Pomegranate peels ameliorate renal nitric oxide synthase, interleukin-1β, and kidney injury molecule-1 in nephrotoxicity induced by acrylamide in rats

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Background and objectives
Acrylamide (AA) is considered a toxic intermediate product of the Millard reaction and liberated in high-carbohydrate foods exposed to high temperatures. AA formed during baking, frying, roasting, or grilling of such food. Various studies have recorded the toxic effects of AA on many biological functions. The aim of our study is to determine such toxic effect on the kidney and the prophylactic role of pomegranate peels (PP) as a waste portion of the fruit.
Materials and methods
In this study, 60 male albino rats were administered 40 mg/kg body weight AA orally for 17 consecutive days. To evaluate the nephrotoxic effects of AA, some biochemical parameters were measured. Also, 200 mg/kg body weight PP were administered orally as a prophylaxis for 31 consecutive days.
Results and conclusion
In the AA group, alterations in renal function were observed, which was evident from significantly high levels of urea, creatinine, and uric acid. Estimation of serum and urine electrolytes (Na^+, K^+, and Cl^-) showed electrolyte imbalance as well. AA-induced renal oxidative stress was expressed as low levels of renal antioxidants (glutathione, glutathione peroxidase, and superoxide dismutase) and high levels of renal oxidants (malondialdehyde and nitric oxide). To clarify the Pathogenesis of AA nephrotoxicity, estimation of renal nitric oxide synthase and interleukin-1β is carried out showing high significant level. Direct damage in renal tissue is resembled in high level of renal kidney injury molecule-1. As stated before, the administration of AA resulted in acute nephrotoxicity, whereas PP played a vital role in reducing this toxicity. Lower levels of urea, creatinine, and uric acid were observed in the AA+PP group and electrolyte balance was achieved, indicating the prophylactic effect of PP. PP showed antioxidant activity as higher levels of glutathione, glutathione peroxidase, and superoxide dismutase recorded and also lower levels of nitric oxide and malondialdehyde controlling oxidative stress of AA. The levels of kidney injury molecule-1, interleukin-1β, and nitric oxide synthase improved significantly. Finally, we can state that PP could ameliorate the nephrotoxic effect of AA.

Keywords:
acrylamide, heated food, interleukin-1β, nitric oxide synthase, kidney injury molecule-1, nephrotoxicity, pomegranate peels

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Introduction

Thermal food processing is a common practice both in homes and in food manufacture for food microbiological safety and preservation, and for enhancing food texture, color, and taste. One of the food reactions, called the Millard reaction or nonenzymatic browning [1], occurs when rich carbohydrate food is exposed to high temperatures (>120°C) [2]. Millard reaction products (MRPs) produces many intermediate and end products called MRPs; some of these have benefits and others do not [3]. One of the MRPs has received more attention because of its adverse impact on humans: acrylamide (AA) [4]. Human exposure to AA is widespread in the world. Previous studies proved that AA is a neurotoxic compound and also exerts carcinogenic effects on many organs, but in humans, it is considered a probable carcinogen that human studies could not approve it [5].

In 2002, Swedish scientists first reported that AA formed in heated foods (during frying, baking, and roasting of some foods) [6]. The levels of AA in heated food are variable according to eating habits and different cooking methods [7]. Potatoes provides about 50% of human AA intake, 20% is counted for

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baking products and bread and the rest is mainly for roasted coffee [8].

AA may be released into drinking water through water flocculant agents and soil stabilizers; thus, drinking water is considered a probable source of AA human exposure, but food is the main source of AA [9]. WHO and FAO have reported that the average AA intake in humans is in the range of 0.3–0.8 μg/kg body weight/day [1], whereas the European Food Safety Authority (EFSA) [10] has reported that the mean AA exposure is 0.4–1.9 μg/kg body weight/day.

Heating food at high temperature (>120°C) leads to the formation of AA through the reaction between asparagine (amino group) and reducing sugar glucose and fructose (carbonyl group), both of which are found naturally in food. On the basis of the amount of asparagine (the precursor) found in food, the level of AA can be determined [11,12].

Cytochrome P450 2E1 can metabolize AA, resulting in the production of an epoxide derivative called glycidamide, which is oversensitive to attach DNA and proteins (especially hemoglobin) than AA forming adducts [13]. AA and glycidamide (its metabolite) can also be metabolized by attacking glutathione (GSH) with the aid of hepatic GSH-S-transferases [14] and are then excreted as byproducts of mercapturic acid in human urine [15]. In the Zamani *et al.* [16] study, the kinetics of AA at various doses (0.5–100 mg/kg) were assessed after intravenous and oral administration in rats. They found that AA is extensively and rapidly absorbed in the gastrointestinal tract and is broadly distributed to tissues throughout the blood stream. The AA distribution is the highest in the muscle tissue, about 50% of the absorbed dose, followed by the liver, ~20%, and then the gastrointestinal tract, kidney, pancreas, testis, brain, and gallbladder, as well as epithelia of the oral cavity, esophagus, and bronchi [17,18]. Obviously, some of the systemic effects exerted by AA exposure lead to known neurotoxicity, genotoxicity, and carcinogenicity [19], but there is a lack of data on the direct effect of AA on kidneys; however, some studies have reported an association between renal cell carcinoma and dietary AA intake [20].

Recently, the global attention is drawn to natural, active biological, and nontoxic treatments especially those of plants. One of the plants that has received more attention in the last few decades is pomegranate because of the proved potent antioxidant, anti-inflammatory [21], and antimutagenic effects of the fruit besides the nonedible part: peels [22]. Pomegranate peels (PP) weigh about 40–50% of the total fruit weight. It produced by a huge amount through food industries causing a difficulty to get rid of [23]. Pomegranate is used as a natural medicine in several cultures, especially in the Middle East. In Egypt, various common unhealthy conditions such as inflammation, gastrointestinal tract disorders, cough, and infertility are treated by PP [24]. Some studies have confirmed this prophylactic role of PP against nephrotoxicity such as in gentamicin-induced nephrotoxicity [25], hexachlorobutadiene-induced nephrotoxicity [26], mercuric chloride-induced nephrotoxicity [27], and carbon tetrachloride-induced nephrotoxicity [28]. The main benefits of PP have been attributed to its unique bioactive compounds including hydrolyzable tannins, containing gallic acid and ellagic acid esters, punicalin, punicalagin, hexahydroxydiphenic acid (HHDP) and its derivatives, and also caffeic acid, vanillic acid, p-coumaric acid, and quercetin [29].

However, many clinical studies have examined the nephroprotective effects of PP as mentioned before; no studies have focused on the eventual benefits of consumption of PP on AA-induced nephrotoxicity. Therefore, the aim of our study is to investigate the nephrotoxicity of AA and the ameliorating effects of PP as it can be added to food naturally, especially heat-treated high-carbohydrate food.

Materials and methods

Acrylamide and pomegranate peels

AA (99% purity) was purchased from Merck-Schuchardt Chemical Company (Hohenbrunn, Germany). PP were purchased from ordinary Egyptian market. PP were ground and soaked in drinking water from the tap the day before administration and were administered without filtration.

Animals and group designing

The present study was carried out on 60 male Albino rats of 150±30 g body weight purchased from the Animal Health Research Institute, Dokki, Giza, Egypt. The rats were kept under observation for 2 weeks before the experiment for acclimatization. They were housed in nine plastic cages at a room temperature of 22±1°C under a 12-h light–dark cycle. Our study was carried out in accordance with the regulations for the use and care of experimental animals.

During the experiment, rats were Kept on tap drinking water and standard normal chewable diet in a free manner.

After 2 weeks of acclimatization, the rats were divided into three groups (20 rats/group) for 31 days:

Control (Cr) group: 20 rats were maintained on tap drinking water in a free manner for 31 days.

AA group: 20 rats were administered AA dissolved in tap drinking water daily through an oral gavage at a dose of (40 mg/kg body weight according to Ghorbel *et al*. [30] study) during the last 17 days of the experiment.

AA+PP group: 20 rats were administered PP (200 mg/kg body weight according to El-Habibi [31] study) dissolved in tap drinking water daily through an oral gavage separately during the first 14 days of the experiment and with AA (40 mg/kg body weight) during the rest of the experiment. PP was administered half an hour before the administration of AA.

Sample collection

Blood samples were taken through the puncture of the medial acanthus of the eye in a nonheparinized capillary tube. Tubes containing samples were handled gently, then chilled and slanted until centrifugation with a Heraeus centrifuge at 3500 rpm for 15 min for serum separation. Serum samples were distributed into Eppendorf tubes and were kept at −8°C for the subsequent analysis of different biochemical measurements.

All rats were killed after about 13 h of fasting. Abdominal incisions were performed immediately after the blood samples were obtained for separation of kidneys. The kidneys were immediately excised, washed with cold physiological saline (0.9%), and then dried from excess saline using a filter paper. After the kidneys were cut into several parts, some parts of the kidney tissue were placed in glass Packard tubes and kept at −8°C for homogenization.

Urine samples of the Cr group were collected in a metabolic cage, whereas those of the AA group and the AA+PP group were collected after the rats were killed by a sterile syringe directly from the urinary bladder where urine retention was observed (Fig. 1).

Serum biochemical analysis

Renal function tests (urea, creatinine, and uric acid) were estimated according to the methods of Patton and Crouch [32]; Jaffe [33]; and Trinder [34], respectively, to illustrate the nephrotoxic effects of AA and the prophylactic role of PP.

Electrolytes (sodium, potassium, chloride, magnesium, phosphorus, and calcium) were estimated according to the methods of Tietz [35]; Feldikamp and Zklin [36]; Bohuon [37]; Daly and Ertingshausen [38]; and Young *et al*. [39], respectively, to evaluate the renal function and electrolyte balance. All serum measurements were carried out by a spectrophotometer using kits purchased from Egychem Biomed diagnostics Company (Cairo, Egypt).

Urine electrolytes analysis

Urine electrolytes (sodium, potassium, and chloride) were measured directly using a Sensa core electrolyte analyzer ST-200 plus device (Andhra Pradesh, India) with an ion-selective electrode technique to evaluate the role of kidney in electrolyte balance.

Renal homogenate oxidants and antioxidants analysis

Estimation of renal oxidants, malondialdehyde (MDA) and nitric oxide (NO), were determined according to the methods of Satoh [40]; Montgomery and Dymock [41], respectively, and renal antioxidant superoxide dismutase (SOD), GSH, and glutathione peroxidase (GPx) were determined according to the methods of Nishikimi *et al*. [42]; Beutler *et al*. [43]; Paglia and Valentine [44], respectively, were carried out to evaluate the renal oxidative stress induced by AA and antioxidant effect of PP on renal tissue.

All measurements were carried out spectrophotometrically using kits purchased from Biodiagnostics Company (Cairo, Egypt).

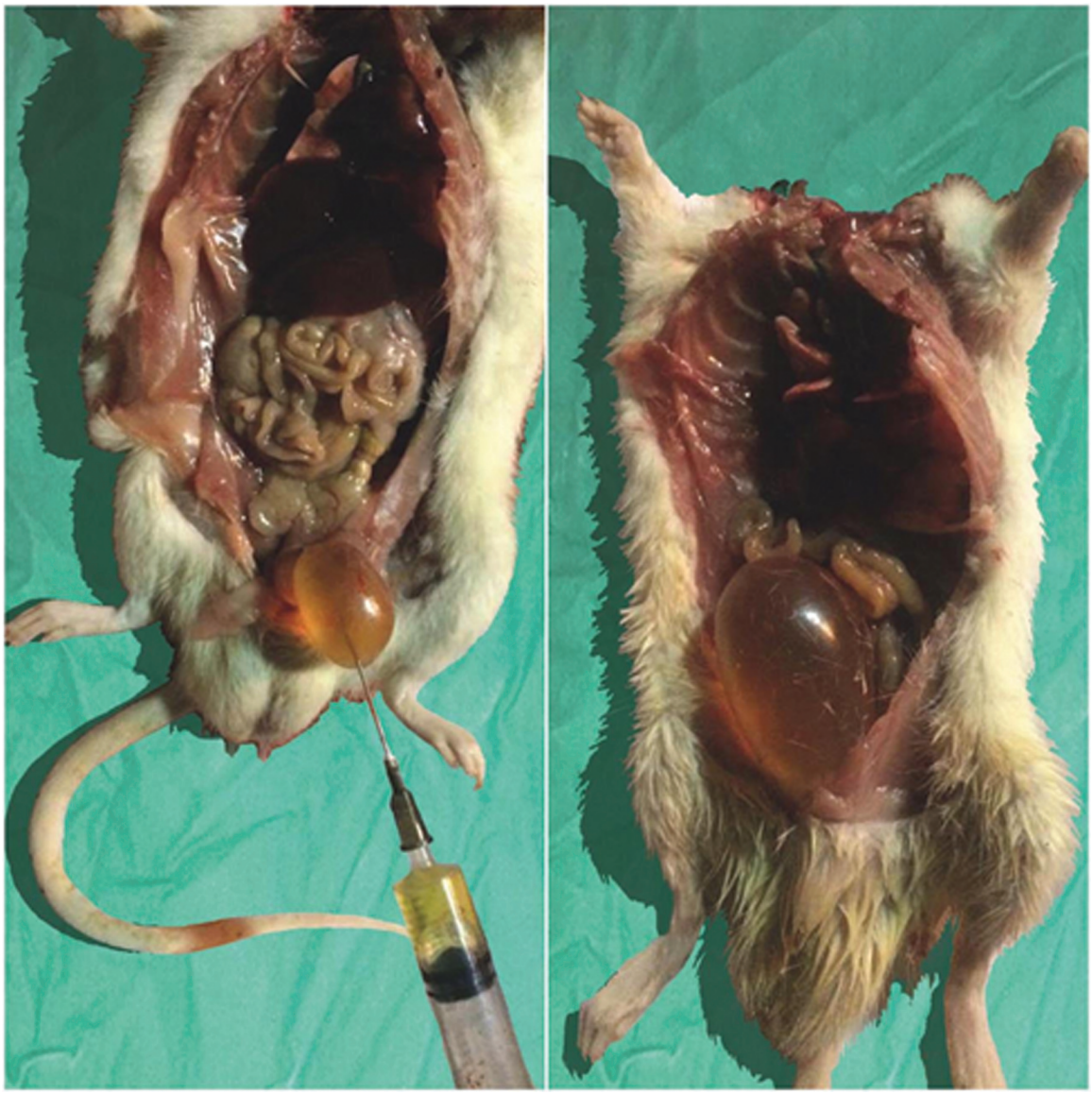
Enzyme-linked immunosorbent assay for the quantitative detection of renal kidney injury molecule-1, nitric oxide synthase, and interleukin-1β

Kidney injury molecule-1 (KIM-1), nitric oxide synthase (iNOS), and interleukin-1β (IL-1β) were measured in kidney homogenate using rat enzyme-linked immunosorbent assay kits according to the manufacturer's protocol (MyBioSource Company, San Diego, California, USA) to evaluate tissue damage, oxidative stress, and inflammation, respectively.

Statistical analysis

Statistical analysis was carried out using GraphPad Instat software (version 3, ISS-Rome, Italy). Unless specified otherwise, groups of data were compared using an unpaired *t* test and one-way analysis of variance (ANOVA), followed by the Tukey–Kramer multiple-comparisons posttest. Values of *P* less than 0.05 were considered to be significant. The data, as

Figure 1



Nervous urine retention in the AA group and collection of urine with a sterile syringe. AA, acrylamide. Motor dysfunction in the AA group (dragging of hind limbs). AA, acrylamide.

indicated clearly, are reported in the tables and figures as mean±SE.

Results and discussion

AA has been classified as a neurotoxic compound as well as a probable carcinogen to humans according to IARC [45]. In our study, AA will be discussed as a nephrotoxic compound. After the administration of AA in rats, motor dysfunction (hind limbs dragging Fig. 1) and coordination impairment were observed. In PM examination, a distended urinary bladder was observed which is called 'nervous retention,' explained by Erkekoglu and Baydar [46] as an extent of neurotoxic effect of AA.

Measurements of urea, creatinine, and uric acid are initial and important markers of kidney function. A highly significant increase in serum urea (48.4±1.503 at $P<0.001$), creatinine (1.56±0.06 at $P<0.001$), and uric acid (7.2±0.35 at $P<0.001$) concentrations was observed in the AA group compared with the Cr group in (Table 1). These results are in agreement with those of Ghorbel *et al.* [30] and Uthra *et al.* [47] as Wistar rats administered 40 mg/kg body weight AA orally for 20 and 10 days, respectively, showed increased levels of urea ($P<0.001$), uric acid ($P<0.001$), and creatinine ($P<0.001$). Raju *et al.* [48], in their AA intoxication study, attributed the increase in serum urea, creatinine, and uric acid to a decrease in glomerular filtration.

Table 1 Serum urea, creatinine, and uric acid (mg/dl) concentrations of the control group, the acrylamide group, and the acrylamide+pomegranate peels group at the end of the experiment (31st day)

	Cr –ve	AA group	AA+PP group
Urea (mg/dl)	30.6 ±1.503	48.4 ±1.503***[a]	33.8±1.28***[b]
Creatinine (mg/dl)	0.57±0.13	1.56±0.06***[a]	1.06±0.06**[a,b]
Uric acid (mg/dl)	3.38±0.16	7.2±0.35***[a]	4.38±0.17*[a,***b]

N=20, values were statistically analyzed by the one-way analysis of variance test, followed by the Tukey–Kramer posttest and reported as mean±SE. AA, acrylamide; PP, pomegranate peels. [a]versus control, [b] versus acrylamide at ****P* value less than 0.001, ***P* value less than 0.01, and **P* value less than 0.05.

Table 2 Serum electrolyte [phosphorus (mg/dl), magnesium (mg/dl), calcium (mg/dl), sodium (mEq/l), potassium (mEq/l), and chloride (mEq/l)] concentrations of the control group, the acrylamide group, and the acrylamide+pomegranate peels group at the end of the experiment (31st day)

	Cr –ve	AA group	AA+PP group
Phosphorus (mg/dl)	5.28±0.1	6.18±0.09***[a]	5.24±0.07***[b]
Magnesium (mg/dl)	3.92±0.12	5.08±0.1***[a]	4.3±0.16**[b]
Calcium (mg/dl)	9.34±0.36	8.58±0.15	9.54±0.19*[b]
Sodium (mEq/l)	141±1.18	130.8±1.16***[a]	143±1.61***[b]
Potassium (mEq/l)	4.36±0.13	5.54±0.25**[a]	4.64±0.19*[b]
Chloride (mEq/l)	75.8±3.9	119.6±6.3***[a]	90.2±2.8***[b]

N=20, values were statistically analyzed by the one-way analysis of variance test followed by the Tukey–Kramer posttest and reported as mean±SE. AA, acrylamide; PP, pomegranate peels. [a]versus control, [b]versus acrylamide at ****P* value less than 0.001, ***P* value less than 0.01, and **P* value less than 0.05.

For more confirmation, estimation of serum and urine electrolytes was carried out, showing electrolyte imbalance as mentioned before in (Tables 2, 3), which is marked by hyponatremia (130.8±1.16 at $P<0.001$) and hyperkalemia (5.54±0.25 at $P<0.01$), indicating renal insufficiency. In contrast to the AA group, we observed an improvement in kidney function and electrolyte imbalance in the AA+PP group, indicating the nephroprotective effect of PP.

In our study, we support two hypotheses. The first hypothesis is affecting cellular redox state, oxidant/antioxidant imbalance as AA either forms conjugates with GSH, yielding N-acetyl-S- (3-amino-3-oxopropyl) cysteine, or reacts with cytochrome P450 to produce the major metabolite glycidamide [49]. Glycidamide further forms conjugates with GSH [50]. During higher intake of AA, more GSH consumed in AA conjugation leading to GSH depletion, which was observed in our study (20.4 ±0.93 at $P<0.001$). However, we observed an increase in cellular oxidants through high levels of MDA (136.4±9.02 at $P<0.001$) and NO (213.5±6.1

Table 3 Urine electrolyte [sodium (mmol/l), potassium (mmol/l), and chloride (mmol/l)] concentrations of the control group, the acrylamide group, and the acrylamide+pomegranate peels group at the end of the experiment (31st day)

	Cr –ve	AA group	AA+PP group
Na^+ (mmol/l)	31±11	44.9±3.01**[a]	29.69±2.41**[b]
K^+ (mmol/l)	121±31	106.1±3.72***[a]	116.04±3.64**[b]
Cl^- (mmol/l)	148±36	125.44±6.43***[a]	140.83±10.44***[b]

N=20, values were statistically analyzed by the one-way analysis of variance test, followed by the Tukey–Kramer posttest and reported as mean±SE. AA, acrylamide; PP, pomegranate peels. [a]versus control, [b]versus acrylamide at ****P* value less than 0.001 and ***P* value less than 0.01.

Table 4 Kidney homogenate oxidants and antioxidant: malondialdehyde (μmol/g tissue), nitric oxide (μmol/g tissue), glutathione (mg/g tissue), glutathione peroxidase (μmol/g tissue), and superoxide dismutase (μmol/g tissue) concentrations of the control group, the acrylamide group, and the acrylamide+pomegranate peels group at the end of the experiment (31st day)

	Cr –ve	AA group	AA+PP group
MDA (μmol/g tissue)	69.12±1.7	136.4 ±9.02***[a]	74.8±4.08***[b]
NO (μmol/g tissue)	103±9.4	213.5±6.1***[a]	131±8.86**[b]
GSH (mg/g tissue)	55.2±3.33	20.4±0.93***[a]	61.4±2.06***[b]
GPx (μmol/g tissue)	22.96 ±0.41	13.62 ±0.39***[a]	25.86±0.92*[a,***b]
SOD (μmol/g tissue)	5.12±0.38	1.44±0.16***[a]	4.8±0.28***[b]

N=20, values were statistically analyzed by the one-way analysis of variance test, followed by the Tukey–Kramer posttest and reported as mean±SE. AA, acrylamide; GPx, glutathione peroxidase; GSH, glutathione; MDA, malondialdehyde; NO, nitric oxide; PP, pomegranate peels; SOD, superoxide dismutase. [a]versus control, [b] versus acrylamide at ****P* value less than 0.001, ***P* value less than 0.01, and **P* value less than 0.05.

at $P<0.001$) as shown in Table 4, indicating renal lipid peroxidation and oxidative stress.

The Bao *et al.* [51] study explained these effects by stating that AA affects the redox state of the renal tissue, causing oxidant/antioxidant disproportion in favor to oxidants, resulting in the subsequent excessive production of reactive oxygen species (ROS), which causes lipid peroxidation and high levels of its end product, MDA. Lipid peroxidation affects cell membrane integrity and permeability, resulting in impairment of cellular vitality ended with apoptosis [52]. Also, NO, the second estimated oxidant, causes cellular apoptosis as NO has an unpaired electron; thus, it reacts with superoxide (one of ROS), producing peroxynitrite anion that oxidizes sulfhydryl groups and thioesters, causing DNA damage [53]. The superoxide is dismutated by SOD into H_2O_2 (one of ROS), which in turn can be eliminated through reduction reaction by aid of GPx enzyme and GSH as a substrate producing H_2O [54].

Table 5 Kidney homogenate nitric oxide synthase (ng/ml homogenate), interleukin-1β (pg/ml homogenate), and kidney injury molecule-1 (pg/ml homogenate) concentrations of the control group, the acrylamide group, and the acrylamide +pomegranate peels group at the end of the experiment (31st day)

	Cr –ve	AA group	AA+PP group
iNOS (ng/ml homogenate)	0.72 ±0.11	6.14±1.2***[a]	1.872±0.25**[b]
IL-1β (pg/ml homogenate)	33.5±1.2	125.62 ±5.1***[a]	66.7±8.01**[a], ***[b]
KIM-1(pg/ml homogenate)	37.7±1.8	192.24 ±7.7***[a]	88.76 ±7.9***[ab]

N=20, values were statistically analyzed by the one-way analysis of variance test, followed by the Tukey–Kramer posttest and reported as mean±SE. AA, acrylamide; IL-1β, interleukin-1β; KIM-1, kidney injury molecule-1; iNOS, nitric oxide synthase; PP, pomegranate peels. [a]versus control and [b] versus acrylamide at ****P* value less than 0.001 and ***P* value less than 0.01.

As shown in Table 4, SOD (1.44±0.16 at $P<0.001$) and GPx (13.62±0.39 at $P<0.001$) decreased significantly; besides the previous noticed GSH depletion causing cellular antioxidant decline and more production of ROS.

In conclusion, oxidative stress resembled in GSH depletion and excessive ROS production is the key factor of AA nephrotoxicity. These results are in agreement with those of Abdel-Daim *et al.* [55]; El-Beltagi and Mahgoub [56] as 20 mg/kg body weight of AA administered in rats orally for 14 and 30 days, respectively, led to decreased levels of GSH ($P<0.05$), GPx ($P<0.05$), and SOD ($P<0.05$). An elevated renal MDA level ($P<0.001$) was recorded by Alturfan *et al.* [57] after the administration of 40 mg/kg body weight AA in rats for 10 days.

Our results showed a highly significant increase in the renal IL-1β level (125.62±5.1 at $P<0.001$), iNOS activity (6.14±1.2 at $P<0.001$), and NO level (213.5 ±6.1 at $P<0.001$), indicating the inflammatory effects of AA. In agreement with our results, Abdel-Daim *et al.* [55] reported high levels of serum IL-1β ($P<0.05$) and renal NO ($P<0.05$) after the administration of 20 mg/kg body weight AA orally in rats for 14 consecutive days. Also, an elevated renal NO ($P<0.05$) level was observed by Aydemir *et al.* [58] after the oral administration of AA in rats by 40 mg/kg body weight for 10 days; however, no study detected renal iNOS activity with AA toxicity.

The Pan *et al.* [59] study attributed the inflammatory effects of AA to GSH depletion and the subsequent increase in intracellular ROS, resulting in the activation of IL-1β production, which is a vital stimulator of the nuclear factor-κB (NF-κB) signaling pathway, inducing an inflammatory response. Also, Zamani *et al.* [16] reported that the increase in NF-κB expression that was induced downstream to the inflammatory effect of AA activated iNOS production and subsequently activated NO generation. On the basis of these evidences, we attempted to clarify the role of the NF-κB signaling pathway in AA nephrotoxicity through determination of IL-1β (one of NF-κB direct stimulators) and iNOS (one of the downstream expressions of the NF-κB pathway). NF-κB is an inducible transcriptional factor activated in stressed cells undergoing cellular apoptosis and also regulates immune and inflammatory responses of the cells [60]. Under normal cellular conditions, NF-κB proteins bound to IκB proteins, forming an inhibitory complex localized in the cytoplasm, blocking nuclear localization. Under stressed or inflamed cellular conditions, nuclear translocation of NF-κB proteins occurred [61]. IL-1β plays a crucial role in this translocation, directly stimulating the NF-κB signaling pathway. IL-1β aids in the phosphorylation of IkB kinases (IKK), converting them into the active form. IKK are responsible for the phosphorylation and ubiquitination of IκB proteins, followed by subsequent degradation separating from NF-κB proteins resulting in nuclear translocation [59]. However, IL-1β activates the NF-κB pathway and also induces the expression of the IL-1β gene, resulting in excessive IL-1β production and so on [62]. With continuous stimulation of the NF-κB pathway, stimulation of iNOS gene expression occurred, leading to excessive production of the iNOS enzyme [63]. Expression of iNOS accompanied by inflammatory conditions resulted in liberating large amounts of NO [64]. Finally, our results support the two hypotheses that AA-induced nephrotoxicity is related to GSH depletion and NF-κB expression as discussed before.

KIM-1 is a newly recognized protein that is released only in damaged renal tissue; thus, it is considered a specific and sensitive marker for proximal tubule injury [65]. KIM-1 is a type-1 transmembrane protein that consists of two portions: a short cytoplasmic tail called endodomain and ectodomain, which has an Ig-like domain and a glycosylated mucin domain [66]. It plays a vital role in apoptotic condition that KIM-1 acts as a receptor for dead cells as well as oxidized lipoprotein, converting normal tubular renal cells into semiprofessional macrophages, in which aid tissue repair [67]. Therefore, it is logically liberated only in damaged tissue according to its function. In our study,

we evaluated the possible role of KIM-1 in association with AA-induced renal apoptosis, which has not been reported before. On the basis of the results shown in Table 5, we observed a significant increase in the level of KIM-1 (192.24±7.7 at $P<0.001$) in the AA group compared with the Cr group (37.7±1.8), indicating renal tubular injury in the AA group.

In our study, AA causes nephrotoxicity but administration of PP could ameliorate such toxicity. As shown in Table 4, an increase in the renal antioxidant levels, GSH (61.4±2.06 at $P<0.001$), SOD (4.8±0.28 at $P<0.001$), and GPx (25.86±0.92 at $P<0.001$), a reduction in the oxidant NO level (131 ±8.86 at $P<0.01$), and a reduction in lipid peroxidation (lowered MDA level (74.8±4.08 at $P<0.001$) indicate the antioxidant effects of PP. The same results were obtained by Ahmed and Ali [68], and Abdel Moneim *et al*. [69] after the administration of 200 mg/kg body weight PP orally for 7 consecutive days in rats to improve renal oxidative damage with a significant level ($P<0.05$). Also, Karwasra *et al*. [70] obtained the same results with a significant level ($P<0.001$) after the administration of the same dose of PP, but for a longer duration in cisplatin-treated rats in addition to low levels of serum creatinine ($P<0.001$) and urea ($P<0.001$), in agreement with our results.

The antioxidant activity of Egyptian PP is counted for 96.24% which determined by Ashoush *et al*. [71] through scavenging 1, 1-diphenyl-2-picrylhydrazyl free radical (DPPH). However, Ibrahium [72] determined the Egyptian PP biological phytochemicals using high-performance liquid chromatography recording the following measurements: total phenolic compounds (867 mg/g) with the most highly component punicalagin (296 mg/g) followed by gallic acid and contained flavonols (quercetin and kaempferol). Punicalagin can be hydrolyzed to punicallin, another ellagitannin, and both can be hydrolyzed to egallic acid under certain conditions such as intestinal microbial fermentation.

All are reported to have a range of biological activities, involving antioxidant and anti-inflammatory activities [73]. The free radical scavenging activity of PP phenolics depends on electron donation to free radicals, converting them into more relative stable compounds [74]. Also, PP could ameliorate renal inflammation induced by AA. This is evidenced by lower levels of renal IL-1β (66.7±8.01 at $P<0.001$), iNOS (1.872±0.25 at $P<0.01$), and NO (131±8.86 at $P<0.01$) after the administration of PP as shown in (Table 5). The ameliorative effect of PP was explained by Dell'Agli *et al*. [75]; Iris and Sissi [76] as punicalagin and its hydrolyzed compounds inhibit the NF-κB pathway through blocking NF-κB-driven transcription, which in turn decreases iNOS production and subsequent NO. In Lee *et al*. study [77] punicalagin and punicalin caused potent inhibition of iNOS activity was recorded. Also, El-Beltagi and Mahgoub [56] study refered to the antioxidant activity of quercetin, one of PP flavonols, in AA toxicity reducing level of NO through scavenging NO radical (electron donation) and inhibition of NF-κB pathway. Viladomiu *et al*. [78] attributed NF-κB downregulation induced by pomegranate to inhibition of IKK and subsequent IκB degradation, preventing NF-κB nuclear translocation.

NF-κB regulates the transcription of multiple pro-inflammatory molecules; thus, its inhibition will inhibit the inflammatory cascade, clarifying the anti-inflammatory role of PP [79]. KIM-1 also shows a significant decrease (88.76±7.9 at $P<0.001$) in the AA +PP group, indicating the nephroprotective effect of PP. The antioxidant and anti-inflammatory activities of PP elucidate the improved results of AA toxicity in our study. Therefore we support the saying of 'Plants afford a cheap source of medicine for majority of human beings' [80].

Conclusion

AA 40 mg/kg body weight induced elevated renal KIM-1, iNOS, and IL-1β, possibly by GSH depletion, oxidative stress, and NF-κB activation. PP 200 mg/kg body weight showed antioxidant and anti-inflammatory effects, ameliorating AA nephrotoxicity through its potent phenolic compounds, possibly by scavenging activity of free radicals, maintenance of antioxidants, and inhibition of the NF-κB pathway. Therefore, supplementation with PP can be useful in individuals who are exposed to AA toxicity.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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