www.ijrps.pharmascope.org

ISSN: 0975-7538
Research Article

Attenuation the Side Effects of Adriamycin- induced Cardiotoxicity and Nephrotoxicity in Rats by Fermented *Punica granatum* (Pomegranate) Peel Extract

Abeer E. Mahmoud*, Mamdouh M. Ali

Biochemistry Department, National Research Center, Cairo, Egypt

ABSTRACT

Most pomegranate (*Punica granatum* Linn., Punicaceae) fruit parts are known to possess enormous antioxidant activity. The present study used *Kluyveromyces marxianus* NRRL Y-8281, as yeast candidate to enhance the antioxidant activities of pomegranate peel by modulating polyphenolic substances during solid state fermentation, in an attempt to examine the protective effects of either methanolic extract of unfermented (UFPP) or fermented (FPP) pomegranate peels on adriamycin-induced myocardial and renal toxicities in rats. Both extracts were found to contain a large amount of polyphenols and exhibit enormous antioxidant activity. UFPP and FPP extracts showed 92% and 93% antioxidant activity in DPPH model system respectively, while the phenolic content recorded 160 and 211 mg gallic acid/ g dry peel for UFPP and FPP respectively at concentration of 125 µg /ml. Administration of adriamycin (10 mg/Kg body weight /day, i.p. for 3 days) caused significant rise in the levels of diagnostic markers [serum creatine kinase (CK) for heart and blood urea nitrogen (BUN) for kidney] as well as lipid peroxidation and total antioxidant status in the heart and kidney tissues. Concomitant decline in the level of tissues reduced glutathione and the activities of antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase) were observed. Administration of either UFPP or FPP extracts at 1/10 of lethal dose, two doses before and two doses after ADR, significantly prevented all the adriamycin adverse effects, through diminution the activity of CK and the level of BUN as well as the level of cardiac and renal lipid peroxidation comparing with ADR group. At the same time, the levels of glutathione, total antioxidant and the activities of antioxidant enzymes were increased. The most significant effects were obtained with FPP extract which could thus relate to their high total phenolic content. In conclusion, the protective effect of pomegranate might be ascribable to its membrane-stabilizing property and/or antioxidant nature which might serve as novel combination chemotherapeutic agent with ADR to limit free radical-mediated organ injury.

Keywords: Adriamycin; Pomegranate; Fermentation; Cardiotoxicity; Nephrotoxicity; Free radicals; Antioxidants

INTRODUCTION

Over the last 3 - 5 decades, the treatment of cancer has relied largely on the use of diverse cytotoxic chemotherapeutic agents. The exploit of chemotherapy in the treatment of cancer has opened new potential for the improvement of the quality of life of cancer patients and for the cure of disease. Regardless of its success, treatments with some of the most effective anticancer drugs can sometimes result in a number of symptoms indicating toxicity (Rajaprabhu *et al.,* 2007).

Adriamycin (ADR) is a quinone-containing anticancer drug that is extensively used to treat a variety of human neoplastic diseases as well as an ample range of solid tumors, including breast, lung and thyroid cancer (Gianni *et al*., 2007). The clinical effectiveness of this drug is limited by its severe cardiotoxic, nephrotoxic and hepatotoxic effects (Deepa and Varalakshmi, 2003; Bryant *et al*., 2007).

There is an increasing evidence for the enhancing effect of free radicals involved in the primary pathogenic mechanism of ADR-induced cardiotoxicity (Li *et al.*, 2000) and nephropathy (Bertani *et al*., 1986) in rats. Adriamycin's toxicity is thought to involve the biological reduction of the drug to semiquinone metabolite with a subsequent reaction with molecular oxygen to form reaction oxygen species and regeneration of the quinine compound. This process can proceed over and over again in a redox cycling process thereby generating substantial quantities of toxic oxygen radicals including hydrogen peroxide (H_2O_2); superoxide anion ($O_2^{\cdot-}$); and hydroxyl radical ($OH^{\cdot}$) are constantly generated in cells, associated with direct oxidative injury to DNA and generates lipid peroxidation and a consequent alteration of cellular membrane integrity (Ali *et al*., 2002; Quiles *et al*., 2002). These highly toxic reactive oxygen species react with cellular molecules including nucleic acids, proteins and lipids, thereby causing cell damage. Several studies have reported that

* Corresponding Author
Email: ab_essam@yahoo.com
Fax: 00202-33370931
Received on: 08-10-2011
Revised on: 05-11-2011
Accepted on: 12-12-2011

adriamycin administration inhibit the activity of the antioxidant enzymes and resulted in imbalance between the generation of free radicals and the antioxidant defense resulted in adriamycin-induced tissue toxicity (Gnanapragasam *et al.*, 2004).

Natural products have been the starting point for the discovery of many important modern drugs. This has led to a worldwide search for pharmacologically important substances derived from natural products (Rajaprabhu *et al.,* 2007). Many naturally occurring compounds with antioxidative action are known to protect cellular components from oxidative damage and prevent diseases. A number of such compounds can activate the antioxidant enzymes, which can remove the toxic elements including reactive oxygen species from our system (Saha and Das 2003).

Punica granatum Linn. (Punicaceae), commonly known as pomegranate, is a small tree native to the Mediterranean region. The plant possesses an immense therapeutic value. A number of biological activities such as antitumor (Afaq *et al*., 2005); antibacterial (Prashanth *et al*., 2001); antidiarrhoeal (Das *et al*., 1999); antifungal (Dutta *et al*., 1998); antiulcer (Gharzouli *et al*., 1999) have been reported with various extracts/constituents of different parts of this plant. Pomegranate, especially flower of pomegranate, has extensively been used in Unani and Ayurvedic systems of medicine (Sivarajan and Balachandran, 1994).

Peels are often the waste part of various fruits. These wastes have not generally received much attention with a view to being used or recycled rather than discharged. This might be due to their lack of commercial application. Interestingly, the peel and seed fractions of some fruits have higher antioxidant activity than the pulp fractions (Jayaprakasha *et al*., 2001).

Solid-state fermentation (SSF) of an edible plant is a biotechnological strategy that may induce health beneficial naturally occurring antioxidant components including polyphenols, tocopherols and ascorbic acid during microbial fermentation (Lee *et al*., 2008; Rashad *et al.*, 2011).

Due to the great importance of ADR in chemotherapy for the treatment of many types of cancer, researchers have expended great efforts trying to prevent or attenuate its side effects. In this sense this work aimed to compare the antioxidant activity of methanolic extract of unfermented and fermented peel of *Punica grana*tum (Pomegranate) *in vitro* and *in vivo* models to reduce the toxic effects of ADR on both heart and kidney.

MATERIALS AND METHODS

Chemicals and facilities

All chemicals were purchased from Sigma and Aldrich (Sigma Chemical Company, St. Louis, MO, USA). Facilities including animal housing and biochemical equipments were available by the National Research Center (NRC), Cairo, Egypt.

Fruits

Fresh pomegranate (*Punica granatum* Linn., Punicaceae) fruits were purchased from the local market; the peels were manually removed, washed with water and cut into small pieces.

Microorganism

Kluyveromyces marxianus NRRL Y-8281 was obtained from Agricultural Research Service, Peoria, Illinois, USA. The strain was maintained on yeast malt agar (Wickerman, 1951), then stored at 4°C and sub-cultured monthly. Inoculum was developed by transferring a loopful of stock culture into a sterile yeast malt medium (Wickerman, 1951) and incubated at 30°C on a shaker at 200 rpm for 24 h.

Animals

The animal care and handling was done according to the guidelines set by the World Health Organization, Geneva, Switzerland, and according to approval from the ethical committee for animals care at the National Research Center, Egypt (Approval No. 11 111). Adult male Sprague-Dawley rats (average body weight of 170 ± 20 g) and adult male Swiss albino mice (6-7 weeks old, weighing 17-20 g) were obtained from the animal house of National Research Center, Egypt. The animals were housed under standard laboratory conditions (12 h light and 12 h dark) in a room with controlled temperature (24±3°C) during the experimental period. The animals were provided with tap water and commercial diets.

Solid state fermentation of pomegranate

Fifty gram fresh pomegranate peel was cooked in an autoclave (121°C, 15 min), cooled and then spraying with 1.0 ml of *K. marxianus* NRRL Y-8281 (10^8/ml) inoculum. After mixing thoroughly, the inoculated pomegranate peel was then incubated statically for three days at 30°C. During the cultivation period, the culture was periodically stirred under sterilized conditions to accelerate the release of fermentation heat (Lin *et al*., 2006; Rashad *et al*., 2011). The unfermented pomegranate peel used as control was prepared without the addition of inoculum. The unfermented pomegranate peel (UFPP) and fermented one (FPP) were collected, dried at 60^{o}C, ground and then used for analysis.

Preparation of extract

The UFPP and FPP powder (100 g) were extracted by stirring using a magnetic stirrer with 600 ml of methanol solution at 30^{o}C for 4 h. The extract was filtered through Whatman no. 1 filter paper for removal of peel particles. The residue was re-extracted with 600 ml of methanol solution and filtered. The extracts were pooled and concentrated under vacuum at 30°C (Singh *et al*., 2002).

Antioxidant activity assay *in vitro*

DPPH radical-scavenging assay

The modified methods of Shimada *et al*. (1992); Mensor *et al*. (2001) were used to study the free radical-scavenging activities of the extracts using 2,2-diphenyl-1-picrylhydrazyl (DPPH). Two ml of methanolic extract at different concentrations (50-1000 µg/ml) were added to 1.0 ml methanolic solution of 0.3 mM DPPH. The mixture was shaken and left in a dark box to stand for 30 min at room temperature (30 ± 1°C). The blank of each sample was prepared with 2.0 ml of sample solution with 1.0 ml of methanol instead of DPPH, while 1.0 ml of methanolic DPPH plus 2.0 ml of methanol served as control. The absorbance of the resulting solution was measured at 517 nm. The inhibitory percentage of DPPH was calculated according to the following equation:

Scavenging activity (%) = [1 - (absorbance sample/absorbance control)] x 100.

Total phenolic assay

Total phenolic was estimated as gallic acid equivalents essentially according to that described by Quettier-Deleu *et al*. (2000) with minor modification. Aliquots of 0.5 ml methanol extract at different concentrations (50-1000 µg/ml) were added to 7.0 ml deionized water and 0.5 ml Folin-Ciocalteu phenol reagent. After 3 min, 2.0 ml of 20% Na_2CO_3 were added and heated in a boiling water bath for 1 min comparatively to gallic acid standard. Absorbance was measured at 750 nm after cooling in darkness and the results expressed in mg of gallic acid equivalent/g dry pomegranate peel.

Determination of extract toxicity

The acute toxicity of methanolic extract of UFPP and FPP was determined according to Prieur *et al*. (1973) and Ghosh (1984). Briefly, the animals were divided into several groups of 6 mice each. Each group was individually injected intraperitoneally (i.p.) with freshly extract of either FPP and UFPP in doses ranging from 0 to 1500 mg/Kg b.w. /day, for 4 day with 24 h intervals. Mortality of the animals was observed up to one week post-extract treatment. Acute LD_{50} of the extract was determined.

Experimental protocol

Adult Sprague-Dawley rats were divided into 6 groups (each of 6 rats) and were treated as follows: Group I served as control group which were given the vehicle alone for the same period of the experiment. The animals in Group II and III were given either UFPP or FPP extracts i.p. at a dose of 100 or 75 mg/Kg b.w./day, for 4 day with 24 h interval respectively (which represents tenth of LD_{50}). Group IV served as ADR treated group, where rats were injected i.p. with 10 mg ADR /Kg b.w./day, for 3 days; the animals of Group V and VI were injected i.p. with 100 mg of UFPP or 75 mg of FPP/Kg b.w./day, 2 doses before and 2 doses following the injection of ADR (10 mg /Kg b.w./day, for 3 days).

Animals were killed by cervical dislocation one week after the last injection. Blood, heart and kidney were collected. Serum was separated from the blood and used in the determination of heart function as assessed by measuring serum activity of creatine kinase (CK). Kidney function was assessed by measuring blood urea nitrogen (BUN) spectrophotometrically according to the manufacturer's instructions, using reagent kits obtained from Biomerieux (France).

Heart and kidney were washed with saline and then homogenized in cold sucrose buffer (0.25 M). All the investigations were carried out on fresh 10% homogenate. After centrifugation at high speed (30,000 xg for 30 min at 4°C) the supernatant was used for antioxidants and protein assay. The protein levels were determined as described by Lowry *et al*. (1951).

Assessment of lipid peroxidation

Malondialdehyde (MDA) is the end product of lipid peroxidation was measured using the method of Ohkawa *et al*. (1979). MDA level was expressed as nmol/mg protein.

Determination of catalase (CAT) activity

CAT activity was adapted from the method of Aebi (1984). The activity was defined as µmol H_2O_2 decreased/min/ mg protein.

Determination of superoxide dismutase (SOD) activity

SOD activity was spectrophotometrically measured using a method developed by Nishikimi *et al*. (1972). The activity was expressed as units/mg protein.

Determination of glutathione peroxidase (GSH-Px) activity

GSH-Px activity was measured using the Paglia and Valentine's method (1967). The activity was expressed as µmol/min/mg protein.

Determination of total antioxidant status

The antioxidant status was assayed as described by Koracevic *et al*. (2001). The total antioxidant activity was expressed as mmol/mg protein.

Determination the level of reduced glutathione (GSH)

The level of GSH was determined as described by Ellman (1959). The level was expressed as nmol/mg protein.

Statistical analysis

Data were expressed as mean ± standard error (S.E.) and analyzed statistically using a one-way analysis of variance (ANOVA) followed by the student *t*-test for comparison between groups. Differences were considered significant at $P < 0.05$.

RESULTS

Extraction yield

The yield of extract obtained from FPP using methanol was found to be 32%, while from UFPP was 30%.

Antioxidant activity assay *in vitro*

DPPH radical-scavenging activity

UFPP and FPP methanolic extracts showed an excellent scavenging activity on DPPH radicals (92 and 93% respectively) at concentration from 125-1000 µg/ml (Fig. 1). While the scavenging activities of both extracts decreased (38.3 and 43.6 % respectively) with increased dilution to 50 µg/ml.

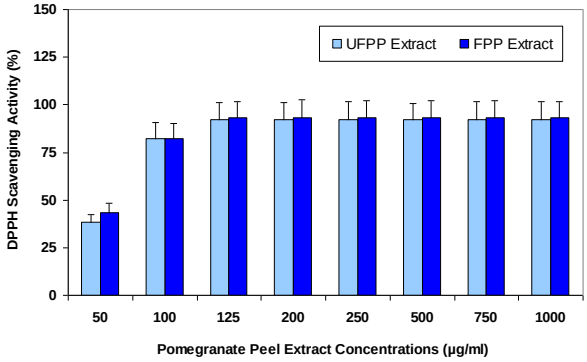


Figure 1: Antioxidant activity of unfermented (UFPP) and fermented (FPP) pomegranate peel extracts at different concentrations. Data are expressed as mean ± S.E. of triplicate measurements

Total phenolic assay

As shown in Fig. 2 the total phenolic content of the methanol extract of FPP with various concentrations (50-1000 µg/ml). All were higher than that of UFPP extract. FPP extract exhibited the highest phenolic content (211 mg gallic acid/g dry peel) at concentration of 125 µg/ml, while the UFPP extract exhibited the highest phenolic content (160 mg gallic acid/g dry peel) at the same concentration. The total phenolic contents of the two extracts decreased gradually with further increasing in the concentration.

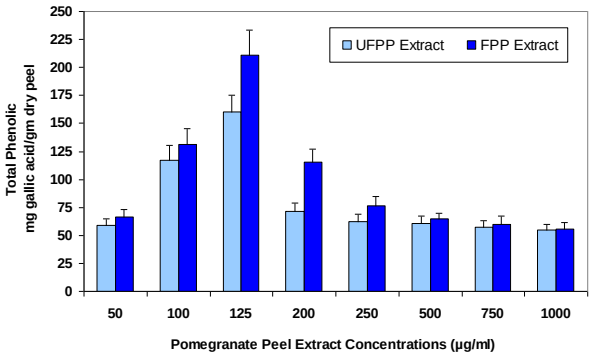


Figure 2: Total phenolic content of unfermented (UFPP) and fermented (FPP) pomegranate peel extracts at different concentrations. Data are expressed as mean ± S.E. of triplicate measurements

Acute toxicity testing of both *UFPP and FPP* extracts

As shown in Fig. 3, the acute toxicity test revealed that administration of the methanolic extract of either UFPP or FPP extracts at doses of 0, 100 and 250 mg/Kg b.w. did not induce any mortality during the whole observation period, while increase the doses to 500 mg/Kg b.w. resulted in mortality of 1 out of 6 mice (16.66%), increasing the dose to 750 mg/Kg b.w. resulted in mortality of 2 out of 6 mice (33.33%) of both UFPP or FPP extracts. However, a further increase in the extract dose to 1000 mg of UFPP Kg/b.w. or 750 mg of FPP extract Kg/b.w., resulted in 50% reduction in the survival of mice. So in this study we used the safe doses of UFPP or FPP extracts as 100 and 75 mg/Kg b.w. respectively which represent 1/10 of the LD_{50}.

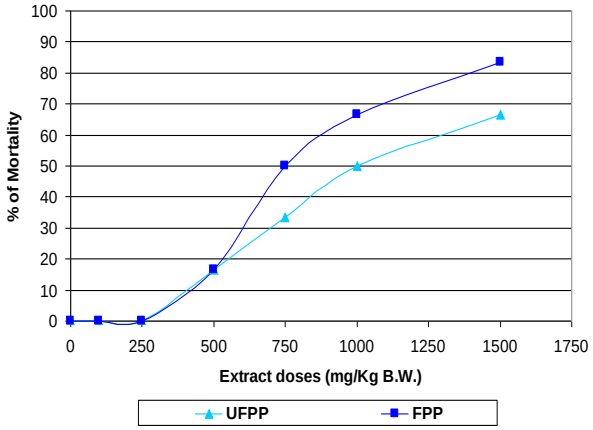


Figure 3: The effect of different doses of unfermented (UFPP) and fermented (FPP) pomegranate peel extracts on the mortality of mice

The effect of ADR alone or in combination with either UFPP or FPP extracts on heart and kidney tissues

Administration of ADR to rats at dose of 10 mg/Kg b.w. for 3 days resulted in an increase in heart and kidney function tests compared to control group (Table 1). Serum activity of CK and level of BUN were significantly ($P < 0.05$) higher in ADR treated rats than in controls by 11 and 3-fold respectively. Changes in lipid peroxidation were measured in the heart and kidney tissues as an index of oxidative stress. MDA levels showed significant increased ($P < 0.05$) up to 2.5 times in both heart and kidney as compared to the control level.

In contrast, co-treatment of ADR with either UFPP or FPP extracts significantly decrease the activity of serum CK and level of BUN as well as remarkably inhibited the rise in lipid peroxidation products in both heart and kidney as compared to adriamycin treated group (Table 1). Also, the results revealed that FPP exerted better effect than UFPP extracts.

Moreover, the results showed that groups treated with either UFPP or FPP extracts only revealed non-significant changes (P>0.05) for CK activity and both level of BUN and MDA as compared to control group.

Table 1: Effect of administration of ADR alone and along with UFPP or FPP extracts on markers of heart and kidney toxicities

Groups	Serum CK (IU/L)	BUN (mg/dl)	Cardiac MDA (nmol/mg protein)	Renal MDA (nmol/mg protein)
Control	50.90±3.66	30.65±2.80	70.00±6.20	30.80±2.90
UFPP	48.60±3.90	29.44±3.20	71.40±6.70	31.20±3.12
FPP	48.30±5.00	30.13±2.62	71.30±6.50	31.82±3.20
ADR	550.00±50.20[a]	95.70±9.00[a]	180.22±20.30[a]	75.70±6.45[a]
UFPP+ADR	120.2±11.60[b]	46.00±4.20[b]	100.80±9.60[b]	50.42±4.22[b]
FPP+ADR	75.40±6.75[b]	39.60±3.21[b]	85.65±7.60 [b]	42.30±3.75[b]

Data are expressed as means ± S.E. of six animals. [a] and [b] indicate significant difference from control and ADR groups respectively, at $P < 0.05$ using ANOVA test.

The effect of ADR alone or in combination with either UFPP or FPP extracts on antioxidant profile

The effect of ADR administration on cardiac and renal non-enzymatic antioxidant GSH and total antioxidant capacity as well as on the antioxidant enzymes SOD, CAT and GSH-Px is shown in Fig. 4 (A-E). Cardiac and renal GSH and total antioxidant capacity levels were reduced in heart and kidney by 70 and 35% for GSH and total antioxidant capacity by 50% and 45% with respect to control (Fig. 4 A-B).

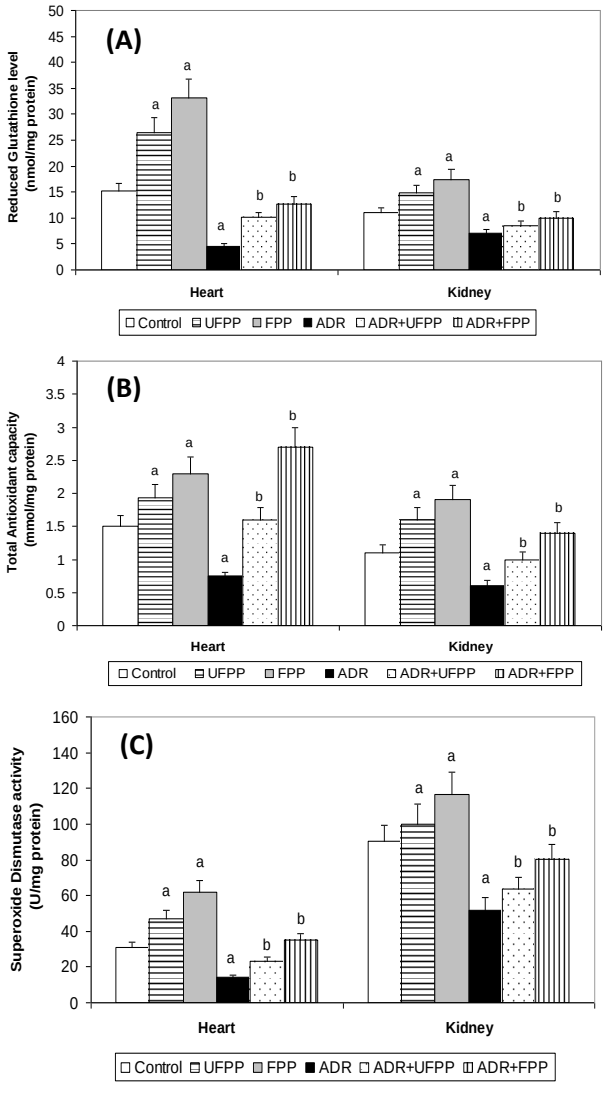
In group treated with UFPP extract plus ADR or FPP extract plus ADR showed significantly increased ($P < 0.05$) in the level of tissues GSH level as compared with the ADR treated group. The level of cardiac GSH was increased 124 and 182% respectively; while the level of renal GSH increased 20 and 41% respectively. Also the level of cardiac total antioxidant capacity was increased 113 and 260% respectively; while the level of renal total antioxidant capacity increased 67 and 133% respectively over the ADR treated group (Fig. 4 A-B).

The animals receiving UFPP or FPP extracts only cause an increase in the level of GSH in both heart and kidney. Cardiac GSH increased by 74 and 118% respectively, while the level of renal GSH increased by 34.5 and 58% respectively over the matched control group. Also, Cardiac total antioxidant capacity increased by 29 and 53% while the level of renal total antioxidant capacity increased by 45 and 73% respectively (Fig. 4 A-B).

In addition Fig. 4 (C-E) shows that in comparison to the corresponding control group there was significant decrease ($P < 0.05$) in all antioxidant enzyme activities in both heart and kidney when the animals treated with ADR alone. SOD activity decreased by 55 and 42.5%; CAT activity decreased by 34 and 50%; GSH-Px decreased by 37 and 24% in both heart and kidney respectively.

In addition data represent in Fig. 4 (C-E) demonstrated significant elevation in all antioxidant enzyme activities comparing with ADR treated group in both heart and kidney when the animals treated with either ADR plus UFPP or plus FPP extracts. Cardiac SOD activity increased by 64 and 151%; CAT activity increased by 37 and 69%; GSH-Px increased by 48 and 55% over the adriamycin treated group. Renal SOD activity increased by 22.5 and 54%; CAT activity increased by 74 and 93%; GSH-Px increased by 36 and 42% over the ADR treated group. These results revealed that FPP exhibited higher activities than UFPP extracts.

Animals receiving UFPP and FPP extracts only causes an increasing in the activities of cardiac and renal SOD, CAT and GSH-Px as comparing with control group (Fig. 4 C-E).



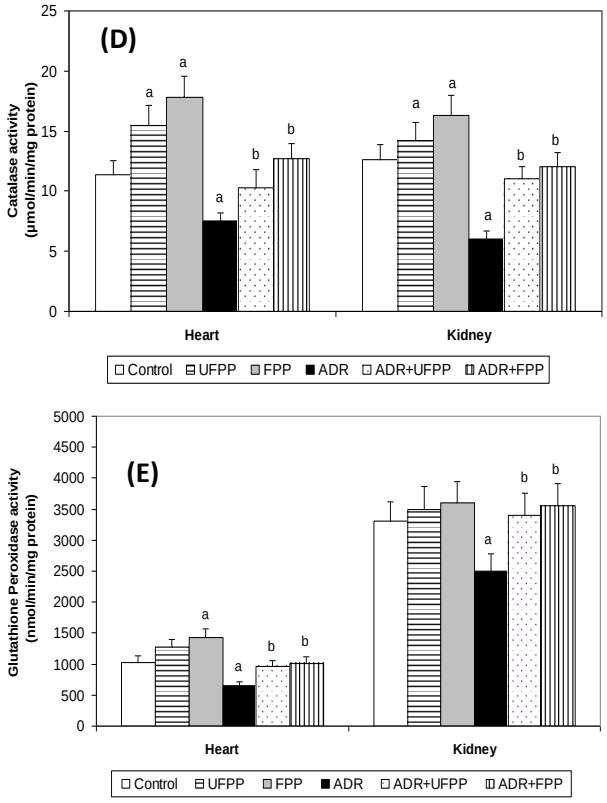


Figure 4: Effect of treatment with UFPP (100 mg/Kg b.w.) or FPP (75 mg/Kg b.w.) extracts alone or in combination with ADR on cardiac and renal reduced glutathione (A) and total antioxidant capacity (B), as well as on antioxidant enzyme activities of superoxide dismutase (C), catalase (D) and glutathione peroxidase (E), in different studied groups. Bars represent means ± S.E. of six animals. [a] and [b] indicate a significant difference from control and ADR groups respectively, at $P < 0.05$ using ANOVA test

DISCUSSION

A lot of scientific research is focused on exploring safe and effective natural antioxidant compounds. Plant extract and plant-derived antioxidant compounds potentiate body's antioxidant defense or act as antioxidant and are antioxidants of choice because of their safety over the synthetic ones. So, several studies are based on discovering plants with antioxidant potential (Kaur *et al*., 2006).

Previous studies have shown that ADR toxicity is associated with oxidative damage. Among strategies to attenuate adriamycin toxicity is the combination of the drug delivery together with an antioxidant in order to reduce oxidative stress.

The antioxidant activity of ethanolic, methanolic and water extracts of pomegranate peels and seeds have been reported in the various *in vitro* models (Singh *et al*., 2002). Hence, in the present study, the methanolic extract of pomegranate peels has been used. The methanolic extraction yield of FPP was higher (32%) than those of UFPP (30%) which is higher than the results of Okonogi *et al*. (2007) who reported that the yield of extract of pomegranate peels were 10.38 and 6.21% respectively. We tested the ability of UFPP and FPP extracts to scavenge free radicals. The results obtained in the present study suggest that UFPP and FPP have an enormous antioxidant activity. Both extracts potently scavenged DPPH radicals. The ability of extracts to scavenge these radicals suggests that it contains compounds that are electron donors, which can react with free radicals to convert them to more stable products and terminate radical chain reaction. Thus, DPPH scavenging may be related to inhibition of lipid peroxidation. Scavenging of DPPH radicals has also been reported for pomegranate juice (Gil *et al*., 2000), peel extract and seed extract (Singh *et al*., 2002).

DPPH scavenging activity of methanolic extracts of UFPP and FPP obtained in this study were 92 and 93% respectively (Fig. 1) is higher than that of ethanolic extracts of peels and flowers (81.6%) as obtained by Kaur *et al*. (2006).

In this study a very high content of polyphenolics was obtained in both UFPP and FPP extracts. At various concentrations of methanolic extracts, FPP showed higher phenolics content than the UFPP (Fig. 2). These results were in agreement with Afaq *et al*. (2005) who stated that pomegranate is a rich source of many phenolic compounds including flavanoids and hydrolyzable tannins, which account for 92% of its antioxidant activity. Fermentation process provides a high concentration and bioactivity of the antioxidant protective substances by breaking the chemical bond between the antioxidant and sugar molecules, making the antioxidants much more bioavailable. Previous studies have shown that fermented pomegranate polyphenols are especially effective in treatment of different diseases (Kim *et al*., 2002; Albrecht *et al*., 2004).

Data presented in our study demonstrated that the high dose administration of ADR induced severe heart and kidney toxicities with massive increase in the activity of serum CK enzyme and the level of BUN respectively, associated with decrease in the antioxidant defense as manifested by the significant increase in lipid peroxidation, reduce the level of GSH and total antioxidant capacity as well as a significant decrease in the antioxidant enzyme activities of SOD, CAT and GSH-Px as compared with control.

Singal *et al.* (2000); DeBeer *et al*. (2001) reported that the mechanism by which ADR-induced cardiotoxicity may be achieved by two different ways. The first way implicates the formation of a semiquinone free radical by the action of several NADPH-dependent reductases that produce a one-electron reduction of the ADR to the corresponding ADR semiquinone. In the presence of oxygen, redox cycling of ADR-derived quinone-semiquinone yields superoxide radicals. In the second way, ADR free radicals come from a non-enzymatic mechanism that involves reactions with iron. Fe^{3+}

reacts with ADR in a redox reaction, the iron atom accepts an electron and a Fe^{2+}- ADR free radical complex is produced (DeBeer *et al*., 2001). This complex can reduce oxygen to hydrogen peroxide and other active oxygen species (Sinha and Polliti, 1990). Heart tissue is very sensitive to free radical damage, because of its lower amount of antioxidant defenses in this organ compared with others like liver and kidney.

The most possible mechanism for the renal toxicity of ADR may be alterations of the permeability of the glomerular capillary wall (Weening and Rennkle, 1983) or may be the consequence of oxidative stress, such as oxidation and cross-linking of cellular thiols and membrane lipid peroxidation (Wu *et al*., 1990). Indeed, Ginevri *et al*. (1990) mentioned that in ADR-induced nephropathy, the glomerular cells produce reactive oxygen species that cause glomerular injury. Reactive oxygen species may directly damage lipid membranes and they may also mediate the activation of genes for some proinflammatory cytokines (TNF-α, IL-1, IL-6) through the stimulation of transcription factor $NF\text{-}_kB$ (Schreck and Baeuerle, 1991).

This study would seem to confirm that the peroxidation of the unsaturated fatty acids in the existing membranes was activated by oxidative stresses, in this case free radicals resulting from ADR administration, thereby accelerating the cell tissue damage accompanied with decreasing the antioxidant enzyme activities in both heart and kidney. Our present results confirmed that administration of ADR reduced GSH content may be due to increase consumption of GSH in the enzymatic and nonenzymatic detoxification of reactive oxygen species generated by ADR.

The results clearly demonstrate that ROS produced by ADR significantly decrease the activities of antioxidant enzymes (SOD, CAT and GSH-Px) in both heart and kidney tissues and as a result decrease the total antioxidant capacity which is the sum of endogenous antioxidants, also elevation of lipid peroxidation may affect cell membrane integrity with subsequent loss of cell enzymes leakage. These results were in agreement with Noriega *et al*. (2000), who have recently reported that enhancement of lipid peroxidation, depletion of GSH content and reduction of catalase and GSH-Px activities seems to be events closely related to ROS production. In addition, Comporti (1985) stated that aldehydes as one of the decomposition product of lipid peroxidation can covalently bind to the active sites of the enzymes with subsequent decrease in the activity of antioxidant enzymes.

On the other hand the present study revealed that preventive administration of methanolic extracts of UFPP or FPP at safe experimental dose level as 1/10 of LD_{50} (Fig. 3) to the animals given ADR, counteracted the development of full-blown heart and kidney toxicities, through restored the biomarker's values of oxidative stress towards normal. In the present study, the administration of the methanolic extracts of UFPP or FPP with ADR resulted in considerable ($P < 0.05$) diminution in the activity of CK and the level of BUN as well as reduction in the level of lipid peroxidation in both heart and kidney as compared with the ADR treated group (Table 1). Also the levels of GSH and total antioxidant status as well as the activities of antioxidant enzymes (SOD, CAT and GSH-Px) were comparatively higher than ADR treated group (Fig. 4 A-E).

From the foregoing results it is clear that, the methanolic extracts of UFPP or FPP inhibited cardio- and renal-toxicities expression, which may suggest that both extracts act possibly by neutralizing the increase of free radicals caused by ADR. It is noteworthy to mention that in this study, the protective effect of UFPP or FPP extracts on ADR-induced cardiac and renal damage but the most significant effects were obtained with FPP extract which could thus relate to their high total phenolic content. The fermentation process appears to improve the antioxidants properties of the extract.

CONCLUSION

The results of present study indicated the cardio- and renal-protective effects of *Punica granatum* peel extract and its fermented one against adriamycin induced heart and kidney toxicities. The fermentation process appears to improve the antioxidants properties of the extract. The overall protective action of *Punica granatum* peel extract was probably due to its membrane stabilizing action, or to a counteraction of free radicals by its antioxidant nature, or to its ability to maintain the normal status activities of free radical enzymes and the level of reduced glutathione, which protect the membrane against peroxidative damage by decreasing lipid peroxidation and strengthening the membrane.

REFERRENCES

Aebi, H. 'Catalase', in Bergemeyer, H.U. (ed.) Method of Enzymatic Analysis, New York: Academic Press, Vol. 2, 1984.

Afaq, F., Saleem, M., Krueger, C.G., Reed, J. D. and Mukhtar, H. Anthocyanin- and hydrolyzable tannin-rich pomegranate fruit extract modulates MAPK and NF-kappa B pathways and inhibits skin tumorigenesis in CD-1 mice. Int. J. Cancer Vol. 113, 2005, pp. 423–433.

Albrecht, M., Jiang, W., Kumi-Diaka, J., Lansky, E.P., Gommersall, L.M., Patel, A., Mansel, R.E., Neeman, I., Geldof, A.A. and Campbell, M.J. Pomegranate extracts potently suppress proliferation, xenograft growth, and invasion of human prostate cancer cells. J. Med. Food Vol.7, 2004, pp. 274–283.

Ali, M.M., Frei, E., Straub, J., Breuer, A. and Wiessler, M. Induction of metallothionein by zinc protects from daunorubicin toxicity in rats. Toxicology Vol. 179, 2002, pp. 85-93.

Bertani, T., Cutillo, F., Zoja, C., Broggini, M. and Remuzzi, G. Tubulo-interstitial lesions mediated renal damage in adriamycin glomerulopathy. Kidney Int. Vol. 30, 1986, pp. 488-496.

Bryant, J., Picot, J., Levitt, G., Sullivan, I., Baxter, L. and Clegg, A. Cardioprotection against the toxic effects of anthracyclines given to children with cancer: A systematic review. Health Technol. Assess. Vol. 11, 2007, pp. 1-84.

Comporti, M. Biology of disease lipid peroxidation and cellular damage in toxic liver injury. Lab. Invest. Vol. 53, 1985, pp. 599-606.

Das, A.K., Mandal, S.C., Banerjee, S.K., Sinha, S., Das, J., Saha, B.P. and Pal, M. Studies on antidiarrhoeal activity of *Punica granatum* seed extract in rats. J. Ethnopharmacol. Vol. 68, 1999, pp. 205–208.

DeBeer, E.L., Bottone, A.E. and Voest, E.E. Doxorubicin and mechanical performance of cardiac trabeculae afteracute and chronic treatment: A review. Eur. J. Pharmacol. Vol. 415, 2001, pp. 1-11.

Deepa, P.R. and Varalakshmi, P. Protective effect of low molecular weight heparin on oxidative injury and cellular abnormalities in adriamycin-induced cardiac and hepatic toxicity. Chem. Biol. Interact. Vol. 146, 2003, pp. 201-210.

Dutta, B.K., Rahman, I. and Das, T.K. Antifungal activity of Indian plant extracts. Mycoses Vol. 41, 1998, pp. 535–536.

Ellman, G.L. Tissue sulfhydryl groups. Arch. Biochem. Biophys. Vol. 82, 1959, pp. 70-77.

Gharzouli, K., Khennouf, S., Amira, S. and Gharzouli, A. Effects of aqueous extracts from *Quercus ilex* L. root bark, *Punica granatum* L. fruit peel and Artemisia herba-alba Asso leaves on ethanol-induced gastric damage in rats. Phytother. Res. Vol. 13, 1999, pp. 42–45.

Ghosh, M.N. 'Toxicity studies' in Ghosh, M.N. (ed.) Fundamentals of Experimental Pharmacology, Calcutta, India: Scientific Book Agency, 1984.

Gianni, L., Salvatorelli, E. and Minotti, G. Anthracycline cardiotoxicity in breast cancer patients: Synergism with trastuzumab and taxanes. Cardiovasc. Toxicol. Vol. 7, 2007, pp. 67-71.

Gil, M.I., Tomas-Barberan, F.A. Hess-Pierce, B., Holcroft, D.M. and Kader, A.A. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. J. Agric. Food Chem. Vol. 48, 2000, pp. 4581–4589.

Ginevri, F., Gusmano, R., Oleggini, R., Acerbo, S., Bertelli, R., Perfumo, F., Cercignani, G., Allegrini, S., D'Allegri, F. and Ghiggeri, G. Renal purine efflux and xanthine oxidase activity during experimental nephrosis in rats: Difference between puromycin aminonucleoside and adriamycin nephrosis. Clin. Sci. (Colch) Vol. 78, 1990, pp. 283–293.

Gnanapragasam, A., Ebenezar, K.K., Sathish, V., Govindaraju, P. and Devaki, T. Protective effect of *Centella asiatica* on antioxidant tissue defense system against adriamycin induced cardiomyopathy in rats. Life Sci. Vol. 76, 2004, pp. 585-597.

Jayaprakasha, G.K., Sigh, R.P. and Sakariah, K.K. Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models *in vitro*. Food Chem. Vol. 73, 2001, pp. 285–290.

Kaur, G., Jabbar, Z., Athar, M. and Sarwar Alam, M. *Punica granatum* (pomegranate) flower extract possesses potent antioxidant activity and abrogates Fe-NTA induced hepatotoxicity in mice. Food Chem. Toxicol. Vol. 44, 2006, pp. 984–993.

Kim, N.D., Mehta, R., Yu, W., Neeman, I., Livney, T., Amichay, A., Poirier, D., Nicholls, P., Kirby, A., Jiang, W., Mansel, R., Ramachandran, C., Rabi, T., Kaplan, B. and Lansky, E. Chemopreventive and adjuvant therapeutic potential of pomegranate (*Punica granatum*) for human breast cancer. Breast Cancer Res. Treat. Vol. 71, 2002, pp. 203–217.

Koracevic, D., Koracevic, G., Djordjevic, V., Andrejevic, S. and Cosic, V. Methods for the measurement of antioxidant activity in human fluids. J. Clin. Pathol. Vol. 54, 2001, pp. 356-361.

Lee, Y.-L., Yang, J.-H. and Mau, J.-L. Antioxidant properties of water extracts from Monascus fermented soybeans. Food Chem. Vol. 106, 2008, pp. 1128-1137.

Li, T., Donelisen, I., Bello-Klein, A. and Singal, P.K. Effects of probucol on changes of antioxidant enzymes in adriamycin-induced cardiomyopathy in rats. Cardiovasc. Res. Vol. 46, 2000, pp. 523-530.

Li, Y.F., Guo, C.J., Yang, J.J., Wei, J.Y., Xu, J. and Cheng, S. Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. Food Chem. Vol. 96, 2006, pp. 254–260.

Lin, C.H., Wei, Y.T. and Chou, C.C. Enhanced antioxidative activity of soybean koji prepared with various filamentous fungi. Food Microbiol. Vol. 23, 2006, pp. 628-633.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. Protein measurement with the folin phenol reagent. J. Biol. Chem. Vol. 193, 1951, pp. 265-275.

Mensor, L.I., Menezes, F.S., Leitao, G.G., Reis, A.S., Dos Santos, T., Coube, C.S. and Leitao, S.G. Screening of Brazilian plants extracts for antioxidants activity by the use of DPPH free radical method. Phytother. Res. Vol. 15, 2001, pp. 127-130.

Nishikimi, M., Roa, N.A. and Yogi, K. Determination of superoxide dismutase. Biochem. Bioph. Res. Common. Vol. 46, 1972, pp. 845-854.

Noriega, C.O., Ossola, J.O., Tomaro, M.L. and Batlle, A.M.C. Effect of acetaminophen on heme metabolism in rat liver. Int. J. Biochem. Cell Biol. Vol. 32, 2000, pp. 983-991.

Ohkawa, H., Ohishi, N. and Yagi, K. Assay of lipid peroxides in animal tissues by thiobarbituric reaction. Anal. Biochem. Vol. 95, 1979, pp. 351-358.

Okonogi, S., Duangrat, C., Anuchpreeda, S., Tachakittirungrod, S. and Chowwanapoonpohn, S. Comparison of antioxidant capacities and cytotoxicities of certain fruit peels. Food Chem. Vol. 103, 2007, pp. 839–846.

Paglia, E.D. and Valentine, W.N. Studies on the quantitative and qualitative characterization of erythrocytes glutathione peroxidase. J. Lab. Clin. Med. Vol. 70, 1967, pp. 158-169.

Prashanth, D., Asha, M.K. and Amit, A. Antibacterial activity of *Punica granatum*. Fitoterapia Vol. 72, 2001, pp. 171–173.

Prieur, D.J., Young, D.M., Davis, R.D., Cooney, D.A., Homan, E.R., Dixon, R.L. and Guarino, A.M. Procedures for preclinical toxicologic evaluation of cancer chemotherapeutic agents, protocols of the laboratory of toxicity. Cancer Chemoth. Rep. Vol. 4, 1973, pp. 1-28.

Quettier-Deleu, C., Gressier, B., Vasseur, J., Dine, T., Brunet, C., Luyckx, M., Cazin, M., Cazin, J.C., Bailleul, F. and Trotin, F. Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum Moench*) hulls and flour. J. Ethnopharmacol. Vol. 72, 2000, pp. 35–42.

Quiles, J.L., Huertas, J.R., Battino, M., Mataix, J. and Ramirez-Tortoso, M.C. Antioxidant nutrients and adriamycin toxicity. Toxicology Vol. 180, 2002, pp. 79-95.

Rajaprabhu, D., Rajesh, R. Jeyakumar, R., Buddhan, S., Ganesan, B. and Anandan, R. Protective effect of *Picrorhiza kurroa* on antioxidant defense status in adriamycin induced cardiomyopathy in rats. J. Med. Plant Res. Vol. 1(4), 2007, pp. 80-85.

Rashad, M.M., Mahmoud, A.E. and Ali, M.M. Hepatoprotective, anti-inflammatory and analgesic potential of methanolic extract of fermented okara in experimental animals. International Journal of Integrative Biology Vol. 11(3), 2011, pp. 117-126.

Saha, P. and Das, S. Regulation of hazardous exposure by protective exposure modulation of phase II detoxification and lipid peroxidation by *Camellia sinensis* and *Swertia chirata*. Teratogen. Carcinogen Mut. Supplement, 1, 2003, pp. 313–322.

Schreck, R. and Baeuerle, P.A. A role for oxygen radicals as second messengers. Trends Cell Biol. Vol. 1, 1991, pp. 39–42.

Shimada, K., Fujikawa, K., Yahara, K. and Nakamura, T. Anti-oxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J. Agric. Food Chem. Vol. 40, 1992, pp. 945-948.

Singal, P.K., Li, T., Kumar, D., Danelisen, I. and Iliskovic, N. Adriamycin-induced heart-failure: Mechanism and modulation. Mol. Cell Biochem. Vol. 207, 2000, pp. 77-85.

Singh, R.P., Chidambara Murthy, K.N. and Jayaprakasha, G.K. Studies on the antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using *in vitro* models. J. Agric. Food. Chem. Vol. 50, 2002, pp. 81–86.

Sinha, B.K. and Polliti, P.M. Anthracyclines. Cancer Chemother. Vol 11, 1990, pp. 45-57.

Sivarajan, V.V. and Balachandran, I. Ayurvedic drugs and their plant sources, London: Oxford and IBH Publishing Co Pvt. Ltd., 1994.

Weening, J.J. and Rennkle, H.G. Glomerular permeability and polyanion adriamycin nephrosis in the rat. Kidney Int. Vol. 24, 1983, pp. 152–159.

Wickerman, L.J. Taxonomy of yeasts: US Department of Agriculture Technical Bulletin, No. 1029, Washington, USA, 1951.

Wu, S.H., Yang, Y.C. and Wang, Z.M. Role of oxygen radicals in adriamycin-induced nephrosis. Clin. Med. J. Engl. Vol. 103, 1990, pp. 283–289.