardiotoxicity and nephrotoxicity in rats (Vora et al., 1996; Deman et al., 2001; Mohamed et al., 2004). Silymarin group was i.p. injected with a dose of 50 mg/kg silymarin dissolved in sodium hydroxide pH less than 7.8 (Skottova et al., 1998), based on the results of previous study (Karimi et al., 2005). ADR + Silymarin group was i.p. injected with 50 mg/kg silymarin 7 days before ADR (10 mg/kg, single i.p. injection) and daily thereafter throughout the study (30 days). Pretreatment with silymarin for 7 days prior to administration of adriamycin was chosen based on a preliminary experiments with silymarin just prior to adriamycin and 7 days prior to adriamycin. Pretreatment with silymarin just prior to adriamycin showed no benefits.

2.3. Sample collection and biochemical analysis

1. On the third day after saline (control) or ADR injection, six animals from each group were decapitated. Blood samples and heart specimens were collected. Plasma was separated for determination of total lactate dehydrogenase (LDH) and total creatine phosphokinase (CPK) activities. Also plasma was used for determination of cholesterol and total lipids levels. Frozen heart specimens were used for estimation of lipid peroxides (measured as malondialdehyde MDA) and reduced glutathione (GSH) contents. Formalin fixed heart specimens were used for histopathological examination.
2. At the end of the experiment period (30 days after saline or ADR injection), the remaining six animals from each group were decapitated. Blood samples and kidney specimens were collected. Plasma was separated for determination of creatinine and urea levels. Frozen kidney specimens were used for estimation of lipid peroxides (MDA) and GSH contents. Formalin fixed kidney specimens were used for histopathological examination.

2.4. Assessment of plasma enzymes

Plasma total LDH and total CPK activities were determined using commercial kits from RANDOX, UK and SPINREACT, Spain, respectively. Total LDH activity was assessed according to the method of Henry (1974). The method depends on the reaction of lactate with NAD, and NADH formed is measured spectrophotometrically at 340 nm. The increase in absorbance is measured at 1-min intervals for 3 min. Plasma total LDH activity was calculated as units per liter (U/L). Total CPK activity was determined according to the method of Abbot et al. (1984). The method is based on the transphosphorylation of ADP to ATP through a series of coupled enzymatic reactions; NADH produced is directly proportional to the CPK activity. The increase in absorbance at 1-min intervals was recorded for 3 min at 340 nm. Plasma total CPK activity was calculated as units per liter (U/L).

2.5. Determination of plasma creatinine and urea levels

Plasma creatinine and urea levels were determined using commercial kits from Biodiagnostic, Egypt. Plasma creatinine was determined according to the method of Bartles et al. (1972). The absorbance of samples was read at 495 nm against a reagent blank. Creatinine concentration was expressed in mg/dl. Plasma urea was determined according to the method of Fawcett and Soctt (1960). The absorbance of samples was read at 550 nm against a reagent blank. Urea concentration was expressed in mg/dl.

2.6. Determination of plasma total cholesterol and total lipids

Plasma total cholesterol and total lipids levels were determined using commercial kits from Biodiagnostic, Egypt. Plasma total cholesterol was determined according to the method of Richmond (1973). The absorbance of samples was read at 500 nm against a reagent blank. Total cholesterol concentration was expressed in mg/dl. Plasma total lipids were determined according to the method of Knight et al. (1972). The absorbance of samples was read at 545 nm against a reagent blank. Total lipids concentration was expressed in mg/dl.

2.7. Determination of cardiac and kidney non-enzymatic antioxidants

Heart and kidney tissues were washed with ice-cold PBS. Tissues were homogenized in approximately 5.0 volumes of ice-cold phosphate buffer (pH 8.0, 0.01 M) using a polytron homogenizer (pt 3100) (five cycles of 10 s at 3000 rpm). Homogenates (20% w/v) were then prepared by sonication in ice-cold phosphate buffer (pH 8.0, 0.01 M). Aliquots were prepared and used for the assessment of different non-enzymatic antioxidants (Arafa et al., 2005).

2.8. Preparation of aliquots for GSH determination

Proteins were precipitated by centrifugation after addition of an equal volume of a 20% trichloroacetic acid solution (TCA).

2.9. Determination of reduced glutathione (GSH)

Reduced glutathione (GSH) was determined according to the method described earlier by Ellman (1959). The procedure is based on the reduction of bis-(3-carboxy-4-nitrophenyl) disulfide reagent by SH group to form 2-nitro-5-mercaptobenzoic acid, which has an intense yellow color that was measured spectrophotometrically at 412 nm. Briefly, aliquots of the supernatant were then mixed with eight times volume of 0.3 M sodium phosphate and an equal volume of DTNB solution prepared by dissolving 4.0 mg of DTNB in 10 ml of 1.0% trisodium citrate solution. The absorbance was measured spectrophotometrically at 412 nm and GSH levels were calculated with reference to the standards (Son et al., 2007).

2.10. Determination of lipid peroxides (measured as MDA)

Malondialdehyde, a reactive aldehyde that is a measure of lipid peroxidation, was determined according to the method of Uchiyama and Mihara (1979). The adducts formed following the reaction of tissue homogenate with thiobarbituric acid in boiling water bath, were extracted with *n*-butanol. The difference in optical density developed at two distinct wavelengths; 535 nm and 525 nm was a measure of the tissue MDA content. Tissue MDA content was expressed as nmol/g tissue.

2.11. Histopathological examination of heart and kidney sections

Formalin fixed heart and kidney sections were embedded in paraffin wax, serially sectioned (3–5 μm), and stained with Hematoxylin and Eosin, for assessment of histopathological changes.

2.12. Statistical analysis

Results are expressed as the mean ± SEM. Comparison between different groups was carried out by one way analysis of variance test (ANOVA). The level of significance was set at $p \leqslant 0.05$.

3. Results

3.1. Effect of silymarin on ADR-induced cardiotoxicity (72 h samples)

Treatments of rats with a single dose of ADR (10 mg/kg) caused a significant increase in both plasma CPK and LDH enzyme activities compared to their respective control values. After 72 h of ADR treatment, the level of this increase was 10- and 2.5-fold their control values, respectively (Table 1).

Pretreatment of ADR-injected rats with silymarin (50 mg/kg) 7 days before ADR and daily thereafter resulted in a significant decrease in plasma CPK and LDH levels by 82% and 43% of their respective ADR-treated rats (Table 1).

Unfortunately, pretreatment of ADR-injected rats with silymarin 7 days before ADR and daily thereafter for 72 h did not reduce either LDH or CPK activity to their respective control values.

Microscopic examination of heart sections after 72 h of ADR treatment revealed sporadic early necrotic fibers, highly oesenophilic cytoplasm, vascular congestion, minimal interstitial edema, minimal mononuclear cellular infiltration and intravascular