Anticancer Effect of Black Raspberry and Apricot Juice against Colon Cancer Induced by Azoxymethane in Male Rats

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Abstract
This study was carried out to evaluate the anticancer effect of black raspberry juice (RJ) and apricot juice (AJ) against colon cancer induced by Azoxymethane (AOM) in rats and to examine the possible mechanisms. Forty two Sprague Dawley male rats were used and randomly distributed into 6 equal groups (n=7). The 1st group was negative control and each rat from the other 5 groups was given a single subcutaneous dose (15 mg/kg b.wt.) of AOM once weekly for 2 weeks to induce colon cancer. The 2nd group was kept positive control and rats in the 3rd, 4th, 5th and 6th groups were orally given RJ and AJ at low (5%) and high (10%) concentrations, respectively, daily for 12 weeks. Serum colon cancer biomarkers [(tumor necrosis factor α-(TNF-α)); nuclear factor-κappaB (NF-κB); interleukin1β (IL-1β) and interleukin and 6 (IL6)] were measured and histopathology of the colon was done to evaluate the anticancer effect. Lipid peroxidation and activities of antioxidant enzymes in colon tissues and content of 8-hydroxy-2-deoxyguanosine (8-OHdG) (apoptosis marker) in colon DNA were measured to examine the possible mechanisms. The results showed that oral administration of RJ and AJ inhibited AOM-induced elevations in serum colon cancer biomarkers levels, antagonized preneoplastic aberrant colon crypts induced by AOM, decreased lipid peroxidation; increased activities of antioxidant enzymes in colon tissues and reduced elevation of 8-OHdG content in colon DNA. These results denoted that RJ and AJ have an anticancer effect against colon cancer. The mechanistic studies demonstrated that RJ and AJ inhibited lipid peroxidation, increased antioxidant enzyme activities (antagonized oxidative stress) and induced of apoptosis. It can be concluded that intake of black raspberry and apricot as fruit salad or juice may be beneficial for patients who suffer from colon cancer.

Keywords: Black Raspberry, Apricot; Colon cancer; Azoxymethane; Cancer colon biomarkers; Lipid peroxidation; Antioxidant; Apoptosis; Histopathology.

Introduction

Bacillary Colon cancer is one of the leading causes of deaths among cancer patients in both underdeveloped and developing countries (Ferlay et al., 2015). Prevention of colon cancer among high-risk volunteers has been long lasting research goals. Curative and palliative therapies of colon cancer have commonly relied upon surgery, chemotherapy and radiotherapy (Johnson and Mukhtar, 2007). Epidemiological studies suggest that high intake of fruits and vegetables in human diets have been linked to a low risk of colon cancer (Poulsen et al., 2011). The incidence of colon cancer is low among Asian countries where the diet is predominantly rich in fruits and vegetables (Haggar and Boushey, 2009). Fruits and vegetables contain many bioactive phytochemicals which reduce oxidative stress, systemic inflammation and other risk factors for chronic diseases such as cancers. Consumption of dietary bioactive compounds found in
fruits and vegetables, such as polyphenols and carotenoids is able to improve the metabolic profile and reduce the risk of chronic diseases such as cancers (William et al., 2017).

Azoxymethane (AOM), the oxide of azomethane, is a potent procarcinogenic that induces colon cancer in rats and mice (Escribano et al., 2004). AOM is a strong inducer of precancerous and dysplastic lesions (aberrant crypt foci, (ACF) in rodent colon and is particularly effective for the induction of colon adenocarcinoma (Dong et al., 2014). In addition, Venning et al., (2013) concluded that Azoxymethane (AOM) enhances early inflammation-induced colon crypt pathology in mice.

Black Raspberry (Rubus occidentalis, Family Rosaceae) has been extensively used to treat a wide range of diseases. Family Rosaceae include strawberry, Black Raspberry and blackberry and apricot. Fruit berries contain many bioactive compounds (Slatnar et al., 2012). Black Raspberries are rich in anthocyanins and carotenoids which are responsible for color of fruit. Black Raspberry is a good source of many bioactive compounds such as phenolic compounds as phenolic acids, flavonoids, anthocyanins, flavonols and tannins (Skrovankova et al., 2015). Previous studies showed that flavonoids in black raspberry are responsible for the antioxidant, anti-inflammatory and anti-proliferative properties (De Souza et al., 2014). It has been shown that the compounds isolated from Black Raspberry are useful for cancer therapy (Cho et al., 2015; Kresty et al., 2016 and El-Bayoumy et al., 2017).

Apricot (Prunus armeniaca, Family Rosaceae) is a delicious summer fruit. Apricot fruits provide a good source of vitamins and minerals, beta-carotene which helps to scavenge free radicals from the body. Phenolic compounds in apricot juice were reported to act as antioxidant, anticarcinogenic, antimicrobial, anti-allergic, antimutagenic and anti-inflammatory (Rice-Evans et al., 1997 and Kim et al., 2003). It was found that apricot juice enhances apoptosis and prevents oxidative stress (Karabulut et al., 2014). Moreover, apricot juice contains lycopene that is known to prevent lung cancer (Shareck et al., 2017). The present study was designed to investigate the anticancer effect and to explore the possible mechanisms of black raspberry and apricot juices against colon cancer induced by Azoxymethane (AOM) in rats.

Materials and Methods

Fruits:

Fully ripe and fresh fruits of black raspberry (Rubus occidentalis, Family Rosaceae) and apricot (Prunus armeniaca, Family Rosaceae) were purchased from local shop of fruits. Black raspberry fruits were hand washed, sliced into small pieces and blinded in an electric mixer to prepare the juice. Apricot fruits were washed with water, kernels were removed and the outer yellow flesh was cut into small pieces and mixed in an electric blender to prepare the juice.

Chemicals and kits:

Azoxymethane (AOM) (CAS Number 25843-45-2) has molecular formula C9H4N2O and molecular weight 74.08. AOM is dispensed as in 1 ml ampoules each containing 25 or 100 mg as clear oily liquid soluble in water. AOM was purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). The stain of Hematoxylin and Eosin was purchased from CellPath, Mochdre, UK. Enzyme linked Immunosorbent assay (ELISA) kits were purchased from Biodiagnostic Company, Dokki, Egypt. The kits were used for the determination of serum colon cancer biomarkers.

Rats:

Forty two adult male Sprague Dawley rats weighing 160-170 g body weight and 7-8 weeks age were used in this study. Rats were obtained from the Laboratory Animal House, Agricultural Research Center, Giza, Egypt and housed in a well ventilated laboratory room under standard conditions of 24 °C temperature, 50-52% relative humidity and 12 hr light/12 hr dark cycles. Rats were fed on basal diet that prepared according to Reeves et al. (1993) and water was provided ad libitum. The experiment on rats was carried out according to the recommendations of National Regulations and Rules for Animal Welfare by Institutional Animal Ethical Committee (IAEC), National Research center, Dokki, Egypt.

Induction of colon cancer:

The preneoplastic lesions of colon cancer (apparent colon crypts) were induced by single subcutaneous dose 15 mg/kg b.wt of AOM once weekly for two subsequent weeks to induce colon cancer (Mohamed et al., 2017).
Experimental design:

Forty two adult male Sprague Dawley rats were randomly distributed into 6 equal groups (n=7). The 1st group was negative control fed on basal diet and received normal saline during the experiment period (12 weeks). Rats of the other 5 groups were given a single subcutaneous dose (15 mg/kg b.wt.) of AOM once weekly for 2 subsequent weeks to induce colon cancer. The 2nd group was kept positive control and rats of the 3rd, 4th, 5th and 6th groups were orally given BJ and AJ at low (5%) and high (10%) concentrations, respectively, for 12 weeks. At the end of experiment period, the rats were euthanized by diethyl ether and blood samples were withdrawn for separating the serum by centrifugation at 8000 rpm for 10 min. Serum samples were kept frozen at -70 °C till biochemical analyses. Serum levels of TNF-α; NF-κB; interleukin1β (IL-1β) and interleukin 6 (IL6) were measured using the corresponding ELISA kits. After sacrificing rats, the colon was carefully removed, opened longitudinally and gently rinsed with saline solution to remove residual bowel contents then the colon was divided into two parts. The 1st part was frozen for biochemical analyses and the 2nd part was preserved in 10% buffered formalin till processed for histopathology.

Biochemical analyses:

Serum levels of colon cancer biomarkers tumor necrosis factor- alpha (proinflammatory cytokine) was quantified as described by Pennica et al. (1985) and nuclear factor-kappa B (transcription factor) was quantified as described by Adams (2009) using ELISA kits (Glory Science Company, Taiwan) according to instructions of kits manufacturers. Other serum colon cancer biomarkers interleukin1β (IL-1β) and interleukin 6 (IL6) were quantified as described by Stanilov et al. (2010) using ELISA kits according to instructions of kits manufacturers.

Tissue lipid peroxidation and activities of antioxidant enzymes:

One gram of the frozen colon tissues was washed with ice-cooled 0.9% NaCl solution and homogenized in 100 ml of ice-cooled 1.5% potassium chloride solution and 50 mmol potassium phosphate buffer solutions (pH 7.4) to yield 1% homogenate (W/V). Colon homogenates were centrifuged at 8000 rpm for 10 min at 4°C. The supernatants were used for estimation of lipid peroxide malondialdehyde (MDA) as described by Ohkawa et al. (1979). Reduced glutathione (GSH) content in colon homogenates was determined colorimetrically described by Bulaj et al. (1998). Activities of antioxidant enzymes glutathione peroxidase (Paglia and Valentaine, 1979); superoxide dismutase (Spitz and Oberley, 1989) and catalase (Sinha, 1972) were colorimetrically determined using commercial ELISA kits.

Quantification of 8-hydroxy-2-deoxyguanosine (8-OHdG):

Genomic DNA in the 1st frozen part of colon tissues was extracted using a commercial DNeasy tissue kit. The content of 8-OHdG in colon DNA was determined using ELISA kits. Briefly, 8-OHdG antibody and the sample were added to ELISA plate reader which had been precoated with 8-OHdG antigen. The content of 8-OHdG in the sample competes with the 8-OHdG antibody binding sites in the plate. The content of 8-OHdG per nanogram (ng) of colon DNA for each group was calculated. The sample DNA assay was performed in triplicate and the average was calculated as described by Plachetka et al. (2013).

Histological procedure:

The 2nd part of colon tissues was preserved in 10 % neutral formalin solution. The fixed specimens were trimmed, washed and dehydrated in ascending grades of alcohol. Tissue specimens were then cleared in xylene, embedded in paraffin, sectioned at 4-6 microns thickness, stained with Hematoxylin and Eosin (H&E stain) and examined under light microscope (Carleton, 1976).

Statistical analysis:

Results were statistically analyzed using computer program Statistical package for Social Sciences (SPSS). One-way analysis of variance (ANOVA), least significant differences (LSD) and Duncan were used. The difference was considered significant at P-value < 0.05 according to Zar (1984).

Results

Subcutaneous injection of Azoxymethane (AOM) at 15 mg/kg b.wt in rats significantly increased serum levels of colon cancer biomarkers [(tumor necrosis factor -α (TNF-α); nuclear factor-kappaB (NF-κB); interleukin1β (IL-1β) and interleukin 6 (IL6)]. Oral administration of BJ and AJ at 5% and 10 % concentrations for 14 weeks significantly lowered the elevated serum levels of colon cancer biomarkers as compared to the positive control group (Table 1).
Table 1. Effect of black raspberry (BJ) and apricot (AJ) juices on serum levels of colon cancer biomarkers in rats injected with Azoxymethane (AOM). (n = 7 rats)

<table>
<thead>
<tr>
<th>Parameters Groups</th>
<th>TNF-α (pg/ml)</th>
<th>NF-κB (pg/ml)</th>
<th>IL-1β (ng/ml)</th>
<th>IL6 (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-Ve control</td>
<td>86.0±3.16d</td>
<td>37.0±2.25a</td>
<td>99.5±3.25d</td>
<td>59.0±1.26a</td>
</tr>
<tr>
<td>+Ve control</td>
<td>140.0±3.23a</td>
<td>165.0±3.54a</td>
<td>149.0±6.17a</td>
<td>76.0±3.11a</td>
</tr>
<tr>
<td>BJ 5%</td>
<td>124.0±3.22e</td>
<td>133.0±2.43e</td>
<td>136.0±4.56e</td>
<td>77.0±2.31c</td>
</tr>
<tr>
<td>BJ 10%</td>
<td>123.0±2.32e</td>
<td>132.0±3.61e</td>
<td>135.0±4.11e</td>
<td>55.0±2.41c</td>
</tr>
<tr>
<td>AJ 5%</td>
<td>134.0±2.22b</td>
<td>140.0±2.44b</td>
<td>141.0±4.56b</td>
<td>65.0±1.12b</td>
</tr>
<tr>
<td>AJ 10%</td>
<td>132.0±3.15b</td>
<td>139.0±3.72b</td>
<td>140.0±3.35b</td>
<td>63.8±1.15b</td>
</tr>
</tbody>
</table>

Means ± SE with different superscript letters (a, b, c, d) in the same column are significant at P < 0.05 or P < 0.01 using one way ANOVA test.

TNF-α = tumor necrosis factor –α; NF-κB=nuclear factor-kappaB; IL-1β= interleukin1β and IL6= interleukin 6.

Rats subcutaneously injected with AOM had a significant high content of malondialdehyde (MDA) and low content of reduced glutathione (GSH) in colon issues when compared with the negative control group. BJ and AJ at 5% and 10 % concentrations when given orally to rats for 14 weeks significantly normalized levels of MDA and GSH in colon tissues as compared to the positive control group (Table 2).

Table 2. Effect of black raspberry (BJ) and apricot (AJ) juices on malondialdehyde (MDA) and reduced glutathione (GSH) contents in colon issues of rats injected with Azoxymethane (AOM). (n = 7 rats)

<table>
<thead>
<tr>
<th>Parameters Groups</th>
<th>MDA (μmol/mg protein)</th>
<th>GSH (mmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-Ve Control</td>
<td>1.44 ± 0.2d</td>
<td>28.40 ± 0.12a</td>
</tr>
<tr>
<td>+Ve Control</td>
<td>4.80 ± 0.3a</td>
<td>19.95 ± 0.15d</td>
</tr>
<tr>
<td>BJ 5%</td>
<td>3.75 ± 0.4b</td>
<td>24.67 ± 0.21c</td>
</tr>
<tr>
<td>BJ 10%</td>
<td>3.50 ± 0.2b</td>
<td>23.89 ± 0.32c</td>
</tr>
<tr>
<td>AJ 5%</td>
<td>2.90 ± 0.3c</td>
<td>25.75 ± 0.25b</td>
</tr>
<tr>
<td>AJ 10%</td>
<td>2.66 ± 0.2c</td>
<td>26.25 ± 0.22b</td>
</tr>
</tbody>
</table>

Means ± SE with different superscript letters (a, b, c, d) in the same column are significant at P < 0.05 or P < 0.01 using one way ANOVA test.

Azoxymethane (AOM) at 15 mg/kg b.wt when subcutaneously injected to rats significantly reduced activities of colon tissue glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) antioxidiant enzymes. Oral administration of BJ and AJ at 5% and 10 % concentrations significantly enhanced activities of SOD, GPx and CAT enzymes as compared with the positive control group (Table3).
Table 3. Effect of black raspberry (BJ) and apricot (AJ) juices on activities of colon tissue glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) in rats injected subcutaneously with Azoxymethane. (n = 7 rats)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>GPx (IU/gm protein)</th>
<th>SOD (IU/gm protein)</th>
<th>CAT (IU/gm protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-Ve Control</td>
<td>42.10 ± 0.21 (^{a})</td>
<td>5.85 ± 0.4 (^{a})</td>
<td>50.60 ± 0.8 (^{a})</td>
</tr>
<tr>
<td>+Ve Control</td>
<td>27.22 ± 0.15 (^{d})</td>
<td>2.47 ± 0.3 (^{a})</td>
<td>33.35 ± 0.9 (^{a})</td>
</tr>
<tr>
<td>BJ 5%</td>
<td>36.13 ± 0.33 (^{b})</td>
<td>3.56 ± 0.2 (^{c})</td>
<td>44.42 ± 0.6 (^{b})</td>
</tr>
<tr>
<td>BJ 10%</td>
<td>37.16 ± 0.35 (^{b})</td>
<td>3.59 ± 0.3 (^{c})</td>
<td>43.58 ± 0.4 (^{b})</td>
</tr>
<tr>
<td>AJ 5%</td>
<td>32.23 ± 0.13 (^{c})</td>
<td>4.77 ± 0.2 (^{b})</td>
<td>37.50 ± 0.4 (^{c})</td>
</tr>
<tr>
<td>AJ 10%</td>
<td>31.34 ± 0.12 (^{c})</td>
<td>4.81 ± 0.2 (^{b})</td>
<td>38.50 ± 0.5 (^{c})</td>
</tr>
</tbody>
</table>

Means ± SE with different superscript letters (a, b, c, d) in the same column are significant at P < 0.05 using one way ANOVA test.

In rats injected with Azoxymethane (AOM), the content of apoptosis marker 8-hydroxy-2-deoxyguanosine (8-OHdG) in colon mucosa was higher (94ng/mg DNA) than in the normal mucosa (12 ng/mg DNA) in the negative control group. Oral administration of BJ and AJ at 5 and 10 % concentrations decreased the high content of 8-OHdG to 80, 70, 63 and 50 ng/mg DNA, respectively versus to 94 ng/mg DNA in the carcinogenic mucosa in the positive control group as shown in figure (1).

![Fig. 1. Effect of black raspberry (BJ) and apricot juices at low (5%) and high (10%) concentrations on content of 8-hydroxy-2-deoxyguanosine (8-OHdG) in colon tissues DNA.](image)

Histopathological examination of colon sections of rats in the negative control group showed normal histological structure of intestinal mucosa (Fig 2a). Colon sections of rats subcutaneously injected with Azoxymethane showed preneoplastic abbrent colon crypts and distortion of crypt architecture (Fig. 2b). In rats given orally large (10%) concentration of Black Raspberry juice (BJ) for 14 weeks revealed less developed abbrent colon crypts as shown in Fig. (2c). Oral administration of apricot juice for 14 weeks in rats injected subcutaneously with Azoxymethane caused less number of abbrent colon foci and less distortion of crypt architecture (Fig. 2d).
Fig. 2a. C.S. of colon of a negative control rat showing normal histological structure of colonic (intestinal) mucosa. (H&E X 400).

Fig. 2b. C.S. of colon of a rat subcutaneously injected with Azoxymethane (AOM) showing preneoplastic aberrant colon crypts (red arrows). (H&E X 400).

Fig. 2c. C.S. of colon of a rat subcutaneously injected with Azoxymethane (AOM) and given orally 10% of black raspberry juice (BJ) for 14 weeks showing less developed aberrant colon crypts (red arrows). (H&E X 400).

Fig. 2d. C.S. of colon of rats subcutaneously injected with Azoxymethane (AOM) and given orally 10% of Apricot juice (AJ) for 14 weeks showing less number of aberrant colon foci and less distortion of crypt architecture (red arrows). (H&E X 400).

Discussion

Colon cancer is one of the leading causes of deaths among cancer patients in both underdeveloped and developing countries (Ferlay et al., 2015). The concept of cancer prevention using diet containing naturally occurring bioactive components is gaining increased attention. In this line, different types of fruits and vegetables have been re-evaluated and recognized as valuable sources of bioactive phytochemicals. Therefore, the present study was carried out to evaluate the anticancer effect of Black Raspberry juice (BJ) and apricot juice (AJ) on colon cancer induced by Azoxymethane (AOM) in rats and to examine the possible mechanisms of action.

Recent epidemiological studies revealed that regular consumption of fruits and vegetables improve overall human health and wellbeing by preventing non-communicable diseases including several types of cancer (Siddiqui et al., 2010; Rajput and Mandal, 2012; Brown, 2012 and Lee et al., 2016). The biological value of fruits and vegetables depends upon presence of bioactive constituents, especially those of antioxidant and anti-inflammatory properties.

The mechanisms underlying the anticancer activity of plant materials are still need for further investigations.

Azoxymethane (AOM), the oxide of azomethane, is procarcinogenic that induces colon cancer in rats and mice (Escribano et al., 2004 and Dong et al., 2014). AOM induces colon cancer via mechanisms that include glutathione depletion and impairing total antioxidant capacity so inducing oxidative stress in colonic cells of rats (Waly et al., 2012). Moreover, AOM-mediated carcinogenesis process involves mutagenicity by initiating chromosomal damage and induction of micronuclei cells (Al-Numair et al., 2011).

Black Raspberry has been extensively evaluated for its impact on human health due to its rich phytochemical contents, efficacy in rodent models and minimal or no toxicity observed in pilot human studies (De Souza et al., 2014). The anticancer activity of Black Raspberry is due to presence of many bioactive compounds including phenolic compounds as phenolic acids, flavonoids, anthocyanins, flavonols and tannins (Skrovankova et al., 2015).
The present study revealed that oral administration of black raspberry juice at 5% and 10% concentration to rats for 14 weeks produced anticancer activity against colon cancer induced by AOM. This result was similar to those reported by Cho et al. (2015); Kresty et al. (2016) and El-Bayoumy et al. (2017) who concluded that Black Raspberry fruits are useful for therapy of the oral cavity, esophagus and colon cancers. The previous authors reported that black raspberry inhibited tumor progression via its antiproliferative, anti-inflammatory and antioxidant activities. In addition, it has been reported that polyphenolic compounds (anthocyanins, flavonoid glycosides) in Black Raspberry prevent the initiation, promotion, and progression of carcinogenesis in digestive tract and esophagus of rats (Medda et al., 2015). Moreover, Derosa et al. (2016) attributed the anticancer activity of black raspberry due to its high content of ellagic acid which exhibits antiproliferative and antioxidant effects.

Concerning apricot juice, the current study showed that it produced anticancer effect against colon cancer induced by AOM in rats. This effect was partially similar to that reported by Rice-Evans et al. (1997) and Kim et al. (2003). The previous authors concluded that apricot juice produces antioxidant, anticarcinogenic, anti-microbial, anti-allergic, antimitogenic and anti-inflammatory activities. In addition, Shareck et al. (2017) reported that apricot juice contains lycopene which is known to help prevention of lung cancer.

In this study, the mechanistic studies demonstrated that black raspberry and apricot juices inhibited lipid peroxidation, increased activities of antioxidant enzymes so antagonized oxidative stress and induced apoptosis in rats injected with colon cancer. These findings partially agreed with those of Derosa et al. (2016) who found that the anticancer activity of black raspberry is due to its high content of ellagic acid which exhibits antiproliferative and antioxidant effects. Vardi et al. (2013) reported that apricot juice significantly increased activities of antioxidant enzymes catalase, superoxide dismutase, and reduced glutathione levels but decreased formation of malondialdehyde (MDA). Previous studies reported that 8-hydroxy-2'-deoxyguanosine (8-OHdG) has been used as a biomarker for the measurement of endogenous oxidative DNA damage and risk of cancers. In the present study, Black Raspberry and Apricot juices decreased tissue level of 8-OHdG in colon. These findings agreed with that of Seeram et al. (2006) who reported blackberry, black raspberry, blueberry, cranberry and red raspberry reduced the level of 8-OHdG in colon tissues and stimulated apoptosis of human cancer cells in vitro. Moreover, Plachetka et al. (2013) concluded that the content of 8-hydroxy-2'-deoxyguanosine in colorectal adenocarcinoma is high as a result of oxidative stress.

In conclusion, the results documented that black raspberry and apricot juices exhibit anticancer effect against colon cancer in rats. The mechanisms of this effect may be due to inhibition of lipid peroxidation, increasing activities of antioxidant enzymes so preventing oxidative stress and induction of apoptosis in colon tissues. The study recommends that intake of black raspberry and apricot as fruit salad or juice or may be beneficial for patients who suffer from colon cancer.

References


التأثير المضاد للسرطان لعصير توت العليق الأسود والمشمش ضد سرطان القولون

المحدث بواسطة أزوكسي ميثان في ذكور الفئران

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المخلص العربي

ملخص

تهدف هذه الدراسة إلى دراسة التأثير المضاد للسرطان لعصير توت العليق الأسود والمشمش مدى قوة تأثير هذا العصير على تأثير سرطان الفم. 

انون إبراهيم

يعمل على تزويد مجموعة من الفئران بعصير توت العليق الأسود والمشمش وذلك بتوزيعهم بطرق عشوائية على ثماني مجموعات. وتركزة إعطاءهم العصير يومياً لمدة 15 يوماً من الزمان. 

عند نهاية هذه الدراسة أظهرت هذه الدراسة أن عصير توت العليق الأسود والمشمش له تأثير متميز ضد سرطان الفم، حيث أن نسبة الفئران المصابين بسرطان الفم بين المجموعة التي استقبلت العصير كان أصغر بكثير من المجموعة التي لم تصلب عصير توت العليق الأسود والمشمش.

أظهرت هذه الدراسة أن عصير توت العليق الأسود والمشمش له تأثير ممتاز ضد سرطان الفم، حيث أن نسبة الفئران المصابين بسرطان الفم بين المجموعة التي استقبلت العصير كان أصغر بكثير من المجموعة التي لم تصلب عصير توت العليق الأسود والمشمش.

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