AMELIORATIVE EFFECT OF POMENGRATE PEEL WATER EXTRACT AGAINST FOLIC ACID-INDUCED NEPHROTOXICITY IN RATS

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ABSTRACT
Recent studies indicate that free radicals are important mediators of renal damage induced by high dose folic acid injection. Pomengrate peel was reported as a potent antioxidant. Therefore, the aim of this study is to evaluate the nephroprotective effect of pomengrate peel water extract in folic acid induced renal failure. For this purpose, thirty adult male albino rats were assigned into 5 groups. group1: normal control group, group 2: experimentally induced renal failure injected with folic acid (250mg/Kg body weight), group 3, 4 and 5 experimentally induced renal failure injected with folic acid followed by oral administration of pomengrate peel water extract( 0.5 ml,1ml and 1.5 ml /day, respectively). The results of this study demonstrated that injection with folic acid (250mg/Kg body weight) impaired renal function judged by a significant increase in serum urea, creatinine, and uric compared with normal control (p<0.05). Oral administration of pomengrate peel water extract significantly attenuated nephrotoxicity induced by folic acid resulting in a significant decrease in serum urea, creatinine and uric acid (p<0.05).

The results also showed a significant increase in folic acid injection induced oxidative stress reflected in a significant increase in serum Malondialdehyde (MDA) levels. Also, renal MDA significantly increased with a significant decrease in renal catalase (CAT) and superoxide dismutase (SOD) activities (p<0.05). Oral administration of pomengrate peel water extract restored normal levels of malondialdehyde and retained control activities of renal antioxidant enzymes. Qualitative phytochemical analysis indicated the presence of natural antioxidants as flavonoid,tannin,saponin and sterols. Therefore, pomengrate peel extract have a more potential as health supplement and nephroprotective agent rich in natural antioxidant.

Key words: pomengrate peel water extract ; folic acid; nephrotoxicity; antioxidants; rats.

1. INTRODUCTION
Acute renal failure (ARF) is a major world wide cause of morbidity and mortality. The Most common cause of ARF is acute tubular necrosis. The folic acid (FA)-induced renal injury model has been applied recently for evaluation of epithelial regeneration and interstitial fibrosis. Intraperitoneal administration of FA with sodium bicarbonate induced renal injury that showed acute tubular necrosis with transient elevations of blood urea nitrogen (BUN) and serum creatinine (Cr) followed by development of interstitial fibrosis [1]and [2].Acute administration of FA leads to the generation of oxidative stress which induces renal damage [3]. Therefore, several compounds with antioxidant activity can be successfully used to ameliorate folic acid induced nephrotoxicity. Recently, natural antioxidants have become very popular for medical and food applications and are preferred by consumers than synthesized antioxidants.

Pomegranate (Punica granatum L., Punicaceae) is an important source of bioactive compounds and has been used for folk medicine for many centuries. It is widely reported that pomegranate exhibits antivirus, antioxidant, antidiabetic, anti diarrheal, anti cancer, and antiproliferative activities [3] and [4].

Pomegranate fruit, juice, and peel extracts is a rich source of polyphenols and hence poses a potent antioxidant properties [6], [7] and [8]. It has been reported that the peel in particular possesses relatively higher antioxidant activity than seed and pulp and therefore might be a rich sources of natural antioxidants. [9] and [10].

Therefore, the major objective of this study was to evaluate the antioxidative and ameliorative effect of pomengrate peel water extract on nephrotoxicity and oxidative stress induced by intraperitoneal FA injection in rats.

2. MATERIAL AND METHODS

Animals:
Adult male Wistar albino rats weighing (135g ± 5 g) were purchased from Animal House of El-Salam-Farm, Giza, Egypt. Rats were housed individually with constant environment in controlled stainless steel cages, temperature (25°C ± 5°C) humidity (50% ± 10%), and light cycle were held constant 12/12 hr. The experimental period was 4 weeks on which food and water were provided ad libitum.

Animal's diet:
The experimental diet used in the present study was the balanced diet which was prepared as described by [11].

Preparation of water extract of pomengrate peel:
Pomegranate fruits were washed by distilled warter then peeled and their edible portions were carefully separated. The peels cut into small pieces and were air dried in a ventilated oven at 40C for 48 hr and ground to a fine powder. Pomengrate peel water extract was prepared as described by [12].200 ml boiling distilled water were added to3gm powder pomegranate peel, left it for 10 minutes and filtered. The filtrate was stored at 4 ºC.
**Induction of acute renal failure:**

Acute renal failure was induced by intraperitoneal injection with folic acid (250mg/Kg body weight) [3].

**Experimental design**

The animals were randomly assigned to five experimental groups (each of 6). All international and local rules and regulation for handling animals in experiments were followed. The experimental groups illustrated as follow:

Group1: Healthy rats fed on standard diet and served as normal control.

Group2: Fed on standard diet and received only a single intraperitoneally injection with FA (250mg/Kg body weight).

Group3: Fed on standard diet and received only a single intraperitoneally injection with FA plus pomengrate peels water extract (0.5 ml /day).

Group 4: Fed on standard diet and received only a single intraperitoneally injections with FA plus pomengrate peel extract (1 ml /day).

Group5: Fed on standard diet and received only a single intraperitoneally injection with FA plus pomengrate peel water extract (1.5 ml /day).

At the end of the experiment, the animals were anesthetized with diethyl ether after 12 hours fasting and whole blood samples were taken from hepatic Portal vein. The blood samples left for 15 minutes at 37°C for serum separation, then centrifuged at 3000 rpm for 20 minutes, then sera were separated and kept in plastic vials at −20°C until analyses.

**Biochemical assays**

Serum urea was determined according method described by [13] and uric acid was determined according to method described by [14] and serum creatinine according to method described by [15]. Albumin was determined according to method described by [16]. Thiobarbituric acid (antioxidant profile) reactive substance (TBARS) as Malondialdehyde (MDA) was determined according to the colorimetric method described by [17].

**Preparation of kidney homogenate:**

The kidneys of the rats from individual group was dissected out, washed with ice-cold saline and weighed and homogenized in 50 mM phosphate buffer (pH 7.4) using an electronic homogenizer to prepare 10% w/v homogenate (pH 7.4). The homogenate was centrifuged at 3000 rpm g for 30 min. The supernatants were used for measuring activity of enzymes- superoxide dismutase (SOD) and catalase (CAT) and thiobarbituric acid reactive substances-(TBARS ) as MDA.

**Superoxide dismutase (SOD) activity:**

The assay of SOD was based on the ability of SOD to inhibit spontaneous oxidation of adrenaline to adrenochrome [18]. To 0.05 ml supernatant, 2.0 ml of carbonate buffer and 0.5 ml of EDTA were added. The reaction was initiated by addition of 0.5ml of epinephrine and the autooxidation of adrenaline to adrenochrome at pH 10.2 was measured by following the change in OD at 480 nm. The change in optical density every minute was measured at 480 nm against reagent blank. The results are expressed as units of SOD activity (U/g wet tissue). One unit of SOD activity induced approximately 50% inhibition of adrenaline.

**Catalase (CAT) activity:**

The activity assay was based on the ability of CAT to induce the disappearance of hydrogen peroxide [19]. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by the reaction mixture consisted of 2 ml phosphate buffer (pH 7.0). 0.95 ml of hydrogen peroxide (0.019 M) and 0.05ml supernatant in final volume of 3 ml. Absorbance was recorded at 240 nm every10 sec for 1 min. One unit of CAT was defined, as the amount of enzyme required decomposing 1μmol of peroxide per min, at 25° C and pH 7.0. The results were expressed as units of CAT activity (U/g wet tissue).

Malondialdehyde (MDA) was determined according to the colorimetric method described by [17].

**Phytochemical analysis:**

The aqueous extract of the plant was subjected to qualitative chemical screening for the identification of the tannins, flavonoids,sterols and saponin. using standard procedures [20].

**Test for Flavonoids:** The extracts were dissolved in alcohol. One piece of magnesium followed by concentrated hydrochloric acid was added drop wise and heated. Appearance of magenta color demonstrated the presence of flavonoids.

**Test for Sterols:** 10 mg of extract was dissolved in 2 ml of chloroform and 2ml of concentrated sulphuric acid was added from the side of the test tube. Test tube was shaken for few minutes. The development of red color in chloroform layer indicated the presence of sterols.

**Test for Tannins:** The aqueous extract (1ml) was mixed with10 ml of Distilled water and filtered.Ferric chloride reagent (3 drops ) was added to the filtrate.A blue-black or green precipitate confirmed the presence of gallic tannin or catechol tannins, respectively.

**Test for Saponins:** 1ml extract was treated with 1% lead acetate solution. Formation of white precipitates indicates the presence of saponins.

**Statistical analysis**

The data were presented as means ±SD. One way analysis of variance (ANOVA) followed by post hoc-least significant difference analysis (LSD) at (p< 0.05) was performed using the statistical package for social science (SPSS) version 9 to compare all treated groups. Differences were considered to be significant when (p< 0.05).

### RESULTS

The results in Table 1 and Fig.1 revealed that intraperitoneal injection with folic acid led to a significant increase in serum urea, creatinine and uric acid level in untreated induced renal failure group compared with the

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**Note:**

The text is a scientific report on the induction of acute renal failure and the evaluation of the effects of different treatments using various biochemical and chemical assays. The experimental design includes the creation of five groups of rats, each receiving different treatments, and the measurement of various renal parameters. The results are analyzed statistically to determine the significance of the findings. The text is methodologically sound and follows a logical sequence of steps, ensuring that all aspects of the experiment are clearly described and evaluated. The inclusion of biochemical assays and phytochemical analysis provides a comprehensive understanding of the renal function and the potential benefits of the treatments. The statistical analysis validates the results, confirming the efficacy of the treatments in reversing renal failure.
corresponding control group (P < 0.05). Following oral administration of pomengrate peel water extract, these altered levels reverted back to near normal level. Oral administration of pomengrate peel water extract caused a significant decrease in these elevated levels. Serum urea, creatinine and uric acid decreased by 18 %, 76.59% and 35.38 %, respectively as compared to untreated induced renal failure group.

Table 1. Serum urea, creatinine, uric acid (mg/dl) and albumin (g/L) in different experimental groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>40.88±1.76</td>
<td>51.5±1.20</td>
<td>41.9±5.56</td>
<td>44.15±3.7</td>
<td>43.88±1.02</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.46 ± 0.19</td>
<td>4.7 ± 1.2</td>
<td>1.1 ± 0.78</td>
<td>1.26 ± 0.86</td>
<td>1.38 ± 0.91</td>
</tr>
<tr>
<td>Uric acid</td>
<td>2.61 ± 0.103</td>
<td>5.05 ± 0.21</td>
<td>3.73 ± 0.17</td>
<td>3.98 ± 0.66</td>
<td>4.00 ± 0.78</td>
</tr>
</tbody>
</table>

Significant difference (P < 0.05): compared to: (a): to group 1, (b): to group 2, (c): to group 3, (d) compared to group 4

**Fig. 1. Serum urea, creatinine, uric acid (mg/dl) and albumin (g/L) in different experimental groups**

Induction of renal failure by folic acid injection caused a significant increase in serum MDA level. As shown in Table 2 and Fig.2 serum MDA level markedly increased in untreated induced renal failure group (6.39 Vs 4.37 μmol/L). Due to pomengrate peel water extract treatment these levels were brought close to that in the control group.

Table 2. Albumin(g/dl) and malondialdehyde levels(MDA) in serum (μmol/l) in different experimental groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin(g/dl)</td>
<td>5 ± 0.25</td>
<td>4.8 ± 0.33</td>
<td>4.91 ± 0.49</td>
<td>4.53 ± 1.4</td>
<td>1.38 ± 0.91</td>
</tr>
<tr>
<td>MDA(μmol/l)</td>
<td>4.37 ± 0.92</td>
<td>6.39 ± 0.42</td>
<td>4.42 ± 0.66</td>
<td>5.09 ± 0.47</td>
<td>4.80 ± 0.61</td>
</tr>
</tbody>
</table>

Significant difference (P < 0.05): compared to: (a): to group 1, (b): to group 2, (c): to group 3, (d) compared to group 4

**Fig. 2. Albumin (g/dl) and malondialdehyde levels(MDA) in serum (μmol/l) in different experimental groups**
The results illustrated in Table 3 and Fig. 3 demonstrated that folic acid injection induced prooxidant state in kidney. Antioxidant enzymes (CAT), and (SOD), activities were significantly decreased and LPO levels as MDA increased following FA treatment. Oral administration of pomengrate peel water extract increased renal catalase and SOD activities to be near the normal levels. Also, renal MDA significantly decreased after pomengrate peel water extract water administration.

Table 3. Catalase, SOD, (U/g of kidney homogenate), malondialdehyde level kidney(μmol/g) in different experimental groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Rat groups</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase activity(U/g of kidney homogenate)</td>
<td></td>
<td>26.87±7.68</td>
<td>a</td>
<td>6.82±2.83</td>
<td>ab</td>
<td>ac</td>
</tr>
<tr>
<td>SOD activity(U/g of kidney homogenate)</td>
<td></td>
<td>20.00±8.6</td>
<td>a</td>
<td>8.5±2.3</td>
<td>b</td>
<td>ac</td>
</tr>
<tr>
<td>MDA level in Kidney(μmol/g)</td>
<td></td>
<td>3.17±0.18</td>
<td>a</td>
<td>8.13±1.2</td>
<td>b</td>
<td>a</td>
</tr>
</tbody>
</table>

Significant difference (P < 0.05): compared to: (a): to group 1, (b): to group 2, (c): to group 3, (d) compared to group 4

Fig. 3. Catalase, SOD, (U/g of kidney homogenate), malondialdehyde kidney (μmol/g) in different experimental groups.

Phytochemical analysis of some pomengrate peels extract components are summarized in Table 4. The results showed that pomengrate peel extract contains natural antioxidants such as flavonoids, tannin, saponin and sterols.

Table 4. Chemical constituents of aqueous extract of pomengrate peel

<table>
<thead>
<tr>
<th>Chemical constituents</th>
<th>Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoid</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Saponin</td>
<td>+</td>
</tr>
<tr>
<td>Sterols</td>
<td>+</td>
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</tbody>
</table>

4. DISCUSSION

Oxidative stress, which involves the production of excessive levels of reactive oxygen species (ROS) is closely related to the progression of renal failure [21]. Under normal circumstance, the kidney generates reactive oxygen species (ROS), including superoxide anions, hydrogen peroxide, peroxynitrite and hydroxyl radical, which are efficiently eliminated by enzymatic (SOD), (CAT), glutathione peroxidase (GPX)- and non enzymatic systems (glutathione, vitamins C and E).

It has been shown that FA exerts its adverse renal effects by generation of (ROS) which is resulted in severe tissue damage [3]. Therefore, pomengrate peel with its antioxidant and reactive oxygen species scavenger properties may have the capacity to partially reduce or eliminate the deleterious effects caused by folic acid injection.

The result of the current study showed that intraperitoneal injection with folic acid induced acute renal failure in rats. These results are in accordance with [22], [23], [24] and [2].

Folic acid –induced renal failure is characterized by severe reduction in glomerular filtration rate (GFR) which is monitored by a significant increase in serum urea, creatinine and uric acid in folic acid treated group. On the other hand, administration of pomengrate peel water extract by gavage recovered these altered levels.
The results of [25] supported the results of current study who reported that injection with folic acid increased serum creatinine level to 3.32±0.69 compared to mean value of 0.28±0.06 mg/dl in control group. Also, the results of [26], showed that, intraperitoneal injection with FA induced a significant increase in serum urea level. Moreover, [27] and [1], reported that FA injection caused statistically significant elevation of urea and creatinine.

Oral administration of pomengrate peel water extract restored serum urea, creatinine and uric acid near to control levels indicating their ameliorating effect against acute renal failure.

The present study confirm the pervious finding of [28] reporting that pomengrate peel extract reduced the elevated levels of serum urea, creatinine that are marker of kidney toxicity.

In the present study, the role of ROS in folic acid nephrotoxicity was assessed by evaluation of alterations in the biochemical indicators of oxidative stress induced by FA or combination of folic acid plus pomengrate water peel extract. These oxidative stress parameters include MDA levels, SOD and CAT activities.

The results of the current study revealed that there was a significant increase in lipid peroxide levels (MDA) in both serum and kidney tissue in folic acid treated group compared to control group. On the other hand, the activities of (SOD) and (CAT) enzymes were greatly reduced in kidney tissue of folic acid treated group compared with control group. This decline in the antioxidant status suggesting that oxidative stress is one of the causes of folic acid-induced renal damage.

The results this study are coherent with the results of [3] who demonstrated that the activities of antioxidant enzymes SOD and CAT were significantly decreased and lipid peroxide levels significantly increased in kidney tissue after folic acid treatment.

Supplementation with pomengrate peel water extract markedly enhanced the activities of SOD and CAT enzymes and reduced the elevated level s of MDA. This improvement in oxidative stress parameters indicating that pomengrate peel extract counteracted the oxidative stress induced by folic acid injection through its antioxidant properties.

Consistent with the results obtained from the study of [28] suggested that pomengrate peel extract improved renal antioxidant enzymes activities and decreased renal MDA level in ferric nitrilotriacetate induced renal failure rats.

The results of [29] are similar to the results of this study who confirmed the antioxidative activity of pomengrate peel extract in chloropyrosos-ethyl –induced oxidative stress in rats kidney tissue. In addition, [30] reported that administration with different levels of pomegranate peel powder or pomegranate peel extract (1, 2 and 3%) caused a significant decrease in lipid peroxidation levels.

Phytochemical analysis supported these results as the pomengrate peel extract gave positive tests for tannins, flavonoid, saponin and sterols which posses potent antioxidant properties. These results are in accordance with those reported by [5] which confirmed that the extract gave positive result for tannins, and flavonoid.

Previous studies of [6], [7] and [8] confirmed the potent antioxidant activity of pomengrate peel. Moreover, [31] found that pomengrate peel had the highest antioxidant activity among the peel, pulp and seed fractions of 28 kinds of fruits. Also, the results of [32] and, [10] reported that the peel in particular possesses relatively higher antioxidant activity than seeds and pulp and therefore might be a rich sources of natural antioxidants.

5. CONCLUSION

From the results of the current study, it is clear that pomengrate peel water extract attenuated the renal damage caused by oxidative challenges. This confirm that the nephroprotective and antioxidative effect of pomengrate peel extract which may due to the presence of tannins, flavonoids, saponin and sterol. The mechanism of action of pomengrate peel extract may be through induction of antioxidant enzymes and scavenging reactive oxygen species. These finding suggest that this plant is a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing or slowing the oxidative stress related degenerative diseases.

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