

ANSWERING REVIEWERS

June 16, 2014

Dear Editor,



Please find enclosed the edited manuscript in Word format (file name: 10653-review.doc).

Title: Chemopreventive Effect of Apple and Berry Fruits against Colon Cancer

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Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 10653

Reviewer no: 02535483

The current review focused on colon cancer and chemopreventive effects of fruit juices against The current review focused on colon cancer and chemopreventive effects of fruit juices against colon cancer. This literature survey assembles the in vitro and in vivo studies on the subject, suggesting that various phenolic phytochemicals present in these fruit juices might have even therapeutic potential against colon cancer. The review is well-written and extensive background work has been performed. I highly recommend the publishing. As a minor comment, I highly recommend references 1-4 to add and discussed in the text. References 1. Soyalan Bet al. Eur J Nutr. 2011 Mar;50(2):135-43. doi: 10.1007/s00394-010-0124-9. 2. Koch TC, et al Mol Nutr Food Res. 2009 Oct;53(10):1289-302. doi: 10.1002/mnfr.200800457 3. Saruwatari A, et al J Med Food. 2008 Dec;11(4):623-8. doi: 10.1089/jmf.2007.0050. 4. Tamura H, Matsui M. Biol Pharm Bull. 2000 Jun;23(6):695-9. .

The manuscript has been improved according to the suggestions of reviewers:

Thank you for the comments and the authors would like to express sincere gratitude for the same. Moreover, as suggested by him some references were added politely to further add value to our paper.

Following changes were made to incorporate the references mentioned by the above-mentioned reviewer:

In addition to consumption of fruits as such, it is also found that the fruits consumed in liquid form as juices also found to exhibit similar chemopreventive effects against colon cancer [6, 7].

- [6] Saruwatari A, Okamura S, Nakajima Y, Narukawa Y, Takeda T, Tamura H. Pomegranate juice inhibits sulfoconjugation in Caco-2 human colon carcinoma cells. J Med Food. 2008;11(4):623-8.[PMID: 19053852][DOI 10.1089/jmf.2007.0050]
- [7] 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(6):695-9.[PMID: 10864017]

The work done by Soyalan *et al* demonstrated the potential of polyphenol-rich apple juices in stimulating the redox-sensitive gene expression in rats for colon cancer prevention [22]. Further 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 [22]. In another independent experiment conducted by Koch *et al* explored the efficacy of apple juice against obese rats showed no cancer preventive bioactivity. Although obesity and colon cancer formation are directly linked, he did not rule out the role of chemopreventive effect of apple juices and suggested future trials with obese human or diabetic individuals [23].

[22] Bulent Soyalan, Jutta Minn, Hans J. Schmitz, Dieter Schrenk, Frank Will, Helmut Dietrich, Matthias Baum, Gerhard Eisenbrand, Christine Janzowski. 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]

[23] Tatiana C. L. Koch, Karlis Briviba, Bernhard Watzl, Christine Fhndrich, Achim Bub, Gerhard Rechkemmer and Stephan W. Barth. Prevention of colon carcinogenesis by apple juice *invivo*: Impact of juice constituents and obesity. *Mol Nutr Food Res*. 2009;53(10):1289-302[PMID: 19753605][DOI 10.1002/mnfr.200800457]

Reviewer no: 02528717

This paper is about the chemoprventive effect of berry and apple juices in colon cancer. However there are many problems: 1-Paper is very cahaotic and it is difficult to understand this paper. All the paper must be reviewed and re-arranged. There must be a plan to present the paper: basic asicence about this effect and then animal studies must be presented 2-Tables 1 and 2 must be in 2 parts: one table for berry and one table for apple juice 3-English is poor and and must be re-written 4-References are true?

First of all, I thank this reviewer for his efforts in making our manuscript more meaningful:

Query 1-Paper is very cahaotic and it is difficult to understand this paper. All the paper must be reviewed and re-arranged. There must be a plan to present the paper: basic asicence about this effect and then animal studies must be presented

The paper is well arranged according to the comments: Now the paper progresses in three subtitles for each fruit juices namely for all juices it starts with bioavailability, then *in vitro* studies and finally *in vivo* for better understanding for the readers of the manuscript.

Query 2-Tables 1 and 2 must be in 2 parts: one table for berry and one table for apple juice

According to reviewer comments table was corrected from the previously given tables: there are two tables one for berry and another for apple juice with the table heading as follows:

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

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

Query 3-English is poor and and must be re-written

The manuscript was given to native speaker and following English corrections were made which is highlighted in redfont.

- Colon cancer is the second most **prevalent** cause of cancer death in men and women after lung cancer
- Chemoprevention is defined as the administration of agents to **restrain** induction, to inhibit or delay the progression of cancer, or as the inhibition or reversal of carcinogenesis at a premalignant stage
- There is always an increasing interest how these agents are linked with colon cancer as colon cancer cells are exposed directly compared to other **cancer cells**.
- The digestive tract cancers, especially the colon cancer is found to be very much **amenable** to dietary modification
- With the advent of technology, it **is** been marketed in the form of juices. Intake of fruits as juices has gained wide acceptance among the young population because it is easier to consume and also intake amount of juices can be increased significantly compared to fruits itself.
- Hence adequate intake of these phytochemicals may **hinder** cancer by enhanced DNA repair and thereby reducing the damage to the DNA via oxidative stress
- This work motivated us further to delineate the role of apple and **berry juices'** chemopreventive effect against colon cancer
- Berry juices are **prepared** by crushing various berries and bottled for marketing
- Anthocyanins in chokeberry extract pass into large intestine and **are degraded** by bacteria. Also, detecting and measuring the trace levels of anthocyanins in urine, plasma, excretion and metabolism is very difficult
- The cumulative total serum concentration of anthocyanins and their metabolites **were reported as** 591.7nmol/L
- Anthocyanin fractions extracted from different cherry and berry extracts effectively inhibited growth of human intestinal carcinoma HCT-15 cells *in vitro* **in comparison to that of flavonoid fractions**
- Cyclin-dependent kinase inhibitors (CDKIs) negatively regulate **the activity of CDK** and play an important role in controlling cell cycle progression. CDKIs, such as p21WAF1 and p27KIP1, are related proteins which bind to cyclin-CDK complexes and **arrest the G1phase of cell cycle**.
- p21WAF1, a CDKI **is adept to** block both the phases of cell cycle by inhibiting G1 as well as G2-phase cyclin-CDK complexes^[35]. Although dual blockage at G1/G0 and G2/M phases of cell cycle by chokeberry ARE-exposed HT-29 cells was demonstrated, **chokeberry ARE neither affected cell cycle nor altered the expression of cell cycle genes in normal colon cells. This evidence substantiates that prevention of cancer cell growth through the inhibition of cell cycle events was ascribed to ARE.**
- ARE may be through a COX-dependent mechanism is **corroborated with** the difference in basal levels of cyclooxygenase expression of the two cell lines.
- Hagiwara *et al*, demonstrated that rats fed with anthocyanin-rich PCC (purple corn color) showed decrease in incidence of **aberrant crypt foci, early colon cancer lesions**.
- In another report, oral administration of lyophilized black raspberries containing anthocyanins in 50 rodents with colorectal cancer and/or polyp tissue **demonstrated inhibitory effects** of raspberries.
- Hence, further studies on the pharmacodynamic effects of bilberry anthocyanins **at various doses are essential** to demonstrate them as potential colorectal cancer chemopreventive agents.

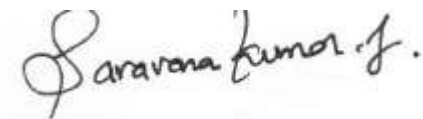
- The objective of this review **is** to document the chemopreventive effects of apple and berry fruit juices against colon cancer.
- **Furthermore, bioavailability and metabolism of these fruit extracts are highlighted.** The summary of **all the discussions is** reported in Tables 1 and 2 **comprising *in vitro* and *in vivo* studies of apple and berry juice respectively against colon cancer.**
- Hence conducting human clinical trials may **validate the potency of these fruit juices** in fighting colon cancer.

Query 4-References are true?

All references were checked and even validated by giving the PUBMED ID for the references used.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,



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