



World Journal of Gastroenterology

Submit a Manuscript: <http://www.wjgnet.com/esps/>
Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>
DOI: 10.3748/wjg.v20.i45.17029

World J Gastroenterol 2014 December 7; 20(45): 17029-17036
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2014 Baishideng Publishing Group Inc. All rights reserved.

MINIREVIEWS

Chemopreventive effect of apple and berry fruits against colon cancer

Saravana Kumar Jaganathan, Muthu Vignesh Vellayappan, Gayathri Narasimhan, Eko Supriyanto, Dyah Ekashanti Octorina Dewi, Aqilah Leela T Narayanan, Arunpandian Balaji, Aruna Priyadarshini Subramanian, Mustafa Yusof

Saravana Kumar Jaganathan, Eko Supriyanto, Dyah Ekashanti Octorina Dewi, Aqilah Leela T Narayanan, Mustafa Yusof, IJN-UTM Cardiovascular Engineering Centre, Faculty of Bioscience and Medical Engineering, Universiti Teknologi Malaysia, Johor Bahru 81310, Malaysia

Muthu Vignesh Vellayappan, Arunpandian Balaji, Aruna Priyadarshini Subramanian, Department of Research and Development, PSNA College of Engineering and Technology, Kothandaraman Nagar, Tamil Nadu, Dindigul 624622, India
Gayathri Narasimhan, Department of Pharmacology, Cinvestav-IPN, Mexico city 07360, Mexico

Supported by (in part) Ministry of Higher Education Malaysia with the Grant Vot, No. R.J130000.7809.4F444 and the Ref No. PY/2014/03167; Part of the grant with the Vot, No. 04H93, Ministry of Education was also used for this study

Author contributions: Jaganathan SK designed the research; Jaganathan SK, Vellayappan MV, Narasimhan G, Balaji A and Subramanian AP collected the literature; Vellayappan MV and Narasimhan G wrote the review in consultation with Jaganathan SK; Narasimhan G, Supriyanto E, Octorina Dewi DE, Narayanan ALT, Arunpandian B and Subramanian AP participated in editing of manuscript by providing suggestions; Yusof M had checked the overall integrity of manuscript during revision by providing suggestions; all the authors verified the final manuscript.

Correspondence to: Saravana Kumar Jaganathan, Senior Lecturer, IJN-UTM Cardiovascular Engineering Centre, Faculty of Bioscience and Medical Engineering, Universiti Teknologi Malaysia, Skudai, Johor Bahru 81310, Malaysia. jaganathaniitkqp@gmail.com

Telephone: +60-7-5558563 Fax: +60-7-5558553

Received: April 11, 2014 Revised: June 16, 2014

Accepted: August 13, 2014

Published online: December 7, 2014

Abstract

Colon cancer arises due to the conversion of precancerous polyps (benign) found in the inner lining of the colon. Prevention is better than cure, and this is very true with respect to colon cancer. Various epidemiologic stud-

ies have linked colorectal cancer with food intake. Apple and berry juices are widely consumed among various ethnicities because of their nutritious values. In this review article, chemopreventive effects of these fruit juices against colon cancer are discussed. Studies dealing with bioavailability, *in vitro* and *in vivo* effects of apple and berry juices are emphasized in this article. A thorough literature survey indicated that various phenolic phytochemicals present in these fruit juices have the innate potential to inhibit colon cancer cell lines. This review proposes the need for more preclinical evidence for the effects of fruit juices against different colon cancer cells, and also strives to facilitate clinical studies using these juices in humans in large trials. The conclusion of the review is that these apple and berry juices will be possible candidates in the campaign against colon cancer.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

Key words: Apple; Berry; Chemoprevention; Colon cancer; Fruit juices; Phytochemicals

Core tip: Colon cancer is one of the leading causes of death worldwide. Chemoprevention of colon cancer using various dietary agents is an area of current interest. This manuscript reviews the chemopreventive potential of apple and berry juices against colon cancer. This work will further promote the importance of dietary fruit juices in colon cancer prevention.

Jaganathan SK, Vellayappan MV, Narasimhan G, Supriyanto E, Octorina Dewi DE, Narayanan ALT, Balaji A, Subramanian AP, Yusof M. Chemopreventive effect of apple and berry fruits against colon cancer. *World J Gastroenterol* 2014; 20(45): 17029-17036 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v20/i45/17029.htm> DOI: <http://dx.doi.org/10.3748/wjg.v20.i45.17029>

INTRODUCTION

Current statistics from the American Cancer Society indicate that in 2014, there will 1665540 new cancer cases in the United States and 585720 cancer deaths, corresponding to 1600 deaths per day. Colon cancer is the second most prevalent cause of cancer death in men and women after lung cancer. Recently, there has been tremendous growth in understanding the fundamentals of carcinogenesis leading to the new mode of cancer prevention known as “chemoprevention.” Chemoprevention is defined as the intake of foreign agents in order to restrain induction, to prevent or slow the progression of cancer, or reversal of carcinogenesis at a premalignant stage^[1]. Chemoprevention makes use of either suitable pharmacologic or dietary agents that are consumed in various forms such as macronutrients, micronutrients, or non-nutritive phytochemicals^[2]. Various dietary agents have been explored for their chemopreventive effects against cancers^[3,4]. There is always an increasing interest for how these agents are linked with colon cancer, as unlike other cancer cells, colon cancer cells are exposed directly. Various epidemiologic studies demonstrated that the oral consumption of fruits and vegetables is inversely associated with the risk of cancer development^[5]. In addition, fruits consumed in liquid form as juices also exhibit similar chemopreventive effects against colon cancer^[6,7]. Digestive tract cancers, especially colon cancer, are amenable to dietary modification. Recent research works show that almost 75% of all the sporadic cases of colorectal cancer are directly affected by dietary intake. Thus, dietary modification is probably one of the best ways for alleviating the risk of developing colon cancer^[8].

Apple and berry fruits have long been widely consumed by various ethnicities because of their beneficial values. Fruit juice intake is gaining in popularity among younger generations due to the comparative ease of consumption. Moreover, the quantity of fruit intake can also be incremented by the intake of juice. In addition to this, fruit juices are available in most retail stores and the various benefits of intake have encouraged many to consume them. The protective nature of fruits against colon cancer may be attributed to the presence of compounds such as dietary fiber, minerals, vitamins and phytochemicals^[9]. Phytochemicals are biologically active non-nutrient components present in fruits that can be divided into alkaloids, carotenoids, polyphenols and other nitrogen-containing components^[10]. The phytochemicals have a chemopreventive property due to their antioxidant activity *via* inactivation of reactive oxygen species, which plays a vital role in the initiation and progression of colon cancer^[11]. Hence adequate intake of these phytochemicals may hinder cancer by enhanced DNA repair and thereby reduce the damage to DNA *via* oxidative stress^[12].

We recently reviewed the role of pomegranate and citrus juices in colon cancer prevention^[4]. As a fair amount of research has been conducted using apple and berry juice phytochemicals as a chemopreventive agent against cancer, we chose to further delineate the chemo-

preventive effects of these juices against colon cancer as well. For this purpose, studies examining the effects of apple and berry juices in colon cancer cell lines and animal models, as well as their bioavailability, are discussed.

APPLE JUICE

The botanical name of the apple is *Malus domestica*. It is the one of the most commonly cultivated tree fruits. Apple juice is the consumable fluid or liquid that is obtained by crushing the apple. The apple juice contains various beneficial polyphenols such as hydroxycinnamic acids, flavan-3-ols/procyanidins, flavonols, dihydrochalcones, and anthocyanins (red peel).

Bioavailability and metabolism of apple juice in colon cancer

In order to determine the protective effect of apple juice on colon cancer, we first must understand the bioavailability of apple products. Research on determination of absorption in ileostomy patients by Feskanich *et al.*^[13] showed that nearly 67%-100% of apple phenolics were digested almost completely in the small intestine itself, while only 0%-33% reached the colon. These authors showed the level of metabolization of the polyphenolic components of the apple juice after digestion and absorption. Other studies have demonstrated the extensive cleavage, isomerization and conjugation of the native polyphenolic compounds occurring during the process. It was observed that 12.7% of the cloudy apple juice compounds get as far as the intestine, however, 22.3% of the apple juice compounds are recovered as metabolites. These studies shed light on the importance of the compounds in the metabolized apple products and indicate the need for the determination of the biologic activity of metabolites of the phytochemicals *in vivo*^[13]. In general, only the native polyphenolic compounds are commonly tested, though they are subjected to substantial metabolic modification. Thus, under physiologic conditions of the tissues, the ingested polyphenolic compounds may be present in a very small quantity or absent. The ingested polyphenols are metabolized by colonic microflora. Hence, it is vital to examine the breakdown products produced at the stomach along with the various metabolites in the blood^[13].

***In vitro* studies of apple juice using colon cancer cell lines**

For the study of *in vitro* effects of apple juice on cancer related processes, various experiments were conducted using cultured colonic cells and cancer-derived cell lines at different developmental stages. Schaefer *et al.*^[14] crushed and extracted apple juice to obtain several polyphenolic mixtures from cider and table apples harvested in Germany, including one extract from apple pomace. A comparison was made among four preparations differing in relative percentages of 14 phytochemicals in apple juice and their effects on oxidative markers in cell lines such

as Caco-2 and HT29. HT29 is a well-developed cell line of colon adenocarcinoma and Caco-2 cells are from human colon cancer cells. It was found that all the extracts reduced the oxidative damage and the presence of butyl hydroperoxide-induced reactive oxygen species. Even though there is difference in the effectiveness observed in the different extract preparations, their efficacy against cancer is similar to the efficacy of phytochemicals identified in the apple juice. In addition, antioxidant capacity determined by Trolox equivalent antioxidant capacity differed for the various extracts with similar chemical compositions. This indicates that there are unknown compounds that account for the antioxidant effects in apple juice. Moreover, prolonged exposure of apple juice results in even greater antioxidant properties for some compounds. From this, it can be deduced that metabolic products obtained over time may have enhanced antioxidant capacity compared to the parent phytochemicals.

A recent study examined the effect of apple juice on cell proliferation. An experiment was done with MCF-7 cells, a breast cancer cell line, and HT29 cells for the study of effect of extracts of ten fruits, including apple peels^[15]. Moreover, a proportion of anthocyanidin-rich parts of the fruit juices were tested. It was found that apple peel extract exhibited the property of significant dose reduction in HT29, but not MCF-7 cells; the MCF-7 cells were less responsive to extract exposure. Thus, there was a differential outcome in the inhibitory effect of anthocyanidin-rich fraction of apple juice in HT29 cells.

Gossé *et al.*^[16] conducted a study to determine whether polymeric or monomeric apple polyphenols are more effective in attenuating the proliferation of SW620 adenocarcinoma-derived metastatic colon cancer cells. This study revealed that larger polymeric molecules are absorbed in a higher segment of the intestine, which reduces their effectiveness in attenuating colon cancer. The absorbed polymeric molecules result in increased residual concentration in the colon. When the SW620 cells are incubated with the procyanidins (polymeric molecules), dose-dependent inhibition of cell growth was observed; 50% inhibition was observed at 45 mg/mL, and total inhibition at 70 mg/mL. Moreover, this extract induced downregulation of various signaling pathways involved in cell proliferation and differentiation, including protein kinase C (PKC), and enzymes involved in polyamine biosynthesis. The polyamines are regulators of cell function and have a significant impact on cancer *via* nurturing cell proliferation or cell death based on the cell type. Finally, flow cytometric experiments revealed that the apple extract increased the number of apoptotic cells and interrupted the cell cycle. In an extension of the above study, the procyanidins found in apple juice have dual effects of downregulating polyamine biosynthesis and stimulating the catabolism or breakdown of these compounds. In addition, the apoptotic effect of apple procyanidins is improved by a compound that disables the activity of polyamine oxidase. Hence, the authors concluded that apple procyanidins are a potential chemopreventive agent

for treating colon cancer.

Some other works have described the molecular mechanisms and cell signaling response to apple juice exposure. A recent work by Kern *et al.*^[17] in 2007 showed that after 24-h exposure at a high concentration (403 mg/mL) of apple juice, PKC activity was decreased by 50% in HT29 cells. When the duration of exposure was longer, extracts in the apple juice targeted the signaling elements upstream of PKC. However, when each compound was segregated from the apple extract, they were ineffective in altering any of the markers under study. Thus, it may be concluded that only the composite mixture of the apple extracts are more important in mediating the observed effects due to the synergy or interaction between different compounds.

The effects of apple juice products on particular enzymes involved in colon carcinogenesis have also been studied. Cytochrome P450 1A1 is one of the most prominent enzymes responsible for activating the chemical carcinogens in the colon. In a study using Caco-2 cells, apple juice without or with reduced levels of acids, carbohydrates, and other native compounds induced the expression of cytochrome P450 1A1 as well as inhibited its catalytic activity. The major phenolics isolated from the apple juice, such as phlorizin, rutin, quercetin and their two glucoside forms, and phloretin, exhibited significant inhibition. It was found that the other types of enzymes relevant to cancer etiology also were favorably affected by apple products^[18]. In a study, polyphenols were extracted from cider apple and a variety of juices. Moreover, an artificial mixture mimicking the natural polyphenolic profile was developed, and its effect on cultured HT29 cells was studied. Cells incubated with the apple juice extract for 24, 48, or 72 h had a decreased growth rate. Furthermore, inhibition of cell growth by the synthetic mixture was less efficient than the natural polyphenolic profile. The isolated components were less effective than both mixtures. Treatment with apple juice triggered an increase in the expression of some vital genes, which includes phase 2 enzymes. These enzymes are associated with chemoprevention (glutathione S-transferases and sulfotransferases). Even though more study is needed, it is intriguing that the apple products have the potential to alter the genetic profiles in a protective manner. All the observed results clearly show that individual isolated components are less effective than the complete mixture of the juice polyphenols.

In 2007, Veeriah *et al.*^[19] examined the effects of apple extracts fermented *in vitro* with human fecal flora on cultured HT29 and LT97 cells. LT97 is a colon adenoma cell line representing the early stage of malignant tumor development. It was found that 99.9% of the parent polyphenols, with the exception of complex structures, were degraded due to fecal fermentation. Furthermore, the short-chain fatty acids (SCFA) increased 1.5-fold in the fermented samples compared to non-fermented samples. Even though a correlation between growth inhibition and SCFA was not assessed, it is known that SCFA stimulates apoptosis, differentiation, and growth arrest pathways. Hence, it can be concluded that the fermented apple juice

extracts have an antiproliferative effect on both types of cells lines, especially LT97 cells.

In vivo effect of apple juice on colon cancer

To determine the impact of apple juice on colon cancer, Barth *et al.*^[20] utilized a rat model of chemically induced colonic damage (using 1,2-dimethylhydrazine). The rats were fed daily with two preparations of apple juice, a clear juice preparation and “cloudy” (higher content of pectin and procyanidin) preparation, obtained from a combination of apples, for seven weeks. The daily consumption of this apple juice protected the mucosa from colonic damage. As there is a possibility for the progression of early lesions into malignancies, the presence of hyperproliferative and aberrant crypts indicated the potential pathogenesis in this rat model. The two methods of apple juice preparation decreased the number of large aberrant crypt foci in the distal colon, DNA damage and hyperproliferation. The same authors further studied the effects of segregated fractions on the various markers and concluded that the juice in the original form has a better efficacy compared to the unique components, which include the polyphenolic-rich extracts^[21]. The work done by Soyalan *et al.*^[22] demonstrated the potential of polyphenol-rich apple juices in stimulating the redox-sensitive gene expression in rats for colon cancer prevention. They also elucidated that the cloudy apple juice has better efficacy as a cancer chemopreventive agent compared to the clear apple juice and smoothie apple juice. In another independent experiment, Koch *et al.*^[23] explored the efficacy of apple juice in obese rats, and found no cancer preventive bioactivity. Although obesity and colon cancer formation are directly linked, they did not rule out the role of chemopreventive effects of apple juices and suggested future trials with obese or diabetic human individuals^[23].

In another study, rats were injected with the chemical carcinogen azoxymethane, which results in various morphologic changes such as carcinoma. It was found that apple extracts given along with the animals’ drinking water for 6 wk had colon cancer protective effects^[16]. Pre-neoplastic lesions in the rats fed with apple phytochemicals were reduced as result of apple intake. There were 50% fewer aberrant crypts observed compared to the non-fed group. The authors predicted that this effect is extendable to humans who are consuming two apples per day. This would provide a sufficient amount of procyanidin (4-10 mg/kg body weight) to attain similar results in humans.

BERRY JUICE

The botanical name of chokeberry is *Aronia melanocarpa* and that of bilberry is *Vaccinium myrtillus*. These fruits are grown in North America, Europe, Asia, and Africa. Anthocyanin-rich extracts (AREs) derived from berries are prepared by certain quantization methods to maximize pigment recovery. The berry extracts contain anthocyanin, β -carotene, a carotenoid and tangeretin, a flavonoid.

Berry juices are prepared by crushing various berries and bottled for marketing.

Bioavailability and metabolism of berry juice in colon cancer

Anthocyanins in chokeberry extract pass into the large intestine and are degraded by bacteria. Detecting and measuring the trace levels of anthocyanins in urine, plasma, excretion and metabolism is very difficult^[24]. Approximately 66% of anthocyanins derived from cyanidine-3-galactoside were detected in urine and serum samples of volunteers who consumed 20 g of chokeberry extract consisting of 1.3 g cyanidin-3-glycosides. Metabolites such as oxidized and methylated derivatives of cyanidin-3-galactoside, cyanidin glucuronide and glucuronide conjugates, were present at levels of 0.011 to 0.013 nmol/L in urine samples. The cumulative total serum concentration of anthocyanins and their metabolites was 591.7 nmol/L^[25]. Wu *et al.*^[26] reported similar results, showing that cyanidine-3-galactoside accounted for 60.7% of the total anthocyanin-based compounds in the urinary excretion of chokeberry-fed pigs. The peak anthocyanin concentration of 2.9 nmol/L in plasma at 1 h and 18 min, including a total renal excretion of 0.02% of ingested anthocyanins over 24 h, which are metabolites of cyanidin, was observed in healthy volunteers as depicted by Wiczowski *et al.*^[27] in 2010. As flavonols are present in very small quantities in chokeberry extracts, there is not much information on its bioavailability. However, a study with rats by Kida *et al.*^[28] in 2000 showed that the monomers of flavan-3-ol were eliminated in the liver from the bloodstream and returned to the small intestine. Flavan-3-ols are highly excreted in urine and recovered in ileal fluid from ileostomy patients^[29]. Biosorption studies revealed that biologic uptake of anthocyanin extracts depends on the structure of anthocyanin. However, Koide *et al.*^[30] demonstrated that HCT-15 cancer cell growth inhibition was affected by various anthocyanin sources and glycosylation modifications. In addition, anthocyanins in bilberry extracts are non-acylated glucoside derivatives, but are cyanidin derivatives in chokeberry extracts.

In vitro studies of berry juice using colon cancer cell lines

Growth of human intestinal carcinoma HCT-15 cells *in vitro* was inhibited efficaciously by anthocyanin fractions extracted from different cherry and berry extracts in comparison to that of flavonoid fractions^[31]. Berry extracts including cowberry, strawberry, blueberry, and bilberry extracts that contain anthocyanins inhibited the growth of HCT-116 colon cancer cells^[32]. Growth of HT29 and HCT-116 cell lines were also inhibited by tart cherry anthocyanins and their aglycon, cyanidin^[33]. Cyanidine-3-galactoside is the predominant anthocyanin in commercialized chokeberry ARE that is available as a mixture of phenolic compounds. Chokeberry AREs rendered 65% growth inhibition and cell viability of HT29 cells within 24 h of exposure, suggesting a cytostatic inhibi-

tion. In the presence of the chokeberry extract, NCM460 cells, which are epithelial cells from normal colon, were found to grow normally^[34].

Cell cycle progression is regulated by the interaction between cyclins and cyclin-dependent kinases (CDKs). Cyclin-dependent kinase inhibitors (CDKIs) negatively regulate the activity of CDKs and play an important role in controlling cell cycle progression. CDKIs, such as p21WAF1 and p27KIP1, bind to cyclin-CDK complexes and halt the G1 phase of the cell cycle. HT29 cell percentage after exposure to chokeberry was increased, and a block in the cell cycle checkpoint in the G1/G0 phase was observed^[35]. Depending on the time of exposure to AREs, an increase in p21WAF1 and p27KIP1 gene expression was in agreement with ARE-induced G1/G0 cell cycle arrest. Phytochemicals, such as β -carotene and tangeretin, mediate cell cycle arrest in colon cancer cells by upregulating the expression of p27KIP1 and p21WAF1, which can block the cell cycle by inhibiting both G1 and G2-phase cyclin-CDK complexes^[35]. Although blocking of cell cycle at both G1/G0 and G2/M phases after exposure of HT29 cells to chokeberry AREs was demonstrated, chokeberry AREs neither affected the cell cycle nor altered the expression of cell cycle genes in normal colon cells. This evidence supports the notion that AREs prevent cancer cell growth through the inhibition of cell cycle events.

Cyclooxygenase (COX) enzymes are involved in prostaglandin formation through the catalysis of arachidonic acid oxygenation. Carcinomas and colorectal adenomas show an increase in COX-2 mRNA compared to normal mucosa^[36]. COX-2 is also upregulated or induced in response to inflammation, whereas COX-1 is expressed in all body tissues and performs a "housekeeping" role. In the NCM460 cell line, derived from normal colon mucosa, expression of COX-2 is low in comparison to the constitutively expressed COX-1. In contrast, high expression of COX-2 is seen in HT29 cells. Hence, chemoprevention of colon cancer is possible by inhibiting COX genes. A plausible method is to use non-steroidal anti-inflammatory drugs to inhibit the COX-2 enzyme, thereby delaying or preventing cancer of the colon. Recent studies revealed that anthocyanin fractions isolated from various berries show an inhibitory effect on COX enzyme activities *in vitro*^[37]. Seeram *et al.*^[37] demonstrated that COX-1 and COX-2 enzymes were inactivated by cyanidin-3-rutinoside and cyanidin-3-glucosylrutinoside anthocyanins extracted from sweet cherries and raspberries. The basal level of COX-2 mRNA after exposure to chokeberry ARE was not altered in NCM460 cells. In contrast, the levels of COX-2 mRNA derived from colon adenocarcinoma were high. It is supposed that the difference in growth response of the two cell lines after exposure to chokeberry extracts may be through a COX-dependent mechanism, as evidenced by the variable expression levels of COX enzymes. However, further experimentation revealed that with an increase in chokeberry extract exposure time, there was an increase in COX-2 expression levels in HT29 cells.

The cells treated with ARE showed an increase in COX-2 protein and prostaglandin E2, indicating that growth inhibition was not ascribed to COX-2 inhibition. HT29 cell growth inhibition after exposure to chokeberry AREs is not mediated through the suppression of COX-2 activity, though the activity of COX enzymes was inhibited by anthocyanins *in vitro*. It may be a COX-independent growth inhibition. A COX-independent effect in growth was also observed in colorectal cancer cells treated with celecoxib^[37]. Furthermore, the mechanism of growth inhibition may involve the NF- κ B pathway inhibition, peroxisome proliferator-activated receptors, or expression of the cell cycle genes^[38]. Thus, it can be concluded that chokeberry AREs upregulated p21WAF1 and p27KIP1 and downregulated cyclin A and cyclin B1 to inhibit colon carcinoma cell growth, with very limited effects on the growth of normal colon cells. This ensures that AREs can be used for treating human colorectal cancers without affecting normal cells significantly.

***In vivo* effect of berry juice on colon cancer**

Hagiwara *et al.*^[33] demonstrated that rats fed with purple corn color extract, which is rich in anthocyanin, showed a decrease in the prevalence of aberrant crypt foci and early colon cancer lesions. This experiment demonstrates that 2-amino-1-methyl-6-phenylimidazol(4,5-*b*) pyridine-induced colon carcinogenesis was abated by anthocyanin-rich fractions^[33]. In another report, oral administration of lyophilized black raspberries containing anthocyanins to rodents produced inhibitory effects on colorectal cancer and/or polyp tissue. In addition, biomarkers of proliferation and angiogenesis were significantly reduced by the berry treatment, while apoptosis was greatly enhanced^[39].

Mirtocyan is an anthocyanin-rich standardized bilberry extract. It causes pharmacodynamic changes commensurate with colorectal cancer chemopreventive efficacy and generates measurable anthocyanin levels in urine, blood and target tissues. Low doses of mirtocyan showed a significant reduction in the levels of circulating insulin-like growth factor-1, which possesses a procarcinogenic property^[40]. Hence, further studies on the pharmacodynamic effects of bilberry anthocyanins at various doses are essential to demonstrate them as potential colorectal cancer chemopreventive agents.

CONCLUSION

The objectives of this review were to document the chemopreventive effects of apple and berry fruit juices against colon cancer and to discuss preclinical and clinical effects of these juices. Furthermore, bioavailability and metabolism of these fruit extracts were highlighted. Summaries of *in vitro* and *in vivo* studies of apple and berry juice against colon cancer represented in Tables 1 and 2, respectively.

This review justifies the need for additional preclinical studies with these juices and extracts. In order to bolster the anticancer effect of these juices/extracts against co-

Table 1 *In vitro* and *in vivo* effects of apple extracts against colon cancer

Apple juice and its Components	Models (cell line/animal)	Observation/result	Ref.
Apple extracts	HT29 and LT97 colon adenoma cell line	SCFA increased 1.5 fold in fermented samples SCFA stimulates apoptosis, differentiation and growth arrest	[19]
Composite mixture of apple extracts	HT29 cells	PKC activity was decreased by 50% after 24 h exposure of 403 mg/mL Upstream signaling elements targeted after longer duration of exposure	[17]
Polyphenolic mixtures from cider, table apples and apple pomace; Phenolics such as phlorizin, rutin, quercetin and phloretin	Caco-2 cells and HT29 colonic cell lines Caco-2 cells	Reduced oxidative damage Reduced the presence of butyl hydroperoxide-induced ROS Induction of p450 1A1 expression and inhibition of catalytic activity of the enzyme	[14]
Anthocyanidin-rich fraction of apple juice	HT29 and MCF-7 breast cancer cell line	Significant dose reduction in HT29 cells and not in MCF-7 cells MCF-7 cells are less responsive to extract exposure	[15]
Procyanidins of apple juice (polymeric polyphenols)	SW620 adenocarcinoma-derived metastatic cells of colon cancer	Dose-dependent inhibition of cell growth 50% of inhibition at 45 mg/mL and total inhibition at 70 mg/mL Downregulation of signaling pathway such as cell proliferation and differentiation, PKC and enzymes involved in polyamine biosynthesis and stimulation of catabolism Increased number of apoptotic cells and interruption in cell cycle	[16]
Apple phytochemicals	Rats injected with azoxymethane	Reduction in preneoplastic lesions and 50% fewer aberrant crypts	
Apple juice extract; Synthetic mixture, isolated components	HT29 cells	Decreased growth rate efficiently for 24, 48 and 72 h Increased the expression of genes which include phase-2 enzymes, including glutathione-s-transferases and sulfotransferases, which are involved in chemoprevention Growth rate inhibition was less efficient comparatively Lesser effectiveness than either mixture	[18]
Two preparation methods of apple juice: clear and cloudy (higher content of pectin and procyanidin)	Rat model (induced with 1,2-dimethylhydrazine)	Protection from dimethylhydrazine-induced genotoxic damage Important markers were reduced including decreased number of large aberrant crypt foci in the distal colon, reduced DNA damage and reduced hyper-proliferation	[20]
Polyphenolic-rich extracts	Rat model (induced with 1,2-dimethylhydrazine)	Juice fractions were found to be more effective in protecting from damage compared to isolated fractions	[21]

PKC: Protein kinase C; ROS: Reactive oxygen species; SCFA: Short-chain fatty acids.

Table 2 *In vitro* and *in vivo* effects of berry extracts against colon cancer

Berry juice and its Components	Models (cell line/animal)	Observation/result	Ref.
Anthocyanin fractions of berry juice	HCT-15 cells HCT-116 cells	Inhibited growth of human intestinal carcinoma cells Inhibited the growth of colon cancer cells	[31,33]
Chokeberry ARE	HT29 cells	Percentage of cells increased and blockade in G1/G0 phase of cell cycle Increased expression of p21WAF1 and p27KIP1 genes Decreased percentage of cells in S phase indicating that cells moved to G2/M phase	[32,33]
Predominant anthocyanin: cyanidin-3-galactoside	HT29 cells	65% growth inhibition after 24 h exposure High cell viability observed indicating cytostatic inhibition Inactivation of COX-1 and COX-2 enzymes	[35] [34]
Anthocyanins (cyanidin-3-glucosylrutinoside and cyanidin-3-rutinoside)	HT29 cells	Growth inhibition of cells may occur in a COX-independent manner	[37]
Phytochemicals of berry juice; Carotenoid (β-carotene) and flavonoid (tangeritin)	HT29 cells	Upregulation of p21WAF1 and p27KIP1 expression p21WAF1 CDKI blocks G1 and G2 phase cyclin-CDK complexes	[35]
Anthocyanin-rich purple corn color of berry juice	Rats injected with PhIP	Decrease in incidence of early colon cancer lesions, decrease in aberrant crypt foci and a protective effect against PhIP-induced colon carcinogenesis	[33]
Anthocyanin-rich black raspberries	Rodents with colorectal cancer/polyp tissue	Biomarkers of proliferation and angiogenesis were significantly reduced while apoptosis of cancer cells was enhanced	
Mirtocyan of bilberry extract	Patients with colorectal adenocarcinoma and colorectal liver metastases	Significant reduction in IGF-1 levels thereby reducing carcinogenesis	[40]

ARE: Anthocyanin-rich extract; CDK: Cyclin-dependent kinase; CDKI: CDK inhibitor; COX: Cyclooxygenase; IGF-1: Insulin-like growth factor-1; PhIP: 2-amino-1-methyl-6-phenylimidazol(4,5-b) pyridine.

lon cancer *in vitro*, experiments using additional cell lines are crucial. Further, *in vivo* studies using Apc^{Min/+} mouse models may provide some valuable insights about the mechanism of action of these juices. The purpose of this work is to highlight the importance of establishing clinical trials in human subjects. It will be noteworthy to perform clinical trials in patients with colon cancer, in which the effects produced by the oral consumption of these fruit juices can be outlined. For instance, colorectal cancer patients must be administered a measured quantity of fruit juices on a regular basis for a specific duration of time. Then, chemopreventive effects produced by these fruit juices against colon cancer may be documented. Hence, conducting human clinical trials may validate the potency of these fruit juices in the fight against colon cancer.

ACKNOWLEDGMENTS

Authors acknowledge University Industry Research Laboratory (UPMU) of UTM for their support.

REFERENCES

- Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. *CA Cancer J Clin* 2007; **57**: 43-66 [PMID: 17237035 DOI: 10.3322/canjclin.57.1.43]
- Liu RH. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *Am J Clin Nutr* 2003; **78**: 517S-520S [PMID: 12936943]
- Jaganathan SK, Mandal M. Anticancer activity of honey and its phenolic components. Honey: Current Research and Clinical Applications. New York: Nova science Publisher, 2012: 39-60
- Jaganathan SK, Vellayappan MV, Narasimhan G, Supriyanto E. Role of pomegranate and citrus fruit juices in colon cancer prevention. *World J Gastroenterol* 2014; **20**: 4618-4625 [PMID: 24782614 DOI: 10.3748/wjg.v20.i16.4618]
- Anon, Joint WHO/FAO Expert Consultation on Diet, Nutrition and the Prevention of Chronic Diseases. Geneva. Switzerland: WHO technical report series, 2002: 916
- Saruwatari A, Okamura S, Nakajima Y, Narukawa Y, Take-da T, Tamura H. Pomegranate juice inhibits sulfoconjugation in Caco-2 human colon carcinoma cells. *J Med Food* 2008; **11**: 623-628 [PMID: 19053852 DOI: 10.1089/jmf.2007.0050]
- Tamura H, Matsui M. Inhibitory effects of green tea and grape juice on the phenol sulfotransferase activity of mouse intestines and human colon carcinoma cell line, Caco-2. *Biol Pharm Bull* 2000; **23**: 695-699 [PMID: 10864017 DOI: 10.1248/bpb.23.695]
- Johnson IT. New approaches to the role of diet in the prevention of cancers of the alimentary tract. *Mutat Res* 2004; **551**: 9-28 [PMID: 15225578 DOI: 10.1016/j.mrfmmm.2004.02.017]
- Lu H, Li J, Zhang D, Stoner GD, Huang C. Molecular mechanisms involved in chemoprevention of black raspberry extracts: from transcription factors to their target genes. *Nutr Cancer* 2006; **54**: 69-78 [PMID: 16800774 DOI: 10.1207/s15327914nc5401_8]
- Liu RH. Potential synergy of phytochemicals in cancer prevention: mechanism of action. *J Nutr* 2004; **134**: 3479S-3485S [PMID: 15570057]
- Duthie SJ, Jenkinson AM, Crozier A, Mullen W, Pirie L, Kyle J, Yap LS, Christen P, Duthie GG. The effects of cranberry juice consumption on antioxidant status and biomarkers relating to heart disease and cancer in healthy human volunteers. *Eur J Nutr* 2006; **45**: 113-122 [PMID: 16032375 DOI: 10.1007/s00394-005-0572-9]
- Freese R. Markers of oxidative DNA damage in human interventions with fruit and berries. *Nutr Cancer* 2006; **54**: 143-147 [PMID: 16800780 DOI: 10.1207/s15327914nc5401_14]
- Feskanich D, Ziegler RG, Michaud DS, Giovannucci EL, Speizer FE, Willett WC, Colditz GA. Prospective study of fruit and vegetable consumption and risk of lung cancer among men and women. *J Natl Cancer Inst* 2000; **92**: 1812-1823 [PMID: 11078758 DOI: 10.1093/jnci/92.22.1812]
- Schaefer S, Baum M, Eisenbrand G, Dietrich H, Will F, Janzowski C. Polyphenolic apple juice extracts and their major constituents reduce oxidative damage in human colon cell lines. *Mol Nutr Food Res* 2006; **50**: 24-33 [PMID: 16317784 DOI: 10.1002/mnfr.200500136]
- Olsson ME, Gustavsson KE, Andersson S, Nilsson A, Duan RD. Inhibition of cancer cell proliferation *in vitro* by fruit and berry extracts and correlations with antioxidant levels. *J Agric Food Chem* 2004; **52**: 7264-7271 [PMID: 15563205 DOI: 10.1021/jf030479p]
- Gossé F, Guyot S, Roussi S, Lobstein A, Fischer B, Seiler N, Raul F. Chemopreventive properties of apple procyanidins on human colon cancer-derived metastatic SW620 cells and in a rat model of colon carcinogenesis. *Carcinogenesis* 2005; **26**: 1291-1295 [PMID: 15790589 DOI: 10.1093/carcin/bgi074]
- Kern M, Pahlke G, Balavenkatraman KK, Böhmer FD, Marko D. Apple polyphenols affect protein kinase C activity and the onset of apoptosis in human colon carcinoma cells. *J Agric Food Chem* 2007; **55**: 4999-5006 [PMID: 17542601 DOI: 10.1021/jf063158x]
- Veeriah S, Kautenburger T, Habermann N, Sauer J, Dietrich H, Will F, Pool-Zobel BL. Apple flavonoids inhibit growth of HT29 human colon cancer cells and modulate expression of genes involved in the biotransformation of xenobiotics. *Mol Carcinog* 2006; **45**: 164-174 [PMID: 16369997 DOI: 10.1002/mc.20158]
- Veeriah S, Hofmann T, Gleis M, Dietrich H, Will F, Schreier P, Knaup B, Pool-Zobel BL. Apple polyphenols and products formed in the gut differently inhibit survival of human cell lines derived from colon adenoma (LT97) and carcinoma (HT29). *J Agric Food Chem* 2007; **55**: 2892-2900 [PMID: 17378580 DOI: 10.1021/jf063386r]
- Barth SW, Fähndrich C, Bub A, Dietrich H, Watzl B, Will F, Briviba K, Rechkemmer G. Cloudy apple juice decreases DNA damage, hyperproliferation and aberrant crypt foci development in the distal colon of DMH-initiated rats. *Carcinogenesis* 2005; **26**: 1414-1421 [PMID: 15802299 DOI: 10.1093/carcin/bgi082]
- Barth SW, Faehndrich C, Bub A, Watzl B, Will F, Dietrich H, Rechkemmer G, Briviba K. Cloudy apple juice is more effective than apple polyphenols and an apple juice derived cloud fraction in a rat model of colon carcinogenesis. *J Agric Food Chem* 2007; **55**: 1181-1187 [PMID: 17261019 DOI: 10.1021/jf063078t]
- Soyalán B, Minn J, Schmitz HJ, Schrenk D, Will F, Dietrich H, Baum M, Eisenbrand G, Janzowski C. Apple juice intervention modulates expression of ARE-dependent genes in rat colon and liver. *Eur J Nutr* 2011; **50**: 135-143 [PMID: 20652274 DOI: 10.1007/s00394-010-0124-9]
- Koch TC, Briviba K, Watzl B, Fähndrich C, Bub A, Rechkemmer G, Barth SW. Prevention of colon carcinogenesis by apple juice *in vivo*: impact of juice constituents and obesity. *Mol Nutr Food Res* 2009; **53**: 1289-1302 [PMID: 19753605 DOI: 10.1002/mnfr.200800457]
- Petko ND, Christo GK, Milan C, Antonin L, Maria GK. Bioavailability and Antioxidant Activity of BlackChokeberry (Aroniamelanocarpa) Polyphenols: *in vitro* and *in vivo* Evidences and Possible Mechanisms of Action: A Review. *Compr Rev Food Sci Food Safety* 2012; **11**: 471-489 [DOI: 10.1111/j.1541-4337.2012.00198.x]
- Kay CD, Mazza G, Holub BJ, Wang J. Anthocyanin metabolites in human urine and serum. *Br J Nutr* 2004; **91**: 933-942 [PMID: 15228048 DOI: 10.1079/BJN20041126]

- 26 **Wu X**, Pittman HE, McKay S, Prior RL. Aglycones and sugar moieties alter anthocyanin absorption and metabolism after berry consumption in weanling pigs. *J Nutr* 2005; **135**: 2417-2424 [PMID: 16177206]
- 27 **Wiczowski W**, Romaszko E, Piskula MK. Bioavailability of cyanidin glycosides from natural chokeberry (*Aronia melanocarpa*) juice with dietary-relevant dose of anthocyanins in humans. *J Agric Food Chem* 2010; **58**: 12130-12136 [PMID: 21067240 DOI: 10.1021/jf102979z]
- 28 **Kida K**, Suzuki M, Matsumoto N, Nanjo F, Hara Y. Identification of biliary metabolites of (-)-epigallocatechin gallate in rats. *J Agric Food Chem* 2000; **48**: 4151-4155 [PMID: 10995329 DOI: 10.1021/jf000386x]
- 29 **Stalmach A**, Mullen W, Steiling H, Williamson G, Lean ME, Crozier A. Absorption, metabolism, and excretion of green tea flavan-3-ols in humans with an ileostomy. *Mol Nutr Food Res* 2010; **54**: 323-334 [PMID: 19937856 DOI: 10.1002/mnfr.200900194]
- 30 **Koide T**, Kamei H, Hashimoto Y, Kojima T, Terabe K, Umehara T. Influence of flavonoids on cell cycle phase as analyzed by flow-cytometry. *Cancer Biother Radiopharm* 1997; **12**: 111-115 [PMID: 10851454 DOI: 10.1089/cbr.1997.12.111]
- 31 **Kresty LA**, Morse MA, Morgan C, Carlton PS, Lu J, Gupta A, Blackwood M, Stoner GD. Chemoprevention of esophageal tumorigenesis by dietary administration of lyophilized black raspberries. *Cancer Res* 2001; **61**: 6112-6119 [PMID: 11507061]
- 32 **Kuntz S**, Wenzel U, Daniel H. Comparative analysis of the effects of flavonoids on proliferation, cytotoxicity, and apoptosis in human colon cancer cell lines. *Eur J Nutr* 1999; **38**: 133-142 [PMID: 10443335 DOI: 10.1007/s003940050054]
- 33 **Hagiwara A**, Miyashita K, Nakanishi T, Sano M, Tamano S, Kadota T, Koda T, Nakamura M, Imaida K, Ito N, Shirai T. Pronounced inhibition by a natural anthocyanin, purple corn color, of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)-associated colorectal carcinogenesis in male F344 rats pretreated with 1,2-dimethylhydrazine. *Cancer Lett* 2001; **171**: 17-25 [PMID: 11485824 DOI: 10.1016/S0304-3835(01)00510-9]
- 34 **Moyer MP**, Manzano LA, Merriman RL, Stauffer JS, Tanzer LR. NCM460, a normal human colon mucosal epithelial cell line. *In Vitro Cell Dev Biol Anim* 1996; **32**: 315-317 [PMID: 8842743 DOI: 10.1007/BF02722955]
- 35 **Cayrol C**, Knibiehler M, Ducommun B. p21 binding to PCNA causes G1 and G2 cell cycle arrest in p53-deficient cells. *Oncogene* 1998; **16**: 311-320 [PMID: 9467956 DOI: 10.1038/sj.onc.1201543]
- 36 **Eberhart CE**, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S, DuBois RN. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. *Gastroenterology* 1994; **107**: 1183-1188 [PMID: 7926468]
- 37 **Seeram NP**, Momin RA, Nair MG, Bourquin LD. Cyclooxygenase inhibitory and antioxidant cyanidin glycosides in cherries and berries. *Phytomedicine* 2001; **8**: 362-369 [PMID: 11695879 DOI: 10.1078/0944-7113-00053]
- 38 **Wang C**, Fu M, D'Amico M, Albanese C, Zhou JN, Brownlee M, Lisanti MP, Chatterjee VK, Lazar MA, Pestell RG. Inhibition of cellular proliferation through IκappaB kinase-independent and peroxisome proliferator-activated receptor gamma-dependent repression of cyclin D1. *Mol Cell Biol* 2001; **21**: 3057-3070 [PMID: 11287611 DOI: 10.1128/MCB.21.9.3057-3070.2001]
- 39 **Wang LS**. Effect of Freeze-Dried Black Raspberries on Human Colorectal Cancer Lesions. American Association for Cancer Research Special Conference in Cancer Research. Cambridge, MA: Advances in Colon Cancer Research, 2007
- 40 **Thomasset S**, Berry DP, Cai H, West K, Marczylo TH, Marsden D, Brown K, Dennison A, Garcea G, Miller A, Hemingway D, Steward WP, Gescher AJ. Pilot study of oral anthocyanins for colorectal cancer chemoprevention. *Cancer Prev Res (Phila)* 2009; **2**: 625-633 [PMID: 19584076 DOI: 10.1158/1940-6207.CAPR-08-0201]

P- Reviewer: Paydas S, Pessi T S- Editor: Qi Y
L- Editor: AmEditor E- Editor: Wang CH





Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: bpgooffice@wjgnet.com

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



ISSN 1007-9327

