Cranberry extract as a supplemented food in treatment of oxidative stress and breast cancer induced by N-methyl-N-nitrosourea in Female Virgin Rats

Sylvia A. Boshra¹ and Mohammed A. Hussein¹*

Abstract

Breast cancer is the most common cancer and a major cause of death in women. The present study was designed to evaluate the antioxidant and anticancer potential of cranberry extract against N-methyl-N-nitrosourea (MNU) induced mammary carcinoma in rats. The tumor was induced in Female virgin rats of age 50 days by single dose of MNU (50mg/kg.b.w i.p.). After 85 days; all rats developed at least one tumor. Animals were treated with cranberry extract (400 and 600 mg/kg.b.w orally) and tamoxifen (2mg/kg.b.w. i.p) for 4 weeks (from day 86 to day 113). MNU treatment resulted in a significant decrease (p < 0.05) in blood hemoglobin (Hb), red blood cells (RBC), platelets (PLTs) as well as blood, liver and breast catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD). However, MNU treatment resulted in a significant increase in White blood cells (WBC) as well as plasma, liver and mammary tissue gamma glutamyl transferase (GGT), lactate dehydrogenase (LDH), hexosamine, sialic acid and thiobarbituric acid reactive substances (TBARs). Upon administration of the cranberry extract, the levels of WBC, GGT, LDH, hexosamine, sialic acid, TBARs, Hb, RBC, PLTs, CAT, GPx and SOD were significantly normalized. Histopathological changes also confirmed the formation of tumor tubules and neovascularization after the MNU treatment. Cranberry extract administration significantly reduces the growth of MNU-induced mammary tumors, and therefore has strong potential as a useful therapeutic regimen for inhibiting breast cancer development. Comparing the beneficial effect of cranberry extract with that of MNU-induced breast cancer, cranberry extract showed antitumor and antioxidant activity indicated by the measured biochemical parameters and the histopathological examination of mammary tissue. The results of the present study indicate that cranberry extract possesses strong anticancer effects through its role in modulating glycoprotein components and the levels of oxidative stress biomarkers. Cranberry exerted a stronger anticancer effect at the dosage of 600 mg/kg body weight than at dosage 400 mg/kg body weight.

Keywords: MNU, tamoxifen, cranberry extract, breast cancer, liver, mammary tissue.

Introduction

Breast cancer is the most common cancer and a major cause of death in women [1]. It is a highly heterogeneous disease represented by tumors that have a diverse natural history, complex histology and a variable response to therapy [2]. The use of specific chemicals to prevent the development or retard the progression of carcinogenesis, a technique known as chemoprevention, offers a promising strategy for cancer prevention [3, 4]. Tamoxifen, a synthetic non-steroidal antagonist of estrogen receptor in breast tissue, used in the treatment and prevention of all stages of hormone dependent breast cancer in patients with early stage breast cancer as well as those with metastatic breast cancer. It reduces level of estrogen by competition with estrogen for binding to its receptor in breast tissue [5]. Many cancer chemotherapeutic agents exert their anticancer properties by inducing apoptosis and oxidative stress through mechanisms that involve mitochondria and nitric oxide (NO) [6]. Tamoxifen leads to oxidative liver damage and it has been elucidated to be a hepatocarcinogen in rodents, many cases of tamoxifen-induced hepatotoxicity have been reported including toxic hepatitis, massive hepatic steatosis, and multifocal
hepatic fatty in filtration, hepatic necrosis, hepatic cirrhosis and even hepatic cell carcinoma [7].
A new group of phytochemicals that has been attracting much attention from both the general public and health professionals is “proanthocyanidins” which are poly-phenols and more specifically are polymers of flavonoids. Their main dietary sources are to be found in cranberry, grapes, cocoa and apples [8, 9]. Proanthocyanidins are famed for their potent antioxidant capacity and free radical scavenging properties. There is substantial evidence that proanthocyanidins intake from grapes or cocoa have anticarcinogenic and anti-inflammatory properties and increase the antioxidant status among hypercholesterolemic, hyperlipidemic, hemodialysis patients and smoker [9]. One of such plants rich in proanthocyanidins [10], Cranberry ranks high among fruit in both antioxidant quality and quantity [11] because of its substantial flavonoid content and a wealth of phenolic acids. Cranberry extracts are rich in these compounds, they reportedly inhibit oxidative processes including oxidation of low-density lipoproteins [12, 13], oxidative damage to at neurons during simulated ischemia [14], and oxidative and inflammatory damage to the vascular endothelium [15]. The antioxidant properties of the phenolic compounds in cranberry fruit may contribute to the observed antitumor activity. Plant-derived fractions are rich sources of phenolic compounds [16]. Phenolics are known to have potential to prevent tumor and have been used in aromatherapy for obese middle-aged women. Flavonoids extracted from plants may have antioxidant activity that could mitigate tumor-related complications, including atherosclerosis and some cancers [17, 18]. Not surprisingly, plants such as cranberry extract contain high levels of unsaturated fatty acids and poly-phenols [10, 17], which are excellent antioxidant and antitumor agent [19, 20]. In vivo tests have been conducted with foods to determine for example, its antitumor [19], hypolipidemic, hypoglycemic and antioxidant activity [20]. As a continued interest of research program in pharmaceutical importance of cranberry extract [19-21], we report here, a facile route to evaluate the antitumor and antioxidant effects of cranberry extract against MNU induced breast cancer in female rats.

Materials and methods

MNU was purchased from Sigma, USA and dissolved in 0.9% NaCl containing 0.05% acetic acid (pH 5). Tamoxifen was purchased from Fresenius Oncology Ltd., India. Cranberry extract was purchased from Virgin Extracts (TM), Chinese.

Rats

This experiment was conducted in accordance with guidelines established by the Animal Care and use Committee of October 6 University. Female virgin rats of age 50 days were purchased from National Cancer Institute, Cairo University, Egypt. They were individually housed in cages in an air-conditioned room with a temperature of 22 ± 2°C, a relative humidity of 60%, and an 8:00 to 20:00 light cycle. During the acclimatization period, each animal was raised on a regular diet ad libitum.

Experimental design

The animals were divided into 5 groups consisting of 8 animals, two controls groups and three treatment groups:
Group (1): 8 rats: Negative control rats received 1ml 0.9% NaCl, i.p.
Group (2): 32 rats: Rats were given single dose of MNU (50mg/kg.b.w.i.p.) (22).
After 85 days, group 2 was divided into 4 subgroups, 8 in each. Subgroup (1): positive control (breast cancer bearing rats) Subgroup (2): Animals were treated with tamoxifen (2 mg/kg/ day, I.P.) for 28 days (from day 86 to day 113).
Subgroup (2): Animals were treated with cranberry extract at 400 mg/kg/ day, orally, for 28 days (from day 86 to day 113) (19).
Subgroup (4): Animals were treated with cranberry extract at 600 mg/kg/ day, orally, for 28 days (from day 86 to day 113) (19).
On day 114, at the end of the study, all rats were sacrificed, blood was collected, one part of blood was collected for hematological parameters such as hemoglobin (Hb), red blood cells (RBC), leucocyte (WBC) and platelet count (PLT) were determined as described by Jain [23]. Also, the other part centrifuged, and plasma was used freshly for estimation of plasma gamma glutamyl transferase [24] and lactate dehydrogenase (LDH) [25]. The levels of hexosamine and sialic acid in plasma, liver and mammary gland were estimated by the methods of Niebes, and Wagner respectively [26, 27].

blood, liver and mammary tissue catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD) and plasma thiobarbituric acid reactive substances (TBARs) levels were estimated by the methods of Sinha [28], Paglia and Valentine [29], Suttle [30] and Nichols and Samulelson [31], respectively. Protein was estimated by the method of Lowry et al [32].

Measurement of antioxidant enzymes activity

Superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) activities were determined using commercially available assay kits (Bio Diagnostics Inc., River Falls, WI, USA). Briefly, tissues were weighed and homogenized with appropriate buffers (provided by the kits). The homogenates were then determined following the procedures provided by the respective manufacturers. The Superoxide Dismutase Assay Kit utilizes a tetrazolium salt for detection of superoxide radicals generated by red formazan dye reduction produced (29). One unit (U) of SOD activity is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. The Glutathione Peroxidase Assay Kit measures GPx activity indirectly by a coupled reaction with GR [33]. Oxidized glutathione, produced upon reduction of hydroperoxide by GPx, is recycled to its reduced state by GR and NADPH. The oxidation of NADPH to NADP+ is accompanied by a...
Statistical analysis

All data were expressed as mean ± SD. All analyses utilized SPSS 13.0 statistical package for Windows (SPSS, 13.0 software, Inc., Chicago, IL, 2009) [36]. A one-way analysis of variance (ANOVA) was employed for comparisons of means of the different groups. A p-value <0.05 was accepted as statistically significant.

Results

Table 1: Effect of cranberry and Tamoxifen on hematological parameters in MNU induce breast cancer in rats

<table>
<thead>
<tr>
<th>No.</th>
<th>Groups</th>
<th>Hb% (g/dL)</th>
<th>RBCs (10^6/μL)</th>
<th>WBCs (x10^3 cells/mm^3)</th>
<th>PLT 10^3 / mm^3</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I)</td>
<td>Normal group</td>
<td>13.45 ± 1.03</td>
<td>5.260 ± 1.44</td>
<td>6.300 ± 0.64</td>
<td>595.25</td>
</tr>
<tr>
<td></td>
<td>(MNU, 50mg/kg.b.w.)</td>
<td>10.73 ± 1.17^a</td>
<td>3.690 ± 0.95^a</td>
<td>13.250 ± 2.30^a</td>
<td>382.46</td>
</tr>
<tr>
<td></td>
<td>Positive control</td>
<td>9.40 ± 0.87^ab</td>
<td>3.540 ± 0.89^ab</td>
<td>8.300 ± 1.09^ab</td>
<td>400.4</td>
</tr>
<tr>
<td></td>
<td>+ Tamoxifen (2mg/kg. b.w.)</td>
<td>12.29 ± 0.51^bc</td>
<td>4.240 ± 1.00^bc</td>
<td>7.250 ± 1.92^abc</td>
<td>490.60</td>
</tr>
<tr>
<td></td>
<td>+ 600mg/kg.w.b. Cranberry</td>
<td>12.60 ± 1.19^bc</td>
<td>4.380 ± 0.97^abc</td>
<td>6.200 ± 1.40^bcd</td>
<td>520.85</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD for groups of eight animals each. a: significant from normal control; b: significant from MNU (50mg/kg.b.w.) supplement group; c: significant from tamoxifen (2mg/kg. b.w.); d: significant from cranberry extract (400mg/kg. b.w.). * Values are statistically significant at P<0.05.
Table 2: Effect of cranberry and Tamoxifen on plasma and liver gamma glutamyl transferase and lactate dehydrogenase in MNU induce breast cancer in rats

<table>
<thead>
<tr>
<th>No.</th>
<th>Groups</th>
<th>Gamma glutamyl transferase</th>
<th>Lactate dehydrogenase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Plasma</td>
<td>Liver</td>
</tr>
<tr>
<td>(I)</td>
<td>Normal group</td>
<td>1.48± 0.19</td>
<td>5.06± 0.41</td>
</tr>
<tr>
<td>(II)</td>
<td>Positive control (MNU, 50mg/kg.b.w.)</td>
<td>3.67± 0.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.37± 0.55&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(III)</td>
<td>Positive control + Tamoxifen (2mg/kg, b.w.)</td>
<td>6.42± 1.38&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>12.70± 1.77&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>(IV)</td>
<td>Positive control + 400mg/kg.w.b. Cranberry</td>
<td>3.17±0.79&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>6.34±0.87&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>(V)</td>
<td>Positive control + 600mg/kg.w.b. Cranberry</td>
<td>2.75±0.77&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>5.76±1.08&lt;sup&gt;abcd&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD for groups of eight animals each.  
<sup>a</sup>: significant from normal control; 
<sup>b</sup>: significant from MNU (50mg/kg.b.w) supplement group; 
<sup>c</sup>: significant from tamoxifen (2mg/kg, b.w.); 
<sup>d</sup>: significant from cranberry extract (400mg/kg, b.w.).  
* Values are statistically significant at P<0.05.  
Liver Gamma glutamyl transferase is expressed as µmoles of p-nitroaniline liberated per minute/ mg of protein; liver lactate dehydrogenase is expressed as µmoles of pyruvate liberated per minute/ mg of protein; plasma Gamma glutamyl transferase lactate dehydrogenase are expressed as (IU/L).

Table 3: Effect of cranberry and Tamoxifen on plasma, liver and mammary tissue glycoprotein in MNU induce breast cancer in rats

<table>
<thead>
<tr>
<th>No.</th>
<th>Groups</th>
<th>Hexosamine</th>
<th>Sialic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Plasma</td>
<td>Liver</td>
</tr>
<tr>
<td>(I)</td>
<td>Normal group</td>
<td>39.27</td>
<td>3.87± 0.25</td>
</tr>
<tr>
<td>(II)</td>
<td>Positive control (MNU, 50mg/kg.b.w.)</td>
<td>61.53± 7.02</td>
<td>9.46± 1.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Positive control + Tamoxifen (2mg/kg, b.w.)</td>
<td>57.39± 3.20&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.35± 0.37&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>(IV)</td>
<td>Positive control + 400mg/kg.w.b. Cranberry</td>
<td>43.14± 3.25&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>5.86± 0.60&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>(V)</td>
<td>Positive control + 600mg/kg.w.b. Cranberry</td>
<td>34.27± 2.73&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>4.18± 0.59&lt;sup&gt;abcd&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD for groups of eight animals each.  
<sup>a</sup>: significant from normal control; 
<sup>b</sup>: significant from MNU (50mg/kg.b.w) supplement group; 
<sup>c</sup>: significant from tamoxifen (2mg/kg, b.w.); 
<sup>d</sup>: significant from cranberry extract (400mg/kg, b.w.).  
* Values are statistically significant at P<0.05.  
Liver and mammary tissues hexosamine and sialic acid are expressed as mg/g tissue and plasma hexosamine and sialic acid are expressed as mg/dL.
### Table 4: Levels of blood catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD) and plasma thiobarbituric acid reactive substances (TBARs) in MNU induce breast cancer in rats

<table>
<thead>
<tr>
<th>No.</th>
<th>Groups</th>
<th>CAT (U/L)</th>
<th>GPx (U/ml)</th>
<th>SOD (U/ml)</th>
<th>TBARs (nmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I)</td>
<td>Normal group</td>
<td>314.77</td>
<td>52.27</td>
<td>26.50</td>
<td>3.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 9.41</td>
<td>± 2.97</td>
<td>± 4.05</td>
<td>± 0.76</td>
</tr>
<tr>
<td>(II)</td>
<td>Positive control (MNU, 50mg/kg.b.w.)</td>
<td>293.38</td>
<td>43.80</td>
<td>19.36</td>
<td>4.88</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 1021</td>
<td>± 4.12</td>
<td>± 2.30</td>
<td>± 0.56</td>
</tr>
<tr>
<td>(III)</td>
<td>Positive control + Tamoxifen (2mg/kg. b.w.)</td>
<td>228.56</td>
<td>35.73</td>
<td>10.80</td>
<td>8.41</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 11.13</td>
<td>± 3.92</td>
<td>± 2.11</td>
<td>± 1.56</td>
</tr>
<tr>
<td>(IV)</td>
<td>Positive control + 600mg/kg.w.b. Cranberry</td>
<td>297.33</td>
<td>49.08</td>
<td>23.39</td>
<td>3.84</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±8.06</td>
<td>±2.44</td>
<td>±2.13</td>
<td>±0.41</td>
</tr>
<tr>
<td>(V)</td>
<td>Positive control + 600mg/kg.w.b. Cranberry</td>
<td>300.74</td>
<td>55.20</td>
<td>26.66</td>
<td>3.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±18.81</td>
<td>±3.11</td>
<td>±3.38</td>
<td>±0.61</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD for groups of eight animals each. a: significant from normal control; b: significant from MNU (50mg/kg.b.w) supplement group; c: significant from tamoxifen (2mg/kg. b.w.) ; d: significant from cranberry extract (400mg/kg. b.w.) . * Values are statistically significant at P<0.05.

### Table 5: Levels of liver catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD) and plasma thiobarbituric acid reactive substances (TBARs) in MNU induce breast cancer in rats

<table>
<thead>
<tr>
<th>No.</th>
<th>Groups</th>
<th>CAT (U/g protein)</th>
<th>GPx (U/ml)</th>
<th>SOD (U/mg protein)</th>
<th>TBARs (nmol/g protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I)</td>
<td>Normal group</td>
<td>46.37</td>
<td>20.37</td>
<td>65.83</td>
<td>6.86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 3.78</td>
<td>± 3.94</td>
<td>± 8.91</td>
<td>± 1.53</td>
</tr>
<tr>
<td>(II)</td>
<td>Positive control (MNU, 50mg/kg.b.w.)</td>
<td>36.58</td>
<td>14.83</td>
<td>43.36</td>
<td>16.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 4.41</td>
<td>± 2.04</td>
<td>± 6.09</td>
<td>± 3.02</td>
</tr>
<tr>
<td>(III)</td>
<td>Positive control + Tamoxifen (2mg/kg. b.w.)</td>
<td>29.80</td>
<td>11.56</td>
<td>33.66</td>
<td>22.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 2.44</td>
<td>± 1.93</td>
<td>± 3.00</td>
<td>± 3.15</td>
</tr>
<tr>
<td>(IV)</td>
<td>Positive control + 400mg/kg.w.b. Cranberry</td>
<td>40.25</td>
<td>17.05</td>
<td>51.28</td>
<td>7.52</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±3.42</td>
<td>±1.71</td>
<td>±4.88</td>
<td>±1.20</td>
</tr>
<tr>
<td>(V)</td>
<td>Positive control + 600mg/kg.w.b. Cranberry</td>
<td>48.45</td>
<td>19.55</td>
<td>57.05</td>
<td>6.68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±2.16</td>
<td>±1.51</td>
<td>±2.72</td>
<td>±2.20</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD for groups of eight animals each. a: significant from normal control; b: significant from MNU (50mg/kg.b.w) supplement group; c: significant from tamoxifen (2mg/kg. b.w.) ; d: significant from cranberry extract (400mg/kg. b.w.) . * Values are statistically significant at P<0.05.

### Table 6: Levels of breast catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD) and plasma thiobarbituric acid reactive substances (TBARs) in MNU induce breast cancer in rats

<table>
<thead>
<tr>
<th>No.</th>
<th>Groups</th>
<th>CAT (U/g protein)</th>
<th>GPx (U/mg protein)</th>
<th>SOD (U/mg protein)</th>
<th>TBARs (nmol/g protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I)</td>
<td>Normal group</td>
<td>608.44</td>
<td>1.37</td>
<td>3.15</td>
<td>3.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 28.82</td>
<td>± 0.28</td>
<td>± 0.58</td>
<td>± 0.26</td>
</tr>
<tr>
<td>(II)</td>
<td>Positive control (MNU, 50mg/kg.b.w.)</td>
<td>528.74</td>
<td>0.85</td>
<td>1.44</td>
<td>4.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 17.63</td>
<td>±0.1</td>
<td>± 0.40</td>
<td>±0.22</td>
</tr>
<tr>
<td>(III)</td>
<td>Positive control + Tamoxifen (2mg/kg. b.w.)</td>
<td>468.25</td>
<td>0.28</td>
<td>0.98</td>
<td>5.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 15.85</td>
<td>±0.05</td>
<td>± 0.3</td>
<td>±0.28</td>
</tr>
<tr>
<td>(IV)</td>
<td>Positive control + 400mg/kg.w.b. Cranberry</td>
<td>570.16</td>
<td>0.93</td>
<td>2.48</td>
<td>3.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±20.07</td>
<td>±0.1</td>
<td>±0.36</td>
<td>±0.71</td>
</tr>
<tr>
<td>(V)</td>
<td>Positive control + 600mg/kg.w.b. Cranberry</td>
<td>666.09</td>
<td>1.44</td>
<td>3.89</td>
<td>2.37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±18.44</td>
<td>±0.07</td>
<td>±0.24</td>
<td>±0.24</td>
</tr>
</tbody>
</table>
Values are given as mean ± SD for groups of eight animals each. a: significant from normal control; b: significant from MNU (50mg/kg.b.w) supplement group; c: significant from tamoxifen (2mg/kg. b.w.); d: significant from cranberry extract (400mg/kg. b.w.). * Values are statistically significant at P<0.05.

Histology of normal mammary gland (Figure 1A) shows the presence of lobules (L) with numerous acini and clear basement membrane (BM). Figure 1B of mammary tissue of treated MNU (50mg/kg.b.w.) group clearly shows marked proliferation of stroma (SP) resulted due to stromal reaction with carcinogen. Figure 1C of mammary tissue treated MNU (50mg/kg.b.w.) + tamoxifen (2mg/kg.b.w.) showed moderate decreased of glandular elements. Figure 1D of mammary tissue treated MNU + cranberry extract (400mg/kg.b.w.) showed mild decrease of glandular elements. Figure 1E of mammary tissue treated MNU + cranberry extract (600mg/kg.b.w.) showed moderate decrease of glandular elements.

Discussion

Animal models are particularly useful for the study of human mammary carcinogenesis. Since the rat mammary gland shows a high susceptibility to develop neoplasms which closely mimic human breast cancer [37]. The carcinogen N-methyl-N-nitrosourea (MNU) induces hormone dependent mammary tumors in rats. This model has previously been used to develop breast cancer [38]. Breast cancer is one of the main life-threatening diseases [39]. Although different anticancer drugs are present in the market, their serious adverse effects still need to identify potent anticancer molecules from natural origin. Herbal medicine has been regarded as one of the most visible fields for cancer chemoprevention and it constitutes the main source of effective new anticancer agents [40, 41]. In the present research, in vivo anticancer and antioxidant activity of cranberry extract against MNU induced mammary cancer...
in female rats was reported for the first time. The induction of mammary tumors in Sprague-Dawley rats has also been well reported [42, 43]. Its effects on Wistar rats were examined by da Silva Franchi et al [44], while Akanni et al. [45] reported its effects on the hematolog of Wistar rats. Results of the present study, showed that oral administration of cranberry can normalize the levels of hematological parameters, which may be due to the presence of antioxidant phytochemicals [46-50]. These results were in agreement with previous studies which concluded that administration cranberry extract provided normalization in hematological parameters in leucopenia rats [21].

Tumor markers are most useful for monitoring response to therapy and early detection of cancer. The result of this study showed elevated plasma and liver GGT and LDH activity in the tumor-bearing female rats. Other study showed that serum ALP concentration increased significantly in cancer patients with metastasis [51]. In our rats, there was evidence of metastasis, suggesting that the increased GGT and LDH may in fact be due to the primary tumor. Liver metastasis [52] and heptotoxicity [53] also can be determined by changes in serum GGT and LDH. Plasma and liver LDH concentrations often increased significantly in MNU treated female rats [54]. According to Perunal et al. [55], the elevated activity of LDH may be due to overproduction by tumor cells, or it may be due to the release of isoenzymes from destroyed tissues. Our results are also consistent with the above reports. The significant high (P < 0.05) plasma and liver LDH concentrations observed in this study were similar to those in human cancer patients with endometrial adenocarcinoma, ovarian cyst adenocarcinomas and breast cancers. However, it was suggested that the plasma LDH concentration is nonspecific for the diagnosis of metastasis [51]. Some studies showed correlations between serum GGT concentration and malignant neoplasm such as cancers of the digestive, respiratory, female genital, lymphoid and hematopoietic organs [56]. Our findings showed that the serum GGT concentrations were significantly different in normal and tumor-bearing rats suggesting that this serum parameter is not a good biomarker for rat mammary gland tumors. These results were hand in hand with other studies [57] that reported that serum GGT significant increase in women with breast malignant neoplasm.

It is quite well known that flavanones, a cranberry flavonoid act as antioxidant molecules [58], which can scavenge the excess free radicals in biological system. Since cranberry has shown antioxidant and free radical scavenging activity [59], the present study primarily ameliorating the effect of cranberry' polyphenols on free radicals accumulation and oxidative damage in the liver of MNU treated rat is studied. Oral administration of cranberry extract significantly inverse the MNU induced peroxidative damage in liver which is evidenced from the lowered levels of GGT and LDH. This may be due to the antioxidative effect of polyphenols [60]. An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reaction can produce free radicals, which start chain reactions that damage cells.

Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. As a result are often reducing agents such as thiols, ascorbic acid or polyphenols [61].

Breast cancer is the third most common malignancy affecting female population, and approximately 19% of cancer mortality was reported worldwide [62]. Glycoproteins; e.g. hexosamine and sialic acid are common components of cell surfaces and are also commonly found as constituents of lysosomes and among the products secreted/exposed by the cell [63]. The cell surface glycoproteins have been shown to play important roles in tumourogenesis [64]. Elevation of glycoprotein contents are useful indicators of carcinogenic process and these changes alter the rigidity of cell membranes [65]. Abnormal increase in the levels of plasma glycoprotein component have been related to the changes in hepatic cells during neoplastic transformation. Sialic acids are widely distributed in nature as terminal sugars in glycoproteins or glycolipids, impart a net negative charge to cell surface and are reported to be important in cell-to-cell and cell-to matrix interactions [66]. It was previously demonstrated that neoplastic transformation leads to elevated plasma sialic acid concentration [67] through the shedding or secreting of sialic acid from the tumor cell surfaces [68]. In the present study increased levels of glycoproteins in plasma, liver and breast tissues of cancer bearing animals were observed.

Flavonoids and other phenolic compounds are well known natural antioxidants. The flavonoids present in cranberry extract are thought to be the cause of their antitumor and anti-inflammatory effects [46-50]. Flavonoids have a chemopreventive role in cancer by means of their effect in signal transduction in cell proliferation and angiogenesis [21]. This important property may be responsible for its antitumor and antioxidant activity against MNU induced breast cancer. Antioxidant and antitumor activity of cranberry extract against different carcinogenic agents has already been established by the present authors [21].

CAT, GPx and SOD are inducible enzymes important in the detoxication of many different xenobiotics in mammals. The antioxidant enzymes achieve detoxication by catalyzing the conjugation of reduced glutathione to various electrophilic substrates [69]. It serves as a marker for hepatotoxicity in rodent system, and also plays an important role in carcinogen detoxification [70]. Consequently, inhibition of antioxidant enzymes activity might potentiate the deleterious effects of many environmental toxicants and carcinogens. Antioxidant enzymes are also engaged in the intracellular transport of variety of hormones, endogenous metabolites, and drugs, by virtue of their capacity to bind these substances [71]. The decreased activity of antioxidant enzymes in group II cancer bearing animals may be due to the excessive utilization of this enzyme in conjugation process and also may be due to the enhancement of the covalent binding of MNU metabolites to cellular DNA and results in an increase in the degree
of cell damage leading to neoplastic growth. Results of the present study, showed that administration of MNU resulted in significant decrease in CAT, SOD and GPx activity as well as significant increase in TBARS level.

Also, in this study it was found that tamoxifen significantly reduced the blood, hepatic and mammary tissue antioxidant enzymes CAT, SOD and GPx accompanied with significant elevation of TBARS level; a product of lipid peroxidation, compared to normal control group [72-73]. The activities of intracellular antioxidant enzymes decreased with the increase of lipid peroxidation levels [74], which were concomitant with the results achieved from this study. More over depletion of the hepatic reduced glutathione GSH results in the reduction of GPx activity as glutathione peroxidase utilizes GSH for H$_2$O$_2$ detoxification into water and organic peroxides (R-OOH) and this would eventually result in H$_2$O$_2$ accumulation which in turn leads to exhaustion of antioxidant superoxide dismutase (SOD) and catalase (CAT) enzymes [75].

Superoxide dismutase, catalase and glutathione peroxidase constitute the major enzymatic antioxidant defenses which convert active oxygen molecules in to non-toxic compounds [76]. Superoxide dismutase is a ubiquitous enzyme with an essential function in protecting aerobic cells against oxidative stress. It is primarily mitochondrial enzyme usually found in the plasma membrane [77]. Catalase is a tetrameric heme protein that undergoes alternative divalent oxidation and reduction at its active site in the presence of hydrogen peroxide [78]. As a substrate for the antioxidant enzyme glutathione peroxidase, reduced glutathione protects cellular constituents from the damaging effects of peroxides formed in metabolism and other reactive oxygen species reaction [79]. Glutathione peroxidase catalyzes the reaction of hydroperoxides with reduced glutathione to form glutathione disulphide and the reduction product of the hydroperoxide [80]. Cranberry extract offers protection against oxidative damage due to the ability of enhanced antioxidant activity [81]. Antioxidant and hepatoprotective effects of cranberry extract might be associated with the structure-antioxidant relationship of its active constituents such as vitamin C, vitamin E and polyphenols (anthocyanins).

Finally, histopathological examination showed marked degree of mammary tissue proliferation in MNU-treated rats (Figure 1B). Comparing the beneficial effect of cranberry extract with that of MNU-induced breast cancer, cranberry showed antitumor activity indicated by the measured biochemical parameters and the histopathological examination of mammary tissue. In addition, group of rats continuously treated with cranberry extract with MNU injection showing mild decrease of glandular elements (fig. 1D&E).

In this study, the most novel and relevant finding was that cranberry extract supplementation was accompanied by the alleviation of mammary tissue proliferation in this model. Antitumor and antioxidant effect of cranberry extract against breast cancer induced by MNU has not been reported earlier to our knowledge, and this study is perhaps the first observation of its kind.

In conclusion, the present study showed that cranberry extract has a powerful antitumor and hepatoprotective activity against MNU induced breast cancer and liver toxicity. These effects could be due to membrane protective action of cranberry by scavenging the free radicals and its antioxidant action. This could serve as a stepping stone towards the discovery of newer safe anticancer and antioxidant agents.

Acknowledgement

Thanks to Ass. Prof. Dr. Amal Haridy, Faculty of Medicine, Cairo University, Egypt, for her involvement in histopathological examination.

References


Gastroenterol Hepatol 2004; 16:593–598.


[21]. Hussein MA and Boshra SA. Antileukopenic and Antioxidant Effects of Cranberry Extract in Benzene and fluorouracil induced leukopenia in Rats. IJARNP, 2015; inpress.


[65]. Selvam S and Nagini S. Administration of the plasticizer di(engihexyl)phthalate


[76]. Lee CP, Shih PH, Hsu CL and Yen GH. Hepatoprotection tea seed oil (Camellia oleifera Abel) against CCl4- induced oxidative damage in rats. Food and chem toxicol 2007; 45: 888-893.


