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Research Article

The Effects of Avocado Fruit Methanolic Extract on Human Gastric Adenocarcinoma Cell Line in Comparison with Peripheral Blood Mononuclear Cells

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Abstract

Objective: Gastric cancer is the second leading cause of cancer-related deaths in worldwide. Adenocarcinomas comprise the majority of stomach cancers that are usually diagnosed in the late stage with low survival rate and have prompted considerable interest in neo adjuvant therapies to improve longevity. It has been reported that neoplasias in the gastrointestinal system are mostly due to change in dietary habits. The therapeutic effect of herbal materials in suppression of cancer cell proliferation was shown. In this study, we evaluated the efficiency of methanolic extract of avocado fruit on the inhibition of growth of gastric adenocarcinoma cell line, in comparison with normal cells.

Material and Methods: The human epithelial gastric cancer cell lines and normal human peripheral blood mononuclear cells were cultured in RPMI supplemented by 10% FCS, penicillin and streptomycin and were treated with increasing concentration of avocado fruit extract. After 48 hours of incubation, cell viability and cell proliferation rate were determined using trypan blue exclusion test and MTT assay.

Results: Significant dose dependent growth inhibition of treated stomach cancer cells was observed compared with the untreated control cells. This extract did not inhibit growth of normal human peripheral blood mononuclear cells $p<0.05$).

Conclusion: These data for the first time revealed potential anti tumoral effect of avocado fruit extract on gastric adenocarcinoma cell line. Therefore, postulated avocado is one of phytochemical rich nutrient factor that can be considered as appropriate complementary treatment of refractory gastric cancers.

Keywords

Methanolic extract; Gastric adenocarcinoma; Avocado; Fruit

Introduction

Avocado

Gastric cancer is the second common cause of cancer-related death worldwide [1-4]. Its 5-year survival rate is low ranging from 10 to 19% [3]. Some genetic and environmental factors were shown to be linked to cancer. Since gastric adenocarcinoma is usually detected in an advanced stage that poorly respond to conventional therapeutic modalities such as palliative surgery, chemotherapy and radiation, thus searching novel therapeutic approaches is a necessity. Some efforts in this field are immunotherapy with monoclonal antibodies that induce apoptosis or inhibit cell proliferation, gene therapy and the use of phytochemicals extracted from some fruits and vegetables such as Avocado (Persea americana) [4-6].

Avocado is a widely grown fruit that has a high content of phytochemicals with potential chemo preventive activity. Studies have shown that phytochemicals extracted from the avocado fruit selectively induce cell cycle arrest, inhibit growth and induce apoptosis in precancerous and cancer cell lines [5]. Other benefits of avocado extracts include liver injury suppressing effect [7], antifungal, anticonvulsant, vaso relaxant [8], hypotensive [9], analgesic & anti-inflammatory [10], antiviral [11], antiulcer [12] and hypoglycemic [13] effects are also reported. Its leaf extract induces necrosis of breast acinar epithelial cells [14] and also reduces body weight [15].

Previous studies about the chemoprotective and anticancer effects of avocado extracts were limited and did not yield proven results. With regard to the high incidence rate of gastric cancer in Iran [16] especially in Mazandaran, for the first time we examined the effects of methanolic extract of avocado fruit on gastric cancer cell line growth in culture media.

Materials and Methods

Preparation and isolation of methanolic extract

First the avocado fruit is peeled, fragmented and dried at 40°C in dark environment. They are milled to 0.1-0.25 mm slices. 200 ml solvent petroleum ether was added to 20 gr dried mill to extract lipid elements of avocado and then filtered. This process was repeated with 400 ml of absolute methanol to extract alcoholic extract. Refuse was exhausted and solvent evaporated with rotary evaporator and freeze drier. According to previous studies [17], 0.01 gr of dried extract was solved into phosphate buffered saline (PBS) and 4 different concentrations of this extract at doses of 5, 10, 15 and 20 µg/ml were prepared.

Cell culture of gastric adenocarcinoma and treatment with extract

The human gastric adenocarcinoma cell line, AGS, prepared from the National Cell Bank of Iran and was routinely maintained in RPM1 1640 supplemented with 10% FCS, 100 IU/ml penicillin and 50 µg/ml streptomycin under 5% CO_2 at 37°C for 48 hours in 15 wells of micro plate. 12 wells were treated with 4 different concentrations of extract; by which each concentration of extract was performed in triplicate. Furthermore 3 wells remained untreated as control.

Isolation and treatment of normal human peripheral blood mononuclear cells

5 ml heparinized blood was added gently to 2.5 ml Ficoll solution

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in a falcon tube and centrifuged at 3000 rpm for 20 minutes. A ring including monocytes and lymphocytes was sampled and washed by RPMI and centrifuged at 3000 rpm for 5 minutes. Then, cell counting was done with a hemo cytometer. Then 100000 of isolated mononuclear cells were cultured in 3 micro plate wells containing RPMI 1640 supplemented with 10% FCS, 100 µg/ml penicillin and 50 µg/ml streptomycin. Then 15 µg/ml of avocado extract was added to micro plates and incubated under 5% CO_2 at 37°C for 48 hours.

Cell proliferation assays

48 hours after treatment, cell viability was determined by trypan blue exclusion test and MTT assay. In trypan blue method, a drop of culture media admixed with a drop of trypan blue and cell counting was done with a hemo cytometer under light microscope. After optimization by trypan blue exclusion test, In MTT method, 200 µl of 3-[4, 5-dimethylthiazolyl]-2,5-diphenyl-tetrazolium bromide(MTT, sigma) solution was added to culture media treated with best efficient concentration of 15 µg/ml extract and maintained for 4 hours, then, 150 µl DMSO was added to it. Cell proliferation responses of experiment and control to 15 µg/ml concentration of avocado extract were triplicate measured by reading their optical absorbance at 540 nm, which is an index of cell proliferation. In MTT assay, percentage of viable cells indirectly calculated with this formula:

Percent of viable cells = (Mean absorbance of treated well)/(Mean absorbance of control well) x 100

Statistical Analysis

Data are presented as mean ± SD. Normal distribution of data was tested by Kormogorov test. The one-way ANOVA is used to compare the results among groups. A p-value less than 0.05 are considered statistically significant. The analysis was performed using SPSS Version 18.

Results

We evaluated the ability of avocado fruit extract in inhibition of gastric adenocarcinoma cell line proliferation and peripheral blood mononuclear cells (PBMC) growth in comparison with control using trypan exclusion blue test and MTT proliferation assay

Gastric cancer cell lines were treated with 4 different concentrations of extract. We examined the morphologic characteristics and growth rate of the cancer cells in the culture media under inverted microscope (Figure 1). As shown in Figure 1A, the untreated cancer cells had grown well and appeared polyhedral, pleomorphic adherent well-preserved cells. By increasing the concentration of extract as shown in Figures 1 B, C, D the growth of cancer cells was inhibited, became shrunken and showed signs of detachment from culture media. PBMC grow well and appear oval-shaped adherent cell in culture media (Figure 1E) the results showed that the high concentrations of extract could significantly inhibit gastric cell line.

Mean cell viability percentage in each of 3 wells was treated with the same concentration of avocado extract determined by trypan blue exclusion test (Figure 2), the best result was achieved by the dosage of 15µg/ml of extract.

MTT assay was done in triplicate on 3 wells treated with 15 µg/ml of extract and 3 control wells. Mean cell viability percentages were 35.46% ± 2.1 and 96.7 ± 3.3 for gastric cancer cell line and PBMC, respectively (Table 1). PBMC co-cultured with avocado extract proliferated at a significantly higher degree compared to cancer cell line (p=0.001)

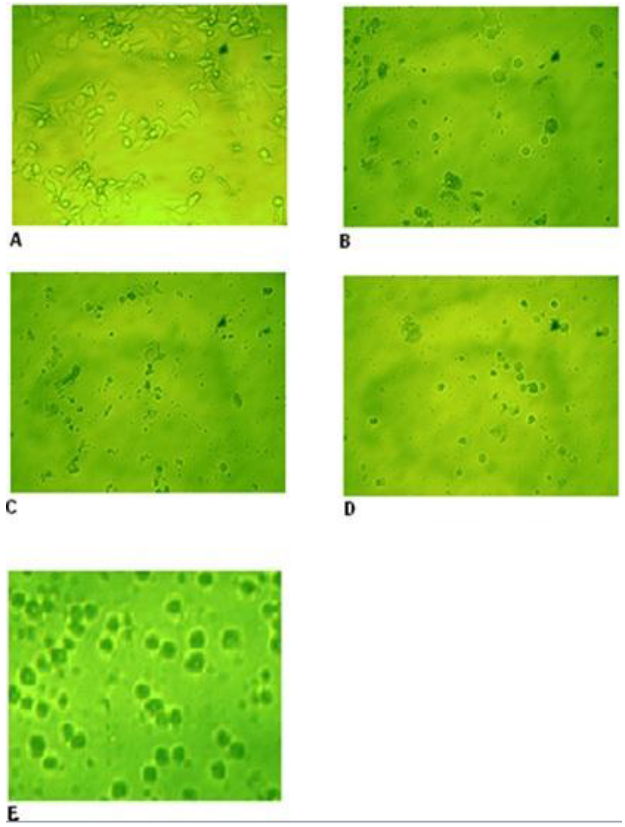

Figure 1: Gastric cancer cell lines were treated with 4 different concentrations of extract, observed by inverted microscope scanning (magnified x 100) after 48h incubation Figure 1 A: untreated control cancer cell line Figure 1B: cancer cell treated with 5 µg/ml avocado extract Figure 1C: cancer cell treated with 10 µg/ml avocado extract Figure 1D: cancer cell treated with 15 µg/ml avocado extract Figure 1F: PBMC treated with 15 µg/ml avocado extract.

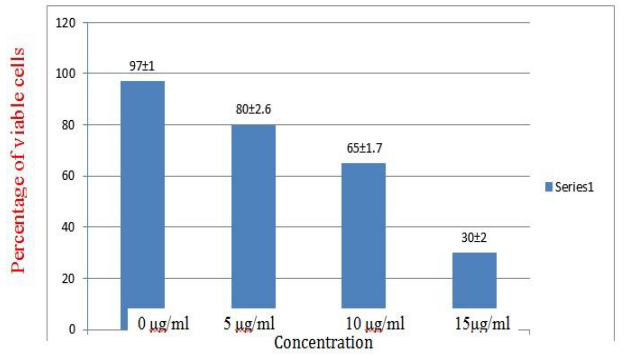

Figure 2: Results of Trypan blue exclusion blue test: mean percentage of viable gastric cancer cells after triplicate treatment with different concentrations of avocado extract.

Discussion

The anticancer effects of phytochemicals of different parts of avocado were previously studied on some malignant cell lines such as human oral epithelial cell, prostate cancer and breast esophagus and

Table 1: The mean of viable stomach cancer cell line (%) treated with 15 µg/ml concentration of avocado extract in comparison with peripheral blood mononuclear cells in culture media evaluated by MTT assay.

Type of cell	Mean ± SD of optical density	Mean of viable cells (%)	P
Treated Cancer cells PBMC	226.33 ± 22.54 631.66 ± 34.96	35.46 ± 2.1 96.7 ± 3.3	0.001
Untreated cancer cells (control)	877±6.6	97±1	-

colon cancer cells [18-20]. However, this is the first study to evaluate the methanolic extract of avocado fruit on gastric carcinoma cell line growth. Our study was performed on malignant gastric epithelial cell line (AGS) which was treated by 4 different concentrations of avocado fruit methanolic extract. In this research, the anticancer effect of avacado in different concentrations was studied; the 15 µg/ml concentration had the best effect in suppression of gastric cancer cell line proliferation but without any obvious effect on PBMC.

Final viability assays show a significant reverse dose-dependent relationship between methanolic extract and cancer cell viability. This extract has no significant effect on normal human PBMC viability. Our findings are compatible with similar studies about anticancer effect of avocado performed on other malignant cell lines. Avocado fruit extract had anti tumoral effects against some malignant cell lines, such as prostatic adenocarcinoma esophageal squamous cell carcinoma and colon adenocarcinoma [21]. An acetone extract of avocado containing carotenoids and tocopherols was shown by Lu Qy et al. [22] to inhibit the growth of both androgen-dependent and androgen-independent prostate cancer cell lines in vitro. Ma ZS et al. [23] described Quercetin, a flavonoid that was found in avocado inhibited prostate tumor growth in mice. Phytochemicals extracted with chloroform from avocado fruits target multiple signaling pathways and increase intracellular reactive oxygen leading to apoptosis [23-25]. Also, Ding H and et al. [17] reported avocado extract selectively induced apoptosis in human oral cancer cells and not in normal oral cells. Our recent preliminary study has revealed avocado fruit extract significantly inhibited esophageal and colonic cancer cell lines growth in culture media. A toxin extracted from avocado leaves, persin, can induce apoptosis in human breast cancer cells.

Mechanism of effect of the extract on these malignant cells is not obvious yet and needs to be recognized. But few hypotheses are present. They include increased ROS levels in malignant cells and resultant susceptibility to apoptosis (proapoptotic effect of plant toxin & cell cycle arrest, inhibition of angiogenesis, polymerization of microtubules and geno toxicity of methanolic extract [25].

Five major groups of chemical constituents of various parts of avocado plant were recognized: alkalones, terpenoid glycosides, various furan rings - containing derivatives and a coumarin. Among them, alkalones had anticancer effects but the active component of methanolic extract of avocado fruit and its chemical structure have not been characterized in the current study and need to be done in the future.

In our study significant dose dependent growth inhibition of treated stomach cancer cells was observed compared with control cells. This extract did not inhibit growth of normal human PBMC.

We concluded that avocado can have an anticancer activity, while cancer cells were more sensitive to avocado than normal peripheral blood mononuclear cells.

We hope that these data provide support for developing a novel chemotherapeutic agent with potential effects on refractory gastric cancers.

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