Evaluation of Antioxidative and Cytotoxic Properties of Ethanolic Extract of Salvia Officinalis on PC3 Human Prostate Cancer and Hela Cervical Cancer Cell Lines

Parizad Sarmast1, Ramesh Monajemi2 Monireh Ranjbar2

1 MA in Animal Physiology, Islamic Azad University, Falavarjan Branch, Isfahan, Iran
2 Biology Department, Islamic Azad University, Falavarjan Branch, Isfahan, Iran
*Corresponding author: sarmastparizad@yahoo.com

ABSTRACT

Background and Objective: Free radicals cause many diseases in human and their neutralizing by antioxidants reduces risk of cancers. Salvia Officinalis from Lamiaceae family is one of the valuable plants in traditional medicine. The present study aimed to evaluate the antioxidative and cytotoxic properties of ethanolic extract of the aerial parts of this plant on human prostate and cervical cancer cells.

Methodology: In this study, in order to evaluate the antioxidative property, diphenylpicrylhydrazyl (DPPH) test was used and to investigate the cytotoxic property, PC3 prostate and Hela cervical cancer cells were cultured in RPMI-1640 medium with 10% bovine serum. The extract of Salvia Officinalis leaf powder was obtained through soaking method with 80% alcohol. The toxicity of different concentrations of extracts (12.5, 25, 50, 100, 250, 500, 750, and 1000) on the cultured cells was measured using MTT assay at three times at 24, 48, and 72 hours.

Findings: In this investigation, the antioxidative property was inversely related to IC50. The results of MTT test indicated that ethanolic extracts of Salvia Officinalis show highly significant differences at different days.

Results: Ethanolic extract of the plant in DPPH method had a very good and the best antioxidative activity, while its cytotoxic property was very poor.

Keywords: Cytotoxic, Salvia Officinalis, MTT test, PC3, Hela

Introduction

Due to ease of access and reduced side effects, medicinal plants were considered as appropriate alternatives for the industrial medicines. Particularity, they gained attentions of researchers during recent decades (Mongelli et al., 2000). Genus of salvia officinalis belongs to the lamiaceae family, which includes 700 to 900 species worldwide. Fifty-seven species of this genus were identified in Iran of which 17 species are native. Its degree of indigenousness in Iran is 29 percent (Hedge, 1982 & Hey, 1978). Salvia officinalis (sage) is an herbaceous perennial plant with square downy stems. Its root is rather thick and it directly penetrates into soil and has many branches (Ölmez et al., 2002). It is considered as one of the most important medicinal plants in the mint family. It is frequently used in Europe for cooking.

Corresponding Author E-mail: sarmastparizad@yahoo.com
It is one of the old medicines, which is extensively used in traditional (folk) medicine. This plant has a protective effect on liver. It contains biological compounds such as rosmarinic acid, ferulic acid, flavonoids, and terpenes. Some of its terpene compounds are anti-cancer agents. Its ursolic compound is a factor effective in angiogenesis, tumor inhibition, and metastasis. One of the mechanisms of cancer treatment is suppression of protease activities and metalloproteinase, which plays a crucial role in tumor invasion and metastasis. It is used for treating wounds, washing skin and hair, and angiogenesis. It is one of the richest sources of antioxidants. Of course, long-term consumption of this plant causes dementia and seizure (Bowles, 2003; Kintzios, 2009; Dagilis et al., 2002). Its phenolic acids have antimicrobial properties, especially against Staphylococcus aureus (Lana et al., 2012). Sage essential oil helps to treat an extensive range of illnesses such as heart, respiratory, gastrointestinal, and endocrine diseases (Ileana et al., 2006). Studies on different components of the plant indicate phenol and flavonoid compounds (Shekarchi et al., 2012). With respect to the extensive uses of the plant in dietary and pharmaceutical industries, here, we have attempted to discuss the effects of ethanolic extract of oregano leaves on the parameters of PC3 cancer cells (prostate cancer), Hela cancer cells (cervical cancer), and antioxidant effects on humans.

**Materials and Methods**

**Cell Culture**

To conduct the present experimental study, PC3 and Hela cell lines in vials were purchased from the Pasteur Institute of Iran (IPI). RPMI-1640 (Bia Idea Iran) culture medium was used, which was enriched by bovine serum 10% (Fetal Bovine Serum-FBS) (Bia Idea Iran) and streptomycin antibiotics (100 mg/ml) and penicillin (100 units/ml, CinnaGen Tehran). The cells were put in the culture medium inside an incubator under 5% carbon dioxide and 37°C. Salvia officinalis was harvested from the surrounding farms of Isfahan in summer 2012. A confirmation code was received by the herbarium of Islamic Azad University of Falavarjan and the plant was dried away from sunlight and cold temperature. The rinsing method was employed to preserve antioxidant components of the plant. First, we weighted 0.01 of the dried extract and diluted it to the intended volume by ethanol 80%. To do so, 2×104 cells were put in each well of a 96-well container. After 24 hours, different concentrations of oregano (12.5 to 1000 mg/ml) were added to the wells for periods of 24 to 72 hours, they were assayed using the MTT method, and they were read at a wavelength of 564 nm by an ELISA set.

**Evaluation of Antioxidant Activity Using Diphenylpicrylhydrazyl (DPPH)**

Concentrations of the extracts of the above six tubes were obtained as 100, 200, 400, 600, 800 and 1000 mg, respectively. We take 3 test tubes for each concentration and pour 1 ml of the diluted extract in each tube. We add 3 ml of DPPH solution that prepared earlier in each tube. We expose the tubes under continues shaking for one hour. After that, we read light absorption of the solutions at the wavelength of 517 nm using a spectrophotometer.

With respect to the obtained absorption solution, we calculated the inhibition percentage of DPPH free radicals by the antioxidants using the following formula:

$$\text{RSC} = \frac{(A_{\text{sampleblank}} - A_{\text{sample}})}{A_{\text{sampleblank}}} \times 100\%$$
Statistical Analysis

In the present research, all the tests were iterated at least three times and the results were shown as the mean of the three iterations ± the standard deviation. SPSS 18 and the one-way analysis of variance (ANOVA) were used for analysis of the statistical data. The level of significance was considered less than 0.001.

Results

Cytotoxic effect of ethanolic extract on cervical cancer cells (Hela)
As shown in Figure 1, the ethanolic extract of salvia officinalis has not a great effect on the cervical cells; with the average percentage of survival decreasing, more cells were damaged. Within 72 hours, number of the cells killed at concentration of 25 was high. Within 24 hours at concentration of 250, a small number of cells were killed. Within these three intervals, the ethanolic extract of salvia officinalis had the weakest toxicity of all within the 24-hour interval and it had the strongest toxicity on the cell line within the 72-hour interval. Within 48 hours, with the extracts concentration increasing, the effect of cytotoxic increased significantly. However, its effect did not depend on concentration at 24 hour and 72 hour intervals.

![Figure 1](http://www.sid.ir)
Cytotoxic Effect of Ethanol Extracts on PC3 Cancer Cells

As Figure 2 shows, ethanolic extract of salvia officinalis has not a considerable effect on PC3 cells and with the average of survival percentage of cells decreasing, more cells were damaged. Within 48 hours, many cells were killed at concentration of 750; within 24 hours, a small number of cells were killed at concentration of 1000. Ethanolic extract of salvia officinalis had the strongest cytotoxic effect of all other intervals within the interval of 48 hours.

Fig 2. Effect of ethanolic extract of salvia officinalis after 24, 48, and 72 hrs on PC3 cell line

Antioxidant Effect

This plant has a strong antioxidant property. It was observed that there is a reversed relationship between antioxidant property and IC50. The stronger the antioxidant property is, the smaller is IC50 amount. As it is noticed, gradient of Diagram 2, percent (%) inhibition, and OD absorption are reversed. Gradient of the diagram of percent inhibition is positive and gradient of diagram of absorption is negative. This indicates that with the concentration of inhibition percent increasing, the absorption decreases. The more color spectrum of the test tubes is shifted from violet to yellow, the more antioxidant property and free radicals inhibition it has, DPPH radical inhibition increases and we see more yellow color in the medium. The diagram with black background shows ic50 of the extracts with each other and with the standard. With the ic50 column height decreasing in the diagrams, the antioxidant property increases. BHT of this substance is used as a standard. It is also used for making comparisons. The antioxidant property of the extracts was determined by inhibition of DPPH radicals.
Diagram 1: Antioxidant effect in terms of optical density (OD)

Diagram 2: Antioxidant effect in terms of inhibitory power
Diagram 3: IC50 content of ethanolic extract of salvia officinalis

Discussion

Using cell culture techniques develops a much deeper understanding of the impact of drugs and medicinal plants on cells (Deshpande et al., 2008). The effects and changes of different components of ethanol extract of saliva officinalis on cells, which were studied in this research, can be studied in a controlled cell culture medium. This way, we may identify accurately the mechanisms, their biological impacts, and their effects on intracellular factors. Such facilities allow us to better identify the processes and intracellular interactions during cancer treatment using medicinal plants, which may lead to promoting treatment methods. However, one of the major candidates for synthesis of anti-cancer drug is medicinal plants, which have cytotoxic effects and their toxicity is possible in cellular culture. On the other hand, pharmacists have focused on the components of plant origin for synthesis of modern drugs to treat
incurable diseases such as cancers, as they are abundant and they have less side effects and drug interactions (Deshpande et al., 2008). With a view to the diagrams and figures presented in the ‘Results’ section, it can be asserted that the ethanolic extract of salvia officinalis has little toxic effects on Hela and PC3 cells lines. The reason might be that the used ethanolic extract was not able to divide polar compounds such as rosmarinic acid, ferulic acid, flavonoids, and luteolin (Dastmalchi et al., 2007) from the non-polar compounds. We attempted to study the antioxidant effect and phenolic property of these plants; however, we should have a deeper understanding of these compounds.

Enzymatic defense system, such as superoxide dismutase, glutathione peroxidase, catalase, and compounds such as the antioxidant found in foodstuffs such as vitamins, polyphenols and carotenes are of the major defensive systems of the living organisms. They regulate the amount of ROS radicals such as hydrogen peroxide, superoxide radicals, and hydroxyl radicals, which are obtained from oxygen metabolism under unstable conditions of oxidation (Gutteridge, 1984 & Therond et al., 2000).

When cells are exposed under these conditions, they cause unbalanced metabolic pathways, which in turn are able to alter metabolic activity of cells and cause oxidative stress. Oxidative stress causes very severe damage to large molecules of cells such as proteins, lipids, and nucleic acids in it (Sies et al., 1995).

By creating active species of oxygen (AOS), oxidative stress leads to peroxidation of membrane lipids and these damages overshadow cell membrane condition. Free radicals of oxygen affect chemical bonds of hydrogen peroxide and convert them into an atom of hydrogen and hydroxide. These free radicals are acceptors of an electron (Yuli et al., 1981). Phenolic compounds are potential antioxidants that are able to inhibit free radicals. Therefore, there is a correlation between antioxidant compounds and phenolic compounds (Guo et al., 2003).

The plants of lamiaceae family, due to having compounds such flavonoids, thymol, carvacrol, and terpenoids, have antioxidant properties. Depending on the type of these compounds, they can inhibit free radicals to some extent. For instance, due to having carvacrol, thymol, and flavonoids compounds, thymus vulgaris extract - which is of the lamiaceae family - has antioxidant properties. When they tested the effect of the plant extract on precancerous lesions and squamous cell carcinomas (SCC) of prostate gland, they found out that these compounds inhibit the growth and metastasis of lung carcinoma in a mouse. Therefore, if all these compounds are present and they work together, they exhibit an extremely considerable carcinogenesis effect (Zhang et al., 2005). The main compounds in salvia officinalis extract are rosmarinic acid, caffeic acid, ferulic acid, which are among the phenolic compounds, and Luteolin -7-glucoside and apigenin -7-glucoside, which are among the flavonoid compounds. One of the compounds known in salvia officinalis is rosmarinic acid (Shekarchi et al., 2012). The phenolic compounds of clove and cinnamon such as eugenol and eugenol acetate have high antioxidant power (Bondet et al., 1997).

Rosmarinic acid is a very phenolic compound, but it enjoys a high antioxidant property. It is also capable of cleaning most free radicals of oxygen and active radicals of nitrite peroxide (Sul et al., 2009). Rosmarinic acid is able to inhibit cholinesterase enzyme through which it plays its protective effect. By reducing oxidative stress and cell death through apoptosis caused by amyloid peptide that causes inflammation in hippocampus section of brain, rosmarinic acid plays its anti-inflammatory and antioxidant roles (Sultana et al., 2005).

Caffeic acid in salvia officinalis acts as an antioxidant, it performs toxicity caused by amyloid peptide through reducing intracellular calcium, it imposes its effect through proteins, which reduces number of proteins and consequently reduces their activity. This process causes proteins not to perform their duties properly (Sul et al., 2009). In a series of essential optimization studies, the results of some researches showed that binary solvents were more effective than single ones in extracting phenolic compounds. With respect to their relative concentration, fraction of polyphenolic masses, rosmarinic acid, and lutein-3-glucuronide as a huge polyphenol is different. As a function of type of solution (ethanol, acetone or water), solution compounds are aqueous/organic. While extraction
temperature had a significant fraction of time of extraction, it was only on a mass of lutein-3-glucuronide fraction. It emphasized that ethanolic and acetonic aqueous solvent 30% at 60°C for 10 minutes had the most effect on extraction of polyphenols from dry leaves of salvia officinalis (Dent et al., 2012). While the ethanolic solvent under test in our study, which was a single solvent, had a considerable amount of polyphenolic compounds and it enabled it to intensify its antioxidant and phenolic power.

Conclusion

The results of this research showed that salvia officinalis has a very little Sytotoxic property. Therefore, it has no effect on PC3 and Hela cell lines; however it has a considerable antioxidant effect and it is able to inhibit free radicals well. Finally, it should be noted that the results of this study are the outcome of a laboratory environment. Meanwhile, conducting further studies is emphasized.

Acknowledgements

This research is concerning a master’s thesis of animal sciences field of study, animal physiology minor, Islamic Azad University, Falavarjan Department, Tel No. 03117420135 and the corresponding author bore its charges. Hereby, I kindly appreciate all those who helped pave the way for this research.

References


Ileana C. Farcasanu _ and Eliza Oprea. 2006. ethanol extract of salvia officinalis exhibit antifungal properties againts.


Ölmez F.N., Kayabaşi N., 2002. A research on the colors obtained from Sage (Salvia officinalis L.) and their fastness.


