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Cardioprotective effect of alcohol extract and seed powder dietary supplementation of quinoa (*Chenopodium quinoa*) in female rats treated with Doxorubicin

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Abstract
Doxorubicin (DOX) is an anthracycline drug used for different type of cancer treatment related to many toxic systemic effects. We examined the cardioprotective potential of Chenopodium quinoa against DOX cardiotoxicity. 30 female Sprague Dawley rats were randomly assorted into 6 groups. Control group I: received normal saline with standard rat feed and water *ad libitum*; DOX group II: received 15 mg/kg b.w. dosage of single intraperitoneal DOX on the eighth day of the 21-day experiment period. Co-treatment groups III, IV and V: received 300 mg/kg b.w. of quinoa seeds ethanol extract, different percentages of (20 and 40%) of dietary supplementation with quinoa seeds powder (QSP) were given orally throughout the trial period in concomitant with single intraperitoneal DOX as in DOX group II respectively. Standard group VI: received vitamin C 250 mg/kg b.w daily as in co-treatment groups. Phytochemical screening and antioxidant activity of quinoa seeds, Biochemical changes and oxidative stress markers in serum and heart tissue extract were examined. DOX caused significant increase in serum LDH and CK activity and lower antioxidant enzymes in serum and heart tissue. Quinoa seed ethanol extract and dietary supplementation with quinoa seed powder ameliorates DOX-prompted changes in serum and tissue heart by enhancing antioxidant enzyme activities. Quinoa seeds possesses a majority of phytochemical classes of compounds . Our results suggest that co-treatment of QSP dietary supplementation markedly improve DOX-induced heart deleterious effects. The potency of co-treatment of QSP dietary supplementation at 40% is similar to vitamin C. These results revealed that dietary supplementation with quinoa seed powder might serve as a potential adjuvant that avoids DOX-induced cardiotoxicity.
***Keywords*:** Doxorubicin; Antioxidant; Chenopodium quinoa; Cardiotoxicity; Enzyme markers , oxidative stress

Introduction

Doxorubicin (Dox) is an anticancer drug, which belongs to anthracycline glycoside antibiotic that is widely used to treat a variety of cancers, including leukemia's, lymphomas, soft-tissue sarcomas, and solid tumors[1,2]. However, its clinical uses are limited by a dose-dependent side effect of cardiotoxicity, which may lead to irreversible cardiomyopathy and eventually heart failure[3,4]. Doxorubicin-induced cardiotoxicity is mediated through different mechanisms including the production of free radicals and alterations of deoxyribonucleic acid (DNA), calcium overloading, mitochondrial dysfunction, and peroxynitrite formation have been proposed by [5,6]. Doxorubicin is enzymatically reduced to its semi Quinone free radical, which can generate reactive oxygen species (ROS) including superoxide, hydroxyl radicals, and hydrogen peroxide [7]. These free radicals limited the using of this drug in chemotherapy and at the same time are critical to Dox-mediated cytotoxicity, including cardiotoxicity and hepatotoxicity [8,9].

Recently, a great interest has been focused on naturally occurring antioxidants, which play important roles in inhibiting the formation of both free radicals and oxidative chain-reactions within tissues and membranes [10]. Several phytochemicals particularly polyphenols like phenolic acids, flavonoids, tannins, anthocyanins, are familiar to be liable for the free radical scavenging and antioxidant activities. These phytoconstituents from plants were responsible for the activity of protecting different body systems [11,12].

Quinoa (Chenopodium quinoa) is a pseudocereal considered as super food since it is a good source of complete protein as it contains all nine essential amino acids, unsaturated fatty acids, minerals, vitamins, fiber, and antioxidants [13]. Its seeds contain significant amounts of bioactive compounds, including polyphenols, flavonoids and tocopherols (Vitamin E), tocotrienols and carotenoids [14,15]. Previous studies observed that bioactive compound of Quinoa could change antioxidant status in the organism by preventing oxidative stress and helps to reduce the risk of various chronic diseases related to free radicals [16]. This research aimed to assesses the ameliorative effects of Quinoa seeds ethanol extract and powder dietary supplementation against cardiotoxicity complicated by doxorubicin therapy.

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Material and Methods

Collection and preparation of plant extract

Chenopodium quinoa seeds were collected from the local markets for grain in the city of Baghdad / Iraq. After crushing it and getting the seed powder, material was extracted using 70% ethanol as solvent. A dry residue was obtained by filtering, concentration and evaporation of the extract.

Chemicals and reagents

Nitro blue tetrazolium (NBT), glutathione (GSH) and 5-50 dithiobis (2-nitro benzoic acid) (DTNB) were purchased from Fluka, Switzerland; thiobarbituric acid from BDH, England; Doxorubicin from Kocak;. Biochemical kits manufactured by Euro Diagnostic Systems, Chennai, India, were used for estimation of Lactate Dehydrogenase (LDH), Creatine Phosphokinase (CPK), all other chemicals and reagents used were of analytical grade.

Phytochemical screening

The prepared alcohol extract of quinoa seeds was used to test various phytochemical components. Different chemical reagents were prepared and specific test for specific phytochemicals was done [17,18].

Determination of total phenol by Folin-reagent method

Total phenol content was determined by Folin-Ciocalteu reagent method with modification [19].

Determination of total flavonoids by colorimetric method

The total flavonoids contents of different crude extracts were estimated by aluminium chloride colorimetric method as described by [20].

DPPH radical scavenging activity

Antioxidant potential of the quinoa seeds alcohol extract was assessed by using 1,1-diphenyl 1-2-picryl-hydrazyl (DPPH) assay. IC50 is the concentration value, which scavenged 50% of the DPPH radicals. Ascorbic acid was used as reference compounds [21].

Experimental animals

Female Sprague Dawley rats (200-250g) were obtained from Louay animal breeding center, , and were maintained at standardized environmental conditions In terms of temperature, humidity, and lighting period and brought up with standard rat feed and water *ad libitum*.

Experimental design

Thirty-six female Sprague Dawley rats (200-250 g body weight) were divided into six groups of five animals each and were kept under the following treatment schedule.

Group I: Normal reference group received normal saline. Group II: Negative control received a single intraperitoneal dose of DOX (15 mg/kg b.w.) on the eighth day of the 21-day experiment period. Groups III, IV and V: co-treatment groups, 300 mg/kg b.w. of quinoa seed ethanol extract, different percentages of (20 and 40%) of dietary supplementation with QSP were given orally daily for the duration of the experiment in concomitant with single intraperitoneal DOX as in-group II respectively (served as positive control). Group VI: Standard group received vitamin C (250 mg/kg b.w) daily as in co-treatment groups.

Induction of cardiotoxicity started from day 8 intraperitonelly, up to day 21, when all the groups (except I), received single intra-peritoneal injections of Doxorubicin at a dose 15 mg /kg of animal body weight.

Evaluation of serum and heart tissue homogenates biochemical parameters

At the end of the experiment, female rats were sacrificed and blood was collected by heart puncture into non-heparinised vials to collect serum. Serum biochemical parameters like CPK, LDH, MDA, GSH, CAT and SOD were analyzed to evaluate the extent of heart damage as a result of DOX treatment and to evaluate the role of quinoa seeds in improving it.

10% homogenates of heart tissue were prepared in ice cold 0.1M Tris-HCl. MDA level analyzed according to the method of [22]. GSH, CAT, SOD levels were analyzed in the cytosolic fraction of the heart tissue homogenate according to the methods of [23,24,25] respectively.

Statistical analysis

The values were expressed as mean ± SD of 5 animals per group. Statistical evaluation of the data was done by one way ANOVA. Significant differences between the groups were determined by Duncan's test [26]. Probability levels of less than 0.05 were considered significant.

Results

Phytochemical analysis

The data shown in Table (1) shows the preliminary phytochemical analysis of quinoa seeds alcohol extract. Results showed that varying amounts of alkaloids, phenols, sterols, tannins, flavonoid, glycosides, terpenoid and saponins were present.

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Table 1: Qualitative phytochemical screening of quinoa seeds alcohol extract

Active Compounds	Test results for alcohol extract of quinoa seeds
phenols	+
flavonoid	+
alkaloids	+
sterols	+
tannins	+
glycosides	+
terpenoid	+
saponins	+

Quantitative determination of phenols and flavonoids contents

Analysis of the total phenolic and flavonoids contents of quinoa seeds ethanol extract was 55.53 mg/100g gallic acid and 9.10 mg/g quercetin respectively as shown in fig (1).

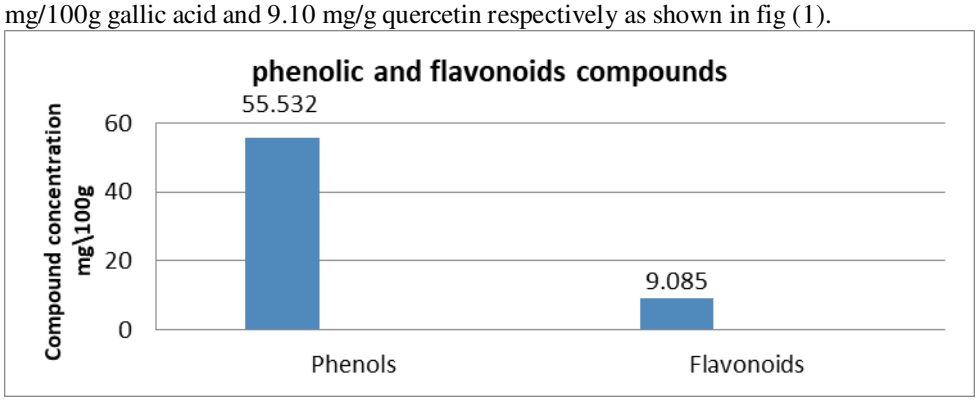

Fig. (1) Quantity of phenolic and flavonoids compounds from quinoa seed alcohol extract

Determination of Antioxidant Activity

Antioxidant properties of quinoa seeds ethanol extract using 2,2-diphenyl-1- picrylhydrazyl (DPPH) assay are shown in Figure (2). It was 0.039 µg/ml for quinoa seed ethanol extract compared to 0.028 for ascorbic acid.

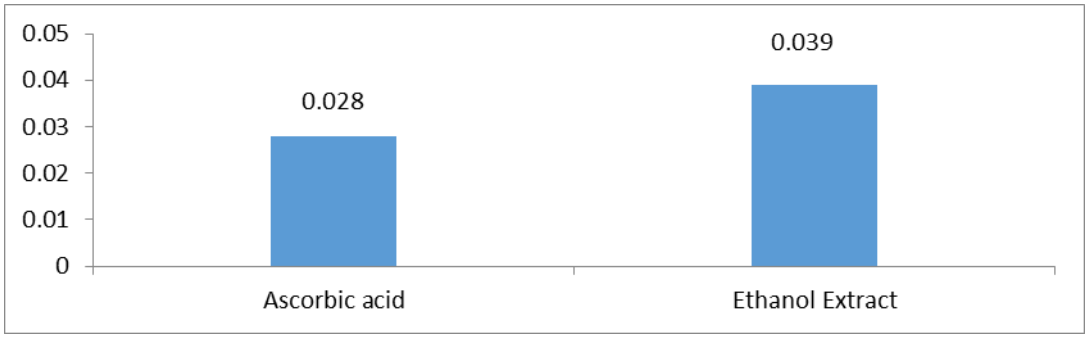

Fig. (2) Antioxidant activity of *quinoa seeds* alcohol extracts on DPPH

Effect on serum enzyme activity

Significant ($p < 0.05$) increase in the level of cardiac marker enzymes CPK, LDH was observed in the doxorubicin alone treated control group II, compared to normal group I. In the co-treated groups III, IV and V and standard group VI, though the values were inclined more towards normalcy. Quinoa seed powder (20 and 40%) had a more significant effect in decreasing the activity of enzymes compared to the quinoa seeds alcoholic extract and vitamin C. (Fig.3).

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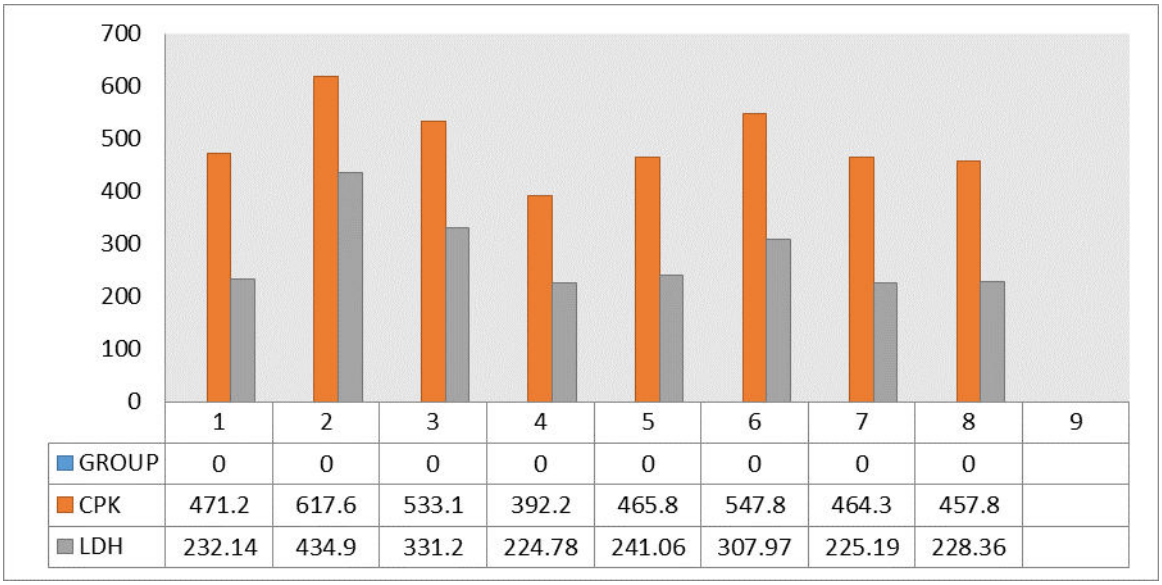

Fig. (3). Effect of doxorubicin, alcohol extract and seed powder of quinoa seeds on serum CPK and LDH enzymes

Effect on serum and heart tissue homogenate oxidant-antioxidant status parameters

The results revealed increasing level of lipid peroxidation marker MDA in serum and heart tissue homogenate of group II compared to the normal group I. However, this was significantly low in the co-treated groups III, IV and V and standard group VI compared to group II. The antioxidant profile including SOD and CAT activities, as well as GSH levels were reduced in the group II. The standard and the co-treated groups III, IV and V, however disclosed a near normal antioxidant profile. The most effective effect was the quinoa seed powder (20 and 40%) (Table2,3).

Table 2: Effect of doxorubicin, alcohol extract and seed powder of quinoa seeds on serum oxidative stress markers

Mean ± SD				
Group	***MDA nm/l***	***GSH nm/l***	***catalase nm/l***	***SOD nm/l***
DOX	2.6100 ± 0.195a	0.3290 ±0.0291e	0.7153±0.1122c	0.5507±0.0271e
Alcoholic Extract+DOX	1.9267±0.1305b	0.4317±0.01301 d	1.0800±0.1087 b	0.6231±0.0265 d
DOX+20%Quinoa	1.5700±0.1253 c	0.4897 ±0.0234 b	1.4797±0.0349 a	0.7147±0.0189 b
DOX+40%Quinoa	1.4567±0.1168 d	0.5210±0.01082 a	1.5333±0.0444 a	0.7563±0.0266 a
Vit C+DOX	1.9667±0.1531b	0.4543±0.0270 c	1.0997 ±0.1688b	0.6673±0.01365c
Alcoholic Extract	1.3267±0.0971e	0.5030 ±0.0174 b	1.5200±0.0281 a	0.7440±0.0358 a
Vit C	1.3933 ±0.1168de	0.5227±0.01601 a	1.4370±0.0286 a	0.7127±0.0204 b

Table 3: Effect of doxorubicin, alcohol extract and seed powder of quinoa on heart tissue homogenate oxidative stress markers

Mean ± SD				
Group	***MDA nm/l***	***GSH nm/l***	***catalase nm/l***	***SOD nm/l***
CONTROL	1.3030±0.0276 c	0.32233±0.01332 a	1.2313±0.0506 b	0.5673±0.01601 c
DOX	1.9540±0.0380 a	0.22976±0.01553 c	0.7293±0.1378 d	0.3253±0.01234 e
Alcoholic Extract+DOX	1.6990±0.1259 b	0.27167±0.01301 b	1.0177±0.1254 c	0.4410±0.0262 d
DOX+20%Quinoa	1.2607± 0.0237 cd	0.33100±0.01249 a	1.2320±0.0406 b	0.6187±0.0237 b
DOX+40%Quinoa	1.2380±0.0460 d	0.32300±0.0183 a	1.3337±0.1298 a	0.6087±0.0299 b

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Vit C+DOX	1.6100±0.0606 b	0.26800±0.0292 b	0. 9413±0.0972 c	0.4223±0.0297 d
Alcoholic Extract	1.2910±0.02262 c	0.33030±0.0176 a	1.2783±0.0339 b	0.5977±0.0182 b
Vit C	1.2337±0.0679 d	0.33730±0.0189 a	1.2653±0.0950 b	0.6463±0.0229 a

Discussion

The aim of the current research was to assess the ameliorative effects of quinoa seeds ethanol extract and powder dietary supplementation against cardiotoxicity induced by doxorubicin therapy by restoration of tissue redox balance. Phytochemical compounds and plant extracts as such, are effective as well in overcoming the doxorubicin-induced cardiac injury. Treatment with doxorubicin recorded a significant decrease in the antioxidant parameters, evidenced by the drop in SOD, CAT and GSH values, giving multi-chances to cause membrane-damage, which resulted in increased level of MDA lipid peroxidation marker and loss of function and integrity of myocardial membrane [27]. Accumulation of free radical generation causes depleted of glutathione and marked decrease in SOD, CAT values promotes the formation of hydroxyl radicals, and initiation and propagation of lipid peroxidation. However, it is suggested that the decrease in the activities of these antioxidative markers is a consequence of a defect in the oxidative – antioxidant status in the cardiac tissues due to the overproduction of active reactive oxygen species. Treatment of the animals with quinoa seeds ethanol extract and powder dietary supplementation regained near normalcy in the SOD, CAT and GSH levels, when compared to control animals treated with doxorubicin alone, indicating the inference that the protective role of quinoa seeds powder and alcohol extract may be credited to its ability in ameliorating the tissue antioxidant status.

These secondary metabolites contribute significantly towards the biological activities of medicinal plants for the co- therapy of diverse global disseminated ailments, supporting the traditional medicine in cases like cardiovascular diseases, Aging diseases like Alzheimer, hypoglycemic, antidiabetic, antioxidant, antiinflammatory, anticarcinogenic, anticholinergic, etc. [28].

The identification of phytochemicals of quinoa seeds is essential onset point for evaluating their biological, nutritional and technological facets. Table 2 presents the qualitative phytochemical screening of alcohol extract of quinoa seeds, It seems clear that the alcoholic extract contained the majority of the secondary metabolism compounds. The biological activity of these compounds effectively contributes to encircling the types of free radicals generated as a result of doxorubicin treatment. As it became clear that the semi Quinone free radical, generated by the enzymatically reduction of Doxorubicin could generate reactive oxygen species (ROS) including superoxide, hydroxyl radicals, and hydrogen peroxide [7].

The antioxidant effect of quinoa seeds is evident in their cardioprotective role by improving and normalizing the values of cardiac enzyme markers, LDH and CK that significantly increased their enzymatic activity after treatment with doxorubicin. This explains its effective role in inhibiting the destructive effect of active radicals and ending their effectiveness in peroxidizing the lipids of the membranes of cardiac muscle cells, and these membranes restored their vital functions and thus their vitality. The results of the current study are in accordance with several studies of cardioprotection against the doxorubicin-induced oxidative stress [29,30,31,32,33]. Several remarkable similarities were found after extrapolating the conclusions of the above studies to the results of the current study.

In conclusion, Quinoa seeds is a good dietary supplementation for health-promoting compounds, meeting concurrently the promising antioxidant activity, vitamins, minerals … etc, that can be utilized virtually as food complements, to tardiness lipid oxidization and healing from particular ailments via its free-radicals scavenging ability. More research is required to examine the role of the various bioactive components responsible for these activities. The findings of the present study will contribute in the heart protection during DOX chemotherapy.

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