Study of the preventive role of cranberry extract on some physiological parameters in male rat induced by sodium fluoride

Shatha Galal Azez Naseer Marza Hamza*
Department of biology, College of Education for Pure Sciences, University of Kerbala, Iraq
*Corresponding author. Email address: naser.m@uokerbala.edu.iq

Abstract

Background: Traditional medicine is still widely practiced today and modern medicine benefits from many compounds. The aim of current study was to determine the biological efficacy of the cranberry plant extract on some blood parameters specific to renal function in laboratory white rats (Rattus norvegicus), orally injected with sodium fluoride.

Methods: Current study was conducted over a period of one month, from February 2019 to July 2019. The laboratory white strain inventory was studied (albino rats, n= 25) inventory of males aged between (2-4) months and weighed between (150-250g). They were divided into five groups randomly; the first group included negative control group, the second group included positive control administered an oral dose of sodium fluoride (20mg/kg body weight) for 30 respective days. Groups III and IV were considered as test groups for the protective role of cranberry extract which were administered oral doses of cranberry extract (225, 150 and 75(mg/kg body weight plus sodium fluoride for 30 respective days. After the end of the experiment, animals were anesthetized with ketamine and the blood was collected by direct heart puncture. The studied parameters included (body weight and serum concentrations of sodium, calcium, potassium, urea and creatinine.

Results: The results of present study showed significant differences in the studied parameters. A significant increase was observed in the concentration of sodium, potassium, calcium, creatinine and urea. Also, a significant decrease was observed in the body weight (P≤0.05) in rats treated with sodium fluoride when compared to the control group. On the other hand, the results of the current study showed that the treatment with cranberry extract was accompanied by some significant changes. A significant decrease was observed in body weight and concentrations of sodium, calcium, potassium and creatine (P≤0.05).

When comparing the fluoride and the cranberry extract groups, the decrease led to return of these parameters closer to the state in the control group. However, the concentration of urea was increased significantly (P≤0.05) when comparing the fluoride group with the cranberry extract group.

Conclusion: It was clear from the results of this study that the extract of the Cranberry plant has a biological effect in resisting the effects of toxic substances to which the organism is exposed.

Keywords: protective role, sodium fluoride, cranberry, renal function, serum electrolytes

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Introduction

Medicinal plants

Medicinal plants have been and are the basis for medical treatment during much of human history. Traditional medicine is still widely practiced today and modern medicine benefits from many compounds. Although plant therapy may not apply with modern standards for testing the pharmacological efficacy of current medications due to different purity and concentration, medicinal plants are used for therapeutic purposes despite the fact that not all these plants have been fully tested. To check how effective they are or to know what interactions they might have with other herbs, supplements, medications and/ or some different foods (1). Cranberry is a small medicinal plant of the genus (Vaccinium) and family (Ericaceae) red fruits edible color evergreen leaves, oval in shape, length less than 1.2cm, appear small flowers in the form of a bell, found small or northern cranberries in marshlands in northern North America and Asia and in northern and central Europe (2). Romans were the first to know the first medical uses of cranberries as the world rose (Herbalist Henry) documented its therapeutic effects in 1578. Since then, cranberry has been a popular folk remedy for a variety of diseases including gout, rheumatism, diarrhea, constipation, scurvy, fever, skin infections and other skin problems such as eczema. Cranberries are known as a remedy for women's health problems such as cystitis, urinary and genital tract infections (3). Many sources suggested that cranberries are rich in organic acids that give the acidic taste of berries and contribute to prevention of renal stones. They are also
rich in vitamins, including vitamin (A), carotenoids, Riboflavin and Niacin. Also, they contain many essential minerals such as sodium, potassium, calcium, magnesium, phosphorus, copper, sulfur, iron and iodine. These vitamins and minerals are powerful antioxidants that enable cranberries to help protect the body from infectious diseases as well as from colds or flu because of high vitamin (C) content. Cranberries have been used in the past to prevent vitamin (C) deficiency, known as scurvy (4). Traditionally, cranberries have been used to treat and prevent urinary tract infections over time and research suggested that their mechanism of action is to prevent bacteria from sticking to the surfaces of the body's lining cells. Therapeutic applications of cranberry documented during the 17th century included relief of blood disorders, stomach diseases, liver problems, vomiting, anorexia, scurvy and cancer. Before the advent of antibiotics, cranberries were considered to be herbal remedies for urinary tract infection (5).

Sodium fluoride

It is one of the first toxic substances entering the body, causing increased concentration in the blood plasma with dysfunction of the majority of body organs where the concentration in the blood plasma is twice as high as in the blood cells (6). It is distributed through the blood plasma to all body tissues. High fluoride for a long period of time has become a major cause of kidney failure and inefficient glomerular filtration (7). Most sources indicated that there is no clear evidence to support the use of cranberries in the treatment or prevention of the risk of toxic substances entering the body in different ways, especially those that cause malfunction of kidneys that are accompanied by urinary tract infection and may exceed that to cause damage to the kidney tissue (8). The sources that give clear evidence of the role of cranberry extract in the treatment of nephrotoxicity and the results are almost nonexistent which encouraged the conduct of this research. Therefore, current study was aimed to show the role of cranberry extract in the prevention of toxic effects of sodium fluoride on renal standards of electrolytes (sodium, potassium, calcium, urea and creatinine) in addition to showing its effect on body weight.

1. Materials and methods of work

Experiment animals

Current study was conducted over a period of one month, from February 2019 to July 2019. The laboratory white strain inventory was studied (albino rats, n= 25) inventory of males aged between (2-4) months and weighed between (150-250g). They were divided into five groups randomly; the first group included negative control group, the second group included positive control administered an oral dose of sodium fluoride (20mg/kg body weight) for 30 respective days. Group III and IV were considered as test groups for the protective role of cranberry extract which were administered oral doses of cranberry extract (225, 150 and 75(mg/kg body weight plus sodium fluoride for 30 respective days. After the end of the experiment, animals were anesthetized with ketamine and the blood was collected by direct heart puncture (9).

Measurement of body weight

The body weight was measured before giving the doses and after the end of the experiment by a German electronic sensitive balance (Sartorius).

Measurement of serum sodium concentration

The level of sodium ions was estimated in the serum using method described by (11) which depends on the preparation of solutions of the substances (Table 1).

<table>
<thead>
<tr>
<th>Reagent type</th>
<th>Material</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>PREC (Precipitant solution)</td>
<td>Uranyl acetate</td>
<td>19.00mmol/L</td>
</tr>
<tr>
<td></td>
<td>Magnesium acetate</td>
<td>140.0mmol/L</td>
</tr>
<tr>
<td>Reagent 1</td>
<td>Ammonium thioglycolate</td>
<td>550.0mmol/L</td>
</tr>
<tr>
<td></td>
<td>Ammonia</td>
<td>550.0mmol/L</td>
</tr>
<tr>
<td>STD.</td>
<td>Standard sodium (Na⁺)</td>
<td>150.0mmol/L</td>
</tr>
</tbody>
</table>

Working method included mixing (20µl) of standard with (2000µl) of PREC and (20µl) of the sample in a closed tube and with well mixing, then it was left for 5 minutes at (25°C), and was shacked for (3min) then left for (30min). Finally the tube was spun in centrifuge at a speed of 6000RPM for (5min).
After that, the blank was prepared by mixing (20µl) of PREC and (1000µl) of Reagent 1. Finally, the results of step (1) and step (2) were mixed well for (5min) by the vortex device and left at room temperature. The absorbance was read at the wavelength (410nm).

1- Sodium concentration was measured based on the following equation:

\[
\text{Sodium concentration (mmol/L)} = N \times \frac{\text{sample absorbance}}{\text{standard absorbance}}
\]

Where \( N \) is the standard concentration.

**Measurement of serum calcium concentration**

The level of calcium was estimated in the serum according to method described by \(^{(12)}\) which depends on the preparation of solutions of the substances (Table 2).

<table>
<thead>
<tr>
<th>Reagent type</th>
<th>Material</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer solution</td>
<td>(2 amino-2methyl-1-propanol)</td>
<td>500.0mmol/L, PH 7.</td>
</tr>
<tr>
<td>Chromogene solution</td>
<td>Cresolphthalein complex</td>
<td>0.620mmol/L</td>
</tr>
<tr>
<td></td>
<td>8-hydroxyquinoline</td>
<td>69.00mmol/L</td>
</tr>
<tr>
<td>Standard</td>
<td>Calcium standard</td>
<td>2.500mmol/L</td>
</tr>
</tbody>
</table>

**The basic principle**

Measurement of calcium ions in serum is based on the composition of the complex chromatography between calcium ions (O- Cresolphthalein) according to the following equation:

\[
\text{Ca}^{+2} + \text{O - Cresolphthalein} \xrightarrow{\text{OH}^+} \text{red complex}
\]

1- The working reagent was attended by mix (1000µl) of the blank and (1000µl) of standard with (1000µl) of the sample. The resulted solution was mixed well in a tube, then it was left for (5min) at room temperature. After that the absorbance was measured at a wavelength of (570nm). Calcium concentration was measured based on the following equation:

\[
\text{Calcium concentration (mmol/L)} = N \times \frac{\text{sample absorbance}}{\text{standard absorbance}}
\]

Where \( N \) is the standard concentration.

**Estimation of serum potassium ions level**

The Level of serum potassium ions was estimated in the serum according to method described by \(^{(13)}\) which depends on the preparation of solutions of the substances (Table 3).

<table>
<thead>
<tr>
<th>Reagent type</th>
<th>Material</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>PREC (Precipitant)</td>
<td>Trichloroacetic acid (TCA)</td>
<td>0.3mol/L</td>
</tr>
<tr>
<td>Reagent 1 (TPB)</td>
<td>Sodium tetraphenylboron (TPB – NA)</td>
<td>0.2mol/L</td>
</tr>
<tr>
<td>Reagent 2 (NAOH)</td>
<td>Sodium hydroxide (NaOH)</td>
<td>2.0mol/L</td>
</tr>
<tr>
<td>STD</td>
<td>Standard potassium (K⁺)</td>
<td>5.0mmol/L</td>
</tr>
</tbody>
</table>
Basic principle

The free potassium ion reacts in the middle with tetra-vinyl sodium boron (Sodium tetraphenylboron) to produce turbid suspension of quaternary vinyl Potassium Euron (Potassium tetraphenylboron). The resulting turbidity is based on a measure of potassium concentration at photosynthesis.

The working method included supernatant preparation by mixing (50µl) of serum with (500µl) of PREC in a glass tube and was spun by a centrifuge at 6000RPM for (5min). In addition, a working reagent was prepared by mixing equal volumes of (R1) and (R2) in a glass tube and it had been left for (15-30min) before use. Finally, the result of step (1) was mixed with the result of step (2) and left for (5min), then the absorbance was read on a wavelength of (578nm) based on the following equation:

\[
\text{Potassium concentration (mmol/L)} = N \times \frac{\text{sample absorbance}}{\text{standard absorbance}}
\]

Where N is the standard concentration.

Estimation of urea level

The Level of urea was estimated in the serum according to method described by (14) which depends on the preparation of solutions of the substances (Table 4).

<table>
<thead>
<tr>
<th>Reagent Type</th>
<th>Material</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent (1) a</td>
<td>Urease</td>
<td>≥5000µ/L</td>
</tr>
<tr>
<td></td>
<td>Phosphate buffer</td>
<td>120.0mmol/L, pH7</td>
</tr>
<tr>
<td></td>
<td>Sodium salicylate</td>
<td>63.40mmol/L</td>
</tr>
<tr>
<td></td>
<td>Sodium nitroprusside</td>
<td>500.0mmol/L</td>
</tr>
<tr>
<td></td>
<td>EDTA</td>
<td>1,500mmol/L</td>
</tr>
<tr>
<td>Reagent (1) b</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sodium Hypochlorite</td>
<td>180.0mmol/L</td>
</tr>
<tr>
<td></td>
<td>Sodium Hydroxide</td>
<td>750.0mmol/L</td>
</tr>
<tr>
<td>Reagent (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Standard</td>
<td></td>
</tr>
</tbody>
</table>

The basic principle depends on hydrolysis of urea in the presence of enzyme urease in which the ammonium ion reacts with (salicylate) and (Hypochlorite) to be a green-colored complex of (2-2 dicarboxylindophenol) based on the following equation:

\[
\text{Urea} + \text{H}_{2}\text{O} \xrightarrow{\text{Urease}} 2 \text{NH}_{3} + \text{CO}_{2}
\]

The working reagent was prepared by mixing R1a with R1b in a tube and incubated for (3min) in a water bath at (37°C). after that, the concentration of urea was calculated depending on the following equation:

\[
\text{urea concentration (mg/dL)} = N \times \frac{\text{sample absorbance}}{\text{standard absorbance}}
\]

Where N is the standard concentration.

Estimation of serum creatinine level

The level of creatinine was estimated in the serum according to method of (13) which depends on the preparation of solutions of the substances (Table 5).

<table>
<thead>
<tr>
<th>Reagent Type</th>
<th>Material</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent (1)</td>
<td>Picric acid</td>
<td>25mmol/L</td>
</tr>
<tr>
<td>Reagent (2)</td>
<td>Alkaline buffer (phosphate buffer)</td>
<td>300mmol/L</td>
</tr>
<tr>
<td></td>
<td>SDS</td>
<td>2.0g/L</td>
</tr>
<tr>
<td>CAL</td>
<td>Standard</td>
<td></td>
</tr>
</tbody>
</table>
It depends on the reaction of creatinine in the base medium with picric acid to be a complex of red color.

\[
\text{Creatinine} + \text{Picric acid} \xrightarrow{pH \geq 12} \text{Red complex}
\]

The preparation of the working reagent included the following steps:

1. The first solution (A1) was prepared by mixing reagent 1 with the sample, and left for (25min) at room temperature, and then the absorbance was read at a wavelength of (510nm).

2. The second solution (A2) was prepared by mixing reagent 2 with the sample, and left for (25min) at room temperature, and then the absorbance was read at a wavelength of (510nm).

3. After that, the concentration of creatinine was calculated depending on calculation of absorption according to the following equation:

\[
\text{creatinine concentration (mg/dL)} = N \times \frac{(A_1 - A_2)}{(B_1)}
\]

Where:

- \(A_1\): The first absorbance of the sample.
- \(A_2\): The second absorbance of the sample.
- \(B_1\): The first absorbance of the standard.
- \(N\): Standard concentration.

**Results and Discussion**

**Toxic effects of sodium fluoride**

The results of the present study showed that there are clear differences in the concentrations of blood electrolytes (sodium, potassium and calcium) in addition to serum urea, creatinine which can be adopted in determining kidney efficiency \(^{(15)}\). There was significant an increase in the concentrations of these parameters at a significant level \((P \leq 0.05)\) after treatment with sodium fluoride at a dose of \((20\text{mg/kg of body weight})\) when compared to control group as the concentrations in the control group were equal to \((39.86\pm0.16; 0.26\pm0.021; 0.50\pm0.04; 3.56\pm0.07; 134\pm0.46)\) while their concentrations were equal to \((43.45\pm0.54; 0.55\pm0.02, 11.29\pm0.07, 4.30\pm0.07 	ext{ and } 141\pm0.37)\) in the fluoride-treated group, respectively (Table 6). The results of present study were consistent with those of \(^{(16)}\) who noted in their study, of the effect of taking fluoride with feeding, that exposure to fluoride leads to an excessive increase in blood urea concentration besides creatinine, This was due to the inefficiency of kidneys in expelling toxic substances and reabsorbing minerals and non-metallic ions. In the same direction, \(^{(17)}\), pointed out that exposure to fluoride leads to a significant increase in the concentration of urea and creatinine and that was caused by the occurrence of renal failure. The results of current study confirmed those of \(^{(16)}\), increased serum creatinine concentration. As a result, the inability of kidneys to get rid of toxic wastes in the blood, which is linked to the occurrence of pathological changes in the kidney tissue after chronic exposure to sodium fluoride, but it did not agree with the results of current study with regard to high sodium, potassium and calcium and an indication of low standards because of reabsorption by the kidneys. Moreover, \(^{(18)}\) noted in their study of the effects of sodium fluoride on oxidative parameters in mice that fluoride leads to renal damage that can be inferred by high concentration of oxidative agents including (reactive oxygen species) malondialdehyde (Protein carbonyls) whose production is directly related to damage to kidneys such as (degeneration, necrosis of tubular cells) The rise of these oxidative parameters will cause high blood electrolytes concentrations and irregular concentration in the serum which is mainly related to the inefficiency of kidneys in getting the body ride of toxic waste. This was an affirmation of results reported by \(^{(19)}\), in his study of the linear relationship between the structure of the kidney and its function, noting that the function of the kidney depends mainly on its histological structure, and that any damage to the kidney tissue is accompanied by a decrease in the efficiency of the kidneys which is reflected negatively on blood electrolytes. In addition, \(^{(20)}\) pointed out that the damage of kidney tissue is accompanied by an increase in the productivity of oxidative stress factors in the blood, of which (reactive oxygen species), which in turn combine with peptides and proteins found in the plasma membrane structure of living cells and that this union works to reduce the liquidity of plasma membrane in addition to disrupting its permeability and thus disturbing the balance of fluids within the body organs and thus reduced the efficiency of the kidneys. In addition, \(^{(21)}\), pointed out that the imbalance between oxidative parameters and antioxidants due to exposure of the body to fluoride leads to tissue damage, in that direction many studies indicated that the exposure of the body to sodium fluoride leads to production of peroxide fat.
which reduces the effectiveness of glutathione peroxidase, catalase and superoxide dismutase in the kidneys leading to kidney damage. On the other hand, the results of present study showed a significant decrease at the level of significant failure and tissue damage \(^{(22)}\). While the results of present study showed significant decrease at \((P \leq 0.05)\) when compared to control group, the mean±SD body weight was equal to \((213.50±5.63 \text{ mg})\) in the control group and was equal to \((193.50±2.99 \text{ mg})\) in the fluoride group. The results of present study agreed with \(^{(23)}\) who, in their study of the effect of taking sodium fluoride with drinking water, found that exposure to sodium fluoride worked to damage tissues, which firstly affected the liver and kidneys, as the kidneys undertake to get rid of the body of all toxic elements which expose them to harm, whether by physiological or histological criteria, which resulted in lack of drinking water in the experimental animals and thus an imbalance in bodily fluids and low rate of metabolism in animals and then low weight of organs (liver, kidney, muscle mass). This in turn was associated with low body weight. While \(^{(24)}\) stated that exposure to sodium fluoride was accompanied by an increase in the weight of kidneys and this was associated with exposure to tissue damage which is the death of cells and the accumulation of body fluids inside.

1.1. Biological activity of Cranberry

The results of current study showed that the use of cranberry extract in the treatment of poisoning, caused by exposure to sodium fluoride, led to a significant decrease \((P \leq 0.05)\) in the concentrations of all blood electrolytes (sodium, potassium and calcium) in addition to creatinine and urea after treatment with cranberry at concentrations of \((150 \text{ and } 75 \text{ mg/kg})\) when compared with the fluoride-treated group as the concentrations in the latter were equal to \((0.55±0.02; 11.29±0.07; 4.30±0.07; 141±0.37; 43.45±0.54)\), while their concentrations in the Cranberry group first treatment appeared to be equal to \((41.99±0.48; 0.37±0.02; 10.76±0.05; 3.94±0.05; 137.80±0.44)\); whereas their concentrations were shown in the Cranberry group the second treatment to be equal \((42.90±0.28; 0.42±0.01; 10.96±0.04; 4.10±0.13; 136.50±0.41)\), respectively (Table 6). These results were comparable to concentrations in the control group but not equal to them. From these results it can be noted that cranberry contributed to significantly reduce the toxic effects of fluoride. The results of the current study were consistent with those pointed out in \(^{(25)}\) who reported the protective role of cranberry against toxic factors exposed to the body. Moreover, cranberry helps regulate the level of sugar and hemoglobin in the blood. The study of \(^{(26)}\) indicated that cranberry extract contains active substances, that contribute to the reduction of damage to the body as a result exposure to toxic substances, including Vitamin C, anthocyanin, Catechin and total phenol) that are combined with antioxidant efficacy, as it works to prevent oxidation that occurs through exposure of the body to metal ions by the work of a complex compound of (co pigment). In the same aspect, \(^{(27)}\) pointed to the antioxidant power of the compound (anthocyanin) which comes from its ability to remove free radicals from the blood. On the other hand, \(^{(28)}\) pointed to the importance of polyphenol compounds, which contain hydroxyl groups, in the removal of toxic elements through the formation of complexes with them. In the same aspect, \(^{(29)}\) pointed to the importance of polyphenol compounds in the structure of cranberry to protect the mortars in cell membranes from free radical reactions and thus preventing the formation of toxic peroxides within the blood that causes violent changes in the structure of cells and tissues. In addition, it can reduce the risk of cardiovascular fat by lowering the concentration of cholesterol in the blood. The study of \(^{(30)}\) indicated that the toxicity of sodium fluoride lies in the production of high concentrations of reactive oxygen species which contribute to the damage of target tissues by combining with the biological elements in the plasma membrane of living cells. These large concentrations produced by reactive oxygen species can cause damage to all living compounds within the cell including proteins, primates, sugars and DNA. Thus, it contributes to the failure of most of the body organs including the kidneys and liver \(^{(31)}\). In this aspect \(^{(32)}\) pointed to the significant role played by polyphenol in reducing the high levels of reactive oxygen species formed in the kidneys by preventing the formation of fat peroxides resulting from exposure to toxic substances and thus protect the body.
Table (6): The effects of sodium fluoride on some blood electrolytes

<table>
<thead>
<tr>
<th>Treatment Parameter</th>
<th>Negative control group (Before treatment)</th>
<th>Positive control group (After transaction NF)</th>
<th>First Preventive group</th>
<th>Second preventive group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>213.5± 5.6 A</td>
<td>193.5± 5 B</td>
<td>187.80± 2.9 CB</td>
<td>184.10± 2.33 CD</td>
</tr>
<tr>
<td>Sodium concentration</td>
<td>134.1± 0.6 A</td>
<td>141.6± 6 B</td>
<td>137.80± 80 C</td>
<td>136.50± 0.41 C</td>
</tr>
<tr>
<td>Potassium concentration</td>
<td>3.56± 0.07 A</td>
<td>4.30± 0.07 B</td>
<td>3.94± 0.05 C</td>
<td>4.10± 0.13 D</td>
</tr>
<tr>
<td>Calcium concentration</td>
<td>10.5± 0.04 A</td>
<td>11.29± 0.07 B</td>
<td>10.76± 0.05 C</td>
<td>10.96± 0.04 D</td>
</tr>
<tr>
<td>Urea concentration</td>
<td>39.86± 0.16 AD</td>
<td>43.45± 0.5 B</td>
<td>14.99± 0.48 C</td>
<td></td>
</tr>
<tr>
<td>Creatinine concentration</td>
<td>0.26± 0.02 A</td>
<td>0.55± 0.02 B</td>
<td>0.37± 0.02 C</td>
<td>0.42± 0.01 D</td>
</tr>
</tbody>
</table>

Similar letters mean non-significant differences. Different letters mean there was significant difference.

2. Conclusion
The low doses of sodium fluoride lead to nephrotoxicity which is associated with damage in renal tissue. Thus, sodium fluoride can be considered as a toxic substance that has cumulative effects in the body. Also, the moderate concentrations of cranberry can be used to decrease the toxic hazards of sodium fluoride.

Ethical Clearance: The research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

Conflict of interest: The authors declare that they have no conflict of interest.

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