The protective role of Pomegranate seed oil (Pometone) on serum protein in sodium fluoride treated female rats

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Summary
This study is designed to determine the protective role of pomegranate seed oil against deleterious effect induced by sodium fluoride on serum proteins in rats. Forty (40) adult female rats were randomly divided into four equal group (10/group) and treated daily for 40 days as following: Group C administered distilled water (control), group T1 received sodium fluoride 120 ppm/liter in drinking water, group T2 received both sodium fluoride 120 ppm/liter in drinking water and Pometone 30 mg/kg B.w. orally and group T3 administered Pometone 30 mg/kg B.w. orally. Fasting blood samples were collected at 0, 20 and 40 days of the experimental periods and serum samples were aliquoted for estimation serum total protein, albumin and globulin concentrations. Also, protein electrophoresis was measured. The results revealed that sodium fluoride caused significant reduction in serum total protein, albumin and globulin concentrations, in addition to changes in the patterns of serum protein fractions % (albumin, α1-globulin, α2-globulin, β-globulin and γ-globulin) as compared with control rats. In conclusion Pomegranate seed oil caused elevation of proteins concentration as compared to group T1 and could potentially be beneficial in preventing the hepatic damage caused by sodium fluoride.

Keywords: Pomegranate seed oil, Sodium fluoride, Total protein, Protein electrophoresis, Rats.

Introduction
Fluorine is notable for its small size; large numbers of fluorine atoms fit around atoms of another element. This, along with its electronegativity, allows the formation of many simple and complex fluorides (1). Sodium fluoride (SF) is a strong, hard, anion and a cumulative toxic agent an inorganic anionic compound, on dissolving gives separated Na and F ions (2). In addition, to drinking water (largest contributor), foods (almonds, dates, rice, wheat's, walnuts), tea, tooth paste and tobacco are rich sources of fluoride (3 and 4). Sodium fluoride is used in various pesticide formulation, including fungicides and insecticides and wood preservative (5 and 6). Other industrial uses for sodium fluoride include glass frosting, stainless and steel pickling (7). Drugs, fluoride dusts and fumes from industries using fluoride containing salts are other ways for fluoride entering the body (8). Inorganic fluorine are used in aluminum production and as a flux in the steel and glass fiber industries (9) and used as anticoagulant (10). Recently, in modern preventive dentistry fluoride was used as a new tool for management of dental caries (11).

Metabolic function and structural damages caused by chronic fluorosis have been reported in many organs (12-14). However, more fluoride intake might cause toxic effect in animals and human being (15).

Oxidative stress (OS) is one of the most important factors that exacerbate damage caused by some drugs and environmental chemicals. Earlier reports have shown that SF induce OS through significant changes in levels of oxidant/antioxidants status, resulting in altered free-radical metabolism in several tissues and red blood cells (16-18). As well as fluoride has the ability to initiate respiratory burst and stimulate the generation of free radicals which change the architecture permeability of cell membranes leading to impairment of the cell function (19). The generation of anion superoxide (O$_2^-$) induced by fluoride is responsible for pathophysiology of some disease - through enhanced generation of reactive oxygen species (ROS) - like cardiac disorders and myocardium injury (20), toxicity of central nervous system (21), alterations in the metabolism of salivary glands (14). Furthermore, SF could inhibit the...
activities of some carbohydrate metabolizing enzymes (22).

The pomegranate fruit have many functional and medicinal effects. Pomegranate flowers attenuate aging-mediated undesirable skin abnormalities (23), in addition to their potent antioxidant and hepatoprotective effects (24) and diminishing cardiac toxicity (25), the pomegranate peel has been found to be strong anti-inflammatory (26) antimutagenic (27) and antifungal (28). Pomegranate products have also been reported to possess antimicrobial, immunosuppressive activities and hepatoprotective effects (29 and 30), improve abnormal cardiac lipid metabolism in diabetic rats (31) and have preventive role for obesity (32). Furthermore, the pomegranate peel extracts also prevents liver fibrosis (33). Recently, AL-Okaily (34) explained that pomegranate seed oil (PSO) have a cardioprotective effects in methionine over load rabbits. It has been well known that natural antioxidants agents could quench free radicals by electron or proton donation and protect body tissues against oxidative stress. Therefore, this study designed to study the role of Pometone (pomegranate seed oil /PSO) in alleviating the deleterious effect of sodium fluoride on serum proteins in adult female rats.

Materials and Methods

Forty adult female Wistar rats, weighed 219.5-250.1 g were used in this investigation. Animals were housed in plastic cages in a conditioned room (22-25 °C) in the animal house of the College of Veterinary Medicine - University of Baghdad. They were left for two weeks for acclimatization with the experimental conditions. Animals had free access to water and standard pellet diet along the experimental period. Rats were divided randomly into four equal group (10/group) and treated daily for 40 days as following: Group C, rats are given distilled water, (control); group T1, rats received sodium fluoride 120 ppm/liter in drinking water; group T2, rats in this group were subjected to sodium fluoride 120 ppm/liter in drinking water and pometone (Vitam Pharma- Germany) 30 mg/kg B.w. orally and group T3 rats were administered pometone 30mg/kg B.w. orally. Fasting blood samples were collected at 0, 20 and 40 days of the experimental periods. Blood was drawn by cardiac puncture from anesthetized rats by using of ketamine 90 mg/kg B.W and xylazin 40 mg/kg B.W. (I/M). Blood samples were kept in gel tubes, and centrifuged at 3000 rpm for 15 minutes, and then serum samples were aliquoted and frozen at -20 °C until analysis of the following parameters: serum total protein and albumin concentrations was measured enzymatically by using total serum protein kit product of Bio system, Spain (35), and serum albumin kit produced by Bio system company (36), as well as, globulin concentration (37). All types of proteins were measured by electrophoresis (SAS1, SAS2) (Helena Bioscience / Europe). Statistical analysis of data was performed on the basis of Two-Way Analysis of Variance (ANOVA) using a significant level of (P<0.05). Specific group differences were determined using least significant differences (LSD) (38).

Results and Discussion

Exposed of female rats to SF (group T1) at dose 120 ppm/L in drinking water for 40 successive days caused a significant (P<0.05) decrease in serum total protein concentration after 20 and 40 day as compared with control, T2 and T3 treated groups. A slight significant (P<0.05) decrease in this parameter was observed in T2 group compared with normal control group (Table, 1). No changes were observed in group treated with PSO at two treated periods when compared to control group.

In (Table, 2) No difference between groups during zero time period. However, after 20 and 40 days of the experiment the results showed a significant (P<0.05) elevation in serum albumin concentration in rats administered pometone (group T3) as compared to control, T1 and T2 treated groups. T1 and T2 treated groups revealed a significant (P<0.05) decrease in the mean concentration of serum albumin in these periods as compared to control group. Also, the result showed a significant (P<0.05) decrease in serum total albumin concentration in T1 and T2 groups at the end of the experiment compared to pretreatment period. Rats received PSO (group T3) showed a significant (P<0.05) increase in
albumin concentration after 40 days of treatment as compared with pretreated period.

There were no differences in the mean value of serum globulin concentration between the experimental groups during pretreated period (Table, 3). A significant (P<0.05) decrease in serum globulin concentration was observed in T1 treated group after 40 day of the experiment as compared to control and T2 groups. Treatment of female rats with SF plus PSO (group T2) showed a significant (P<0.05) increase in this parameter at two treated groups compared to other groups. The mean concentration of serum globulin in T2 after 20 and 40 day were 3.16 ±0.12 and 3.52 ±0.08 respectively. At the end of the experiment, the serum globulin concentration significantly (P<0.05) decreased after administration of SF (group T1) and PSO (group T3) compared with control group. The results showed a significant (P<0.05) increase in serum globulin concentration in group T2 at two treated periods compared to pretreated period, while a significant (P<0.05) reduction was observed in the two treated group T1 and T3 group after 20 and 40 day compared to the pretreated period.

Table 1: Effect of pomegranate seed oil (PSO) on total serum protein concentration (mg/dl) in female rats treated with sodium fluoride (SF).

<table>
<thead>
<tr>
<th>Days</th>
<th>Groups</th>
<th>Control (C)</th>
<th>T1 (SF120ppm)</th>
<th>T2 (SF + PSO)</th>
<th>T3 (PSO 30/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Control (C)</td>
<td>6.32±0.14</td>
<td>6.22±0.13</td>
<td>6.16±0.13</td>
<td>6.16±0.17</td>
</tr>
<tr>
<td></td>
<td>A a</td>
<td>6.20±0.08</td>
<td>4.78±0.15</td>
<td>5.46±0.09</td>
<td>6.24±0.16</td>
</tr>
<tr>
<td></td>
<td>B b</td>
<td>6.28±0.11</td>
<td>4.16±0.05</td>
<td>5.64±0.05</td>
<td>6.60±0.07</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE, n=10/group. Capital letters denote significant (P<0.05) differences between groups. Small letters denote significant differences (P<0.05) within time.

Table 2: Effect of pomegranate seed oil (PSO) on serum albumin concentration (mg/dl) in female rats treated with sodium fluoride (SF).

<table>
<thead>
<tr>
<th>Days</th>
<th>Groups</th>
<th>Control (C)</th>
<th>T1 (SF120ppm)</th>
<th>T2 (SF + PSO)</th>
<th>T3 (PSO 30/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Control (C)</td>
<td>3.38±0.20</td>
<td>3.54±0.16</td>
<td>3.54±0.10</td>
<td>3.32±0.12</td>
</tr>
<tr>
<td></td>
<td>A a</td>
<td>3.54±0.19</td>
<td>2.32±0.30</td>
<td>2.30±0.16</td>
<td>3.92±0.14</td>
</tr>
<tr>
<td></td>
<td>B b</td>
<td>3.40±0.07</td>
<td>1.78±0.12</td>
<td>2.12±0.06</td>
<td>4.04±0.12</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE, n=10/group. Capital letters denote significant (P<0.05) differences between groups. Small letters denote significant differences (P<0.05) within time.

Table 3: Effect of pomegranate seed oil (PSO) on serum globulin concentration (g/dl) in female rats treated with sodium fluoride (SF).

<table>
<thead>
<tr>
<th>Days</th>
<th>Groups</th>
<th>Control (C)</th>
<th>T1 (SF120ppm)</th>
<th>T2 (SF + PSO)</th>
<th>T3 (PSO 30/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Control (C)</td>
<td>2.80±0.10</td>
<td>2.86±0.09</td>
<td>2.66±0.05</td>
<td>2.84±0.06</td>
</tr>
<tr>
<td></td>
<td>A a</td>
<td>2.66±0.12</td>
<td>2.46±0.16</td>
<td>3.16±0.12</td>
<td>2.32±0.07</td>
</tr>
<tr>
<td></td>
<td>B b</td>
<td>2.68±0.48</td>
<td>2.38±0.08</td>
<td>3.52±0.08</td>
<td>2.56±0.06</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE, n=10/group. Capital letters denote significant (P<0.05) differences among groups. Small letters denote significant differences (P<0.05) among times.

Changes in the concentration and patterns of serum protein fractions (%) (albumin, α₁-globulin, α₂ –globulin, β-globulin, γ-globulin) for normal and treated female rats at the end of experimental period were manifested in (Table, 4) and (Fig. 1 - 4). The results revealed that albumin concentration in rats received sodium fluoride in drinking water (group T1) and SF + PSO (group T2) were 16% and 17% of total albumin, compared to the control value.
(22.5%). No considerable changes in albumin concentration were observed between group T3 and control, the values were 22.5% and 23.0%, respectively. Rats received sodium fluoride in drinking water (group T1) showed a decrease in the percentages of $\alpha_1$ and $\alpha_2$-globulins as compared to the control and other treated groups. The recorded value were 6.33, 3.29, 9.66 and 4.23% for T1 and control, respectively.

Table, 4: Effect of pomegranate seed oil (PSO) and sodium fluoride (SF) on serum proteins electrophoresis (protein fraction %) in female rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Albumin</th>
<th>$\alpha_1$-globulin</th>
<th>$\alpha_2$-globulin</th>
<th>$\beta$-globulin</th>
<th>$\gamma$-globulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>22.5</td>
<td>9.66</td>
<td>4.23</td>
<td>10.82</td>
<td>10.27</td>
</tr>
<tr>
<td>T1 SF(120ppm/L)</td>
<td>16.0</td>
<td>6.33</td>
<td>3.29</td>
<td>7.18</td>
<td>9.19</td>
</tr>
<tr>
<td>T2 (SF + PSO)</td>
<td>17.0</td>
<td>9.2</td>
<td>5.75</td>
<td>11.41</td>
<td>12.13</td>
</tr>
<tr>
<td>T3 (PSO 30mg)</td>
<td>23.0</td>
<td>10.21</td>
<td>5.39</td>
<td>12.58</td>
<td>13.31</td>
</tr>
</tbody>
</table>

Values are expressed as mean, n= 2

Treatment of rats with PSO (group T3) afforded an increase in $\alpha_1$ and $\alpha_2$-globulins concentration as compared to group T1. On the other hands the highest concentration of $\beta$-globulin was recorded in SF + PSO treated group (T2) and PSO treated group (T3) compared to T1 group. The values were 11.41%, 12.58% and 7.18% for T2, T3 and T1 respectively. Rats received SF for forty successive days caused a decrease in serum $\gamma$-globulin level (9.19%) comparing to control (10.27%), group T2 (12.13%) and group T3 (13.31%) of $\gamma$-globulin.

In the present study, a significant decrement in total protein, albumin and globulin concentration in group T1 rats treated with SF was observed. This results in agreement with (39-42). The liver is considered as a major organ that plays an important role in various cases of toxication (43), and excretes the end products of some metabolites. However, a
reduction in liver protein content induced by SF might be due to increased protein breakdown, decreased protein synthesis or fluoride induced osmotic imbalance caused by lipid peroxidation. Fluoride also, inhibits oxidative decarboxylation of branched chain amino acid and simultaneously promotes protein breakdown (44), or it could affect the rate of cellular protein synthesis, which is mainly due to impairment of peptide chain initiation (45). However, the disturbance of protein synthesizing systems caused by sodium fluoride may be attributed to suppress Na-K-activated ATPase, an essential enzyme for the uptake of amino acid by tissues and inhibited incorporation of amino acids into protein (42).

Hordyjewska and Pasternak (46) suggested that sodium fluoride generated free radicals down-regulate the activity of enzymes important in the polymerization of amino acids, thus inhibiting process of elongation of peptides, whereas (17) reported that fluoride exposure caused a reduction in protein content in rats which might be due to either direct effect on protein synthesis or indirectly through DNA and RNA damage. During fluoride intoxication, both calcium and magnesium ions are decreased (47), and the depletion of both ions might be the reason for a decrease in synthesis of DNA, RNA and protein (48). Free radicals also a major source for DNA damage, which could cause strand breaks and base alteration in the DNA (49). Furthermore, the decrease in protein content might be explained by the reduction in insulin level since insulin has an anabolic effect on protein metabolism in that it stimulates protein synthesis and retards protein degradation (50).

Experimental evidence indicated that exposure to high fluoride levels could be induce deleterious effects on the cell and organelles due to general inhibition of different enzymes, like superoxide dismutase, glutathione peroxidase and catalase, causing oxidative stress and then activation of apoptotic cell (51). Reactive oxygen species ROS generated due to fluoride intoxication could lead to inactivation of several enzymatic proteins involved in biosynthesis and metabolism (52).

The results revealed that the pomegranate seed oil caused an increase in serum total protein and albumin concentration in T2 and T3 treated groups compared to SF treated group (T1) which is in consistent with (53). While (54) reported elevation in protein in rat treated with PSO. Globulin concentration did not significantly change in the current study, which seems to agree with (53). Polyphenols present in pomegranate including ellagic and tannic acids caused an increase in total serum protein and protein synthesis in the body (55) and thus elevated its serum concentration. Present results suggest a profound ameliorative effect of PSO in SF treated group, through increased considerably total serum protein and its fractions, which might be due to the presence of anthocyanins, ellagic, gallic and vitamin C (56 and 57) and recently (58 and 59) showed that pomegranate juice could effectively protect liver from oxidative damage. Present findings, concluded that SF could reduce the serum proteins and its fractions, which could be effectively reversed by PSO.

References


الدور الوقائي لزيت بذور الرمان على بروتينات مصل الدم في إناث الجرذان المعاملة بفلوريد الصوديوم

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الخلاصة

سمحت هذه الدراسة لتحديد التأثير الوقائي لزيت بذور الرمان في تقليل التأثيرات الضارة للفلوريد الصوديوم على بروتينات بلازما الدم في إناث الجرذان. استخدمت (40) من إناث الجرذان البالغة، قسمت عشوائيا على أربعة مجموعات متساوية وعملت لمدة أربعين يوما. أعطيت المجموعة الأولى الماء المقطط (كمجموعة对照)، في حين أعطيت المجموعة الثانية (T1) فلوريد الصوديوم مع ماء الشرب وذكرت 120 جزء بالمليون /لتر بالأضافة إلى زيت بذور الرمان بجرعة 30 ملغم/كجم من وزن الجسم في حين جرعت حيوانات المجموعة الرابعة (T3) زيت بذور الرمان بجرعة 30 ملغم/كجم من وزن الجسم. جمعت عينات الدم من المد 0 و40 يوم من التجربة لغرض تقييم تركيز كل من البروتينات الكلية، الكليوبيولين والألومنيوم في مصل الدم، فضلاً عن الحوادث الأخرى، الفطر، البروتينات الكبيرة، والألومنيوم والكليوبيولين. أظهرت النتائج أن إعطاء فلوريد الصوديوم مع ماء الشرب أدى إلى ازائض في تركيز البروتينات الكلية، والألومنيوم والكليوبيولين، فضلاً عن انخفاض في نسبة تأثير الالتهاب الكهربائي لبروتينات مصل الدم. كما أدى الالتهاب الكهربائي لبروتينات مصل الدم إلى زيادة في كميات الكليوبيولين في المجموعة المختبرية. كما أدى الالتهاب الكهربائي لبروتينات مصل الدم إلى ارتفاع في تركيز البروتينات، واستنتج الدراسة أن زيت بذور الرمان دوراً في حماية الكبد من التلف المسبب بلفوريد الصوديوم.

الكلمات المفتاحية: زيت بذور الرمان، فلوريد الصوديوم، البروتينات، الترحل الكهربائي للبروتينات، جرذان.