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Research Article

Ameliorative Effects of *Punica Granatum* Juice and Extracts against 7,12-Dimethylbenz (a) Anthracene and Carbon Tetrachloride-Induced Cardiorenal Toxicity in Albino Rats

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Abstract

Background: Both 7,12-dimethylbenz (a) anthracene and carbon tetrachloride are xenobiotics that are implicated in various toxicological effects including carcinogenesis. *Punica granatum* constituents have antioxidant and anti-inflammatory activities. This study is conducted to assess the preventive effects of pomegranate (*Punica granatum*) juice, seeds extract, husk extract and their mixture on 7,12-Dimethylbenz (A) Anthracene (DMBA) and Carbon Tetrachloride (CCl_4)-induced perturbed kidney and heart function and integrity.

Methods: Fifty male albino rats received single oral dose of DMBA (50 mg/kg b.w.) followed by consecutive oral doses of CCl_4 (3 mg/kg b.w. /week) beginning from the third week for thirteen weeks and ten animals kept as normal control. Pomegranate (*Punica granatum*) juice (10 ml/kg b.w.), seeds extract (400 mg/kg b.w. /day), husk extract (400 mg/kg b.w. /day) and their mixture (10 ml/kg b.w. /day) were orally administered to DMBA/CCl_4-treated rats beginning from the 1st day of the experiment.

Results: The obtained data revealed a significant elevation in serum urea, uric acid, creatinine and K levels and decrease in Na concentration in DMBA/CCl_4-administered animals. In addition, there are an elevation of lipid peroxidation (MDA) and Nitric Oxide (NO) in kidney tissue homogenate and depletion in antioxidant namely GSH, GPx, GST, SOD and Total Antioxidant Capacity (TAC). Furthermore, the activity of serum enzymes related to heart functions as CK, CK-MB, LDH and AST were increased in DMBA/CCl_4-administered animals. These data reflect a cardiorenal toxicity which was confirmed by histopathological perturbations in the kidney and heart tissues. These deleterious changes of DMBA/CCl_4 were significantly improved by treatment with *Punica granatum* juice and fruit extracts.

Conclusion: Based on the obtained result, it can be concluded that pomegranate has antioxidant properties and synergistic effects of its bioactive compounds in juice and extract mixture may prevent DMBA/CCl_4-induced heart and kidney impaired function and integrity.

Introduction

Pomegranate, *Punica granatum* (*P. granatum*), belonging to family punicaceae, is rich in antioxidant of polyphenolic class which includes tannins [1]. As indicated from previous publications, *P. granatum* fruit, juice and peel possess a marked antioxidant capacity [2] while about 92% of the total fruit antioxidant activity and polyphenolic compounds is concentrated in the edible parts, juice and peels [3,4]. The fruit has a rich variety of polyphenols such as anthocyanins, gallotannins, hydroxycinnamic acids derivatives, hydroxybenzoic acids and hydrolysable tannins (as Punicalagin) and gallagyl esters [5]. *P. granatum* has been widely used for several centuries in traditional medicine for a wide variety of diseases including upper respiratory tract infections and influenza [6]. Other previous investigations found that *P. granatum* peel extracts have a free radical scavenging and potent antioxidant capacity [7]. Antioxidants have an increasing role in the protection against exogenous oxidative stress by two basic categories of antioxidant specifically natural and synthetic ones [8].

P. granatum Juice (PJ) has been proposed to have chemopreventive, chemotherapeutic, antiatherosclerotic and anti-inflammatory activities [9,10]. As well, *P. granatum* peel (husk) extract (PHE) was utilized in Egyptian culture, as stated by Ismail, et al. in several common ailments such as inflammation, diarrhea, intestinal worms, cough and infertility [11]. The most researches have also been directed towards using of total plant extracts mainly because of the synergistic effects of the mixture of plant metabolites and the multiple points of intervention in oxidative stress chemoprevention. Tzulker, et al. [12] reported that the homogenates prepared from the whole

fruit exhibited an approximately 20-fold higher antioxidant activity than the level found in the arils or seed sacs juice. Furthermore, the phenolic profile differs greatly from the two resources [13]. Hassanpour Fard, et al. mentioned that the whole fruit extract of *P. granatum* has the ability to reduce oxidative stress in doxorubicin-treated animals and show cardioprotective action [14]. Safety of *P. granatum* and its constituents has been confirmed by Aviram, et al. both experimentally and clinically and no evidence was noticed for toxic effects on body organs including kidney and heart [15].

The aromatic hydrocarbon 7,12- Dimethylbenz (A) Anthracene (DMBA) which is abundantly present in cigarette smoke, polluted air, and charcoal-broiled and smoked food, has been implicated in various toxicological effects, including atherogenesis and carcinogenesis [16,17]. The ultimate DMBA carcinogen is so called dihydrodiol epoxide, produced during cellular metabolism [18]. The created active metabolites after exposure to DMBA produce stable DNA carcinogenic adducts which may be found in various tissues including the kidney, liver and mammary gland [19]. While DMBA is known to break down many enzymes involved in DNA repair, it is normally used by many researchers to induce liver and kidney cancer in experimental animal models [20].

Carbon tetrachloride (CCl_4) is a potent hepatotoxin [21] and is rapidly biotransformed by cytochrome P-450 to the highly lethal trichloromethyl ($^{\bullet}CCl_3$) radical and chloride (Cl) [22]. Consequently, formed peroxy trichloromethyl ($^{\bullet}OOCCl_3$), leading to oxidative stress, is the direct cause of many pathological conditions such as hypertension, diabetes mellitus, cancer, kidney damage, liver damage and death [23,24]. In addition, CCl_4 induced cardiotoxicity by the creation of free radicals which is a rate limiting process in tissue peroxidative damage [25]. In the present study, the chemopreventive and therapeutic effects of *P. granatum* juice and fruit extracts against the toxicity of DMBA/CCl_4 in kidney and heart tissues were investigated.

Materials and Methods

Chemicals

The toxic agent 7,12-Dimethylbenz(a)Anthracene (DMBA) powder 95% (TLC) and mineral oil were purchased from Sigma Aldrich Company 3050 Spruce St. Saint-Louis, MO, United States. Carbone Tetra-chloride (CCl_4) 99.0% was purchased from Laboratory Chemical Trading Company (Egypt for laboratory fine chemicals). All other chemicals used in this experiment with high analytical grade.

Experimental animals

Sixty male albino rats weighing approximately 100-130 grams and aging 8-9 weeks were used in this study. Rats were obtained from the National Research Center (NRC), Dokki, Giza, Egypt. They were housed in the animal house of Zoology Department, Faculty of science, Beni-Suef University, Egypt. Along the experiment, they were fed standard diet and water *ad libitum* and were kept at 23-24 °C and 12 hrs-dark/light cycles in good aerated cages. The supplemented standard diet consists, as percent of total, of 19% protein, 7.5% fat and 60% carbohydrate and 13.5% vitamins and minerals. All animal procedures are in accordance with rules and regulations of Experimental Animals Ethics Committee of Zoology Department, Faculty of Science, Beni-Suef University, Egypt. All efforts were done to decrease the suffering of animals.

Preparation of juice and aqueous extracts doses

P. granatum fruits were purchased from the local market in Beni-Suef, Egypt. Fruits were washed with distilled water and manually peeled and fresh *P. granatum* crude Juice (PJ) from seeds' sacs was prepared and orally gavaged to rats at 10 ml/kg b.w. /day according to Adukondalu, et al. [26]. Seeds and peels were collected and dried in the shadow. They were separately grinded with mechanical grinder to fine powder and extracted in boiled distilled water at concentration of 4% (400mg/10ml) for fifteen minutes then filtered in clean bottles as infusion of *P. granatum* Seed Extract (PSE) and *P. granatum* Husk Extract (PHE). Doses adjusted at 400 mg/kg b.w. /day for PSE and PHE according to Agha, et al. [27] and Akter, et al. [28] respectively. On the other hand, *P. granatum* Mixture of Extracts and juice (PME) was prepared by adding equal volumes of PJ, PSE and PHE and orally gavaged at 10 ml/kg b.w. /day according to Patel, et al. [29]. In addition, DMBA solution was prepared as 50 Mg/kg b.w. in mineral oil [30]. As well, CCl_4 solution 99.0% was injected at 3 ml/kg b.w./ week [31] subcutaneously in the thoracic area.

Experimental design

After two weeks of acclimatization period, the animals were subsequently divided to six groups (ten in each). Group 1 served as normal control and received distilled water, saline and mineral oil as vehicles of aqueous extracts, CCl4 and DMBA respectively. Group 2 (DMBA/CCl_4 control group) was orally gavaged a single dose of DMBA 50 mg/kg b.w. and after three weeks of DMBA intake, administered a consecutive doses of CCl_4 at 3 ml/kg b.w./week for thirteen weeks. Group 3 was administered DMBA and CCl_4 as group 2 and orally treated with PJ at 10 ml/kg b.w. /day from the first day to the end of the experiment (sixteen weeks). Group 4 was managed as group 2 and orally treated with PSE at 400 mg/kg b.w. /day to the end of the experiment. Group 5 was managed as group 2 and orally treated with PHE at 400 mg/kg b.w. /day to the end of the experiment. Group 6 was managed as group 2 and orally treated with PME at 10 ml/kg b.w. /day to the end of the experiment.

Blood and tissue sampling

At the end of the experiment, all groups were sacrificed. Blood samples were collected from jugular arteries and clear non-haemolysed serum were immediately separated then frozen at −30 °C. One of the two kidneys and heart tissues were quickly removed and fixed in neutral buffer formalin for histopathological studies. Another 0.5 gm of the other kidney homogenized in 5 ml of 0.9% sodium chloride solution. Homogenate was centrifuged at 3000 rpm and clear supernatant was frozen till used for oxidative stress and antioxidant defense system markers detection.

Biochemical investigations

Serum urea level was determined according to Chaney, et al. [32] by using kits purchased from BIOMED Diagnostic (Egypt). Furthermore, determination of serum uric acid level was based on the method of Fossati, et al. [33] using reagent kit purchased from Spinreact (Spain). Serum creatinine level also was detected according to the method of Murray, et al. [34] using reagent kits obtained from Diamond Diagnostic Chemical Company (Egypt). Measurement of sodium and potassium concentrations was carried in accordance with Henry, et al. [35] and Hillmann, et al. [36] respectively, using

Table 1: Effects of *P. granatum* juice, fruit extracts and their mixture on serum creatinine, urea, uric acid potassium and sodium levels in DMBA/CCl_4-administered rats.

Parameters / Treatment	Creatinie (mg/L)	% change	Urea (mg/dl)	% change	Uric acid (mg/dl)	% change	Potassium (mMol/L)	% change	Sodium (mEq/L)	% change
Distilled water	4.91 ±0.079^{b}	-	26.93 ± 1.20^{bc}	-	1.57 ± 0.046^{d}	-	4.83 ± 0.095^{b}	-	149.14 ± 0.355^{a}	-
DMBA/CCl_4	6.35 ±0.114^{a}	29.32	38.67 ± 1.53^{a}	43.59	4.23 ± 0.337^{a}	169.42	6.95 ± 0.452^{a}	43.89	146.43 ± 1.517^{b}	-1.81
DMBA/CCl_4 and PJ	5.06 ±0.207^{b}	-20.31	26.06 ± 2.76^{c}	-32.6	2.46 ± 0.287^{bc}	-41.84	4.93 ± 0.240^{b}	-29.06	150.51 ± 0.716^{a}	2.78
DMBA/CCl_4 and PSE	5.05±0.080^{b}	-20.47	19.92 ± 1.48^{c}	-48.48	2.40 ± 0.195^{bc}	-43.26	5.55 ± 0.078^{b}	-20.14	149.98 ± 0.445^{a}	2.42
DMBA/CCl_4 and PHE	4.6 ±0.432^{b}	-27.55	22.56 ± 3.29^{c}	-41.66	2.97 ± 0.184^{b}	-29.78	5.46 ± 0.398^{b}	-21.43	149.34 ± 0.621^{a}	1.98
DMBA/CCl_4 and PME	5.2±0.423^{b}	-18.11	34.33 ± 3.92^{ab}	-11.22	2.15 ± 0.267^{cd}	-49.17	5.13 ± 0.190^{b}	-26.18	149.46 ± 0.298^{a}	2.06
F-probability	P<0.01		P<0.001		P<0.001		P<0.001		P<0.05	
LSD at 5% level	0.7776		7.448		0.6896		0.8101		2.242	
LSD at 1% level	1.0472		10.03		0.9288		1.091		3.0194	

Data are expressed as mean ± mean standard error. Number of animals in each group is six.
Means, which share the same superscript symbol(s), are not significantly different.
Percentage changes were calculated by comparing DMBA/CCl_4- administered control group with normal group (dist. water) and DMBA/CCl_4- administered treated groups with DMBA/CCl_4-administered control group.

reagent kits purchased from Spectrum Company for Biotechnology, Obour City, Cairo, Egypt. As well, Lactate Dehydrogenase (LDH) activity was assayed as described by Pesce, et al. [37] using reagent kits purchased from Spinreact. In addition, Creatine Kinase (CK) and Creatine Kinase-MB (CK-MB) activities in the serum were detected according to the method of Young [38] using purchased kits from Spinreact. The activity of AST in serum was measured using reagent kits purchased from Biosystems S.A (Spain) according to the method of Young [39].

Oxidative stress and antioxidants detection

GSH content in kidney was determined according to Beutler, et al. [40] chemical method by using tissue homogenate supernatant instead of blood samples and readjusted volumes. GST activity in kidney homogenate was measured according to the method of Mannervik and Gutenberg [41]. GPx and SOD activities were determined according to methods of Matkovics, et al. [42] and Marklund and Marklund [43] respectively. Total Antioxidants Capacity (TAC) was measured using assay kit purchased from Abcam Company (El Emam Aly St. Heliopolis, Cairo, Egypt) according to Csillag, et al. [44]. Lipid peroxidation (MDA) was determined according to the method of Preuss, et al. [45]. Nitric oxide (NO) was measured according to Montgomery and Dymock [46] method using reagent kit purchased from Biodiagnostic Company 29 Tahreer St. Dokki, Giza, Egypt.

Histopathological examination

Animals were scarified under mild diethyl ether anesthesia and dissected. Kidneys and heart were rapidly excised and perfused in saline solution. One kidney and longitudinal half of heart of each rat were taken and fixed in 10% neutral buffer formalin for twenty four hours. Fixed organs were sent to Histopathology Department of National Cancer Institute (NIC), Cairo, Egypt for further processing, blocking in wax, sectioning and staining with Haematoxylin and Eosin (H&E). The slides of different groups then examined by Prof Dr. Mahmoud Badwey Mohamed Al-Begawy, Professor of Histopathology, Faculty of Veterinary, Beni-Suef University, Egypt and Dr. Kawkab A Abd El Aziz, Professor of Histopathology, Faculty of Veterinary, Cairo University, to make comments on the histological architecture and lesions.

Statistical analysis

Results were expressed as mean± Standard Error (SE). PC-STAT program was used for data analysis [47]. One-Way Analysis of Variance (ANOVA) followed by LSD at 5% and LSD at 1% were applied to compare between the different groups. Percentage of changes were calculated by comparing DMBA/CCl_4-administered group with normal group and comparing DMBA/CCl_4-administered groups treated with various *P. granatum* infusions and juice with DMBA/CCl_4-administered control group. Values of P>0.05 were considered statistically non-significantly different, while values of P<0.05, P<0.01 and P<0.001 were significantly and very highly significantly different respectively.

Results

Effects on serum parameters related to kidney functions

The obtained data revealed a highly significant increase (P<0.01; LSD) in concentrations of serum creatinine, urea, uric acid and potassium in DMBA/CCl_4-administered control group recording percentage changes of 29.32, 43.59, 169.42 and 43.89% respectively as compared with normal rats. In contrast, sodium concentration was significantly decreased (P<0.05; LSD) in DMBA/CCl_4-administered control group recording percentage change of -1.81% in comparing with normal group (Table 1).

The treatment of DMBA/CCl_4-administered rats with *P. granatum* produced a highly significant decrease (P<0.01; LSD) in the elevated serum creatinine concentration recording percentage changes of -20.31, -20.47, -27.55 and -18.11% as a result of treatment with PJ, PSE, PHE and PME respectively as compared with DMBA/CCl_4-administered control group. The elevated serum urea concentration was also highly significantly (P<0.01; LSD) reduced in DMBA/CCl_4-administered treated groups; the recorded percentage changes were -32.60, -48.48, -41.66 and -11.22% as a result of treatment with PJ, PSE, PHE and PME respectively. The increased uric acid level was highly significantly improved (P<0.01; LSD) in DMBA/CCl_4-

Table 2: Effects of *P. granatum* juice, fruit extracts and their mixture on kidney glutathione, total antioxidants capacity, MDA and NO content in DMBA/CCl_4-administered rats.

Parameters / Treatment	**Glutathione (nmole/100 mg tissue)**	% change	Total antioxidants capacity nmole/mg tissue	% change	MDA (nmole/100mg tissue/hr)	% change	NO nmole/g tissue	% change
Distilled water	38.42 ± 1.57^{a}	-	11.52 ± 0.99^{a}	-	30.23 ± 1.17^{b}	-	61.93 ± 11.96^{bc}	-
DMBA/CCl_4	26.42 ± 3.42^{b}	-31.23	5.95 ± 0.47^{c}	-48.35	42.21 ± 5.36^{a}	39.62	118.13 ± 7.08^{a}	90.74
DMBA/CCl_4 and PJ	42.24± 3.04^{a}	59.87	8.44 ± 0.66^{bc}	41.84	19.26 ± 0.52^{c}	-54.37	47.26 ± 11.00^{c}	-59.99
DMBA/CCl_4 and PSE	38.07 ± 3.62^{a}	44.09	10.40 ± 1.74^{ab}	74.78	18.06 ± 1.05^{c}	-57.21	70.76 ± 13.26^{bc}	-40.09
DMBA/CCl_4 and PHE	45.50 ± 1.86^{a}	72.21	11.09 ± 1.00^{ab}	86.38	28.35 ± 1.06^{b}	-32.83	60.80 ± 2.85^{bc}	-48.53
DMBA/CCl_4 and PME	45.20 ± 1.89^{a}	71.08	9.85 ± 0.86^{ab}	65.54	32.67 ± 1.78^{b}	-22.6	84.30 ± 7.98^{b}	-28.63
F-probability	P < 0.001		P < 0.01		P < 0.001		P < 0.001	
LSD at 5% level	7.792		2.9955		7.059		27.994	
LSD at 1% level	10.494		4.0341		9.506		37.7	

Data are expressed as mean ±mean standard error. Number of animals in each group is six.
Means, which share the same superscript symbol(s) are not significantly different.
Percentage changes were calculated by comparing DMBA/CCl_4-administered control group with normal group (dist. Water) and DMBA/CCl_4-administered treated groups with DMBA/CCl_4-administered control group.

administered groups treated with PJ, PSE, PHE and PME recording percentage changes of -41.84, -43.26, -29.78 and -49.17% respectively as compared with DMBA/CCl_4-administered control. The sodium concentration was highly significantly elevated (P<0.01; LSD) in DMBA/CCl_4-administered groups treated with PJ, PSE and PME while it was only significantly increased (P<0.05; LSD) in PHE treated group. On contrary, potassium concentration was highly significant decreased (P<0.01 LSD) in DMBA/CCl_4-administered groups treated with PJ, PSE, PHE and PME recording percentage decreases of 29.06, 20.14, 21.43 and 26.18 respectively (Table 1).

Changes in oxidative stress and antioxidants molecules

DMBA/CCl_4 administration induced a highly significant decrease (P<0.01; LSD) in the kidney content of GSH and TAC in control group recording percentage changes of -31.23 and -48.35 respectively. Moreover, DMBA/CCl4 administration highly significantly increased (P<0.01; LSD) the tissue content of MDA and NO with percentage changes of 39.62 and 90.74 in that order (Table 2).

On the other hand, PJ treatment to DMBA/CCl_4-administered rats produced a highly significant increase in kidney GSH content and decrease in MDA and NO (P<0.01; LSD). As well, PSE highly significantly elevated (P<0.01; LSD) GSH level and TAC while it decreased MDA and NO content. Moreover, PHE highly significantly increased (P<0.01; LSD) GSH content and TAC while diminished tissue concentrations of MDA and NO. Furthermore, PME highly significantly (P<0.01; LSD) increased GSH and decreased MDA while it significantly (P<0.05; LSD) elevated TAC and reduced NO.

Effects on antioxidants enzymes

The administration of DMBA/CCl4 induced a highly significant decrease (P<0.01; LSD) in GST activity with percentage change of -32.49% but it non- significantly (P>0.05; LSD) affected GPx and SOD activity (Table 3). The treatment of DMBA/CCl4-administered rats with PJ significantly elevated GST and SOD (P<0.05; LSD) and non-significantly increased GPx. PSE and PME treatments significantly elevated (P<0.01; LSD) GPx and GST while it induced a non-

Table 3: Effects of *P. granatum* juice, fruit extracts and their mixture on kidney glutathione peroxidase, glutathione-S-transferase and superoxide dismutase activities in DMBA/CCl_4-administered rats.

Parameters / Treatment	**Glutathione Peroxidase (mU/100 mg tissue)**	% change	**Glutathione-S-transferase (U/100 mg tissue)**	% change	**Superoxide dismutase (U/g).10**	% change
Distilled water	86.31 ± 3.44^{bc}	-	144.28 ± 10.35^{ab}	-	125.86 ± 4.10^{abc}	-
DMBA/CCl_4	77.03 ± 2.04^{c}	-10.75	97.40 ± 3.05^{d}	-32.49	116.44 ± 4.78^{bc}	-7.48
DMBA/CCl_4 and PJ	87.78 ± 4.55^{bc}	13.95	116.27 ± 7.67^{c}	19.37	135.99 ± 3.34^{a}	16.78
DMBA/CCl_4 and PSE	94.86 ± 4.52^{ab}	23.14	129.40 ± 5.19^{bc}	32.85	112.35 ± 6.05^{c}	-3.51
DMBA/CCl_4 and PHE	86.27 ± 5.44^{bc}	11.99	157.36 ± 3.01^{a}	61.56	112.66 ± 7.82^{c}	-3.24
DMBA/CCl_4 and PME	101.11 ± 3.32^{a}	31.26	159.74 ± 6.05^{a}	64	132.62 ± 9.16^{ab}	13.89
F-probability	P < 0.01		P < 0.001		P < 0.05	
LSD at 5% level	11.671		18.567		17.985	
LSD at 1% level	15.717		25.005		24.221	

Data are expressed as mean ±mean standard error. Number of animals in each group is six.
Means, which share the same superscript symbol(s) are not significantly different.
Percentage changes were calculated by comparing DMBA/CCl_4-administered control group with normal group (dist. Water) and DMBA/CCl_4-administered treated groups with DMBA/CCl_4-administered control group.

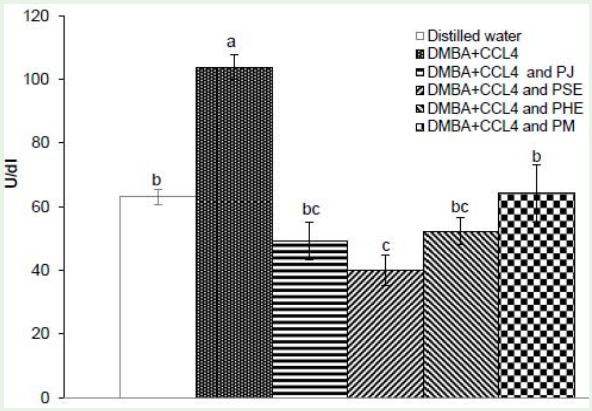


Figure 1: Effect of *P. granatum* juice, seeds aqueous extract, husk aqueous extract and their mixture on AST activity in DMBA/CCl_4-administered rats. Means, which share the same superscript symbol, are not significantly different.

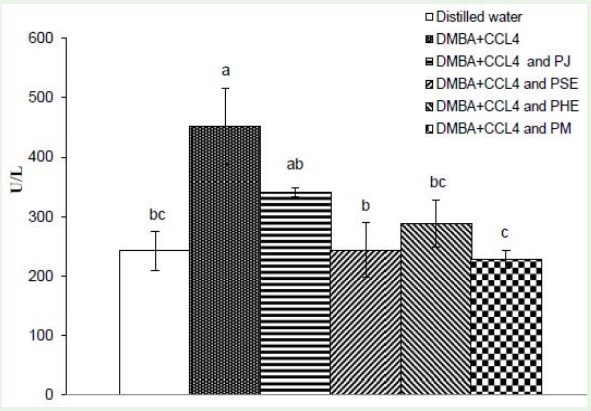


Figure 3: Effect of *P. granatum* juice, seeds aqueous extract, husk aqueous extract and their mixture on total serum CK activity in DMBA/CCl_4-administered rats. Means, which share the same superscript symbol, are not significantly different.

significant change (P>0.05; LSD) of SOD activity. PHE administration highly significantly enhanced (P<0.01; LSD) the activity of GST but it non-significantly increased GPx and SOD activities.

Effects on the activities of serum enzymes related to heart function

The obtained data in figures 1-4 showed a significant elevation (P<0.01; LSD) in the activities of AST, LDH, CK and CK-MB enzymes of DMBA/CCl_4-administered control as compared to the corresponding values of normal group.

The elevated activities of AST, LDH, CK-MB in serum of DMBA/CCl_4-administered rats was highly significantly (P<0.01; LSD) decreased as a result of treatment with PJ, PSE, PHE and PME (Figures 1, 2 and 4). The elevated serum CK activity was significantly improved DMBA/CCl_4-administered rats treated PSE, PHE and PME while it was not significantly affected (p>0.05; LSD) as a result of treatment with PJ (Figure 3).

Effects on the kidney and heart tissues

Concerning photomicrographs of kidney sections, the normal rats exhibited normal architecture and integrity of Bowman's capsule, glomerulus and tubules (Figure 5). On the other hand, the kidney sections of DMBA/CCl_4-administered rats exhibited destructive alterations of kidney tissues as glomerulitis, focal interstitial nephritis, thickening of parietal layer of Bowman's capsule and periglomerular inflammatory cells infiltration. The photomicrographs also depicted necrobiotic changes of epithelial lining renal tubules (Figure 6). Furthermore, examination of kidney tissues of DMBA/CCl_4-administered rats treated with PJ showed only some congestion of glomerular tuft and necrobiotic changes of epithelial lining renal tubules and atrophy of glomerular tuft (Figure 7). The treatment with PSE showed necrobiotic changes of epithelial lining renal tubules (Figure 8). Additionally, animals of group PHE showed no histopathological changes (Figure 9). Treatment of DMBA/CCl_4-injected rats with PME revealed no histopathological changes in kidney tissue (Figure 10).

Examination of stained sections of heart of DMBA/CCl_4-administered animals showed marked deteriorations that appeared as congestion of myocardial blood vessels, multifocal myocarditis, focal hemorrhage and noticed inflammatory cell infiltration (Figures

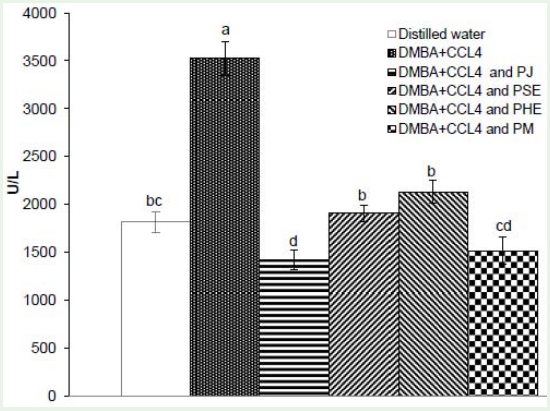


Figure 2: Effect of *P. granatum* juice, seeds aqueous extract, husk aqueous extract and their mixture on serum LDH activity in DMBA/CCl_4-administered rats. Means, which share the same superscript symbol, are not significantly different.

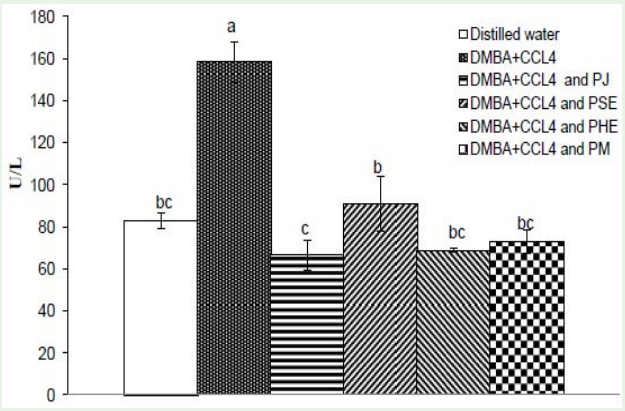


Figure 4: Effect of *P. granatum* juice, seeds aqueous extract, husk aqueous extract and their mixture on serum CK-MB activity in DMBA/CCl_4-administered rats. Means, which share the same superscript symbol, are not significantly different.

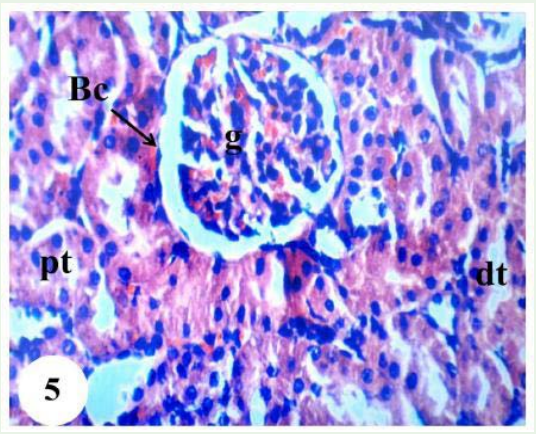


Figure 5: Photomicrograph of kidney section of normal group showed the normal histological structure of the renal parenchyma with Bowman's capsule (Bc), glomerulus (g), proximal tubule (pt) and distal tubule (dt). **(H&E; X 400).**

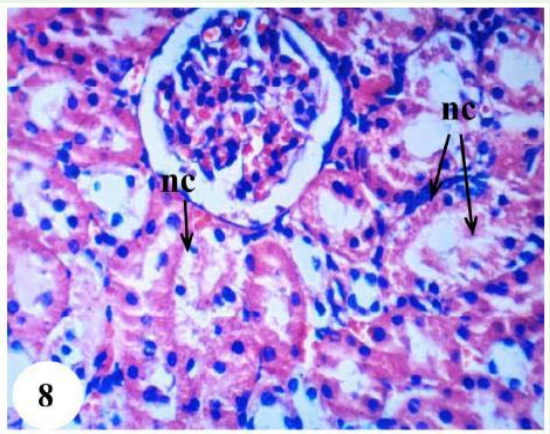


Figure 8: Photomicrograph of kidney section of DMBA/CCl_4-administered rats treated with *P. granatum* seeds extract showed Necrobiotic Changes (nc) of epithelial lining renal tubules. (H&E; X 400).

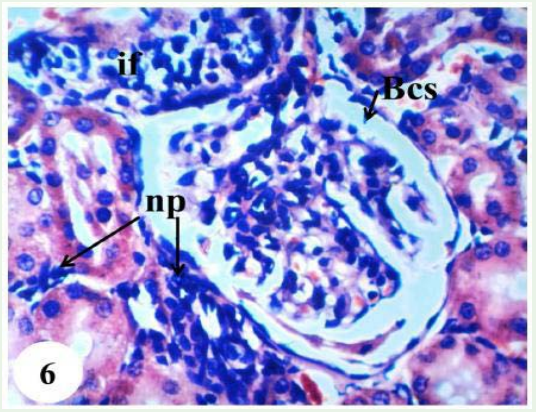


Figure 6: Photomicrograph of kidney section of DMBA/CCl_4-administered rats showed glomerulitis (gt), dilated Bowman's Capsule Space (Bcs) and focal interstitial nephritis (np) with notice inflammatory cells infiltration (if). (H&E; X 400).

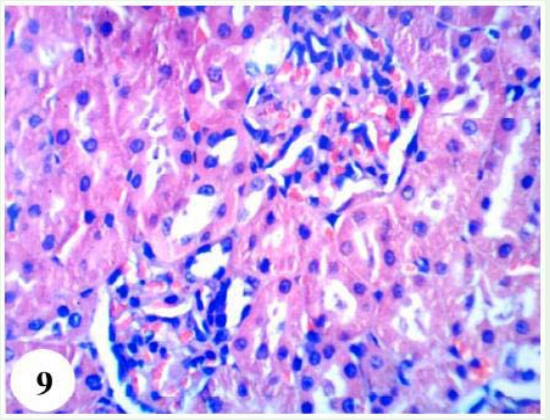


Figure 9: Photomicrograph of kidney section of DMBA/CCl_4-administered rats treated with *P. granatum* husk extract revealed no histopathological changes. (H&E; X 400).

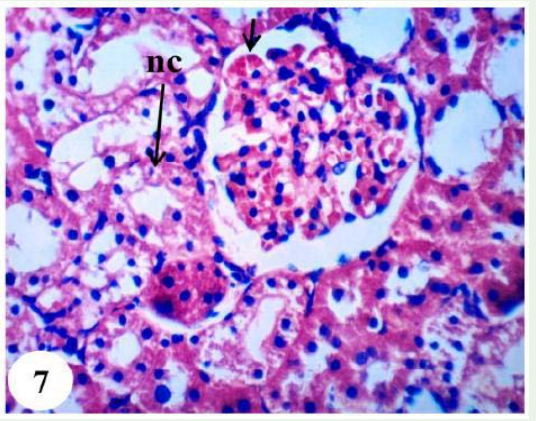


Figure 7: Photomicrograph of kidney section of DMBA/CCl_4-administered rats treated with *P. granatum* juice showed Congestion of Glomerular Tuft (cgt) and Necrobiotic Changes (nc) of epithelial lining renal tubules. (H&E; X 400).

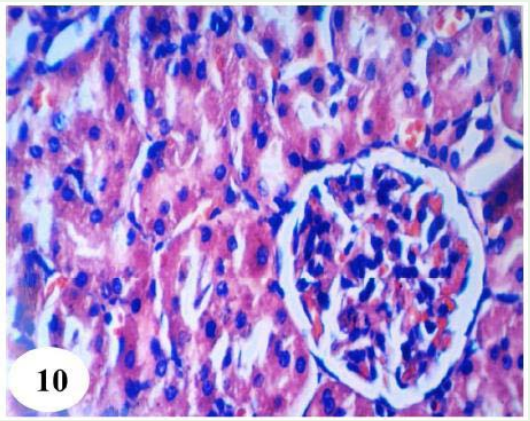


Figure 10: Photomicrograph of kidney section of DMBA/CCl_4-administered rats treated with *P. granatum* mixture of extracts revealed no histopathological changes. (H&E; X 400).

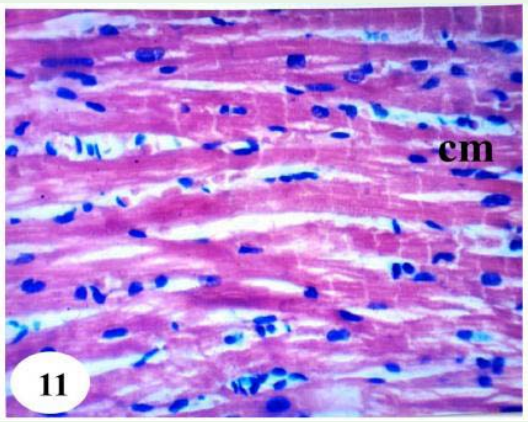


Figure 11: Photomicrograph of heart section of normal group showed the normal texture of heart muscle with cardiac myocytes (cm). (H&E; X 400).

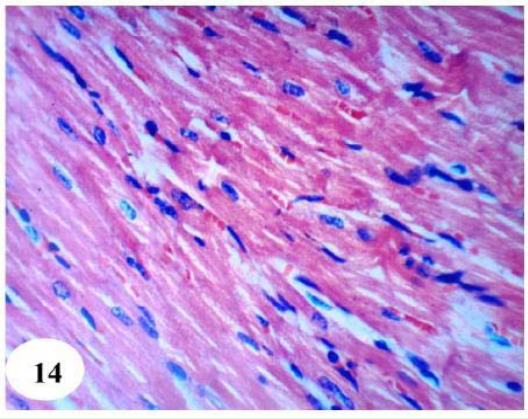
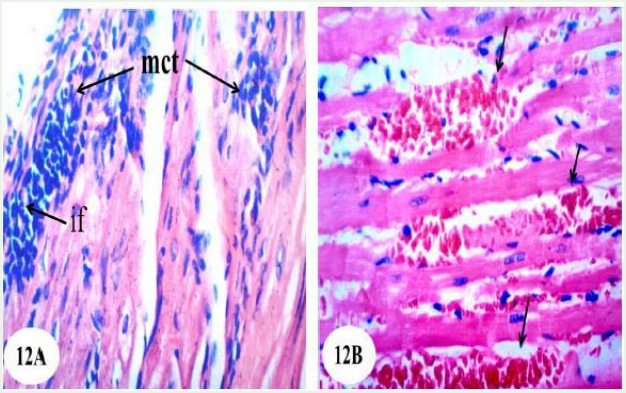


Figure 14: Photomicrograph of heart section of DMBA/CCl_4-administered rats treated with *P. granatum* seeds extract showed no histopathological changes. (H&E; X 400).



Figures 12A & 12B: Photomicrographs of heart sections of DMBA/CCl_4-administered rats showed multifocal myocarditis (mct), inflammatory cell infiltration (if) and congestion of myocardial blood vessels (arrow). (H&E; X 400).

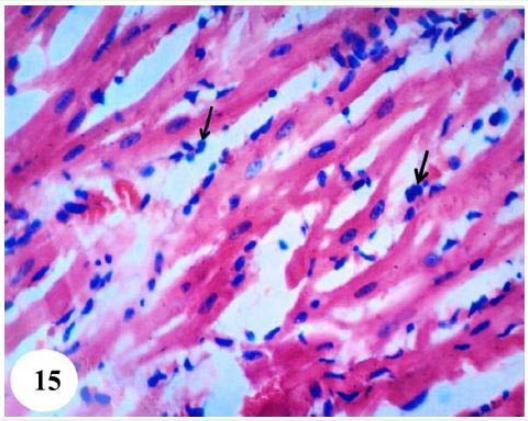


Figure 15: Photomicrograph of heart section of DMBA/CCl_4-administered rats treated with *P. granatum* husk extract showed few inflammatory cells infiltration (if) between cardiac myocytes. (H&E; X 400).

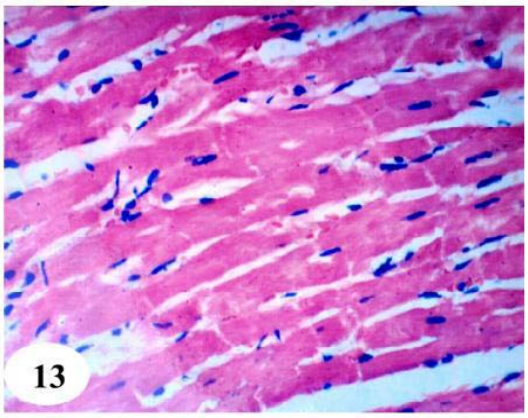


Figure 13: Photomicrograph of heart section of DMBA/CCl_4-administered rats treated with *P. granatum* juice showed no histopathological changes. (H&E; X 400).

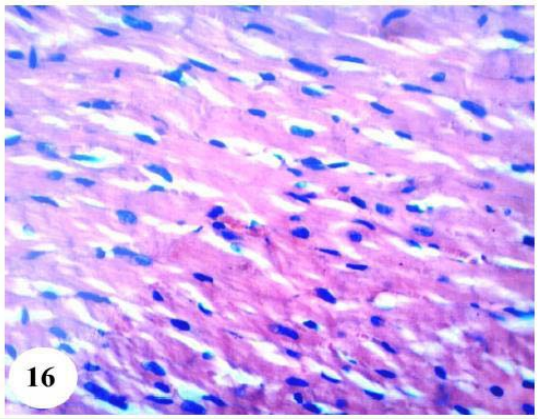


Figure 16: Photomicrograph of heart section of DMBA/CCl_4-administered rats treated with *P. granatum* mixture of extracts and juice showing no histopathological changes. (H&E; X 400).

12A and 12B). DMBA/CCl_4-administered animals treated with PJ and PSE illustrated congestion of myocardial blood vessels in heart tissue and diminutive histopathological changes (Figures 13 and 14). Similarly, DMBA/CCl_4-administered group treated with PHE markedly reduced heart tissue deteriorations; however, there is still few inflammatory cells infiltration between cardiac myocytes as shown in figure 15. In the same way, the treatment of DMBA/CCl_4-administered rats with PME reduced heart tissue deteriorations and improved these deteriorations, so that no histopathological changes appeared in this group as illustrated in figure 16.

Discussion

Administration of DMBA and CCl_4 induced an elevation of serum urea, uric acid and creatinine levels. This biochemical abnormality is one of the clinical manifestations of the renal disorders [48] and reflects renal damage [49]. As so persistently, increased serum creatinine is stated to be a risk factor for progression of chronic kidney disease to kidney failure [50]. These results are in accordance with Dakrory, et al. [51]. Moreover, potassium concentration increased while sodium decreased in serum of DMBA/CCl_4-administered control group which confirmed the nephrotoxic effect of DMBA and CCl_4. Lehotsky, et al. [52] has been showed that, generated ROS could inhibit Na^+, K^+ ATPase of *in vitro* membrane ion transport systems which may be the origin of Na and K variations in serum of DMBA/CCl_4-administered animals. These disturbances of the serum ions revealed kidney dysfunction and toxicity were evidenced by the histopathological examination which depicted destructive alterations of kidney tissues.

In the present study, oxidative stress indices as lipid peroxidation (MDA) and Nitric Oxide (NO) were increased in kidney homogenate which confirmed the suggestion that DMBA and its metabolites are able to generate reactive oxygen species [53]. It is worth mentioning that carbon tetrachloride can damage kidney tissues since it is distributed at higher concentrations in the kidney [54]. It was also stated that CCl_4 shows a high affinity to the kidney cortex which predominantly contains cytochrome P-450 [55]. Accumulation of toxic metabolites of DMBA and CCl_4 exhausted antioxidant defense system markers as GSH, GPx, GST, SOD and Total Antioxidant Capacity (TAC) in kidney tissues of albino rats so that the observed decrease in enzymatic and non-enzymatic antioxidants may be attributed to ROS that diminished the activities of enzymes [56] and causes GSH reduction in kidney tissues. Depletion of GSH molecules that competing ROS led to increasing accumulation of free radicals and generating oxidative stress with a cascade of effects. This imbalance between antioxidants and oxidative stress is disturbing the functional as well as the structural integrity of cell and organelle membranes [57]. These destructive events led to necrobiotic changes and inflammatory cells infiltration as inflammatory response to the chemocytokines in kidney tissue of DMBA/CCl_4-administered group. Treatment of DMBA/CCl_4-administered rats with *P. granatum* juice and fruit extracts (seeds and husk) and their mixture has been shown to exhibit improvement in kidney tissue, normalization of its functions and excess antioxidants content in the renal tissue and decline oxidative stress indicators. These results are in accordance with other early studies revealed the *P. granatum* potent nephroprotective effects against ethylene glycol-induced nephrolithiasis [58] and ferric nitrilotriacetate-induced renal damage [59]. Moreover, it was found that co-treatment of aqueous extract of *P. granatum* attenuated gentamicin-induced renal oxidative damage in rats [60]. The nephroprotective effect of *P. granatum* extracts may be exerted by different mechanisms. One of these mechanisms is the antioxidant property of *P. granatum* during scavenging of free radicals released as outcome of oxidative damage as described in numerous studies [59,61]. This belief also confirmed by remarkable decrease in MDA and NO concomitant with an improvement of kidney tissues of pomegranate juice and fruit extracts.

On the other hand, DMBA and CCl_4 administration led to severe cardiomyopathy as evidenced from the increase in serum activities of cardiac enzymes such as AST, LDH, CK and CK-MP. Leakage of these cardiac specific enzymes serves as diagnostic markers of cardiac damage [62]. Destructive effects of produced ROS induced elevation of serum CK-MB since mechanism of the release of this enzyme seems to be from ongoing myocyte degeneration [63]. DMBA have a high toxicity on heart muscle and can induce neoplasma after being metabolically activated [64]. As well, carbon tetrachloride is a well-known model compound for generation chemical tissue toxicity by creation of free radicals in many tissues [65] such as liver, kidneys, heart, lung, testis, brain and blood [23,66]. These results are greatly reinforced by Eshaghi, et al. [67] who noticed that CCl_4-induced cardiotoxicity in albino rats.

In this study, we find that treatment of DMBA/CCl_4-administered rats with *P. granatum* juice, fruit extracts and their mixture recovered heart tissue and prevent dangerous effects of DMBA and CCl_4. The synergistic consequence of active ingredients normalized the activity of LDH, CK-MB, CK and AST in heart muscle. These results come in agreement with Hassanpour Fard, et al. [14] who used whole fruit extract as a cardioprotective against doxorubicin-induced cardiotoxicity. Treatment with *P. granatum* juice, fruit extracts and their mixture improved heart function and histological integrity via architecture of heart muscle while imparts free radical scavenging and antioxidant effects thereby prevent the myocardium damage [68].

Conclusion

This study can be concluded that *P. granatum* juice and fruit aqueous extracts and their mixture improved kidney and heart tissues and prevent DMBA/CCl_4-induced cardiorenal toxicity by over-coming oxidative stress and associated biochemical and structural distortions.

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