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The Cytotoxic Activity of Rosemary Essential Oil on PANC-1 Cells

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Abstract

BACKGROUND/AIMS: The goal of this research was to analyze the cytotoxic impacts of rosemary essential oil on pancreatic cancer.

MATERIALS AND METHODS: The human pancreatic cancer cell line (PANC-1) was used. The leaves of rosemary cultivated in North Cyprus were collected during the flowering stage. Rosemary essential oil was obtained after air-dried leaves (100 g) were distilled with water for 3 hours. Rosemary essential oil solutions were prepared and diluted in culture medium using various concentrations (100, 200, 300, 400, 500, and 600 µM) for 24 hours and 48 hours incubation periods. The analysis of cytotoxicity was conducted using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide].

RESULTS: Rosemary oil at 500 µM was more effective in diminishing the proliferation of PANC-1 cells than other concentrations during 48 hours. Cell viability was significantly greater at 100 µM for 24 hours (100%) than 600 µM for 48 hours (81.48%). It was determined that substantially greater cell viability was observed at 200 µM for 24 hours (97.05%) when compared to 600 µM for 48 hours (81.48%). In addition, when various concentrations were evaluated during 48 hours, significant differentiation was found between 100 µM and 600 µM. It was shown that there was a significant differentiation between 200 µM and 600 µM for 48 hours.

CONCLUSION: Cell viability was substantially less at 600 µM than at 100 µM and 200 µM, 81.48%, 95.18%, and 90.23%, respectively.

Keywords: Cytotoxic, essential oil, pancreatic cancer, rosemary

INTRODUCTION

Pancreatic cancer has a five-year survival rate of less than 5%, having the worst prognosis. The maximum survival rate has been reported to be approximately 2 years, even in individuals in the early stages, who have undergone surgery or chemotherapy treatment. The reasons for this situation can be explained by various factors. First, because of the lack of effective tools for early diagnosis, most patients present with metastatic disease when diagnosed. In addition to metastasis, early recurrence is a specific feature of pancreatic cancer and worsens the prognosis of the disease. Resistance to chemotherapy or radiation treatments also increases the risk of mortality.[1]

A large number of spices and medicinal plants are reported in the literature. Among these, *Rosmarinus officinalis L.*, known as rosemary, is highlighted for its various potential activities, including anti-cancer

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activity.[2] Rosemary is one of the most popular herbs used for culinary purposes. Native to the Mediterranean region, this herb is grown in different regions around the world.[3] Rosemary essential oil is obtained from the leaves of the rosemary plant. Rosemary oil is rich in certain compounds. These include carnosol, carnosic acid, and rosmarinic acid.[4] Rosemary essential oil is obtained by the hydrodistillation extraction method. There may be variation depending on the age of the plant and harvest time. Its volume is generally stated as 10 mL/kg of dry plant material. Various effects (anti-inflammatory, anti-proliferative, anti-obesity, antioxidant, anti-tumor, anti-microbial, and neuroprotective) of rosemary have been shown in the literature.[5]

Rosemary and its derivatives may show potential anti-cancer effects owing to their anti-oxidant, anti-angiogenic, and anti-inflammatory features, as well as increasing the expression of onco-suppressor genes.[6] Studies have been conducted in the literature on rosemary essential oil and various types of cancer.[7-12]

There have been a limited number of studies in the literature examining the relationship between rosemary oil and pancreatic cancer. Therefore, the objective of this study was to interpret the cytotoxic impacts of rosemary essential oil on pancreatic cancer cells.

MATERIALS AND METHODS

Cell Culture

Human pancreatic cancer cell line-1 (PANC-1) was used for this study. Medium containing Dulbecco's modified Eagle's medium (DMEM), 10% heat-inactivated fetal bovine serum, 1% penicillin-streptomycin, and 1% L-glutamine was used for maintaining the PANC-1. Since this was a cell study, not a human study, informed consent was not obtained.

Plant and Essential Oil Isolation Process

The storage and identification of samples were carried out according to the study by Becer et al.[9] The leaves of rosemary cultivated in North Cyprus were collected during the flowering stage. Rosemary essential oil was obtained after air dried leaves (100 g) were distilled with water for 3 hour. A temperature of 4 °C was preferred for storing the essential oil until analysis.

Cell Viability

MTT was used for the cytotoxicity analysis. The protocol for MTT was adopted according to the study by Hoca et al.[13] Various concentrations of rosemary essential oil (100-200-300-400-500-600-µM) were used to treat PANC-1 cells for two incubation periods (24 hours and 48 hours). Absorbance values were evaluated at 570 nm finally.

Statistical Analysis

Data were presented as mean and standard deviation values. The outcomes were evaluated using Statistical Package for the Social Sciences 18.0. Kruskal-Wallis test and/or Mann-Whitney U tests were employed for differences among groups. When evaluated statistically, $p<0.05$ was considered significant.

RESULTS

The cell viability was analyzed with MTT. Rosemary oil, in all concentrations, reduced the growth of PANC-1 cells in a time- and/or dose-dependent manner. It was indicated that rosemary oil at 500 µM, was more efficient in decreasing the proliferation of cells than other concentrations for 48 hours (Figure 1).

When diverse concentrations (100-200-300-400-500-600 µM) and incubation conditions (24 and 48 hours) were examined, the cell viability was substantially greater at 100 µM for 24 hours (100%) than 600 µM for 48 hours (81.48%) ($p<0.05$). It was also determined that cell viability was substantially greater at 200 µM for 24 hours (97.05%) than at 600 µM for 48 hours (81.48%), ($p<0.05$). Moreover, when various concentrations were examined during 48 hours, significant differentiation was found between 100 µM and 600 µM ($p<0.05$). There was a significant difference between 200 µM and 600 µM for 48 hours ($p<0.05$). When the viability of cells was examined, cell viability was substantially less at 600 µM (81.48%) than 100 µM (95.18%) and 200 µM (90.23%) (Table 1).

DISCUSSION

Rosemary and its components can show anti-cancer activity due to their apoptotic characteristics.[14] Santos et al.[7] showed that rosemary essential oil exhibited no substantial effect on cell viability in cervical cancer cells (HeLa) compared with human liver cells (HepG2). This study emphasized that rosemary oil at a concentration of 500 µM was more effective in declining PANC-1 cell proliferation than other concentrations during the 48-hour period. Al-otaibi[15] showed that 50% decline of human breast adenocarcinoma cell growth when breast cancer cells were treated with 1% (v/v) nano-emulsified rosemary oil + 1.49 µM of mitomycin C. Thus, this study stated that rosemary oil can increase the effect of the anti-neoplastic agent mitomycin C.

Rosmarinus officinalis white extract showed a significant effect on human colon cancer HT-29 cells: a 50% reduction was achieved at 49.6231 µg/mL after 48 hours.[16] Rosemary extracts have been shown to be more powerful in reducing cell viability of colon cancer cells

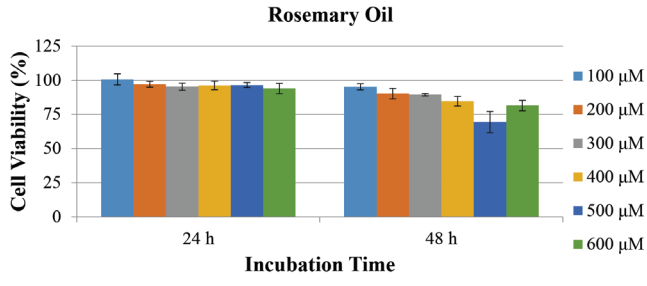

Figure 1. Effects of different doses of rosemary oil on pancreatic cancer cell viability.

Table 1. Cell viability percentages at different concentrations and incubation periods

	Cell viability-24 hour	Cell viability-48 hour
100 µM	100%	95.18%
200 µM	97.05%	90.23%
300 µM	95.30%	89.41%
400 µM	96.20%	84.61%
500 µM	96.47%	69.41%
600 µM	93.96%	81.48%

than pancreatic cancer cells. Thus, it has been demonstrated that colon cancer cells are more susceptible, and it has been demonstrated that pancreatic cancer cells, especially the PANC-1 cell line, are more resistant.[17] In this research, when the effect of rosemary oil on their viability was evaluated, it was observed that there was no decrease below 50% in cell viability, supporting the idea that the PANC-1 cell line may be resistant.

In a study examining cell viability using the MTT test, it was found that the use of rosemary reduced the viability of glioblastoma cells by approximately 57.2%. However, when 500 µg/mL acyclovir and rosemary were used in combination, they had a 10% greater reductive effect on the viability of glioblastoma cells, unlike their separate effects.[18] In this study, especially after 48 hours, rosemary oil reduced cancer cell viability more at higher concentrations (500 µM) compared to other concentrations, supported its cytotoxic effect. The number of live cells was determined by metabolic activity using the MTT method through a series of steps. It was found that the number of live cells was the least at a concentration of 500 µM after 48 hours.

Due to its antioxidant properties, it can act as a defense against oxidative damage by neutralizing free radicals. It can also have a cytotoxic effect through the release of reactive oxygen species. In addition, it has an effect on the antioxidant defense system by activating nuclear factor erythroid 2-related factor 2 and increasing glutathione levels.[6] Moreover, it was emphasized that rosemary essential oil affected the mitotic phase and slowed down mitosis significantly.[19] In an experimental study using the human hepatoma HepG2 cell line, when rosemary essential oil was applied at high concentrations, apoptotic substances were observed in the cells. In addition, when the cell cycle was analyzed, after 24 hours of application, it was found that cells in the G1 phase accumulated, accompanied by a decrease in the number of cells in the S phase. In this context, it was observed that cell division slowed down or paused and eventually cell death occurred.[20]

Different cell lines, have been mainly examined in the above paragraphs due to the limited number of studies in the literature on pancreatic cancer and rosemary oil. At the same time, this situation shows the strength of the study.

Study Limitations

The reliance on cell viability analysis alone is a limitation of the study. In addition, higher doses and incubation periods can be used to show greater or additional effects. The use of specific markers or methods for cell viability is essential to represent the effects more clearly.

CONCLUSION

The cytotoxic activity of rosemary oil was tested in PANC-1 cells using diverse concentrations. Rosemary oil has been shown to reduce cell viability in pancreatic cancer cells. To clarify the specific anti-oncogenic effects of rosemary oil on pancreatic cancer cells, relevant signaling pathways need to be defined. Further studies on various pancreatic cancer cell lines, animals, and humans are needed to elucidate the impacts of rosemary oil on pancreatic cancer, which is one of the rapidly progressive types.

MAIN POINTS

- Rosemary oil at 500 µM was more effectual in diminishing the proliferation of pancreatic cancer cell line-1 cell than another concentrations during 48 hour.
- A significant differentiation was found between 100 µM and 600 µM after 48 hours.
- It was shown that there was a significative differentiation between 200 µM and 600 µM for 48 hour.
- Rosemary oil has been shown to reduce cell viability in pancreatic cancer cells.

ETHICS

Ethics Committee Approval: Not applicable.

Informed Consent: Not applicable.

Footnotes

Authorship Contributions

Concept: M.H., E.B., A.Y., H.S.V., Design: M.H., E.B., A.Y., H.S.V., Data Collection and/or Processing: M.H., E.B., A.Y., H.S.V., Analysis and/or Interpretation: M.H., E.B., A.Y., H.S.V., Literature Search: M.H., E.B., A.Y., H.S.V., Writing: M.H., E.B., A.Y., H.S.V.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

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