Protective Effect of Pomegranate Juice Against Radiation-Induced Histological and Ultra structural Changes in Kidney of Male Albino Rats.

Rezk RG
Health Radiation Research Department, National Center for Radiation Research and Technology

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ABSTRACT

Ionizing radiation is a potent mutagenic and carcinogenic agent due to the liberation of free radicals. It is therefore essential to research for radioprotective measures. The aim of the present study is to evaluate the effect of pomegranate juice on radiation-induced damage in kidney tissues.

Male albino rats were whole body gamma irradiated with 5 Gy delivered as a single shot dose. Pomegranate juice was orally administered to rats (200 ml /Kg body weight) for 60 consecutive days before irradiation. Histopathological investigations of kidney tissues showed with light microscope (x400) showed different distortion in the renal corpuscles and renal convoluted tubular epithelial cells. These distortions varied from swelling, vaculation to necrosis and complete degeneration of the epithelial cells of proximal and distal tubules. The kidney glomeruli were shrunken and obvious lesions in the fine structures of renal tissue were detected by electron microscopy (x 6000) as swelling and crystalysis of mitochondria. The rough endoplasmic reticulum exhibited various degrees of damage, dilatation, fragmentation, degranulation and destruction. Lysosomes were abundant and destruction of the brush border was evident. The nucleus showed irregular nuclear membrane besides clumped marginated chromatin. Oral administration of pomegranate juice before irradiation exerted marked improvement of the histological and ultrastructural changes induced by gamma irradiation. It could be concluded that pomegranate juice might protect kidney tissues from radiation-induced histological and ultrastructural damage due to its antioxidant activity and its capacity to scavenge free radicals and decrease oxidative stress.

Key words: Pomegranate juice, gamma radiation, kidney, rats.

INTRODUCTION

Antioxidants and nutrients play beneficial role in free radical scavenging thus sustaining health and preventing chronic and degenerating diseases and aging syndromes\(^1\). Phenolic compounds are important components of many fruits, vegetables and beverages contributing to their color and sensory properties. Epidemiological studies have demonstrated that the composition of phenol-rich food retards the progression of arteriosclerosis and reduces the incidence of heart diseases by preventing oxidation stress\(^2\).

The ‘superfruit’ pomegranate is gaining tremendous importance because of its potent antioxidant properties attributed primarily to polyphenolic constituents, with anthocyanins being one of the most important\(^3\). Anthocyanin pattern consists of four major peaks corresponding to delphinidin-3,5-diglucoside, delphinidin-3-glucoside, cyanidin-3,5-diglucoside, and cyanidin-3-
glucoside. Minor peaks corresponding to pelargonidin-3,5-diglucoside and pelargonidin-3-glucoside. These components along with hydrolyzable tannins (ellagitannins and gallotannins) and condensed tannins (proanthocyanidins) could contribute in some way to the antioxidant activity of pomegranate juice.

The antioxidant activity of pomegranate surpasses that of red wine, green tea, tomatoes and vitamin E. Pomegranate extracts and constituents exert numerous beneficial activities, e.g. protection against and/or treatment of cancer, neurological damages, inflammation, ulcers, diabetes, dental disorders, high cholesterol, cardiovascular disease, obesity, bacterial infections, erectile dysfunction and male infertility. Pomegranate suppresses the proliferation of human prostate, breast, lung and colon cancer cells in vitro. In vitro studies have also shown that pomegranate products prevent and/or reduce chemically induced tumors in skin. Pomegranate juice clean up free radicals, lessen oxidative damage and protects the kidney against oxidative toxicity.

The kidney has been reported as an important dose-limiting organ. Experimental studies demonstrated that kidney irradiation clearly leads to a progressive reduction in function associated with glomerulosclerosis and/or tubulointerstitial fibrosis associated with a chronic and persistent oxidative stress. In view of these observations, the present study has been undertaken for the assessment of the role played by pomegranate juice for reducing the radiation injury on the histological as well as ultrastructural alterations of rat’s kidney tissue.

MATERIAL AND METHODS

Experimental animals:

Male Swiss albino rats were used with a body weight ranged between 120-140 g purchased from the Egyptian Organization for Biological Products & Vaccines. All animals were kept at room temperature and fed on a well balanced diet and supplied with water ad libitum.

Radiation facility:

Whole body gamma irradiation was performed with a Canadian Gamma cell-40 (137Cs) at the National Center for Radiation Research and Technology, Cairo, Egypt, at a dose rate 0.64 Gy/min. Animals were exposed to 5Gy applied as a single shot dose.

Pomegranate juice treatment:

Pomegranate juice (Punica granatum) was obtained from local market. Animals received pomegranate juice by gavage 200ml/Kg body weight daily for 60 consecutive days before irradiation according to Sentikumaran et al., (2012).

Experimental design:

Animals were divided into 4 groups of 6 rats each. Control: rats neither exposed to radiation nor treated with pomegranate juice. Pomegranate: rats received 200ml/Kg body weight pomegranate juice for 60 consecutive days. Irradiation: rats exposed to 5Gy whole body gamma irradiation. Pomegranate + Irradiation: rats receiving pomegranate juice (200ml/Kg body weight for 60 consecutive days) before irradiation, the last dose given 60 minutes before irradiation. Six rats of each group were sacrificed on the 3rd day after irradiation, and kidneys were quickly removed.

Histological investigations:

Kidney slices were perfused with phosphate buffer (pH7.2), well washed then fixed in neutral formalin, dehydrated in graded ethanol, cleared and embedded in parablast. Sections of 5 micrometers.
thickness were stained with haematoxylin and eosin. Sections were examined by Olympus light microscope to detect the histological changes induced by any of the above treatment (Humanson, 1979).

Electron microscopy investigations:

Small samples of rats’ kidney were fixed in glutaraldehyde followed by osmium tetroxide and processed for electron microscopy according to Hayat (1972). Sections were stained with uranyl acetate and lead citrate according to Reynolds (1963) and examined at 70 kv in electron microscope (Joel JEM100cx) belonging to Ain Shams University.

RESULTS

Histological examination using light microscope revealed that the kidney structures of rats of control and pomegranate groups are of normal architecture (Figures 1 and 2).

Rats irradiated with 5 Gy, showed abnormal architecture of kidney manifested by collapsed glomeruli, necrotic and degenerated renal tubular epithelial cells and widened subcapsular space. Also the cortical region showed large hemorrhagic area with fibroblastic invasion between the degenerated tubules. Most of the convoluted tubules appeared necrotic, some nuclei appeared pyknotic and the glomerular tuft was expanded (Figures 3 and 4).

Rats receiving pomegranate juice before irradiation showed that most of the glomeruli and renal convoluted tubules tended to be normal (Figures 5 & 6).

Through transmission electron microscopy, the cells of proximal convoluted tubules of control and pomegranate rats showed normal structure (Figures 7, 8, 9 and 10). Brush borders are observed at the apical surface of the cells. The cytoplasm contains numerous rod-shaped mitochondria which were longitudinal in the basal part of the cytoplasm. Rough endoplasmic reticulum is observed scattered in the cytoplasm with few lysosomes. The Golgi complex was usually found close to the nucleus and adjacent to the nuclear envelop and it also showed some chromatin clumping in nucleus. The nucleus showed round pattern and the luminal was provided with short microvilli.

Animals exposed to a single dose of whole body gamma irradiation at a dose level 5 Gy and sacrificed on the 3rd day showed degenerated brush border (Figure 11) beside active lysosomes, spherical swollen mitochondria and atrophied nucleus. The lumina of the distal convoluted tubules were constricted and filled with debris derived from dissociated microvilli, dissociated apical plasma membrane and extrusion of cytoplasmic inclusion of cells (Figure 12). Swollen mitochondria with markedly deteriorated cristae, complete destruction in the basal enfolding and irregular nuclear membrane, severe damages of brush border, atrophied nuclei were also seen. Furthermore, the mitochondria were swollen with crystalysis and fused limiting membrane and also the cisternae of the rough endoplasmic reticulum were dilated and scattered throughout the cytoplasm.

Administration of pomegranate juice at dose 200 ml/Kg b wt 60 days prior to irradiation revealed great amelioration in the renal damage induced by gamma irradiation at 5 Gy. Most glomeruli and renal convoluted tubules were approximately normal and a normal renal architecture pattern was observed (Figures 13 and 14). Investigation with electron microscope proved that the epithelial cells lining the cortical tubules were mostly normal in their appearance. They exhibited the common cytoplasmic electron density and normal cell organelles. The nuclei in certain cells exhibited regular shape and normal structural appearance. The basal enfold membrane appeared normal in most of the cells.
Fig 1: Photomicrograph of a section in the renal cortex of control rat showing normal renal cortex architecture with normal 1) glomeruli 2) glomerular capsule 3) urinary space between the glomerulus and the capsule 4) proximal convoluted tubules 5) distal convoluted tubules 6) macula densa. (H&E) (X 400).

Fig 2: Photomicrograph of a section in the renal cortex of pomegranate juice treated rats showing normal renal cortex architecture with normal 1) glomeruli 2) glomerular capsule 3) urinary space between the glomerulus and the capsule 4) proximal convoluted tubules 5) distal convoluted tubules 6) macula densa. (H&E) (X 400).
Fig 3: Photomicrograph of a section in the renal cortex 3rd day post-irradiation at 5Gy showing 1) wide space between the glomerulus and the capsule 2) degenerated tubules and necrotic tissue with infiltrating cells (H&E) (X 400).

Fig 4: Photomicrograph of a section in the renal cortex 3rd day post-irradiation at 5Gy showing 1) severe haemorrhage in both glomeruli and tubules with fibroblastic invasion between degenerated tubules 2) glomerulus sclerosis and fibrosis 3) degenerated distal and proximal tubules (H&E) (X 400).
Fig 5: Photomicrograph of a section in the renal cortex of rats administered pomegranate juice pre-irradiation showing 1) improved glomeruli structure 2) regeneration of distal and proximal convoluted tubules (H&E) (X 400).

Fig 6: Photomicrograph of a section in the renal cortex of rats administered pomegranate juice pre-irradiation showing 1) well-defined structure of glomeruli 2) some proximal and distal convoluted tubules were regenerated and 3) some still with ill-defined structure. (H&E) (X 400).
Fig 7: Electron micrograph of part of the proximal convoluted tubules cell of a control rat kidney showing brush border (bb) at its apical surface and deep infolding (be) at the base of the cell enclosing elongated mitochondria (m), normal lysosomes (Ly). The nucleus (n) is large & rounded (X6000).

Fig 8: Electron micrograph of part of the distal convoluted tubules cell of a control rat kidney showing elongated mitochondria (m) and deep infolding of the plasma membrane (be) and rounded nucleus (n). (X6000).
Fig 9: Electron micrograph of part of the proximal convoluted tubules cell of a rat kidney treated with pomegranate for 60 days showing brush border (bb) at its apical surface. Elongated mitochondria (m), normal lysosomes (ly), and normal basement membrane (bm). The nucleus (n) is large & rounded (X6000).

Fig 10: Electron micrograph of part of the distal convoluted tubules cell of a rat kidney treated with pomegranate for 60 days showing short microvilli (mv) at its surface, elongated mitochondria (m) and deep infolding of the plasma membrane (pm) and rounded nucleus (n). (X6000).
Fig 11: Electron micrograph of part of the proximal convoluted tubules cells of rat kidney 3rd day post - irradiation at 5Gy showing dissociation of brush border (bb), electron dense & sticking mitochondria (m) with cristalisis, nuclear envelope degeneration of the basal infolding (be), prominent active lysosomes (ly), lipid granular (g), atrophied nucleus (n), irregular nuclear envelope, absence of Golgi apparatus. (X6000).

Fig 12: Electron micrograph of part of the distal convoluted tubules cell of a rat kidney 3rd day post - irradiation at 5Gy showing degeneration of the most cytoplasmic organelles, abnormal mitochondria (m), damaged microvilli (mv), abnormal nucleus (n) with irregular nuclear membrane and clumped chromatin, degeneration of the basal infolding (be), absence of Golgi apparatus (X6000).
Fig 13: Electron micrograph of part of the proximal convoluted tubules cells of a rat kidney treated with pomegranate juice before whole body gamma – irradiation at 5 GY showing amelioration of architectural tubular cells, normal nucleus (n), normal mitochondria (m) and intact basement membrane (bm) with normal basal infolding, normal brush border (bb) and normal lysosomes (ly) (X6000).

Fig 14: Electron micrograph of part of distal convoluted tubules cell of a rat kidney treated with pomegranate juice before whole body gamma – irradiation at 5 GY showing improvement of architectural tubular cells, normal intracellular organelles with large numbers of elongated mitochondria (m), normal nucleus (n), normal lysosomes (ly) and intact basement membrane (pm) (X6000).
DISCUSSION

Radiation is an important inducer of oxidative stress. Chronic oxidative stress after total body irradiation is thought to be the cause of radiation nephropathy in rats\(^{(16)}\). Reactive oxygen species are important mediators exerting toxic effects on various organs, including kidney during ischemia–reperfusion injury\(^{(17)}\). Also Oxidative stress has a role as a mediator of injury in chronic allograft tubular atrophy and interstitial fibrosis in rat kidney\(^{(18)}\).

It is evident that exposure to ionizing radiation induces an increase in the free radicals generated in various tissues, especially radiosensitive ones. These free radicals would attack the biological membranes and cause their peroxidation. The kidney is an organ highly vulnerable to damage caused by reactive oxygen species, likely due to the abundance of long chain polyunsaturated fatty acids in the composition of renal lipids\(^{(19)}\). Shaheen and Hassan (1994)\(^{(20)}\) found that cells injury following irradiation was associated with disturbance in the cell membrane permeability induced after radiation exposure mostly attributed to peroxidation of membrane lipids that could disturb the anatomical integrity of the membrane.

Light and electron microscopy were used in this investigation to evaluate the nephrotoxic effects of whole body gamma-irradiation (5 Gy) in rats. The deleterious changes varied from swelling, vacuolation to complete degeneration of the lining epithelial cells of the convoluted renal tubules. The glomerular tufts were converted into structures. Hemorrhage within renal tissues appeared beside to necrotic convoluted tubules with pyknotic nuclei. These degenerative changes are probably caused by hydrogen peroxide and hydroxyl free radicals, which are formed within the cell during irradiation, and can directly cause kidney damage. These results are in agreement with the observations of Down \textit{et al.} (1990)\(^{(21)}\) that radiation induced glomerular injury and renal fibrosis.

The ultrathin section of the proximal and convoluted tubules of kidney of rats exposed to whole body gamma irradiation (5 Gy) showed destruction of brush border that could be attributed to the degenerative changes involving the whole tubules as well as thickening of tubular basement membrane which are probably caused by the peroxidation on membrane lipids\(^{(22)}\).

Most of the mitochondria showed different forms of mitochondrial damage such as swelling, destruction of their cristae, together with fusion of their limiting membrane and rupture of mitochondrial membranes. The results corroborate the findings of Al Karaz \textit{et al.} (1990)\(^{(23)}\) that gamma irradiation caused severe damage of mitochondria and their cristae. Moreover, Percy \textit{et al.} (2008)\(^{(24)}\) found that proximal tubular cells contain large numbers of mitochondria and are the most susceptible to oxidant – induced apoptosis and mutations. Ghadially (1988)\(^{(25)}\) considered that the mitochondrial swelling and destruction of their cristae were due to the influx of water into the inner and outer mitochondrial membranes. Since mitochondria are the site of the main energy production in the cell damage may result in lowered energy output. Therefore, the failure of the mitochondrial activity in the damaged cells may be regarded as factor causing cell degeneration.

The present results revealed that lysosomes were abundant and with dense internal inclusions which are in agreement with the observations of Rene \textit{et al.} (1971)\(^{(26)}\) that radiation induced internal ultrastructural and biochemical changes in lysosome. The abundance and accumulation of lysosomes play an active phagocytic function that could be a defense mechanism against the damaging effect of gamma radiation.
In the present study, administration of pomegranate juice before irradiation to rats was found to ameliorate the damage produced in renal tissue which could be attributed to its polyphenolic constituents, with anthocyanins being one of the most important\(^{(3)}\). The presence of delphinidin-3,5-diglucoside, delphinidin-3-glucoside, cyanidin-3,5-diglucoside, and cyanidin-3-glucoside in addition to pelargonidin-3,5-diglucoside and pelargonidin-3-glucoside (Krueger, 2012)\(^{(4)}\), along with hydrolyzable tannins (ellagitannins and gallotannins) and condensed tannins (proanthocyanidins) could contribute in some way to the antioxidant activity of pomegranate juice\(^{(3)}\).

It could be concluded that pomegranate juice might protect kidney tissues from radiation-induced histological and ultrastructural damage due to its antioxidant activity and its capacity to scavenge free radicals and decrease oxidative stress.

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