

# Evaluating the Effect of Four Extracts of Avocado Fruit on Esophageal Squamous Carcinoma and Colon Adenocarcinoma Cell Lines in Comparison with Peripheral Blood Mononuclear Cells

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**Abstract-** Most patients with gastrointestinal cancers refer to the health centers at advanced stages of the disease and conventional treatments are not significantly effective for these patients. Therefore, using modern therapeutic approaches with lower toxicity bring higher chance for successful treatment and reduced adverse effects in such patients. The aim of this study is to evaluate the effect of avocado fruit extracts on inhibition of the growth of cancer cells in comparison with normal cells. In an experimental study, ethanol, chloroform, ethyl acetate, and petroleum extracts of avocado (*Persea americana*) fruit were prepared. Then, the effects if the extracts on the growth of esophageal squamous cell carcinoma and colon adenocarcinoma cell lines were evaluated in comparison with the control group using the MTT test in the cell culture medium. Effects of the four extracts of avocado fruit on three cells lines of peripheral blood mononuclear cells, esophageal squamous cell carcinoma, and colon adenocarcinoma were tested. The results showed that avocado fruit extract is effective in inhibition of cancer cell growth in comparison with normal cells ( $P<0.05$ ). Avocado fruit is rich in phytochemicals, which play an important role in inhibition of growth of cancer cells. The current study for the first time demonstrates the anti-cancer effect of avocado fruit extracts on two cancers common in Iran. Therefore, it is suggested that the fruit extracts can be considered as appropriate complementary treatments in treatment of esophageal and colon cancers.

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**Keywords:** Esophageal squamous cell; Cancer; Colon Adenocarcinoma; Avocado; Fruit

## Introduction

Cancer is a major health problem around the world. It is the second cause of death in the USA; such that one out of four deaths is caused by cancer. Among the cancers, 19% is squamous cell carcinoma that has poor prognosis with the mortality rate of approximately 50% (1). Cancer is the third cause of death in Iran (2) and almost 50,000 new cases of cancer are annually diagnosis in the 70-million populations, which leads to 14% of the population mortality. With regard to the organs affected by cancer, gastrointestinal (GI) cancers are the most common cancers (38%) (3,4). Absence of public screening tests for the disease leads to referral of the patients in advanced stages, which consequently leads to very low survival rate of the disease after diagnosis (5). Considering the frequency of GI cancers,

more than half million new cases of GI cancers, including esophageal and colon cancers, were diagnosed around the world in 2007 (6).

The most prevalent form of esophageal cancer throughout the world is squamous cell carcinoma, with different incidence rates in various geographical regions. The disease prevalence is higher in men and regions with high prevalence rates include China, India, North Iran, North and East of the Caspian Sea, and South Africa (7).

Different factors involve in incidence of esophageal cancer. Some of these factors are genetic factors, alcohol abuse, smoking, fungal toxins in vegetables and pickles, physical factors such as drinking very hot tea, alkaline diet, and radiation; and chronic achalasia (7). Colon cancer is the second most commonly diagnosed malignancy in male and the third in female and ranks fourth and third as a cause of cancer related

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death in these groups, respectively (8). The risk factors of colon cancer are genetic disorder and environmental agents (9).

Many patients with GI cancer refer to the health center at advanced stages of the disease and thus conventional treatments are not significantly effective in prognosis of these patients. In these regard, neoadjuvants with lower toxicity and other similar therapeutic approaches have a higher chance for successful treatment of these patients. For instance, phytochemicals are effective and accessible sources of such therapeutic factors for cancer treatment (10).

Phytochemicals present in the ethanol extract of the avocado leaf and fruit are effective in blocking the transmission of growth signals inside tumor cells, and by enhancement of intracellular oxygen radicals facilitate apoptosis of these cells (10). Furthermore, it has been observed that the anti-tumoral effect of a plant toxin present in avocado induces apoptosis in breast cancer (11), prostate cancer (12), and tumor cells of premalignant and malignant oral lesions, and play an important role in elimination of tumoral cells from normal cells (13). Avocado leads increased of reactive oxygen species (ROS) in cancer cells, while it does not have such effect on normal cells (10). The fruit contains large amounts of antioxidants such as vitamins C and E, folate, and unsaturated fatty acids (12). The plant grows in many parts of the world (12). In Iran, avocado grows in Mazandaran province and is available in the local market. The fruit does not contain sodium, and has rather few amounts of saturated fatty acids and sugar, and is low in calorie (12). Thus, it is considered as a healthy food. Although the useful healthy properties of avocado have been known since many years ago, the molecular and cellular mechanisms of its phytochemicals in cancer prevention are still unknown (12). Some studies have shown the protective role of avocado fruit against hepatic injury, fungal infections, and convulsion (14,15). The phytochemicals present in the avocado extract inhibits the growth of oral cancer and pre-cancer cells and induces apoptosis, without affecting the normal cells (13). Furthermore, polyunsaturated fatty acids (PFA) derived from avocado show anti-inflammatory effect and protective role against UV radiation in skin cells (16).

Since there are few studies on anti-cancer effects of avocado and there is no comprehensive study in this respect, and sometimes the results are controversial in this respect, the current study was performed to compare the anti-tumoral and of the extracts of avocado fruit against esophageal and colon cancer cell lines. The

extracts studied were ethanol, ethyl acetate, chloroform, and petroleum extracts of the fruit.

## Materials and Methods

This experimental study was performed in the Department of Microbiology and Immunology, Faculty of Medicine, Mazandaran University of Medical Sciences. Firstly, we prepared the ethanol, ethyl acetate, petroleum and chloroform extract of avocado (*Persea americana*) fruit (17), and then its effect on the cellular growth of esophageal squamous cell carcinoma, colon adenocarcinoma and mononuclear cell of peripheral blood in comparison with the control group (the same cell lines without applying the extract) in the cell culture media was evaluated.

After optimization, we prepared 20 µg/ml concentrations of various avocado fruit extracts from 0.1 g/ml concentration, and then the esophageal and colon cancer cell lines were exposed to the extracts in the cell culture media for 48 hours. With regard to the esophageal squamous cell carcinoma, the cell line (KJSE-30) and colon adenocarcinoma cell line were obtained from the cell bank of Pasteur Institute of Iran. The cell lines were then cultured according to the standard method in 6-well plates on the RPMI medium using fetal calf serum (FCS) 10% together with penicillin (100 U) and streptomycin (100 µg) under CO<sub>2</sub> 5%, at 37 °C for 48 hours. Then, the cells were divided into five groups (experiment and control groups). No extract was added to the control group, while experiment groups were treated by the extract. Then, in day 3 after the treatment, the populations of viable cells were counted according to the methyl thiazol diphen-tetrazolium bromide (MTT) method. To this end, 200 µl MTT was added to the culture mixture, the mixture was incubated at 37 °C for 2 hours, and then 200 µl DMSO was added to it.

Finally, optical absorbance of experiment and control wells were measured at the wavelength of 540 nm and the growth and proliferation rates of the viable cells were determined (10).

Proliferation rate and growth inhibition of the control and experiment groups were compared using analysis of variance (ANOVA). *P*-values less than 0.05 were considered statistically significant. The analyses were performed using SPSS software, version 16.

## Result

In the current experimental study, four types of

extracts were prepared from avocado fruit and then their effects on monocytes, esophageal squamous cell carcinoma, and colon adenocarcinoma were evaluated.

The data were tested using the Kolmogorov test. The data distribution was not significantly different from the normal distribution, and thus could be tested by the two-way ANOVA. The difference between the effect of ethanol extract and other extracts was determined using the LSD post-hoc test.

In the study, the effect of ethanol extract was significantly different from other extracts, while the effects of other extracts were not significantly different from each other. In this respect, the *P*-values obtained for comparison of the ethanol extract with chloroform, ethyl acetate, and petroleum extracts were 0.008, 0.008, and 0.004, respectively. The mean difference between ethanol and chloroform extracts was 7.7. The mean difference between ethanol on the one hand and ethyl acetate and petroleum extracts on the other hand were 7.8 and 8.4, respectively.

The mean cell viability rate was  $70.89\% \pm 1.89$  for the ethanol extract, while the values obtained for chloroform, ethyl acetate, and petroleum extracts were  $63.22\% \pm 1.89$ ,  $63.11\% \pm 1.89$ , and  $62.44\% \pm 1.89$ , respectively.

#### Mean cell viability rate with regard to the cell line studied

The mean cell viability rate of different cells in the culture medium containing mononuclear blood cells was  $98.27\% \pm 1.63$  for the above-mentioned extracts. The

values were  $34.82\% \pm 1.64$  and  $58.6\% \pm 1.64$  for the esophageal and colon cancer cells, respectively. The mean difference between the cell viability rates of normal cells and esophageal cancer cells was  $66.5\% \pm 2.3$ , which is statistically significant ( $P=0.001$ ).

The difference between normal cells and colon cancer cells was  $42.75\% \pm 2.32$ , which was statistically significant ( $P=0.001$ ).

Furthermore, the difference between cell viability rates of colon and esophageal cancer cells was  $23.75\% \pm 2.3$ , which was statistically significant ( $P=0.001$ ).

#### The mean cell viability rates with regard to the type of extract were as follows

1. Mean cell viability rates for the ethanol extract of avocado fruit were  $96.73\% \pm 3.27$ ,  $42\% \pm 3.27$ , and  $70.3\% \pm 3.3$  for mononuclear blood cells, esophageal cancer cells, and colon cancer cells, respectively.

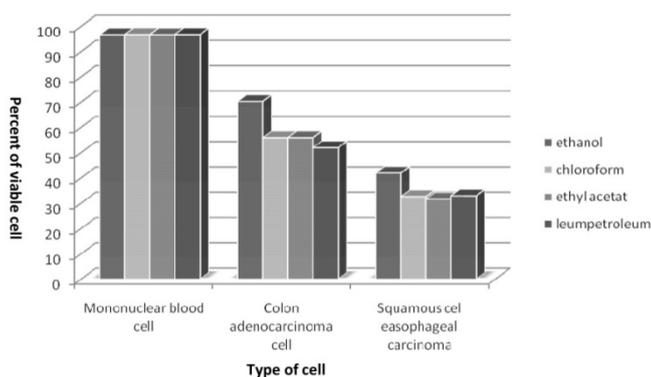
2. Mean cell viability rates for the chloroform extract were  $96.7 \pm 3.3$ ,  $32.7 \pm 3.3$ , and  $56 \pm 3.27$  for normal, esophageal cancer, and colon cancer cells, respectively.

3) Mean cell viability rates for the ethyl acetate extract were  $96.7\% \pm 3.3$ ,  $31.7\% \pm 3.2$ , and  $56\% \pm 3.3$  for normal, esophageal cancer, and colon cancer cells, respectively.

4) Mean cell viability rates for the petroleum extract were  $96.8\% \pm 3.2$ ,  $33\% \pm 3.3$ , and  $52\% \pm 3.27$  for normal, esophageal cancer, and colon cancer cells, respectively (Table 1 and Figure 1).

**Table 1. The mean of viable cells (%) based on extracts and type of cells**

Type of cell	Ethanol	Chloroform	Ethyl acetate	Petroleum
Mononuclear blood cell	$96.7 \pm 3.3$	$96.8 \pm 3.2$	$96.7 \pm 3.3$	$96.8 \pm 3.2$
Colon Adenocarcinoma Cell	$70.3 \pm 3.3$	$56.0 \pm 3.3$	$56.0 \pm 3.3$	$52.0 \pm 3.3$
Esophageal Squamous Cell Carcinoma	$42.0 \pm 3.3$	$32.7 \pm 3.3$	$31.7 \pm 3.2$	$33.0 \pm 3.3$



**Figure 1.** The percent of viable cells based on extracts and type of cells

## Discussion

In the recent decade, herbal medicine and applications of phytochemicals in prevention of cancer have found global importance. Considering the increasing prevalence of different types of cancer, late referral of patients, and lack of response to conventional treatments such as chemotherapy and radiotherapy in advanced stages of the disease, seeking for novel approaches in cancer treatment is reasonable. These approaches deal with employment of neoadjuvants such as phytochemicals, which have less adverse effects and improves the patients' survival. Conventional cancer treatments such as chemotherapy that kills the cancer cells are accompanied by many adverse effects on normal cells, which may consequently affect the patients' life quality (10).

Avocado fruit is rich in such phytochemicals. The plant is cultivated in many parts of the world. However, previously it had medical applications only in the Indian traditional medicine. The effectiveness of phytochemicals of the fruit in treatment of cancers has been confirmed in three studies. However, they have investigated the fruit effects only on prostate (12), breast (11), and oral cancers (13). The present study evaluates effectiveness of the fruit on cell viability of two common cancers in Iran; esophageal and colon cancers. Furthermore, in the study, we evaluated the anti-cancer effects of four fruit extracts including ethanol, ethyl acetate, chloroform, and petroleum extracts in esophageal and colon cancers.

Ding *et al.* evaluated the effect of fruit extract on prostate cancer. This is caused by cell cycle suppression in dividing cells and also apoptosis caused by increased amount of reactive oxygen species (10). Butt *et al.* evaluated the effect of avocado fruit on breast cancer. They explained that the anti-cancer effect of the fruit depends on the concentration of the extract used. The extract inhibits proliferation of cancer cells (11).

Lu *et al.* worked in prostate cancer (12). They showed the fruit contain lipid soluble components such as tocopherols and carotenoids with anti-cancer activities; it can significantly reduce the risk of androgen-dependent and -independent prostate cancer (12).

In another study, the effect of avocado fruit on apoptosis of oral cancer cells was observed, while such effect was not seen for normal cells. D'Ambrosio *et al.* explained that the fatty components of avocado fruit inhibit proliferation and development of oral cancer cells

(13).

Pau *et al.* reported that the highest chemoprotective effect of avocado fruit extract is observed in higher concentrations; such that it can reduce the genotoxic effects of cyclophosphamide in cancer patients. In other words, they demonstrated that the phytochemicals present in the fruit have chemoprotective effects, which can reduce the adverse effects of chemotherapy drugs such as cyclophosphamide in treatment of different types of cancers, particularly at higher doses (200 mg/kg bodyweight) (17).

In the current study, we firstly evaluated the effect of the extracts on non-tumoral peripheral blood mononuclear cells and no fatal effect was observed. The highest effect was observed for esophageal squamous cell carcinoma, and the number of tumoral cells killed was statistically significant, when compared with normal cells. With regard to the colon adenocarcinoma cells, the difference between the number of viable cells and that of the normal non-tumoral cells was statistically significant. The effect of avocado extracts on the two cancer cell lines was different, and the extracts had a higher effect on esophageal squamous cell carcinoma than colonic adenocarcinoma. This indicates that the effect of avocado extracts used may depend on the type of tumoral cells.

Furthermore, we used four different extracts of avocado. It was shown that the ethanol extract had lower fatal activity in comparison with other extracts. The findings of the current study could be helpful for other studies on different extracts and on various cancer cells, so that to determine the most effective extract with the optimal effect on cancer cells with minimal adverse effects. The study was performed on esophageal and colon cancer cells, and no similar study was found in the literature. Considering the prevalence of these two types of cancer in our country, delayed treatment of the diseases, and the adverse effects of conventional treatments such as chemotherapy and radiotherapy, such studies could be helpful in finding more effective treatments with less adverse effects.

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