The Study of Anticancer Activity of Satureja Khuzestanica Alcoholic Extracts On Expression of Bcl2 And Bax Genes In The Pc3 Cell Line

Saman Kazemi
Islamic Azad University Bonab Branch
asghar tanomand
Maragheh University of Medical Sciences
Hossein Soltanzadeh (hossien4040@gmail.com)
Maragheh University of Medical Sciences
Gholamreza Shahsavari
Lorestan University of Medical Sciences

Research Article

Keywords: Satureja khuzestanica, PC3 cell line, Prostate cancer

Posted Date: March 19th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-296598/v1

Abstract

Introduction: Prostate cancer is the most common cancer among men after lung cancer. It has grown in Iran in recent years. The use of medicinal plants is one of the most useful ways that causes the least side effects. Due to high levels of antioxidant compounds, Satureja khuzestanica is a good source for drug use to treat and prevent the development and progress of cancers. The aim of the present study was to evaluate the anti-cancer property of Satureja khuzestanica extract on the expression of Bcl2 and Bax genes in prostate cancer cell lines.

Methodology: After collecting the plant in spring, the chloroform extract was prepared by rotary device. PC3 cancer cells were incubated at different concentrations of the extract for 24 hours. The inhibitory effect of the extract was evaluated using MTT assay as IC50. To evaluate apoptosis, the level of expression of Bax and BCL-2 genes after RNA extraction and transformation to cDNA were evaluated using Real Time PCR. All data were analyzed using REST software.

Results: The results revealed a direct and significant relationship between the two variables of drug composition and rate of PC3 cell death. This composition increased Bax gene expression and decreased BCL-2 gene expression and induced apoptosis ($P < 0.05$).

Discussion and Conclusion: Based on the results, Satureja khuzestanica extract is likely to have anticancer properties and seems to be a new drug for killing prostate cancer cells.

Introduction

Cancer is a fatal and dangerous disease whose treatment is costly and ineffective in most cases. In most countries, chemotherapy is used to control the cancer, but chemotherapy and drugs used to treat cancer have side effects that are sometimes more dangerous than cancer itself, such as liver and kidney failure, nerve damage, and so on. Hence, modern methods to prevent and treat cancer patients are needed to reduce the rate of the side effects (1). In developed countries, prostate cancer is considered as the second most common cancer after skin cancer in men. Prostate cancer indicates the presence of malignancy in men over 50 years. Hereditary history of prostate cancer is an important factor in the development of this cancer. The androgen receptor gene plays a major role in the development and progression of prostate cancer. HSD3B2, HSD3B1, SRD5A2, CYP17 AR genes also play a major role in androgen metabolism and cell proliferation in the prostate. Some polymorphisms in these genes are associated with an increased risk of prostate cancer. Epigenetic changes, especially DNA hyper-methylation in promoter regions, play a major role in the prevention and treatment of prostate cancer. A number of molecular and genetic changes have been observed in prostate cancer. Metastasis inhibitor genes have also been observed in prostate cancer (2). The main cause of non-apoptosis in most cancers is the high expression of anti-apoptotic proteins such as Bcl-2. Increased expression of these proteins increases resistance to chemotherapy. Therefore, inhibition of expression or function of these proteins in cancer cells can induce apoptosis (3). There are various treatments for cancer, including surgery, chemotherapy, radiotherapy, and

anticancer drugs (4). One of the biggest limitations of anticancer drugs is the resistance of cancer cells to the drug, which can be due to intrinsic resistance to the drug and they might act in a way that resistant cells can be selected among the heterogeneous cells. As a result, with increasing the number of resistant cells, the treatment process will become more difficult. Nowadays, the use of medicinal plants is increasingly considered more than chemical drugs due to their fewer side effects (5). As a result, as the number of resistant cells increases, the healing process becomes more difficult (6). Today, the use of medicinal plants has received less attention than chemical drugs due to its side effects (7).

Based on the studies conducted in this regard, it has been concluded that no study has been conducted on the chloroform extract of the studied plant and the effects of chloroform extract on the prostate cancer cell line have not been investigated, and no similar study has been conducted on the effects of S. khuzestanica species on prostate cancer cell line in Iran. Hence, it was decided to investigate the effect of chloroform extract of this plant in inhibiting the growth and expression of Bax and BCL-2 apoptotic genes in PC3 cancer cell line.

Methodology

This study was an experimental study whose all steps were performed in cell culture laboratory of Maragheh University of Medical Sciences. All of its steps were briefly as follows: Satureja khuzestanica was identified and approved by the herbalists of Khorraman Herbal Plants Company in mid-spring of 2018. After direct sending of dried leaves of Satureja khuzestanica, they were powdered by mill. Next, 100 g of Satureja khuzestanica powder was mixed with one liter of chloroform, and after 2 weeks, the mixture of Satureja khuzestanica with chloroform solvent was passed through filter paper and extraction was performed through a vacuum distillation method using a rotary device. The concentrated extract at value of 9.53 g was kept at refrigerator away from heat and light until cell culture(8).

Gas chromatography/ mass spectrometry

Fid- GC was carried out using a Hewlett-Packard 6890 with HP-5 capillary column (phenyl methyl siloxane. 25 m, 0.25 mm i.e., ratio, 1:25, and flame ionization detector. Temperature programmer: 60 ºC (2 min) rising to 240 ºC at 4 ºC/min: injector temperature 250 ºC, detector temperature, 260 ºC. GC-MS was performed using a Hewlett-Packard 6859 with a quadruple detector, on a HP-5 column, operating at 70 eV ionization energy, using the same temperature programmer and carrier gas as above. Retention indices were calculated by using retention times of n-alkanes that were injected after the oil chromatographic.

Cell culture: PC3 cancer cells were used as cell lines in the current study. Prostate cell lines were obtained from the cell bank of Pasteur Institute of Iran and cultured in RPMI1640 with 10% fetal bovine serum (FBS) along with 100 µl of penicillin streptomycin antibody in an incubator at 37 ° C with sufficient humidity and 5% carbon dioxide. The cell culture medium was replaced every two days on average, when needed, and it was passaged when reached to 85% confluency. To perform different tests and when the cells reached at least 80% cell growth, they were removed from the bottom of the flask by trypsin / EDTA enzyme and centrifuged at 1000 rpm for 7 min. Cell deposition was prepared in suspension in 1 ml

culture medium and percentage of cells viability in cell suspension was determined by mixing equal ratio of trypan blue using a hemocytometer slide and by examining the light microscope. MTT assay was used to investigate the cytotoxic effect of Satureja khuzestanica chloroform extract on PC3 cell line. The MTT assay is a metabolic test of mitochondrial competition and based on the breakdown of tetrazolium salt by the mitochondrial succinate dehydrogenase enzyme, living cells is transformed into purple water-insoluble crystals in formazan water (9).

Then, cell suspension was prepared at a concentration of 1 10^4 cells / ml and incubated in 96-well micro-plates for 24 hours. Then, concentrations (1000, 500, 250, 125, 62.5 and 31.25 µg / ml) of the extract were added. The cell group of cell suspension was considered as having no extract. The micro-plates were incubated for 24 h under the same conditions. Then concentrations of 10 µl (5 µg / ml) of MTT solution were added to each well and incubated for four hours. The supernatant was replaced with 100 µl DMSO (dimethyl sulfoxide) and dissolved the formazan crystals. Then, it was measured by ELISA (Statfex. 2100) at 560 nm.

Cell viability for each concentration was calculated using the following formula:

$$\%\ \text{cell toxicity} = \left(\frac{(100\times(\text{control}-\text{sample})}{\text{control}}\right)$$

After obtaining IC50, cells treated with that concentration were used for RNA extraction and Bax and BCL-2 genes` examination. After extraction of RNA from PC3 cancer cells treated with chloroform extract and untreated cells, cDNA synthesis was performed and then, it was used along with the preparation of Bax and BCL-2 and actine-β primers in reaction with 25 µl Real Time PCR to understand the mechanisms involved in the induction of cell death. After 24 hours, their RNA was extracted according to common guidelines (9). Then, the steps of RNA transformation to cDNA were performed. C-DNA synthesis kit (Sinagen Company) was used to transform the RNA into cDNA. After cDNA synthesis, Bax and BCL-2 genes were selected to evaluate the expression of genes involved in apoptosis. To validate the test, actine – control gene (expressed in all cells) was used. For this purpose, primers shown in Table 2 were used in RT-PCR test

Based on the guidelines, after preparing the substances in a 0.2 ml microtube, along with the control and treatment group, they were transferred to Thermo Cycler device. After completion of the Thermo cycler work, RT-PCR product was applied to 1% agarose gel. Then, Real time PCR was used to evaluate the expression of the desired genes. In this method, the primers of Table 2 with program Table 4 were used and the contents of Master mix-Real Time PCR were added according to Table 3.

Statistical analysis of data

The results of this study were analyzed using REST software and ANOVA tests.

Results

Identification of components

The linear retention indices for all the compounds were determined by conjection of the sample with a solution containing the homologous series of C8-C22 n-alkanes. The individual constituents were identified by their identical retention indices, referring to known compounds from the literature and also by comparing their mass spectra with either the known compounds or with the Wiley mass spectral database Table 1.

Analysis of MTT results

Results of MTT assay showed that chloroform extract of the studied plant at different concentrations inhibited the proliferation of cancer cells. Figure 1 shows the cytotoxic effect of chloroform extract of Satureja khuzestanica on PC3 cell line. Cell viability was determined by MTT assay. The absorbance was measured by ELISA device at 560 nm. The viability rate of the control group was considered 100 and the level of significance was considered at $p < 0.05$. Comparison of cell growth percentages at different doses showed that rosemary extract at 125 mg / ml of Satureja khuzestanica extract had more effect in 24 hours and reduced the growth of cancer cells.

Real Time PCR test results

The expression levels of BCL-2 and BAX genes are shown in the following diagrams. As seen, the expression of BAX gene as a promoter of apoptosis was higher at 24 hours after treatment with chloroform extract at 125 mg / ml than the other groups, which was in accordance with MTT assay. At the same dose, the expression level of BCL-2 gene as an apoptotic inhibitor gene was significantly lower Figure 2 and Table 5.

RNA electrophoresis results

To evaluate the accuracy of the quality of the extracted RNA used in Real Time, 4 samples were randomly electrophoresed in 1% agarose gel whose results were in the form of 5s and 28s bands. According to this Figure3, the extracted RNAs had almost good quality.

In order to confirm the results of PCR Real Time, the sample was electrophoresed on 1% Electrophoresis gel Figure 4. The presence of the desired band indicated that all bands have been proliferated and the results were correct.

Discussion

Environmental factors such as air pollution, stress, lifestyle and diet are among the causes associated with the rising incidence of cancer. Eating foods that have antioxidant properties have been shown to be effective in preventing and reducing the incidence of cancer (10)(11).

Despite the use of treatment strategies such as surgery, chemotherapy and radiotherapy, the mortality rate in cancer patients is still high, which indicates the ineffectiveness of these treatment strategies. In addition, the detrimental effect of chemotherapy and radiation therapy on dividing normal cells is another disadvantage associated with these therapeutic processes (12).

S.khuzestanica is one of the medicinal plants used in traditional medicine. No research has been conducted to investigate the effect of this plant cytotoxicity on PC3 prostate cancer cells. In this study, the effect of different concentrations of S.Khuzestanica extract on the growth rate of prostate cancer cells in cell culture medium was investigated. The results showed that the extract of Satureja khuzestanica at 125 mg / ml at 24 hours after treatment had the highest effect in inhibiting the growth of prostate cancer cells. The results also showed high level of BAX gene as an apoptotic promoter gene at the same dose and time. The anti-cancer effects of Satureja khuzestanica have been observed in a limited number of studies conducted on some cancer cells (13). Satureja khuzestanica belongs to Lamiaceae family and it is a well-known aromatic and medicinal plant (14). Studies have shown that the alcoholic extract of Satureja khuzestanica has anti-cancer activity against MCF-7 cells (15). Research conducted by Loizzo on the anticancer effects of the essential oil of Satureja khuzestanica showed that the cytotoxicity of this essential oil has anti-cancer activity on a variety of cancer cells, including breast cancer, and it is able to inhibit breast cancer cells and this ability increases with increasing concentration (16). In 2013, Fathi et al. Examined the antioxidant activity of Satureja hortensis extract and the results showed that the antioxidant activity of Satureja hortensis extract increases with increasing concentration and is able to prevent oxidation processes (17).

By examining the antifungal activity of the oil and the methanolic extract of Satureja khuzestanica against Aspergillus phallus, Dikbas N et al. (2008) concluded that the essential oil of Satureja khuzestanica has high antifungal activity against the mentioned pathogen (18). Studies conducted at (2015) have shown that carvacrol is a poly-phenolic compound that has very powerful antioxidant ability such as vitamin E and ascorbic acid. Carvacrol and many of its antioxidants have a significant role in preventing diseases such as cancer. Through genome degradation, they reduce the viability of cancer cells and genome degradation is much more pronounced at concentrations close to IC50 (19). Based on the studies conducted by Shirali, 43 compounds were identified in the essential oil of Satureja khuzestanica, which make up 99.96% of all essential oils. Then, to determine its antimicrobial effects, they used disk diffusion method against two microorganisms of Escherichia coli and Candida albicans. They concluded that the presence of phenolic compounds such as carvacrol, thymol and Gamma-terpinene in the essential oils also caused antimicrobial activity in this plant. Increasing phenolic compounds of essential oils is also directly associated with increased antimicrobial activity. This property prevents the oxidation of lipids and coronary heart disease and cancer (20).

In a study conducted by Sazghar et al. (2017) to investigate the anti-cancer properties of hydroalcoholic extract of Celeriac and savory on Hela cancer cells, it was found that BAX mRNA expression level increased significantly. However, no change was seen in BCL-2 expression. BAX protein expression level was also increased significantly in Real Time PCR, while it had no effect on BCL-2 expression. In this

study, the cytotoxic effect of this extract on Hela cancer cells was confirmed, but this extract had no toxic effect on fibroblast cell line. In the present study, the same results (significant expression of BAX gene) were obtained using chloroform extract of Satureja khuzestanica. In the current study, the highest expression of BAX gene was observed at 125 mg / ml after 24 hours, which was in accordance with MTT assay results (21).

Declarations

Funding Info

This article was adapted from the MS.c. project(14430513951001) of Saman Kazemi, where Hossein Soltanzadeh supervised, and Asghar Tanomand advised this project.

This study was supported in a MS.C program by bonab Islamic Azad University

Conflicts of interest/Competing interests

We confirm that this manuscript has not been published elsewhere and is not under consideration by another journal. All authors have approved the manuscript and agree with submission to Molecular Biology Reports *journal.* The study was supported in a Master program by Bonab Islamic Azad University. The authors have no conflicts of interest to declare.

Ethics approval

1) This material is the authors' own original work, which has not been previously published elsewhere.

2) The paper is not currently being considered for publication elsewhere.

3) The paper reflects the authors' own research and analysis in a truthful and complete manner.

4) The paper properly credits the meaningful contributions of co-authors and co-researchers.

5) The results are appropriately placed in the context of prior and existing research.

6) All sources used are properly disclosed (correct citation). Literally copying of text must be indicated as such by using quotation marks and giving proper reference.

7) All authors have been personally and actively involved in substantial work leading to the paper, and will take public responsibility for its content.

Ethical principles in writing the article have been Compliance according to the instructions of the National Ethics Committee and the COPE regulations. And due to we used the cancer cell line and didn’t have a human sample, according to the National Ethics Committee, there is no need for a code of ethics.

Authors' contributions

Saman kazemi : Writing- Original draft preparation, Reviewing and Editing, Visualization, Investigation, Validation

Dr Hossein Soltanzadeh: Conceptualization, Methodology, Software, Reviewing and Editing, Supervision

Dr Asghar Tanomand: Supervision

Dr Gholamreza Shahsavari: Methodology identified and approved Satureja khuzestanica

Availability of data and material

Authors can confirm that all relevant data are included in the article and/or its supplementary information files.

References

1- Scholars Research Library J. Nat. Prod. Plant Resour., 2011, 1 (4): 131-136

2- Nouri Dalavi, M., Ebrahimzadeh. Molecular genetics, diagnosis, prevention and gene therapy in prostate cancer. Journal of Medical School, Tehran University of Medical Sciences, 2009, No. 1. Page 131

3- Molecular mechanisms of caspase regulation during apoptosis Stefan J. Riedl & Yigong Shi Nature Reviews Molecular Cell Biology volume 5, pages897–907(2004)

4- Landis, S.; Bolden, T.; Wang, P. (1999). “Cancer statistics”. Ca-A- Cancer Journal for Clinicians. 49(1): 8-31.

5- Raff, M.C., Social controls on cell survival and cell death. Nature, 1992.356(6368): p. 397 400.

6- Cory, S. and J.M. Adams, The Bcl2 family: regulators of the cellular life-or-death switch. Nat Rev Cancer, 2002. 2(9): p. 647-56.

7- Sakar, A.; Dshmukh, V. (2011). “Ethno Pharmacological review of traditional medicinal plants for anti-cancer activity”. 3(4): pp.298-430.

8- Mokhtari MJ, Motamed N, Shokrgozar MA.Evaluation of silibinin on the viability,

migration and adhesion of the human prostateadenocarcinoma (PC-3) cell line. Cell Biol Int

2008; 32(8): 888-92.

9- K Girish, B N Gangadhar, N Janakiramaiah, E S M Sarvanan EEG Seizure duration during ECT : Effect Of Concurrent Psychotropic Drugs ,2003.6(2): p .17-20

10- Ren, W., et al., Flavonoids: promising anticancer agents. Med Res Rev, 2003.23(4): p. 51934.

11-Baumeister, P., M. Reiter, and U. Harreus, Curcumin and other polyphenolic compounds in head and neck cancer chemoprevention. Oxid Med Cell Longev, 2012. 2012: p. 902716.

12-Chabner, B.A. and M.A. Friedman, Progress against rare and not-so-rare cancers. N Engl J Med, 1992. 326(8): p. 563-5.

13-Hossein S, Leyla A, Mustafa T, Massoud H, Gholamreza Sh . Antimicrobial, antioxidant, cytotoxic and apoptic activities of Satureja khuzestanica. Medical journal. Gmi 2018; 99: 264-270

14- Baytop, T (1997). Turkce Bitki Adlari Sozlugu (A dictionary of vernacular names of wild plants of Turkey), Ankara, Publication of the Turk Dil Kurumu (The Turkish Language Society). No.: 578.

15- Zin al-Abedin Nazari. Seyyed Sepehr. The effect of safflower ethanolic extract on MCF-715 cells in breast cancer. Journal of Medical School, Qazvin University of Medical Sciences, 2017

16- Loizzo MR, Tundis R, Menichini F, et al. (2007), Cytotoxic activity of essential oils from labiatae and lauraceae families against in vitro human tumor models. Anticancer Research; 27: 3293-3299.

17- Fathi A, Sahari MA, Barzegar M, Naghdi Badi H . Antioxidant Activity of Satureja hortensis L. Essential Oil and its Application in Safflower Oil. Journal of Medicinal Plants, Volume 12, No. 45, Winter 2013

18- Dikbas N, Kotan R, Dadasoglu F, Sahin F. Control of Aspergillus flavus with essential oil and methanol extract of Satureja hortensis. Int J Food Microbiol 2008; 124: 179-82.

19- Saffari- Chaleshtori J, Nikoukar M, Heidarian E, Keloushadi M, Ghasemi- Dehkordi P, Gholami M. (2015), The effect of carvacrol on growth inhibition and genomic destruction in prostatic cancer cells using comet assay technique. J Shahrekord Univ Med Sci; 17(3): 57-65

20-Shirali R, Alizadeh A, BabadaeiSamani R (2013) Extraction andevaluation of phenolic compounds and antioxidantproperties ofessential medicinal herb savory Bakhtiari(Saturejabachtiarica(Bunge in Fars). National Conference on medicinal herbs, IslamicAzad University, Science and Research-Ayatollah Amoli.

21- Behdarvand Shoshtar Anahita, Sazgar Hossein, Ghasemi Pirbalout Abdollah . Investigatig Cytotoxic effect of hydroalich extract from combination of Kelussia odoratissma mozaff and Satureja bachtiarica bung on Hela cancer cell line :2017 , Volume 27 , Number 3; Page(s) 178 To 186.

Tables

Table 1. **Identification of components**

The linear retention indices for all the compounds were determined by conjection of the sample with a solution containing the homologous series of C8-C22 n-alkanes. The individual constituents were identified by their identical retention indices, referring to known compounds from the literature and also by comparing their mass spectra with either the known compounds or with the Wiley mass spectral database.

NO	Compound Name	Ret.Time	Similarity	KI_{STD}	KI_{CAL}	Area (%)
1	α-Thujene	9.5	96	930	931	0.61
2	α-Pinene	9.8	98	939	940	0.42
3	Camphene	10.4	97	954	959	0.05
4	β-Pinene	11.3	97	979	988	0.12
5	Myrcene	11.5	94	991	995	1.16
6	6-Methyl-3,5-heptadien-2-one	11.7	86	-	1001	0.38
7	3-Octanol	12.0	96	991	1011	0.04
8	3-Carene	12.2	93	1031	1017	0.1
9	α-Terpinene	12.5	95	1017	1026	0.25
10	para Cymene	12.9	93	1025	1038	3.35
11	β-Ocimene	13.4	95	1050	1053	0.02
12	γ-Terpinene	13.9	97	1060	1068	0.67
13	cis Sabinene hydrate	14.5	92	1070	1087	0.44
14	Terpinolene	14.8	95	1089	1096	0.07
15	Linalool	15.3	96	1097	1112	1.02
16	4-Terpineol	18.1	97	1177	1201	1.94
17	α-Terpineol	18.7	95	1189	1218	0.17
18	Thymol	21.6	93	1290	1315	0.28
19	Carvacrol	22.7	89	1299	1353	86.29
20	β-Caryophyllene	25.2	93	1419	1443	0.13
21	Geranyl acetone	25.7	97	1455	1463	0.18
22	α-Franesene	27.1	95	1506	1512	0.33
23	β-Bisabolene	27.3	96	1506	1522	0.97
24	α-Bisabolene	28.1	89	1507	1553	0.15
25	Caryophyllene oxide	29.7	94	1583	1616	0.53
26	β-Udesmol	30.0	89	1651	1630	0.25
27	α-Bisabolol	32.0	89	1686	1711	0.08

Table 2. Nucleotide sequence used for examining of the studied genes extracted from the Takapozist gene bank

Primer Name	Primer Sequence	Annealing Temperature	Base pair length
Bax- F	GGTTGTCGCCCTTTTCTA	48.84	108
Bax- R	CGGAGGAAGTCCAATGTC	49.1	
β -Actin-F	GCGAGAAGATGACCCAGAT	50.87	88
β -Actin-R	GAGGCGTACAGGGATAGC	50.97	
Bcl-2-F	GATGTGATGCCTCTGCGAAG	65	93
Bcl-2-R	CATGCTGATGTCTCTGGAATCT	64	

Table 3. Contents of Real Time PCR-Master mix per sample for examining a gene as performed according to the time and temperature schedule of Table 3 of Real Time PCR

Value based on µl	Substance
12.5	Master mix
8.5	Distilled water
0.5	Forward Primer
0.5	Reverse Primer
3	cDNA
25	Total volume

Table 4. Real-time PCR test schedule and temperature for genes

Gene	step	Temperature	Time	Number of cycles
Bax & Bcl-2 & β-actine	Initialization		15min	
	Denaturation	95°c	10sec	
		95°c		40
β-actine	Annealing	51°c	15sec	
Bax	Annealing	49°c	15sec	
Bcl-2	Annealing	64°c	15sec	
Bax & Bcl-2 & β-actine	Extension	72°c	20sec	

Table 5. Results of relative expression of BAX to BCL-2

Relative Expression Results							
Parameter	**Value**						
Iterations	2000						
Gene	**Type**	**Reaction Efficiency**	**Expression**	**Std. Error**	**95% C.l.**	**P(H1)**	**Result**
Bax	TRG	0/76	4/733	3/934-6/130	3/910-6/167	0/000	UP
Bcl2	TRG	0/8	0/889	0/879-0/900	0/870-0/908	0/024	DOWN
Interpretation							
Bax is UP-regulated in sample group (in comparison to control group) by a mean factor of 4/733 (S.E range is 3/934-6/130)							
Bax sample group is different to control group P(H1)=0/000							
Bcl2 is DOWN-regulated in sample group (in comparison to control group) by a mean factor of 0/889 (S.E range is 0/879-0/900)							
Bcl2 sample group is different to control group P(H1)=0/024							

Figures

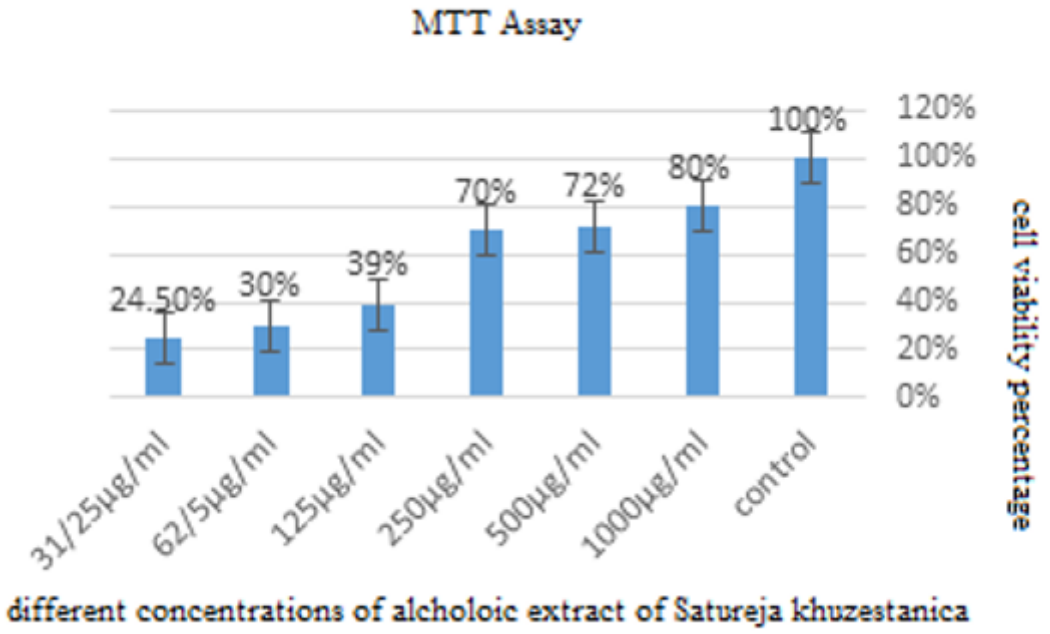

Figure 1

Cytotoxic effect of chloroform extract of S.khuzestanica plant on PC3 cancer cell line within 24 hours

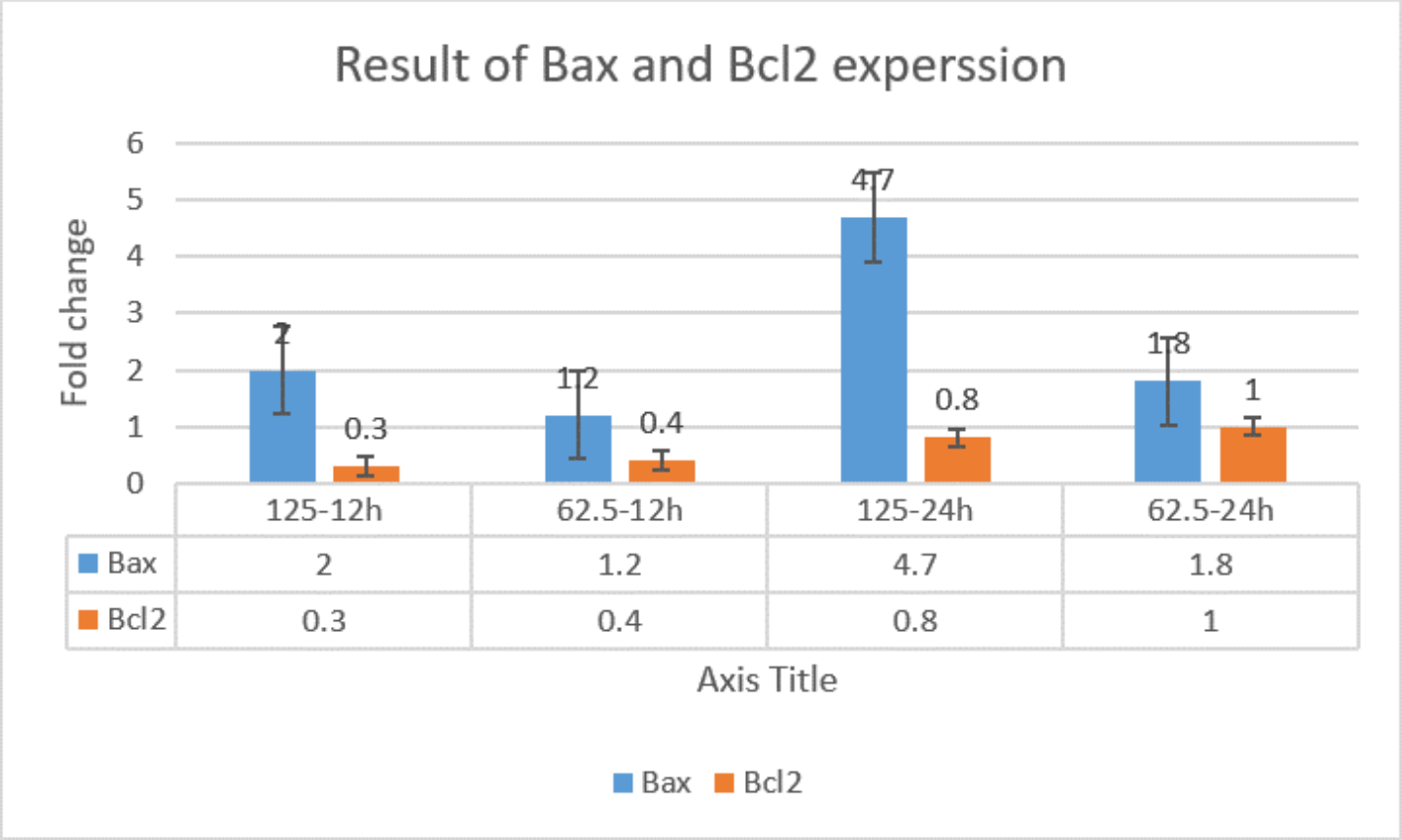

Figure 2

Results of Bax and Bcl-2 genes expression changes in terms of concentration and time.

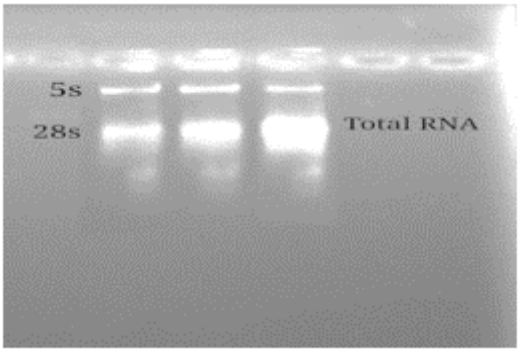

Figure 3

Total RNA Electrophoresis results of β-actine, Bax, and BCL-2 genes to ensure correct operation of Real time PCR

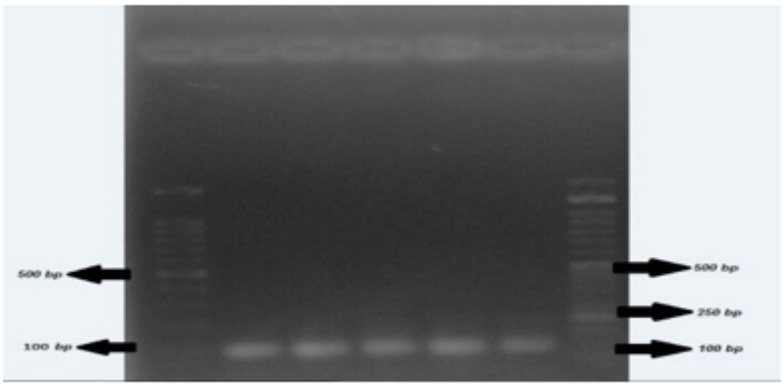

Figure 4

Total RNA Electrophoresis results of β-actine, Bax, and BCL-2 genes to ensure correct operation of Real time PCR

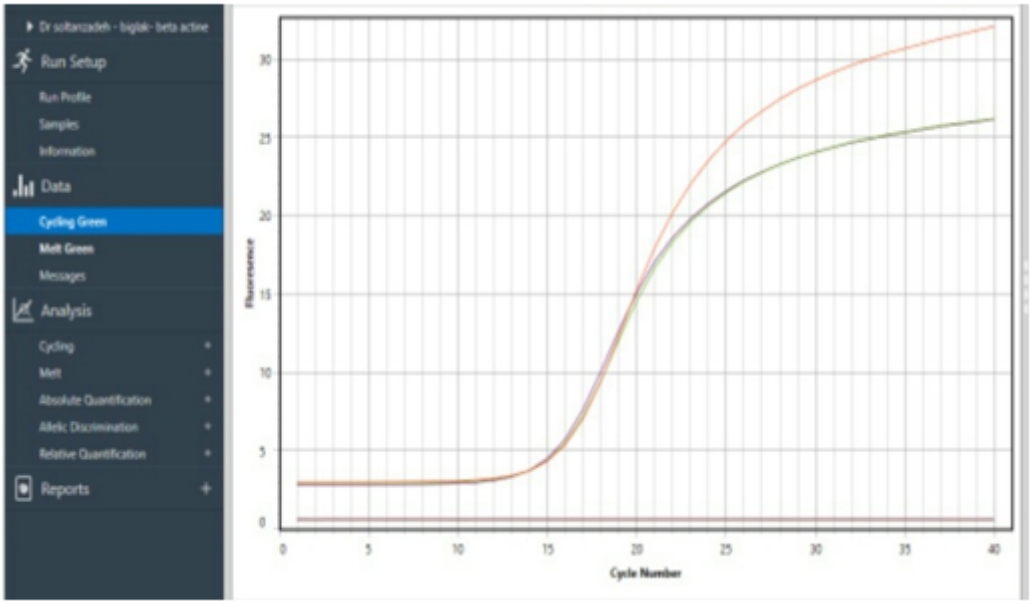

Figure 5

Diagram 2. Real time PCR proliferation curve for actin - β genes after treatment with Satureja khuzestanica

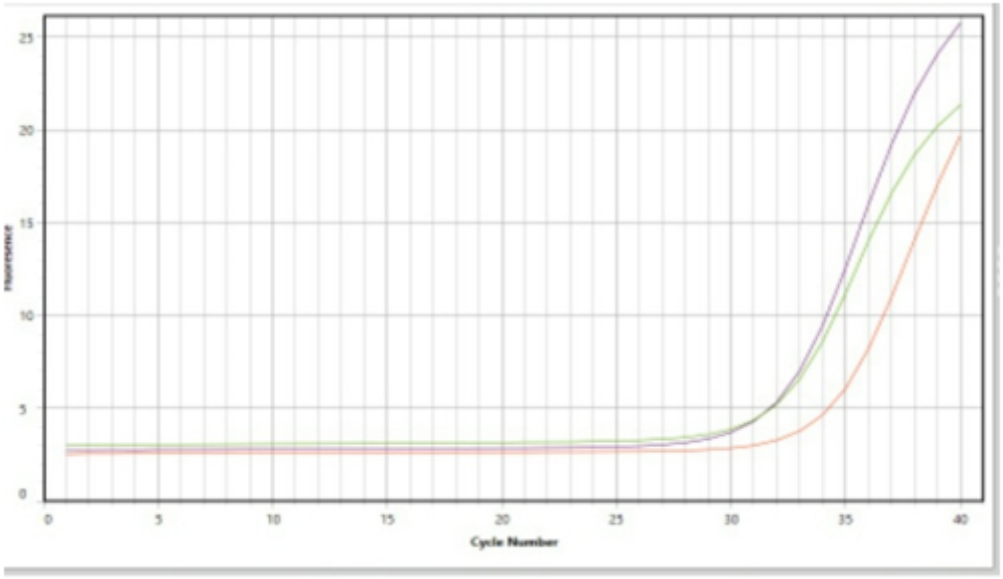

Figure 6

Diagram 3. Real time PCR proliferation curve for BAX genes after treatment with Satureja khuzestanica

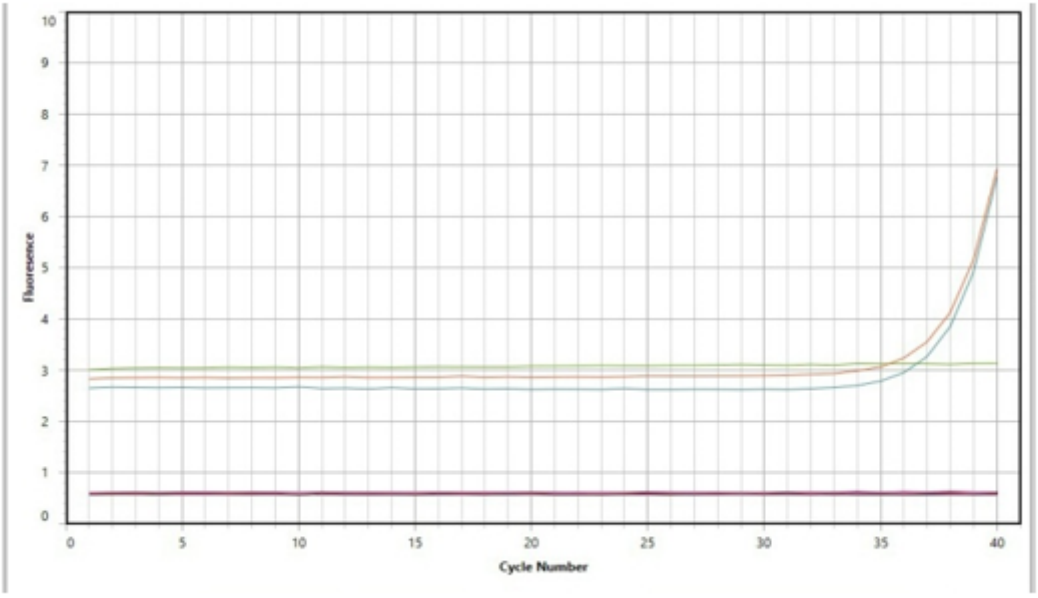

Figure 7

Diagram 4. Real time PCR proliferation curve for BCL-2 genes after treatment with Satureja khuzestanica

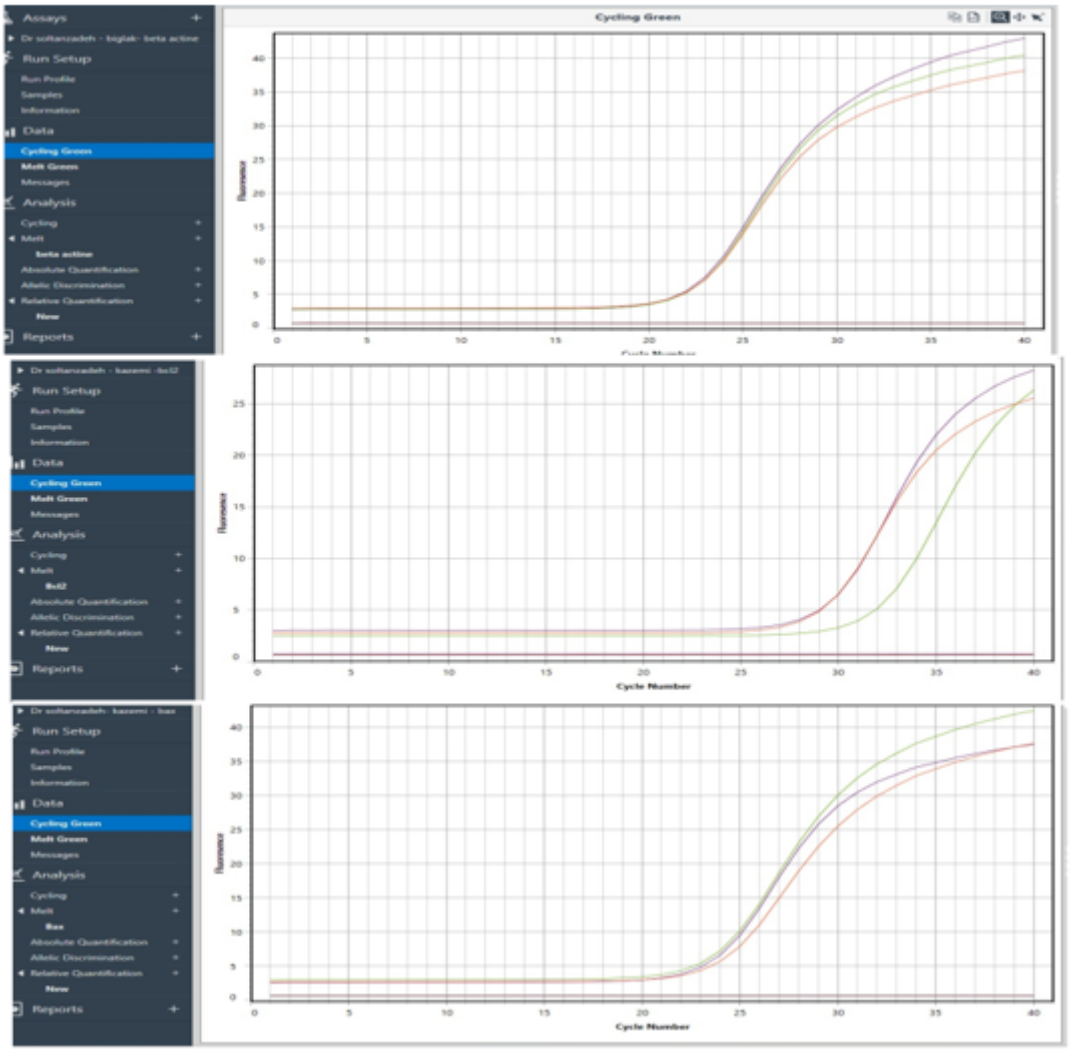

Figure 8

Diagram 5. Real time PCR proliferation curve for actin – β, BAX, and BCL-2 genes for control group cells

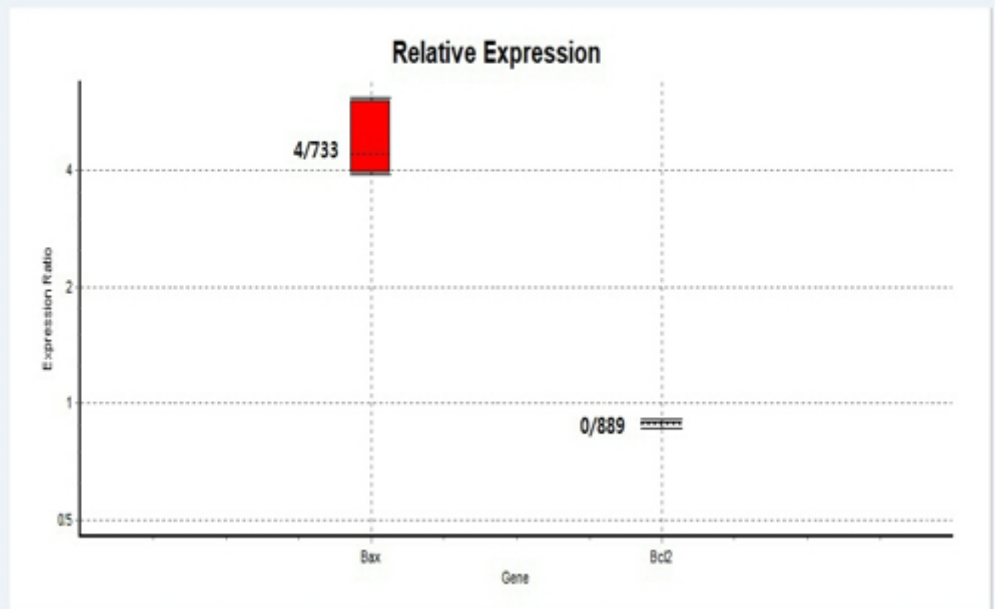

Figure 9

Diagram 6. Relative expression to BCL-2