Antitumor and Antioxidant Activity of Honey in Mice Bearing Ehrlich Ascites Carcinoma

Ahmed Hegazi, Ragaa H.M. Al Tahtawy, Fyrouz Abd Allah and Amr M. Abdou

Abstract: Antitumor and antioxidant activity of coriander honey was investigated in mice bearing Ehrlich ascites carcinoma (EAC). The administration of coriander honey in a dose of (500 mg/kg/mouse) to EAC bearing mice causes decrease in ascitic fluid volume and viable tumor cell count and increases in nonviable tumor cell count. Administration of coriander honey to EAC bearing mice increases the life span of EAC bearing mice. The administration of coriander honey reduces the level of both lipid peroxidation and superoxide dismutase (SOD) while it increases the level of glutathione (GSH) in comparison to EAC control group and shifting them back to near the normal values. These results suggest the possibility of using coriander honey as an effective antioxidant to prevent or interrupt the process of cancer progression.

Key words: Coriander Honey • Antioxidant Activity • Antitumor Activity • Ehrlich Ascites Carcinoma

INTRODUCTION

Honey has been used since long time in human tradition particularly in medical and nutritional applications. The medical importance of honey is a reflection of its unique composition which is rather variable and depends on the floral source and external factors including seasonal variations, environmental conditions and processing methods [1-3]. Cancer continues to represent a major cause of mortality in the world claiming over 6 million lives every year [4]. Earlier studies highlighted the role of oxidative stress in the pathogenesis and complications of degenerative and chronic diseases including cancer [5, 6]. Antioxidants acting as free radical scavengers may inhibit the cancer process in vivo. The antioxidant capacity of honey contributes to the prevention of several acute and chronic disorders such as diabetes [7], inflammatory disorders [8, 9], cardiovascular diseases [10] and cancer [11]. Flavonoids, terpenoids and steroids have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and antitumor activity [12, 13]. The phenolic acids and flavonoids are responsible for the well-established antioxidant activity of honey [9, 14] as well as stimulation of immunity [15]. The present study was carried out to evaluate the influence of antioxidant of coriander honey on the antitumor activity against Ehrlich ascites carcinoma in Swiss albino mice.

MATERIALS AND METHODS

Honey: Coriander honey was purchased from local market in Egypt. Sterile distilled water was used to dilute honey immediately before administration by a stomach tube. Honey was used in a final concentration of 500 mg/kg/mouse.

Animals: Studies were carried out using a total of 210 male Swiss albino mice weighting 22-25 g obtained from Animal House of National Research Center, Giza, Egypt.

Ehrlich Ascites Carcinoma: Ehrlich ascites carcinoma (EAC) cells were obtained from Cancer Biology Section, National Cancer Institute, Cairo, Egypt. The Ehrlich tumor line was maintained, till the time of the experiment in female Swiss albino mice by serial intraperitoneal passage of $2 \times 10^6$ cells/mouse at 7-10 days intervals.
Table 1: Experimental Groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Group Name</th>
<th>Saline</th>
<th>Coriander honey</th>
<th>EAC</th>
<th>5-fluorouracil</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal control</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Coriander control</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>5-fluorouracil control</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>EAC control</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Coriander + EAC</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>5-fluorouracil + EAC</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**Standard Anticancer Drug:** 5-Fluorouracil (Calbiochem, USA) was used as a standard anticancer drug. 5-Fluorouracil was injected intraperitoneally to mice at a dose of 20 mg/kg body weight [16, 17].

**Experimental Design:** A total of 210 male Swiss albino mice were divided into 6 groups (n=35) (Table 1) with an average weight 22-25 gm each as follows:

The first group (normal control) received a daily dose of 50 µl/mouse normal saline through oral administration for 14 days. The second group (coriander control) received a daily dose of 500 mg/kg/mouse through oral administration for 14 days. The third group (5-fluorouracil control) received a daily dose of 20 mg/kg/mouse of 5-fluorouracil as standard anticancer drug for 14 days. The fourth group (EAC control) was inoculated intraperitoneally with EAC cell line (2×10^6 cells/mouse). The fourth group received also the same treatment of normal saline like the normal control group. The fifth and sixth groups (Coriander + EAC and 5-fluorouracil + EAC, respectively) were inoculated intraperitoneally with EAC like the EAC control group but, the fifth group further received the same treatment of coriander honey like the coriander control group while the sixth group also received 5-fluorouracil like the 5-fluorouracil control group.

After 14 days of the experiment 5 mice from each group were subjected to 18 h fasting before sacrificing. Anti-tumor effect of coriander honey was assessed by observation of changes in body weight, ascetics volume, viable and nonviable tumor cell count. The remaining animals in each group were kept to check the mean survival time (MST) of the tumor bearing hosts and increase in life span percentage (ILS %). MST of each group containing five mice were monitored by recording the mortality daily for 7 weeks and ILS % was calculated using the following equation: MST= (Day of first death + Day of last death)/2. ILS % = [(Mean survival time of treated group/mean survival time of control group) -1] X 100 [18, 19].

The liver of sacrificed mice was excised, rinsed in ice-cold normal saline followed by cold 0.15 mol/L Tris-HCl buffer (pH 7.4), blotted dry and weighed. A 10 % w/v homogenate was prepared in 0.15 mol/L Tris-HCl buffer and a portion utilized for the estimation of lipid peroxidation [20]. Another portion of the same liver tissue after precipitating proteins with trichloroacetic acid (TCA) was used for the estimation of glutathione [21]. The remaining homogenate was centrifuged at 1500 rpm for 15 min at 4°C. The supernatant thus obtained was used for the estimation of superoxide dismutase [22, 23] and protein content [24].

**Statistical Analysis:** The results obtained in the present study were represented as means ± standard error and were analyzed using analysis of variance (ANOVA). The significance of difference between means at P<0.05 was calculated using the Duncan Multiple Range Test [25].

**RESULTS**

Administration of repeated daily dose of 500 mg/kg/mouse coriander honey for 14 days did not show any abnormal behavioral responses. Coriander honey increased the body weight of the mice in coriander control group (Table 2). In the EAC control group the mean survival time was 22 days with a decrease of 44.3% in expected life span compared to normal control group, while the treatment with coriander honey increased the mean survival time to 34.5 days in coriander + EAC group with a decrease of 12.7% in expected life span compared to normal control group. Meanwhile, the mean survival for 5-fluorouracil+EAC group was 36.5 days with a decrease of 7.5% in expected life span compared to normal control group. Treatment with coriander honey reduced the ascites volume (1.60±0.01mL) and viable tumor cell count (8.84±0.06 x10^6 cells/L) as compared to that of EAC control group (3.37±0.07mL and 12.30±0.07 x10^6 cells/L respectively). On the other hand nonviable tumor cell counts in coriander + EAC were increased when compared with the EAC control (Table 2).
Table 2: Effect of coriander honey on body weight, MST, % ILS, ascites volume, viable and non-viable tumor cell count in EAC bearing mice.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal Control</th>
<th>Coriander control</th>
<th>5-flourouracil control</th>
<th>EAC (2×10⁶ cells/mouse) + EAC</th>
<th>Coriander honey + EAC</th>
<th>5-flourouracil + EAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>25.70±0.16</td>
<td>28.22±0.16</td>
<td>20.27±0.09</td>
<td>36.70±0.16</td>
<td>34.60±0.19</td>
<td>31.20±0.14</td>
</tr>
<tr>
<td>Mean survival time (d)</td>
<td>39.5</td>
<td>42</td>
<td>38.5</td>
<td>22</td>
<td>34.5</td>
<td>36.5</td>
</tr>
<tr>
<td>Increase life Span %</td>
<td>0</td>
<td>6.3</td>
<td>-2.5</td>
<td>-4.3</td>
<td>-12.7</td>
<td>-7.5</td>
</tr>
<tr>
<td>Ascites volume (mL)</td>
<td>0</td>
<td>0</td>
<td>3.37±0.07</td>
<td>1.60±0.01</td>
<td>1.20±0.01</td>
<td></td>
</tr>
<tr>
<td>Viable tumor cell count (x10⁶ cells/L)</td>
<td>0</td>
<td>0</td>
<td>12.30±0.07</td>
<td>8.84±0.06</td>
<td>5.04±0.04</td>
<td></td>
</tr>
<tr>
<td>Non-Viable tumor cell count (x10⁶ cells/L)</td>
<td>0</td>
<td>0</td>
<td>0.89±0.06</td>
<td>1.62±0.06</td>
<td>1.57±0.05</td>
<td></td>
</tr>
</tbody>
</table>

Mean ± SE  P<0.01 vs EAC control group.

Table 3: Effect of coriander honey on hematological parameters, lipid peroxidation, glutathione content and superoxide dismutase in the liver of EAC bearing mice

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal Control</th>
<th>Coriander control</th>
<th>5-flourouracil control</th>
<th>EAC (2×10⁶ cells/mouse) + EAC</th>
<th>Coriander honey + EAC</th>
<th>5-flourouracil + EAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin g/dl</td>
<td>13.40±0.14</td>
<td>13.95±0.18</td>
<td>11.02±0.17</td>
<td>10.51±1.15</td>
<td>11.95±0.18</td>
<td>11.87±0.19</td>
</tr>
<tr>
<td>RBCs (x10¹²/L)</td>
<td>6.53±0.11</td>
<td>7.95±0.06</td>
<td>4.92±0.02</td>
<td>3.71±0.09</td>
<td>5.95±0.06</td>
<td>5.47±0.03</td>
</tr>
<tr>
<td>WBCs (x10¹²/L)</td>
<td>4.73±0.09</td>
<td>5.02±0.06</td>
<td>12.51±0.08</td>
<td>17.20±0.03</td>
<td>5.02±0.06</td>
<td>8.97±0.03</td>
</tr>
<tr>
<td>Monocyte %</td>
<td>1.80±0.01</td>
<td>1.80±0.01</td>
<td>1.20±0.03</td>
<td>1.10±0.02</td>
<td>1.80±0.01</td>
<td>1.40±0.01</td>
</tr>
<tr>
<td>Neutrophil %</td>
<td>17.8±0.15</td>
<td>25.10±0.12</td>
<td>53.50±0.19</td>
<td>65.40±0.17</td>
<td>35.10±0.12</td>
<td>39.90±0.12</td>
</tr>
<tr>
<td>Lymphocyte %</td>
<td>80.4±0.23</td>
<td>73.10±0.43</td>
<td>45.30±0.35</td>
<td>33.50±0.42</td>
<td>63.10±0.43</td>
<td>58.70±.33</td>
</tr>
<tr>
<td>Lipid peroxidation (nmol MDA/mg protein)</td>
<td>0.96±0.04</td>
<td>0.98±0.05</td>
<td>0.94±0.02</td>
<td>1.40±0.01</td>
<td>1.20±0.01</td>
<td>1.07±0.02</td>
</tr>
<tr>
<td>Glutathione content (mg/g wet tissue)</td>
<td>2.35±0.12</td>
<td>2.45±0.17</td>
<td>2.31±0.28</td>
<td>1.63±0.07</td>
<td>1.75±0.06</td>
<td>1.90±0.09</td>
</tr>
<tr>
<td>Superoxide dismutase (U/mg protein)</td>
<td>4.49±0.18</td>
<td>4.60±0.37</td>
<td>4.53±0.27</td>
<td>2.89±0.26</td>
<td>3.27±0.23</td>
<td>3.58±0.29</td>
</tr>
</tbody>
</table>

Mean ±SE  P<0.01 vs normal group, P<0.01 vs EAC control group.

Hematological status in mice bearing Ehrlich carcinoma were evaluated by measuring some hematological parameters including hemoglobin concentration, red blood cell (RBCs) count, total and differential leukocytic count (Table 3). Hemoglobin concentration showed an increase in coriander control group while it showed a decrease in both EAC control and 5-flourouracil control groups. The EAC control group was subjected to a significant increase in both WBCs count and percentage neutrophil while it was subjected to a significant decrease in RBCs count. Administration of coriander honey brought back all hematological parameters near the normal range.

The level of lipid peroxidation, glutathione and superoxide dismutase was measured to assess the antioxidant activity of coriander honey in EAC bearing mice (Table 3). The levels of lipid peroxidation in liver tissue were increased in EAC control group (1.40±0.01 nmol MDA/mg protein) as compared to the normal control group (0.96±0.04 nmol MDA/mg protein). After administration of coriander honey to EAC bearing mice the level of lipid peroxidation was reduced (1.20±0.01 nmol MDA/mg protein) in comparison to EAC control group. Inoculation of EAC decreased the GSH content in EAC control group (1.63±0.07 mg/g wet tissue) when compared with normal group (2.35±0.12 mg/g wet tissue), while the administration of coriander honey to the EAC bearing mice increased GSH levels (1.75±0.06 mg/g wet tissue) when compared with EAC control group. The level of superoxide dismutase (SOD) in the liver of EAC bearing mice was decreased (2.89±0.26 U/mg protein) in comparison with normal control group (4.49±0.18 U/mg protein), while the administration of coriander honey increased the level of SOD (3.27±0.23 U/mg protein) as compared to that of EAC control group.

**DISCUSSION**

The present study was carried out to evaluate the antitumor and antioxidant activity of coriander honey on EAC bearing mice. The administration of coriander honey at a daily dose of 500 mg/kg/mouse decreased ascites volume, tumor cell count and also brought back the hematological parameters to near the normal levels. However, the administration of coriander honey caused an increase in body weight during the course of experiment. On the other hand coriander honey restored the hepatic lipid peroxidation and the free radical scavenging enzyme GSH as well as the antioxidant enzyme SOD in tumor bearing mice to near normal levels.
A reliable criterion for judging the value of any anticancer agent is the prolongation of life span of animals [26]. A statistically significant antimetastatic effect of honey was documented earlier and found to be achieved by oral application. The findings indicated that honey activates the immune system and its administration may be advantageous with respect to cancer and metastasis prevention. Oral administration of honey before tumor cell inoculation was found to decrease spreading of tumor [27, 28].

In cancer chemotherapy, the major problems are myelosuppression and anemia [29, 30]. The anemia encountered in EAC bearing mice is mainly due to reduction in RBCs count or hemoglobin percentage and this may occur either due to iron deficiency or due to hemolytic or myelopathic conditions [31]. Treatment with coriander honey brought back the hemoglobin content, RBCs and WBCs counts to near the normal values.

Coriander honey was found to stimulate mammalian tissue regeneration, as it caused strong activation of mitosis of cells cultured in vitro and it enhanced protein biosynthesis [32, 33]. Administration of honey increased the body weight of both male and female rats. In female mice, honey exhibited an estrogenic activity resembling estradiol and caused an increase in uterine weight, while in male mice it showed androgenic activity (acetylcholine like action) and stimulated the parasympathetic terminal [34].

Malignant diseases may cause predisposition of the host to bacterial infection. The immunosuppressive effect of Ehrlich ascites carcinoma was documented earlier. This effect was due to the presence of low molecular weight factors in the ascetic fluid that can cause an impairment of macrophages function [35]. The antibacterial activity of honey due to the presence of some active compounds including flavonoids [36, 37] is advantageous to prevent the bacterial infection in mice which become vulnerable to infection due to EAC. Honey as well as other bee products was found to modulate the immune response against infection. Hegazi et al., [38] studied the effect of some bee products on immune response of chicken infected with virulent Newcastle Disease Virus (NDV). They found that, the mortality rate was reduced in groups infected with virulent NDV and subsequently treated either with propolis or honey when compared with the infected groups only.

Excessive production of free radicals resulted in oxidative stress, which leads to damage of macromolecules such as lipids which can induce lipid peroxidation in vivo [39]. Increased lipid peroxidation would cause degeneration of tissues. Lipid peroxide formed in the primary site would be transferred through the circulation and provoke damage by propagating the process of lipid peroxidation [40]. MDA, the end product of lipid peroxidation was reported to be higher in carcinomatous tissue than in non-diseased organs [39]. There is a significant correlation between the antioxidant activity, the phenolic content of honey and the inhibition of the in vitro lipoprotein oxidation of human serum [41]. Honey caused an increase of both the antioxidant and the reducing serum capacity [42]. Honey increased the body antioxidant agents including blood vitamin C concentration by 47%, ß-carotene by 3%, uric acid by 12% and glutathione reductase by 7% [43]. Ahn et al., [44] suggested that, through the synergistic action of its antioxidants, honey by reducing and removing ROS, may lower the risks and effects of acute and chronic free radical induced pathologies in vivo. Furthermore, glutathione, a potent inhibitor of neoplastic process plays an important role as an endogenous antioxidant system that is found particularly in high concentration in liver and is known to have key function in the protective process [40]. Coriander honey at the dose of 500 mg/kg/ mouse reduced the elevated levels of lipid peroxidation and increased the glutathione content in EAC bearing mice. On the other hand the free radical scavenging system, SOD and catalase are present in all oxygen-metabolizing cells and their function is to provide a defense against the potentially damaging reactivity of superoxide and hydrogen peroxide. The inhibition of SOD activity as a result of tumor growth has also been previously reported [45]. Sun et al., [46] reported a decrease in SOD activity in EAC bearing mice which might be due to loss of minor SOD activity in EAC cells and the loss of mitochondria, leading to a decrease in total SOD activity in the liver. Similar findings were observed in the present investigation with EAC bearing mice. The administration of coriander honey increased the SOD level which may indicate the antioxidant and free radical scavenging property of coriander honey.

The antioxidant activity of honey is generally attributed to its content of phenolic compounds and flavonoids [47-49]. Honey has been also found to contain other antioxidants including glucose oxidase, catalase, ascorbic acid, carotenoid derivatives, organic acids, amino acids and proteins [50, 51]. The flavonoid derived from coriander honey showed cytotoxicity towards tumor cells [52] and antitumor activity in experimental animals [53]. The decrease of lipid peroxidation and increase in levels of GSH and SOD following the administration of coriander honey indicates its potential as an inhibitor of EAC...
induced intracellular oxidative stress. The results of the current study propose that the antitumor activity of coriander honey which can be inferred from the increased life span of EAC bearing mice is due to its antioxidant activity. Further investigations are in progress in our laboratory to identify the active principles involved in this antitumor and antioxidant activity.

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REFERENCES


