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MINIREVIEWS

# Role of pomegranate and citrus fruit juices in colon cancer prevention

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## Abstract

Colorectal cancer is the second leading cause of cancer-related deaths in the United States. Recent studies prove that though chemotherapeutic agents are being used for the treatment of colon cancer, they become non-effective when the cancer progresses to an invasive stage. Since consumption of certain dietary agents has been linked with various cancers, fruit juices have been investigated for their consistently protective effect against colon cancer. The unique biochemical composition of fruit juices is responsible for their anticancer properties. In this review, the chemo-preventive effect of fruit juices such as pomegranate and citrus juices against colon cancer are discussed. For this purpose, the bioavailability, *in vitro* and *in vivo* effects of these fruit juices on colorectal cancer are highlighted. Moreover, there is a scarcity of studies involving human trials to estimate the preventive nature of these juices against colon cancer. This review will support the need for more preclinical tests with these crude juices and their constituents in different colorectal cancer cell lines and also some epidemiological studies in order to have a better understanding and promote pomegranate and citrus juices as crusaders against colon cancer.



**Key words:** Colon cancer; Fruit juices; Pomegranate; Citrus fruits; Chemoprevention

**Core tip:** Recent studies prove that though chemotherapeutic agents are being used for the treatment of colon cancer, they become non-effective when the cancer progresses to an invasive stage. This problem can be minimized by the regular intake of fruit juices. The unique biochemical composition of fruit juices is responsible for their chemo-preventive properties. In this review, the chemo-preventive effects of fruit juices such as pomegranate and citrus juices against colon cancer are discussed.

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## INTRODUCTION

Colorectal cancer is the third leading cause of cancer-related deaths in the United States when men and women are considered separately, and the second leading cause when both sexes are combined. It is expected to cause

about 51690 deaths during 2012[1]. The American Cancer Society's most recent estimates for the number of colorectal cancer cases in the United States for 2012 are: 103170 new cases of colon cancer and 40290 new cases of rectal cancer. Duke's classification helps to identify the severity of the disease in different stages of colon cancer. Such a classification enables us to understand the degree of disease progression and the best treatment that is possible. Even after spending decades of years in studies related to treatment and cure for colon cancer, conventional cancer treatments offer little promise to patients. The main drawback lies in the fact that even after various cancer treatments, the disease is found to recur and this time will exacerbate the previous symptoms. A conventional treatment does not aspire to treat the root cause but only its symptoms. The use of chemotherapeutic agents and radiation exhausts the anti-oxidants available and induces oxidative stress, which increases with disease progression. Hence, it is high time to look at alternative yet completely curative measures for treating colon cancers.

In this scenario, various epidemiological studies have shown that a diet which is rich in fiber can minimize the risk of developing colon cancer[2-4]. Similar studies also proved that a phytochemical-rich diet which is absorbed by the body from fruit and vegetable sources can decrease the risk of developing colon cancer[5,6]. Further reports have shown the inhibition of colon carcinogenesis by dietary supplements[7]. Moreover, other reports have also shown that colon cancer is one of the most preventable forms of cancer and have depicted the importance of dietary modification for preventing colon carcinogenesis[8]. Fruits, nuts, vegetables and grains contain major non-nutrient components called polyphenols which have chemo-preventive properties against colon cancer[9]. The major mechanisms through which they exert this activity are through the combination of properties such as antiproliferative, pro-apoptotic and antioxidant properties of the polyphenolics[10].

Consumption of fruit juices by various ethnic groups is prevalent and there is a good market-share between real fruits and the fruit juices. Intake of fruits as juices has gained wider acceptance among the young population because it is easier to consume, and also the intake amount of juices can be increased significantly compared to fruits itself. Further, the availability of 100% fruit juices in the retail market and also the functional claims of such juices further motivate people to consume fruit juices. Since fruit juices contain polyphenolics which help in reducing the growth of colon cancer, they can be consumed as dietary intake regularly to reduce the incidence of colon cancer. Furthermore, there are no side-effects as seen in the conventional treatments as the treatment is aimed at the molecular level. Moreover, since fruit juices alkalize the body and provide an abundance of enzymes, vitamins, minerals, phytochemicals and other nutrients, they prove to be a better alternative for preventing the colon cancer. A review article summarizing the effect of pomegranate on various cancers was recently published[11]. However, till now the effect of pomegranate juice against colon cancer has not been reviewed extensively. Hence, we are discussing the effects of fruit juices such as pomegranate and citrus against colon cancer in this article. For this purpose, we summarize the effect of these fruit juices on colon cancer cell lines and animal models along with their bioavailability studies.

## POMEGRANATE JUICE

The botanical name of pomegranate is *Punica granatum*. The native source of this fruit is Iran and now it has been cultivated in Asian areas such as the Caucasus and the Himalayas in Northern India. The number of seeds present in a pomegranate can vary from 200 to 1400, but some believe that all pomegranates have an equal number of seeds. The pomegranate juice is obtained by crushing the seeds of the pomegranate. This pomegranate juice contains different types of polyphenols such as gallo, ellagitannin and flavonoid classes.

### *Bioavailability and metabolism of pomegranate juice in relation to colon cancer*

As mentioned above, pomegranate juice is rich in polyphenol compounds such as gallo, ellagitannin and flavonoid classes. The commercially available pomegranate juice which is obtained by hydrostatic pressing of whole fruit contains cyanidin 3,5-diglucoside, pelargonidin-3,5-diglucoside, flavonols such as kaempferol and quercetin, flavones such as luteolin, anthocyanins such as cyanidin-3-glucoside, delphinidin-3-glucoside, ellagitannins such as the punicalagins and punicalins, which exist as β-anomers and R- and acyclic hydroxylaldehyde[12]. A significant portion of the pomegranate juice contains the pomegranate polyphenols called ellagitannins and they often coexist with ellagic acid, the main product obtained through hydrolysis of the class tannins. Besides ellagitannins, pomegranate juice also contains variable amounts of the polyphenol called egallic acid. This ellagic acid is obtained by the metabolization of the ellagtanins by the intestinal bacteria. Ellagic acid is found to be analogous to urolithins. The urolithins are reported to be systematically bioavailable where they accumulate in organs such as colon, prostate and intestine.

The modulation of chemical carcinogenesis induced by dietary carcinogens can be achieved using drug-metabolizing enzymes, through cytochrome P450 (CYP) enzyme inhibition and/or by induction of phase-2 conjugating enzymes. It was found that ellagic acid prevents cancer initiation and inhibits the CYP1 activation of procarcinogens[13]. Moreover, the ellagic acid also induces phase-2 enzymes like glutathione *S*-transferase. However, the urolithins and ellagitannins were not tested regarding whether they have anti-carcinogenic activity through inhibition of induction of phase II conjugating enzymes and/or inhibition of CYP1. Thus, the above-mentioned mechanisms are some of the potential mechanisms by which pomegranate juice consumption might inhibit co-

lon cancer formation.

The pomegranate (*Punica granatum* L.) is consumed in various forms such as pomegranate juice, wine and jam. Pomegranate juice exhibits some artheroscelerotic as well as antioxidant properties due to its high content of polyphenols such as ellagic acid, ellagitannins, and other flavonoids (luteolin glycosides, quercetin, and kaempferol)[14]. Among these polyphenols, punicalagin is present in a great amount and is responsible for greater than 50% of the juice's potential antioxidant activity.

### *In vitro* effect of pomegranate juice on colon cancer

Kasimsetty *et al*[15] investigated the action of ellagitannins and urolithins against HT-29 human colon cancer cells. It was found that urolithins A and C inhibited 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, dioxin)-induced CYP1-mediated ethoxyresorufin-O-deethylase activity *in vitro* with $IC_{50}$ values ranging from 56.7 to 74.8 μmol/L. Both of these compounds inhibited the HT-29 cell proliferation in a time- and dose-dependent manner by inducing apoptosis. Hence, they concluded that drinking pomegranate juice in considerable amounts may hinder the colon cancer progression.

Studies done by Seeram *et al*[16] on the effect of pomegranate juice and purified ellagitannins on colon cancer have shown that they inhibit the induction and proliferation of colon cancer cell lines. It was also found that their results are in accordance with the reported anti-proliferative activity of pomegranate polyphenols in breast and prostate cancers[17]. Moreover, this recent study depicts proliferation inhibition by treatment of HT-29 cancer cells with a cyclooxygenase-2 (COX-2) specific inhibitor and NS398. Other studies also show the correlation between increased cell proliferation and enhanced COX-2 expression. Hence, it is hypothesized that COX-2 expression modulation by pomegranate juice might be an important mechanism for the colon cancer anti-proliferative activity of the pomegranate juice. The COX-2 expression in HT-29 cells is found to be decreased by pre-treatment with the pomegranate juice and punicalagin in a dose-dependent manner. Besides, it was proven that pomegranate juice has better potential in decreasing the COX-2 expression. This is mainly because of the important interactions with other bioactive polyphenols in pomegranate juice such as flavonols and anthocyanins. Thus, this result has led to a conclusion that when the individual polyphenols are separated from the pomegranate juice it can decrease the overall activity due to the requirement of other components. Signaling pathways such as mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT)/nuclear factor-kappa B (NFκB) mediate COX-2 expression. The modulation of NFκB activity is mediated by P13K *via* AKT. In the case of mesangial cells, P13K activation resulted in increased cell proliferation as well as COX-2 expression[18]. Hence, this has illustrated the specific relationship between COX-2 and P13K. In accordance with the above observations, other works have depicted that pretreatment with pomegranate juice inhibits NFκB activation, AKT activity and expression of COX-2 in HT-29 cells[19].

Even though some studies have concluded that the COX-2 expression in HT-29 cells depends on NFκB activity, studies done by Jobin *et al*[20] in 1998 have demonstrated that NFκB inhibition by wortmannin decreased the COX-2 expression only partially. Thus, this leads to a conclusion that other signaling pathways may influence the modulation of COX-2 expression in HT-29 cells in collaboration with the NFκB activity. MAPK pathways (SAPK, p38 and ERK1/2) are potential candidates for this role. This is because MAPK was found to be mediating COX-2 expression in a large number of studies[21]. Besides, *in vitro* studies have shown that p28 and ERK modulate the NFκB activity[22]. Moreover, other studies also have shown that both MAPK and NFκB may mediate COX-2 expression, but the inter-relationship between these protein signaling pathways is yet to be determined. The application of pomegranate extracts 30 minutes before TPA treatment of mice resulted in JNK1/2 activity, p38, ERK1/2 and COX-2 expression inhibition.

The availability of the flavonoids in various food materials is a relatively unexplored field. On the other hand, various studies show that they are poorly absorbed in the upper gastrointestinal tract. The rate of absorption of this component in the small intestine ranges from 0% to 60% of the ingested dose, which relies on the food source[23]. Hence, the flavonoids reach the colon in an unabsorbed form or they are secreted as absorbed conjugates where ultimately they are secreted in the bile. However, the inhibition of NFκB, AKT and COX-2 provides us with greater knowledge about the anticancer mechanism actions of the pomegranate juice in colon cancer. These works have also presented us with direction for future studies on the role of pomegranate juice in prevention and treatment of colon cancer.

It was found that COX-2 expression, indicative of an inflammatory signaling process leading to the initiation and progression of colon cancer in HT-29 colon cancer cells, was inhibited by the pomegranate juice. Moreover, the whole juice was found to be more powerful in the inhibition process (79% suppression) than its individual components[24]. Larrosa *et al*[25] have shown the induction of apoptosis of colon cancer cells by punicalagin and ellagic acid from pomegranate juice. The intrinsic pathway of apoptosis occurred when mitochondrial cytochrome c leakage in the cytosol was caused by punicalagin and ellagic acid. A downregulation of the anti-apoptotic Bc1-XL protein was achieved with 30 μmol/L ellagic acid and 100 μmol/L punicalagin. It was found that procapase 3 and caspase 9, which are members of the caspase family of proteases, were induced by punicalalgin and ellagic acid. However, caspase 8, which is related to extrinsic pathways (*e.g.*, induced by cytokines) of apoptosis, was not activated by ellagic acid and punicalalgin. Likewise, the incubation of ellagic acid or punicalagin with anti-Fas ZB4 antibody resulted in no inhibitory effect on apoptosis. Hence, this antibody was utilized for inhibition of

**Figure 1 Pomegranate and Citrus juices are depicted in this figure.**

interaction of ellagic acid or punnicalagin with the Fas receptor. Thus, all this data supports the intrinsic mechanism of a pomegranate juice-induced apoptotic effect in colon carcinogenesis.

A positive effect on COX-2 expression has been observed due to action of the PI3K/AKT/NFκB pathway. Initially, the P13K activates the AKT. Later, this AKT will phosphorylate and activate IκB kinase, ultimately leading to the activation of NFκB. It was found that P13K is associated with colon cancer. Here, phosphatase and tensin homologue gene (PTEN) mutations occur where PTEN inhibits P13K[26]. Moreover, an increased level of P13K activity is observed in adenocarcinoma cell lines and colon cancer cell lines[27]. Inhibition of P13K activity in ovarian cancer cell lines as well as colon cancer lines leads to inhibition of cell proliferation[28].

### *In vivo* effect of pomegranate juice on colon cancer

For the purpose of analyzing the changes associated with colon cancer, Boateng *et al*[29] conducted a study on the effect of pomegranate juice on aberrant cryptic foci (ACF). This study revealed that the pomegranate fruit juice reduced the number of ACF of the colon by 91% in male F-344 rats. The animals utilized in this study were given 20% pomegranate juice before and after treatment of the rats with a specific colon carcinogen called azoxymethane. Later, histopathology of the rat colon was studied after the 17$^{th}$ week of treatment. It was found that there was a significant decrease in the number of large crypts in pomegranate juice-fed rats. Moreover, the observed number of crypts/ACF was also few in these animals. Compared to fruit juices such as cranberry and water melon, the pomegranate juice showed better inhibition of ACF in rat colon. The pomegranate juice-fed rats' food intake and weight gain increase indicates the possibility of the protective effect of the pomegranate juice against cancer cachexia. This is because the activity of hepatic glutathione *S* transferase (GST) was found to be three times higher in the case of rats being fed with pomegranate juice. GST is well known for the scavenging of free radicals that are produced from oxidative stress. When this enzyme activity is induced, it supports the mechanism of pomegranate anti-oxidative actions in other experimental models[30].

The intake of pomegranate seed oil in the diet was found to cease the multiplicity of colonic adenocarcinomas, but dose-dependent variation was not observed. The tumor incidence was found to be coupled with enhanced expression of peroxisome proliferator-activated receptor gamma protein in the normal non-tumor mucosa[7]. Hence, all these results depict the useful effects of pomegranate, which acts against the growth of colonic tumors in mice.

## CITRUS JUICES

The botanical name of orange is *Satsuma mandarin*. It is generally seedless with thin skin (Figure 1). The fruit is grown in cool subtropical regions of Japan, Spain, and central China, Korea, Russia, Turkey, southern South Africa, South America, central California and northern Florida. The pulp and juice of the citrus fruit contain flavonoids such as apigenin, naringenin, hesperidin, nobiletin and limonoids, and cryptoxanthin, a carotenoid. Also, the peel of citrus fruits contains a phytochemical called tangeritin. All these components act as chemo-preventive agents. Bio-availability and metabolism of citrus juice and its effect on colon cancer cells and animal models are discussed below.

### Bio-availability and metabolism of citrus juice in colon cancer

*Satsuma mandarin* (Citrus unshiu Mar.) juice contains β-cryptoxanthin, a carotenoid, and hesperidin, a flavonoid, which are potential chemo-protective compounds. A pulp (CHRP) containing high amounts of β-cryptoxanthin and hesperidin made from *Satsuma mandarin* inhibited chemically induced colon carcinogenesis in rats[31]. CHRP and citrus juices suppress the expression of pro-inflammatory cytokines and inflammatory enzymes in colon. β-Cryptoxanthin with non-substituted β-ionone cycles and pro-vitamin A possesses several biological activities including scavenging of free radicals, enhancement of gap junctions, immune-modulation, and regulation of enzyme activity involved in carcinogenesis and inhibition of tumorigenesis[32]. Hesperidin is found in various vegetables and fruits, and it is shown to exhibit antioxidant activity, anti-inflammatory effect and an inhibiting effect on prostaglandin biosynthesis. This flavonoid inhibits chemically induced carcinogenesis in several organs[33].

In response to CHRP treatment in rats, GST and quinone reductase (QR) levels are elevated by limonin in the colon. CHRP and citrus juices also exhibit suppressing effects on hyper-cell proliferation activity induced by carcinogens in the colon, thereby inhibiting carcinogenesis[34]. They also suppress mRNA expression of several cytokines [tumour necrosis factor-alpha, interleukin (IL)-1β, IL-6] and inflammatory enzymes [COX-2 and inducible nitric oxide synthase (iNOS)] and enhance mRNA expression of Nrf2 in colon that received a carcinogen.

Nrf2 is a transcription factor and a key regulator of the inducible expression of enzymes such as GST and QR. GST and QR are involved in catalyzing the detoxification of reactive electrophiles and oxidants that contribute to the formation of mutations leading to cancers. Nrf2 also regulates the cytoprotective transcriptional response leading to prevention of damage to DNA, proteins and lipids, as well as recognition, repair, and removal of macromolecular damage and tissue renewal following toxicity. With cancer development in tissues there is an association of chronic inflammation regulated and mediated by cytokines. Any imbalance in their levels of production results in tumor invasion and metastasis. In addition, inflammatory bowel disease is an important risk factor for the development of colorectal cancer (CRC). Inflammation is also likely to be involved with other forms of sporadic as well as heritable CRC. Thus, Nrf2 is one of the targets for cancer chemoprevention in the colon, and the positive effects of CHRP and citrus juices are attractive for reducing tumor formation when considering the relationship between inflammation and cancer development[35].

### *In vitro studies based on the effect of citrus juice in colon cancer cell lines*

The anti-proliferative effects of naringenin have also been demonstrated in HT-29 colon cancer cells[36]. Cell culture experiments have reported anti-proliferative effects for hesperetin, the aglycone form of hesperidin, nobiletin, apigenin, and a limonoid glucoside mixture[37]. Citrus flavonoids mainly interact with cyclooxygenase and protein tyrosine kinases. Tangeritin, containing five methoxy groups, is a more potent phytochemical than flavonoids with free hydroxyl groups. Tangeritin is shown to inhibit cancer cell growth by increasing the gap junctional intracellular communication. A study by Pan *et al*[38] on human colon cancer cell lines was performed to determine the effects of flavonoids like tangeritin, nobiletin, quercetin, apigenin, luteolin and rutin on the growth of colon cancer cells. Levels of cyclin, p53 protein levels, the activities of some kinases and phosphorylation state of Rb were measured. It was found that growth of colon cancer cells was inhibited mainly by tangeritin, but luteolin and nobiletin also contributed to the inhibition. The mechanism underlying the inhibition of growth of colon cancer by tangeritin is the blockade of the cell cycle in the $G_0/G_1$ phase, reduced levels of cyclins (A, D1 and E) and the decreased phosphorylation of Rb. Production of p53, p27 and p21 was increased further by tangeritin. Thus, these results indicate that tangeritin inhibits growth of colon cancer by increasing levels of cyclin-dependent kinase inhibitors (p21, p27 and p53) and decreasing the activity of some cdks.

### *In vivo studies related to effect of citrus juice on colon cancer*

Ornithine decarboxylase activity and ACF numbers were reduced by apigenin, and it reduced tumor formation in azoxymethane-induced CF-1 mice[39]. ACF numbers in 1,2-dimethylhydarzine-treated Wistar rats were also reduced by diets containing hesperitin (the aglycone of hesperidin)[40]. A mixture of apigenin and epigallocatechin gallate suppressed colon neoplasia recurrence in human subjects with resected colon cancers[41]. Isolated limonin and naringin suppressed the high multiplicity aberrant crypt foci (HMACF) because of lower levels of proliferation and enhanced apoptosis. Lower levels of iNOS and COX-2 in response to limonin in the diet, and a lower level of iNOS in response to naringin in the diet, suggest that changes in nitric oxide and/or prostaglandin synthesis may be mediating the benefits derived from these dietary interventions[42]. Kohno *et al*[43] found that nobileten decreased prostaglandin $E_2$ ($PGE_2$) production in rats. This strengthens the hypothesis that citrus flavonoids (hesperidin, nobiletin, apigenin, naringenin) and limonoids (a limonin glucoside/obacunone glucoside mixture) could act as chemo-preventive agents at the promotion stage of colon carcinogenesis.

Rats treated with naringenin showed a reduced proportion of proliferating colon cells and smaller expansion of the proliferative zone. Hanske *et al*[44] recently demonstrated that apigenin-7-glucoside is metabolized to not only the aglycone form of apigenin, but also to low levels of naringenin (and other compounds) in *in vivo* studies. Therefore, apigenin, which is involved in reducing proliferation *in vitro,* possibly may not show the same *in vivo* effect due to its metabolism within the intestinal tract. Surface cell apoptosis of colon cells was enhanced in rats provided with naringenin and apigenin. Since naringenin and apigenin up-regulated apoptosis, they could inhibit HMACF[45].

The pro-inflammatory enzymes, COX-2 and iNOS, are expressed in high levels in human colorectal adenomas and adenocarcinomas. A positive correlation was shown between COX-2 level and proliferative zone in rats provided with naringenin; this was expected based on the literature linking $PGE_2$ and cell proliferation[46]. Naringenin and apigenin thus prove to be naturally occurring chemo-preventive agents against colon carcinogenesis.

## CONCLUSION

The main purpose of our work is to consolidate the various chemo-preventive effects of two different types of juices - pomegranate juice and citrus juice - on colon cancer. This review article mainly discusses the *in vitro* and *in vivo* effect of these juice varieties on colon cancer, as well as bioavailability and metabolism of these juices which is relevant to colon cancer. Tables 1 and 2 summarize the *in vitro* and *in vivo* effects of the above juices against colon cancer.

The motive of our work is to address the need for more preclinical tests to be carried out on different colon cancer cell lines other than the commonly used type of cell lines such as HT-29 and Caco-2. In addition to that, in most of the work done on animal studies, normal rats and mice were utilized as a subject instead of transgenic

**Table 1** ***In vitro*** **summary of action against colon cancer by fruit juices and their components**

| Juice and its components | Cell line tested | Observation/result | Ref. |
|---|---|---|---|
| Whole/crude pomegranate juice | HT-29 human colon cancer cells | Inhibition of NFκB activation, AKT activity and COX-2 expression | [18,19] |
| | | Inhibition of COX-2 expression leading to the prevention of initiation and progression of colon cancer | [24] |
| Ellagitannins of pomegranate juice | Caco-2 cells | Apoptosis of Caco-2 cells through the mitochondrial pathway | [16] |
| Punicalagin of pomegranate juice | HT-29 colon cancer cells | Down regulation of the anti-apoptotic Bc1-XL protein was achieved with 30 μmol/L ellagic acid and 100 μmol/L punicalagin | [25] |
| | | Induction of intrinsic pathway of apoptosis in colon cancer cells | |
| Urolithins A and C of pomegranate juice | HT-29 human colon cancer cells | Inhibition of 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced CYP1-mediated ethoxyresorufin-O-deethylase activity | [15] |
| | | IC50 values range: 56.7-74.8 μmol/L | |
| | | Induction of apoptosis in a time- and dose-dependent manner | |
| Naringenin of citrus juice | HT-29 colon cancer cells | Inhibition of cell proliferation at doses greater than 0.71 mmol/L demonstrated using colorimetric assay | [36] |
| Limonoids of citrus juice | Human colon cancer cell lines | Induction of apoptosis and cytotoxic effects in MCF-7 and SKOV-3 cells at high concentrations | [37] |
| Flavonoids of citrus juice | | Cell cycle blockade in $G_0/G_1$ phase | |
| Tangeritin | Human colorectal carcinoma-COLO 205 cells | Reduced levels of cyclins (A, D1 and E) | [38] |
| | | Decreased phosphorylation of Rb | |
| | | Increased production of cyclin-dependent kinase inhibitors, p53, p21, p27 | |
| Luteolin | | Inhibition of growth of colon cancer cells | [39] |
| Nobiletin | | Inhibition of growth of colon cancer cells | |

NFκB: Nuclear factor-kappa B; AKT: Protein kinase B; COX-2: Cyclooxygenase 2; CYP: Cytochrome P450; MCF: Human breast cancer cell line; SKOV-3: Human colorectal cancer cell line.

**Table 2** ***In vivo*** **summary of action against colon cancer by fruit juices and their components**

| Juice and its components | Animal model used | Observation/result | Ref. |
|---|---|---|---|
| Whole/crude pomegranate juice | Male F-344 rats | Histopathological studies of azoxymethane-induced rat colon | [29] |
| | | Significant decrease in number of large cryptic foci | |
| | | Increase in feed intake and weight gain | |
| | | Protective effects against cancer cachexia | |
| | | Three times higher activity of GST | |
| | | Anti oxidative actions by scavenging free radicals | |
| | Mice | Inhibition of JNK1/2 activity, p38, ERK1/2 and COX-2 expression | [30] |
| Apigenin of citrus juice | Azoxymethane-induced CF-1 mice | Reduced ODC activity and ACF numbers | [39] |
| | | Reduced tumor formation | |
| Mixture of apigenin and epigallocathechin-gallate | Patients with colorectal neoplasia | Suppressed colon neoplasia recurrence rates | [41] |
| Hesperitin of citrus juice | DMH-treated male Wistar rats | Reduced number of ACF at a dose of 20 mg/kg | [40] |
| Nobiletin of citrus juice | Azoxymethane-treated male F344 rats | Decreased PGE2 production in rats | [43] |
| | | Chemo-preventive agents at the promotion stage of colon carcinogenesis | |
| Naringenin of citrus juice | Azoxymethane-injected Sprague Dawley rats | Reduced levels of COX-2 and iNOS | [45,46] |
| | | Decrease in proliferation of colon cancer cells | |

GST: Glutathione S transferase; JNK: c-Jun N-terminal kinase; COX-2: Cyclooxygenase2; ODC: Ornithine decarboxylase; ACF: Aberrant crypt foci; PGE2: Prostaglandin E2; iNOS: Inducible nitric oxide synthase; DMH: 1,2-dimethylhydrazine.

animals. Hence, the transgenic animals have to be utilized for animal studies involving the efficacy determination of citrus and pomegranate juices against colon cancer to improve the reliability of the results. It would be appropriate for testing the efficacy of the above juices to use the $Apc^{Min/+}$ mouse (colon cancer model with a dominant germ-line mutation at codon 850 of the homolog of the human adenomatous polyposis coli gene) in order to confirm their colon cancer prevention potential.

Besides that, our work is also aimed at throwing light on the importance of carrying out more clinical trials in human beings with the pomegranate and citrus juices. To assess whether these juices have preventive effects against colon cancer, a study could be initiated with 25 healthy participants or 25 participants with increased risk for colon cancer to assess its predictive efficiency. However, phase Ⅱ and phase Ⅲ clinical trials involving larger groups of participants who are at high risk for colon cancer may validate the effect of these fruit juices and provide information whether these agents have protective effects against the colon cancer biomarkers. However, these research proposals demand large research grants which makes the study a costly and impracticable thing. Moreover, cancer prevention using dietary agents is still

a promising field of oncology where scientists in both basic and clinical sciences face great challenges.

In the current scenario, there are no human clinical trials that have been done to study the effect of pomegranate and citrus juices on colon cancer. However, some recent human clinical trials evaluated the effect of pomegranate juice against prostate cancer. In one of these trials, it was found that regular pomegranate juice consumption by prostate cancer patients decreased the disease progression by increasing prostate specific antigen doubling time from 15 to 54 mo. The researchers demonstrated that post-treatment serum analysis showed a decrease in cell proliferation and increase in cancer cell death[47]. Hence, there is supporting evidence for the chemo-preventive potential of fruit juices which may be extended positively against colon cancer.

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