Original Research

Anti-Leukopenic and Antioxidant Effects of Cranberry Extract in Benzene and Fluorouracil induced Leukopenia in Rats

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Summary. The present study was to evaluate anti-leukopenia and antioxidant effects of cranberry extract (222mg/kg.b.w, orally) in 400mg/kg.b.w., orally benzene and/or 20mg/kg.b.w., I.P 5-Flourouracil-induced leukopenia rats. Two weeks after induction of leukopenia in rats, cranberry extract was administrated for 30 consecutive days. On the 31st day, the rats were sacrificed for the estimation of hemoglobin (Hb%), complete blood cell count Leucocyte (WBC) and platelet count (PLT), as well as biochemical parameters; alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), lipid peroxides (TBARS), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), total cholesterol (TC), triglycerides (TG), HDL-C, LDL-C, p53gene expression, nitric oxide (NO) and tumor necroses factor-α (TNF-α). The results of this study showed that administration of cranberry extract to leukopenia induced rats demonstrated a significant (P<0.01) increase in Hb%, WBCs and PLT as well as a significant (P<0.01) improvement in biochemical parameters and life span as compared to benzene and/or 5-Flourouracil control rats. The histological examinations of this study revealed damage and degeneration in the lung of benzene and/or 5-Flourouracil treated rats. Also, lung of cranberry treated rats showed significant improvement and protection against benzene and/or 5-Flourouracil harmful effect. On the other hand, the results clearly suggested that the oxidative stress of benzene was higher than 5-Flourouracil.

Industrial relevance. Our observations have clearly demonstrated that the cranberry extract has significant antioxidant and anti-leukopenia activity due to presence of phenolic compounds. Cranberry extract possessed a capability to inhibit the lipid peroxidation and activate the antioxidant markers (GSH, SOD and CAT) in leukopenia-induced by 5-Flourouracil and benzene in rats. Also, industrial relevance of the present results showed that cranberry extract can be used as an antioxidant and anti-leukopenia therapeutic agent and deserves clinical trial in the near future as an adjuvant therapy in leukopenic patients. This could serve as a stepping stone towards the discovery of newer safe and effective antitumor agents.

Key words: Cranberry extract; 5-Flourouracil; Benzene; Leukopenia; Antioxidants.

INTRODUCTION

Cancer is an unnatural cell growth, where they can loss their natural function and spread through of the blood, at all the body. Breast cancer is the more commonly diagnosed in industrialized countries and has the highest death toll. Oxidative stress is involved in the process development of cancer and tumors; due to that ROS can damage the macromolecules as lipids which react with metals (as free iron and copper) and produce aldehydes and synthesize malondialdehyde inducing mutations or cause breaks in the double chain, produce modifications in guanine and thymine bases, and sister chromatid exchanges. Human exposure to benzene occurs primarily via inhalation in the workplace, from gasoline vapors, tobacco smoke, and automotive emissions. Individuals exposed to benzene exhibit bone marrow depression, as evidenced by anemia (decreased RBC count), leukopenia (decreased WBC count), and/or thrombocytopenia (decreased platelet count). A depression of all three elements is called pancytopenia, and the simultaneous depression of RBCs, WBCs, and platelets, accompanied by necrosis of the bone marrow, is diagnostic of aplastic anemia. Patients with aplastic anemia also have exhibited mild bilirubinemia, changes in osmotic fragility of erythrocytes, shortened erythrocyte survival time, increased fecal urobilinogen, and mild reticulocytosis. 5-Fluorouracil (5-FU) is an antimetabolite that acts during the S phase of the cell cycle. The toxicity of 5-FU, which includes leukopenia, diarrhea, stomatitis, nausea, vomiting, and alopecia, differs with its schedule of administration. Plants vegetables and spices used in folk and traditional medicine have gained wide acceptance as one of the main sources of prophylactic and chemopreventive drug discovery and development. It is widely accepted that a diet rich in fruits and plants are rich sources of different kinds of antioxidants, phenolic compounds are the most studied and have been recognized to possess a wide range of properties including antioxidant, antibacterial, anti-inflammatory, hepatoprotective and anticarcinogenic actions. Many of the biological functions of flavonoids, phenolic, catechins, curcumin, resveratrol and genistein compounds have been attributed to their free radical scavenging, metal ion chelating and antioxidant activities. Several medicinal plants have been implicated in the mechanisms of chemoprevention which refers to the use chemical substances of natural origin or synthetic to reverse,
retard or delay the multistage carcinogenesis process. One of such plants, Cranberry ranks high among fruit in both antioxidant quality and quantity because of its substantial flavonoid content and a wealth of phenolic acids. Cranberry extracts rich in these compounds reportedly inhibit oxidative processes including oxidation of low-density lipoproteins, oxidative damage to neurons during simulated ischemia, and oxidative and inflammatory damage to the vascular endothelium. The antioxidant properties of the phenolic compounds in cranberry fruit may contribute to the observed antitumor activities of cranberry extracts. Plant-derived fraction are rich sources of volatile terpenoids and phenolic compounds. Terepenoids are known to have potential to prevent obesity and have been used in aromatherapy for obese middle-aged women. Phenolic compounds extracted from plants may have antioxidant activity that could mitigate tumor-related complications, including atherosclerosis and some cancers. Not surprisingly, plants such as cranberry extract contain high levels of polyphenols, which are excellent scavengers of reactive and represent a promising anti-tumor effects. In vivo tests have been conducted with cranberry extract to determine for example, its hepatoprotective, hypolipidemic, hypoglycemic, antioxidant and antitumor activity. But there are no reports about antioxidant of cranberry extract against benzene and/or 5-fluorouracil induced leucopenia in female albino rats.

## MATERIALS AND METHODS

**Chemicals.** 5-fluorouracil and benzene were from Merck Ltd., Germany. All the other reagents used were of analytical grade and were obtained commercially.

**Induction of Leukopenia.** Leukopenia has been demonstrated to occur in rats by oral administration of 400mg/kg.b.w. benzene and/or intraperitoneally injection of 20mg/kg.b.w. 5-Flourouracil/day after day for 2 weeks.

**Dose of Cranberry.** Cranberry extract was purchased from Virgin Extracts (TM), Chinese. Cranberry was given to female mice with 1/50 LD50 (222mg/kg.b.w.), daily for 4weeks (2 weeks after benzene doses) by oral gastric gavage tube.

**Rats.** This experiment was conducted in accordance with guidelines established by the Animal Care and use Committee of Faculty of Pharmacy, October 6 University, Egypt. Adult rats weighing around 180 ± 20gms were purchased from National Cancer Institute, Cairo, 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, three control groups and two treatment groups:

- **Group (1):** Negative control.
- **Group (2):** Positive control-A (400mg/kg.b.w. benzene, orally)
- **Group (3):** Positive control-B (20mg/kg.b.w. 5-fluorouracil, I.P.)
- **Group (4):** Leukopenia bearing rats (400mg/kg. benzene, orally)+ 222mg/kg.b.w.cranberry daily for 4 weeks.
- **Group (5):** Leukopenia bearing rats (20mg/kg.5-fluorouracil,l.I.P)+222mg/kg.b.w.cranberry extract daily for 4 weeks.

On 31th day, 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), complete blood cell count Leucocyte (WBC) and platelet count (PLT) were determined as described by Jain, and the other part centrifuged, and plasma was used freshly for estimation of plasma transaminases (L-alanine and L-aspartate), alkaline phosphatase (ALP), also, lactate dehydrogenase (LDH), Nitric Oxide (NOx), tumor necroses factor-α (TNF-α) and GSH levels in blood and hepatic were done by the methods described by Buhl and Jackson, Miranda and Espey, Beyaert and Fiers, Koster, et al., and Chanarin, respectively. Blood and lung Superoxide dismutase (SOD) and catalase (CAT) activities were carried out Paglia and Valentine, Sinha, respectively. Plasma triglyceride, total cholesterol and HDL-cholesterol were determined using commercially available kits (Asan and Youngdong Pharmaceutical Co.,Korea). Plasma LDL-cholesterol level was calculated from Falholt et al formula (LDL-cholesterol = totalcholesterol – triglycerides/5 – HDL-cholesterol). Finally, lung p53 gene were determined according to the method described by Tribukait.

**Determination of lung P53 gene.** Primer Design. Primers were designed based on the genomic and mRNA sequences retrieved from gene sequence databases such as National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). The location of every intron and exon was determined within the gene sequences based on the mRNA sequence in order to design the primers at exon-exon junctions to avoid the false positive results arising from amplification of possible contaminating genomic DNA. Primers were checked by BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to ensure that they did not have any non-specific binding sites on either the same gene or similar sequence sites in other species. The primer sequences and appropriate annealing temperatures are shown in the following Table.

<table>
<thead>
<tr>
<th>Official name</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta Actin</td>
<td>5′ CGTGCCTAGACATTTAACAGAA 3′</td>
<td>5′ CGCTCATATGGCCGATAGTGAT 3′</td>
</tr>
<tr>
<td>P53</td>
<td>5′ GGAGAATATTTTCACCCCTAAGATCC 3′</td>
<td>5′ GAGTGAGGGCCTGTGCTCTCC 3′</td>
</tr>
</tbody>
</table>

**Gene-specific PCR for amplification of the P53 gene.** PCR was performed using GoTaq Green Master Mix (Promega, Madison, US) in order to set up the optimum annealing temperature for P53 gene. A 25μl PCR reaction was set up as follows:
12.5μl of 2X GoTaq Green Master Mix, which contains GoTaq® DNA polymerase that is supplied in 2X Green GoTaq® Reaction Buffer (pH 8.5), 400μM dATP, 400μM dGTP, 400μM dCTP, 400μM dTTP and 3mM MgCl2; 400nM of 10μM forward (5′GGAGAATATTTCACCCTTAAGATCC3′) and reverse primers (5′ GAGTGAGCCCTGCTGTCTCCT 3′), 200ng cDNA template, and appropriate volume of nuclease free water was added to a 0.2ml nuclelease free PCR tubes and centrifuged for 10sec. The thermal cycler was adjusted as follows: initial denaturation step at 95°C for 2min, subsequent denaturation step at 95°C for 30s, optimization of the annealing conditions by performing the gradient reaction starting approximately 5°C below the calculated melting temperature of the primers and increasing the temperature in increments of 3°C to the annealing temperature for 30sec, followed by 72°C for 25sec for template extension and a final extension of 5 minutes at 72°C, following a 4°C incubation for 10min. All the PCR reaction preparation steps were performed on ice.

**Agarose Gel Electrophoresis.** The GoTaq® Reaction Buffer contains yellow and blue dyes, the blue dye migrates at the same rate as 3–5kb DNA fragments, and the yellow dye migrates at a rate faster than primers (<50bp), in a 1% agarose gel. The PCR product was separated on 2% agarose gel containing 0.1% ethidium bromide (EtBr) and the DNA bands were visualized with a UV transilluminator containing an EtBr filter. The image system was a Gel Doc EZ System imager.

**Analysis of the Relative Expression level of the P53 gene.** The relative levels of expression of each gene were analyzed by taking the band intensity using Quantity One software, Biorad. The ratios of desired genes/β-actin product were subsequently calculated after subtraction of the background pixel intensity for each gene of interest and used to assess the differences in expression levels between control and different groups.

**Histological assessment.** Lungs from rats of different groups were fixed in 10% neutral formalin solution, dehydrated in graded alcohol and embedded in paraffin. Fine sections obtained were mounted on glass slides and counter-stained with Hematoxylin Eosin (H&E) for light microscopic analyses according to the method of Bancroft and Steven 37. The slides were coded and examined by a histopathologist who was ignorant about the treatment groups after which photographs were taken.

**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, 2003)38. 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. Benzene and/or 5-fluorouracil administrated positive control rats were compared with normal control rats as well as experimental groups (cranberry extract + benzene and/or 5-fluorouracil treated rats were compared with positive controls A & B, respectively.

**RESULTS**

Oral administration of benzene at 400mg/kg.b.w. and/or 20mg/kg.b.w. 5-fluorouracil resulted in a significant decrease in blood hemoglobin, complete blood cell count Leucocyte (WBC) and platelet count (PLT) compared with the normal control group(p<0.01). Group of rats received benzene at 400mg/kg.b.w. and/or 20mg/kg.b.w. 5-fluorouracil and treated with cranberry extract at 222mg/kg resulted in a significant increase in hemoglobin, WBCs and PLT compared with the positive controls A and B respectively (p<0.01).

**Tables 2-4** showed that oral administration of benzene and/or 5-fluorouracil resulted in a significant increase in plasma AST, ALT, ALP, LDH and TBARs as well as decrease in blood and lung GSH, SOD and CAT compared to the normal control group (p< 0.01). Administration of cranberry extract at 222mg/kg.b.w. rats resulted in a significant decrease in plasma AST, ALT, ALP, LDH and TBARs as well as increase in blood and lung GSH, SOD and CAT compared with the positive controls A and B respectively (p<0.01).

**Tables 5** showed that oral administration of 400mg benzene and/or 20mg/kg/b.w. 5-fluorouracil resulted in a significant increase in plasma cholesterol (TC), triglycerides (TG) and LDL-C and significant decrease in HDL-C compared with the normal control group (p< 0.01). Administration of cranberry extract at 222mg/kg.b.w. rats resulted in a significant decrease in plasma TC, TG and LDL-C and an increase in HDL-C compared with the positive controls A and B respectively (p<0.01).

**Tables 6** showed that oral administration of 400mg benzene and/or 20mg/kg/b.w. 5-fluorouracil resulted in a significant increase in plasma and lung nitrous oxide (NO) and tumor necroes factor-α (TNF-α) compared with the normal control group (p<0.01). Administration of cranberry extract at 222mg/kg.to rats resulted in a significant decrease in plasma and lung nitrous oxide (NO) and tumor necroses factor-α (TNF-α) compared with the positive controls A and B respectively (p<0.01).

**Table 1.** Effect of cranberry, 5-fluorouracil and there combination on hematological parameters in benzene induce leukopenia in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hb% (g/dL)</th>
<th>WBCs (x10⁶ cells/mm³)</th>
<th>PLT (10⁷/mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (1)</td>
<td>12.15 ± 1.45</td>
<td>9.7 ± 0.65</td>
<td>506 ± 7.68</td>
</tr>
<tr>
<td>Group (2)</td>
<td>9.9 ± 0.87</td>
<td>3.8 ± 0.90</td>
<td>433 ± 5.35</td>
</tr>
<tr>
<td>Group (3)</td>
<td>9.46 ± 0.68</td>
<td>3.2 ± 0.84</td>
<td>457.4 ± 6.90</td>
</tr>
<tr>
<td>Group (4)</td>
<td>13.45 ± 1.15</td>
<td>6.50 ± 1.50</td>
<td>613 ± 4.60</td>
</tr>
<tr>
<td>Group (5)</td>
<td>13.65 ± 1.33</td>
<td>6.60 ± 0.75</td>
<td>676 ± 7.00</td>
</tr>
</tbody>
</table>

Group (1): Negative control; Group (2): Positive control-A (400mg/kg.b.w. benzene, orally); Group (3): Positive control-B (20mg/kg/b.w. 5-fluorouracil, I.P.); Group (4): Leukopenia bearing rats (400mg/kg, benzene, orally) + 222mg/kg/b.w. cranberry daily for 4 weeks; Group (5): Leukopenia bearing rats (20mg/kg. 5-fluorouracil, I.P.) + 222mg/kg/b.w. cranberry extract daily for 4 weeks. Values are given as mean ± SD for groups of eight animals each. * Significantly different from normal group at p< 0.05.a: significant from Group (1); b: significant from Group (4); c: significant from Group (3).
Table 2. Level of plasma alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) in normal and experimental groups of rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALP (U/L)</th>
<th>LDH (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (1)</td>
<td>12.5± 2.30</td>
<td>16.39±1/55</td>
<td>80.67±5.47</td>
<td>176.49±18.90</td>
</tr>
<tr>
<td>Group (2)</td>
<td>24.60±2.46*</td>
<td>29.43±4.20*</td>
<td>184.16±14.65*</td>
<td>286.28±15.40*</td>
</tr>
<tr>
<td>Group (3)</td>
<td>35.46±4.88*</td>
<td>30.90±3.09*</td>
<td>210.76±15.87*</td>
<td>310.66±17.34*</td>
</tr>
<tr>
<td>Group (4)</td>
<td>22.25±1.67*</td>
<td>19.57±2.76*</td>
<td>110.50±10.78*</td>
<td>226.78±20.98*</td>
</tr>
<tr>
<td>Group (5)</td>
<td>18.70±1.98*</td>
<td>16.45±2.10*</td>
<td>80.05±8.77*</td>
<td>170.57±17.60*</td>
</tr>
</tbody>
</table>

Group (1): Negative control; Group (2): Positive control-A (400mg/kg b.w. benzene, orally); Group (3): Positive control-B (20mg/kg b.w. 5-fluorouracil, I.P.); Group (4): Leukopenia bearing rats (400mg/kg benzene, orally) + 222mg/kg b.w. cranberry daily for 4 weeks; Group (5): Leukopenia bearing rats (20mg/kg 5-fluorouracil, I.P) + 222mg/kg b.w. cranberry extract daily for 4 weeks. Blood samples were collected. Values are given as mean ± SD for groups of eight animals each. * Significantly different from normal group at p< 0.05. a: significant from Group (1); b: significant from Group (4); c: significant from Group (3).

Table 3. Level of blood reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and plasma thiobarbituric acid reactive substances (TBARs) in normal and experimental groups of rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH (mg%)</th>
<th>SOD (U/ml)</th>
<th>CAT (U/ml)</th>
<th>TBARs (µmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (1)</td>
<td>33.44± 2.85</td>
<td>251.30±16.54</td>
<td>35.22±2.25</td>
<td>3.54± 0.81</td>
</tr>
<tr>
<td>Group (2)</td>
<td>11.36± 1.64*</td>
<td>132.71±8.44*</td>
<td>20.23±2.86*</td>
<td>6.88± 0.57*</td>
</tr>
<tr>
<td>Group (3)</td>
<td>17.95± 2.25*</td>
<td>177.78±13.28*</td>
<td>22.52±1.27*</td>
<td>5.49± 0.50*</td>
</tr>
<tr>
<td>Group (4)</td>
<td>25.8± 1.96*</td>
<td>225.96±11.82*</td>
<td>30.17±3.00*</td>
<td>3.10± 0.85*</td>
</tr>
<tr>
<td>Group (5)</td>
<td>35.48± 3.48*</td>
<td>249.06±22.37*</td>
<td>37.68±2.88*</td>
<td>3.25± 0.66*</td>
</tr>
</tbody>
</table>

Group (1): Negative control; Group (2): Positive control-A (400mg/kg b.w. benzene, orally); Group (3): Positive control-B (20mg/kg b.w. 5-fluorouracil, I.P.); Group (4): Leukopenia bearing rats (400mg/kg benzene, orally) + 222mg/kg b.w. cranberry daily for 4 weeks; Group (5): Leukopenia bearing rats (20mg/kg 5-fluorouracil, I.P) + 222mg/kg b.w. cranberry extract daily for 4 weeks. Activity is expressed as: 50% of inhibition of pyrogallol auto oxidation per min for SOD. Values are given as mean ± SD for groups of eight animals each. * Significantly different from normal group at p< 0.05. a: significant from Group (1); b: significant from Group (4); c: significant from Group (3).

Table 4. Level of lung reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and thiobarbituric acid reactive substances (TBARs) in normal and experimental groups of rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH (mg/100g tissues)</th>
<th>SOD (U/100gm tissue)</th>
<th>CAT (U/ml)</th>
<th>TBARs (mg/100g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (1)</td>
<td>145.61± 12.44</td>
<td>345.24±14.56</td>
<td>48.58±5.30</td>
<td>0.79± 0.11</td>
</tr>
<tr>
<td>Group (2)</td>
<td>68.37± 8.72*</td>
<td>150.54±10.30*</td>
<td>17.48±2.84*</td>
<td>1.83± 0.08*</td>
</tr>
<tr>
<td>Group (3)</td>
<td>81.25± 6.97*</td>
<td>202.22±16.49*</td>
<td>16.47±3.60*</td>
<td>1.43± 0.14*</td>
</tr>
<tr>
<td>Group (4)</td>
<td>113.70± 8.46*</td>
<td>298.60±22.48*</td>
<td>37.25±4.58*</td>
<td>0.97± 0.08*</td>
</tr>
<tr>
<td>Group (5)</td>
<td>135.60± 11.80*</td>
<td>333.76±17.14*</td>
<td>41.60±4.00*</td>
<td>0.72± 0.07*</td>
</tr>
</tbody>
</table>

Group (1): Negative control; Group (2): Positive control-A (400mg/kg b.w. benzene, orally); Group (3): Positive control-B (20mg/kg b.w. 5-fluorouracil, I.P.); Group (4) : Leukopenia bearing rats (400mg/kg benzene, orally) + 222mg/kg b.w. cranberry daily for 4 weeks; Group (5): Leukopenia bearing rats (20mg/kg 5-fluorouracil, I.P) + 222mg/kg b.w. cranberry extract daily for 4 weeks. Activity is expressed as: 50% of inhibition of pyrogallol auto oxidation per min for SOD. Values are given as mean ± SD for groups of eight animals each. * Significantly different from normal group at p< 0.05. a: significant from Group (1); b: significant from Group (4); c: significant from Group (3).

Table 5. Level of plasma total cholesterol (TC), triglycerides (TG), HDL-C and LDL-C of normal and experimental groups of rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (1)</td>
<td>117.39± 5.47</td>
<td>98.09±7.00</td>
<td>37.80±2.50</td>
<td>59.97± 5.47</td>
</tr>
<tr>
<td>Group (2)</td>
<td>287.50±22.74*</td>
<td>239.70±11.80*</td>
<td>29.56±3.00*</td>
<td>210.00±11.38*</td>
</tr>
<tr>
<td>Group (3)</td>
<td>246.23±21.48*</td>
<td>220.00±13.75*</td>
<td>30.16±2.88*</td>
<td>175.07±9.80*</td>
</tr>
<tr>
<td>Group (4)</td>
<td>223.65±18.79*</td>
<td>187.29±9.83*</td>
<td>34.75±5.49*</td>
<td>151.44±11.87*</td>
</tr>
<tr>
<td>Group (5)</td>
<td>188.40±8.00*</td>
<td>150.77±7.49*</td>
<td>36.28±3.07*</td>
<td>121.97±9.68*</td>
</tr>
</tbody>
</table>

Group (1): Negative control; Group (2): Positive control-A (400mg/kg b.w. benzene, orally); Group (3): Positive control-B (20mg/kg b.w. 5-fluorouracil, I.P.); Group (4): Leukopenia bearing rats (400mg/kg benzene, orally) + 222mg/kg b.w. cranberry daily for 4 weeks; Group (5): Leukopenia bearing rats (20mg/kg 5-fluorouracil, I.P) + 222mg/kg b.w. cranberry extract daily for 4 weeks. Values are given as mean ± SD for groups of eight animals each. * Significantly different from normal group at p< 0.05. a: significant from Group (1); b: significant from Group (4); c: significant from Group (3).
Table 6. Levels of plasma and lung nitrous oxide (NO) and tumor necrosis factor-α (TNF-α) of normal and experimental groups of rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Plasma (umol/ml)</th>
<th>Lung (u mol/g tissue)</th>
<th>Plasma (U/ml)</th>
<th>Lung (U/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (1)</td>
<td>42.88 ± 4.35</td>
<td>66.93 ± 2.25</td>
<td>237.55 ± 11.25</td>
<td>15.17 ± 1.07</td>
</tr>
<tr>
<td>Group (2)</td>
<td>57.86 ± 5.40</td>
<td>98.91 ± 7.46</td>
<td>342.39 ± 10.87</td>
<td>33.36 ± 3.28</td>
</tr>
<tr>
<td>Group (3)</td>
<td>44.83 ± 2.87</td>
<td>84.14 ± 2.76</td>
<td>301.74 ± 8.58</td>
<td>32.13 ± 4.66</td>
</tr>
<tr>
<td>Group (4)</td>
<td>30.80 ± 3.80</td>
<td>64.80 ± 4.87</td>
<td>240.98 ± 13.47</td>
<td>18.30 ± 3.70</td>
</tr>
<tr>
<td>Group (5)</td>
<td>25.45 ± 3.11</td>
<td>57.59 ± 5.07</td>
<td>233.68 ± 20.98</td>
<td>15.64 ± 3.27</td>
</tr>
</tbody>
</table>

Group (1): Negative control; Group (2): Positive control-A (400mg/kg.b.w. benzene, orally); Group (3): Positive control-B (20mg/kg.b.w. 5-fluorouracil, I.P.); Group (4): Leukopenia bearing rats (400mg/kg. benzene, orally) + 222mg/kg.b.w. cranberry daily for 4 weeks; Group (5): Leukopenia bearing rats (20mg/kg. 5-fluorouracil, I.P) + 222mg/kg.b.w. cranberry extract daily for 4 weeks. Values are given as mean ± SD for groups of eight animals each. * Significantly different from normal group at p< 0.05. a: significant from Group (1); b: significant from Group (4); c: significant from Group (3).

Reverse transcriptase PCR (Figure 1) results showed that lung P53 mRNA expression level was significant increased (p< 0.01) in benzene and/or 5-fluorouracil treated groups into 215±22.5 and 162±8.9, respectively when compared to negative control group (100±5.2). However, reverse transcriptase PCR results shown that P53 mRNA expression level was significant decreased (p< 0.05) in cranberry extract treated group (150±15.4) and (198±23.3) when compared to benzene and/or 5-fluorouracil positive controls A and B, respectively.

Figure 1: Reverse transcriptase PCR of lung P53 mRNA expression level. (G1) Negative control; (G2) Positive control-A (400mg/kg.b.w. benzene, orally); (G3) Positive control-B (20mg/kg.b.w. 5-fluorouracil, I.P.); (G4) Leukopenia bearing rats (20mg/kg. 5-fluorouracil, I.P) + 222mg/kg/b.w. cranberry extract daily for 4 weeks.  (G5): Leukopenia bearing rats (400mg/kg. benzene, orally) + 222mg/kg/b.w. cranberry daily for 4 weeks

Figure (2): Analysis of lung P53 transcript by semi-quantitative PCR in lung tissues A) P53 amplicon B) GAPDH amplicon from (1) Negative control; (2) Positive control-A (400mg/kg.b.w. benzene, orally); (3) Positive control-B (20mg/kg.b.w. 5-fluorouracil, I.P.); (4) Leukopenia bearing rats (20mg/kg. 5-fluorouracil, I.P) + 222mg/kg/b.w. cranberry extract daily for 4 weeks. ; (5) Leukopenia bearing rats (400mg/kg. benzene, orally) + 222mg/kg/b.w. cranberry daily for 4 weeks
Histopathology examination of the lung tissues:
Group (1) control negative group showed micrograph of lung section is normal the bronchial veolar unit parenchyma, is normal (Figure 2 (G 1)). Group 2 (control positive- A, rats treated with 400mg/kg/b.w. benzene) showed cloudy swelling, medullary haemorrhage, emphysematous changes and showing thickened septa and marked dilated congested blood vessel in the alveolar septa.marked irregular air spaces (Figure 2 (G 2). Group (3) control positive- B, rats treated with 20mg/kg/b.w. 5-flurouracil) showed moderate cloudy swelling (Figure 2 (G 3). Group (4) rats treated with 400mg/kg/b.w. benzene + 222mg/kg/b.w. cranberry) showed parenchyma nearly normal and normal appearance of interalveolar septa (Figure 2 (G 5). Group 5 rats treated with 20mg/kg/b/w.5-flurouracil + 222mg/kg/b.w. cranberry) showed less normal appearance of interalveolar septa with mild infiltration of inflammatory cells, the congestion of lung was mild with no emphysematous changes and most of air space appeared normal and regular.(H & E) (Figure 2 (G 5).

DISCUSSION
The present article aimed at studying the antitumor activity of administration of cranberry extract at 222mg/kg. in benzene and/or 5-flurouracil induce leukopenia in rats. Our results showed that administration of benzene and/or 5-flurouracil induces leukopenia in rats. This result also was confirmed by Aksoy and Sobrero.

Reduced hemoglobin, WBCs and PLT, i.e. leukopenia. In this study, we observed and reported that cranberry can normalize the levels of hematological parameters, which may be due to the presence of antioxidant phytochemicals.

Lung is considered to be the one of main organ of drug detoxifying organ, some lung marker enzyme levels were measured from plasma. AST, ALT, ALP, LDH, NO, TNF-α, and TBARs levels were increased in benzene and/or 5-flurouracil induced leukopenia groups, whereas GSH, SOD and CAT levels were decreased in blood and lung tissue. Reactive oxygen species (ROS), like superoxide anions, are under normal physiological conditions cleared by antioxidant defense system such as GSH, SOD and CAT. Superoxide anion is dismutated to hydrogen peroxide (H₂O₂) in a process catalyzed by SOD, and H₂O₂ is then eliminated by catalase or GSH-Px. The activities of SOD and GSH-Px were lowered in 5-FU-treated guinea pigs demonstrating a reduced antioxidant capacity. If not eliminated by cellular antioxidant systems, superoxide anions can generate the highly reactive and toxic hydroxyl radicals (−OH) through the Haber–Weiss reaction, which is catalyzed by iron. Increased ROS levels inside cells lead to oxidation of macromolecules, including lipids, nucleic acids, and proteins, thereby disturbing cellular functions. MDA is a frequently used marker of lipid peroxidation, and MDA levels were elevated in guinea pig after 5-FU-treatment, and slightly elevated (but not significantly) in isolated rat organs after 5-FU-treatment. These
findings indicate that some degree of oxidative stress and cellular damage takes place in animal organs during 5-FU-treatment. Our study suggested that 5-FU-induced damage to the arterial endothelium may be due to generation of free radicals, resulting in lipid peroxidation. Phanolic compounds of cranberry increase SOD and CAT activities in animals, hereby improving antioxidant potential.

In the present study, administration of benzene and/or 5-flourouracil resulted in a significant decrease in blood GSH, SOD and CAT as well as plasma TC, TG, HDL-C and LDL-C with a significant increase in plasma TBARS compared to the normal control group. These results were in agreement with Gupta et al., and Raju & Arockiasamy, who reported that the consumption of free amino acid for building the proteins of rapidly dividing tumor cells might result in the disturbance of the enzyme activity in the lung. On treatment with cranberry altered lung enzyme level was restored as that of the normal group.

Alterations of cholesterol metabolism, including increased cholesterol synthesis and accumulation of cholesterol esters in tumor tissues associated with decrease of high density lipoprotein cholesterol in serum, were previously observed in different models of neoplastic cell proliferation including hematological malignancies. A number of studies had indicated that reactive oxygen species (ROS) are involved in a variety of different cellular processes ranging from apoptosis and necrosis to cell proliferation and carcinogenesis.

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. Flavonoids have a chemopreventive role in cancer by means of their effect in signal transduction in cell proliferation and angiogenesis. This important property may be responsible for its leukopanic activity against benzene and/or 5-flourouracil. Antioxidant activity of cranberry extract against different reactive oxygen and nitrogen species has already been established by the present authors.

The present work showed that benzene and/or 5-flourouracil administration caused increase of lung P53 mRNA expression level when compared with normal control rats. Furthermore, cranberry induces apoptosis in p53-null lung cells (Figure 1). Cranberry proanthocyanidins can block cell cycle progression or even apoptosis in a p53-independent manner as well, especially in the cells that lack functional p53.

**CONCLUSIONS**

The present study is concluded that cranberry showed anti-leukopenia and antioxidant potential in benzene and/or 5-flourouracil induced leukopenia which can be attributed to its flavonoids content. This could serve as a steppingstone towards the discovery of newer safer and effective antitumor agents.

**CONFLICT OF INTEREST**

The authors declare no conflicts of interest.

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