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Gene expression of selected apoptotic markers in human oral squamous carcinoma HSC-3 cell line treated with *Myrmecodia pendans* plant extract

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Abstract

Background: *Myrmecodia pendans* (*M. pendans*), or Sarang Semut, is an epiphyte with anticancer potential. It was recently reported that it induces apoptotic activity in the human oral squamous carcinoma (HSC-3) cell line. This study aimed to investigate the effect of *M. pendans* treated samples on the expression of apoptotic markers, Bax and Bcl-2. **Methods**: *M. pendans* was purchased from West Papua, Indonesia. The hypocotyl was dried thoroughly and then extracted aqueously. The apoptotic activity was detected via flow cytometry. Bax and Bcl-2 expression was analyzed by quantitative real-time polymerase chain reaction. **Results**: Results of our cell cycle analysis reveal that aqueous extract of *M. pendans* induced apoptosis in 2.5 and 5 mg/mL but no change between these two concentrations. Apoptosis was observed at 24 h but not at 48 h. Bax and Bcl-2 expression in HSC-3 cells was affected by *M. pendans*. At 24 h, upregulation of Bax was observed at 2.5 mg/mL. However, after 48 h, Bax expression showed no changes at any concentration. Bcl-2 was significantly downregulated after 48 h of treatment. **Conclusions**: *M. pendans* extract induced apoptosis in HSC-3 cells, which might occur via the proapoptotic (Bax) and antiapoptotic (Bcl-2) pathways.

Keywords: apoptosis, cell cycle, flow cytometry, real-time polymerase chain reaction

Introduction

Cancer is a disease condition that affects many people worldwide, it is the state of uncontrolled cellular proliferation that occurs as a result of genetic mutation driven by environmental and host factors. Oral squamous cell carcinoma is a pernicious tumor of epithelial origin with high incidences of metastases and poor prognosis. It is reckoned to be one of the deadliest neoplasms with low survival rates. The World Health Organization reported an increase in oral cancer deaths in Malaysia to 1587 (1.55% of total deaths) in 2011. The HSC-3 cell line is derived from a moderately differentiated primary tumor, with metastatic properties, located in the tongue.[1]

Surgery, radiotherapy, and chemotherapy are some of the complex treatment methods for oral cancer that cause side effects and permanent deformities (after resection) and are often expensive. Furthermore, the intrinsic resistance of cancer cells to chemotherapeutic drugs presents another challenge in its managemen.[2] Nowadays, several plant species are being researched as alternative drugs in an effort to kill cancer cells via various cell death pathways. *M. pendans*, known by locals as Sarang Semut, belongs to the *Rubiaceae* family. It is an epiphyte that primarily grows on other plants including the Cajeput plant (*Melaleuca*), Kaha plant (*Castanopsis*) of the beech family, Casuarina (*Casuarinaceae*) and Beech (*Nothofagus*). Originally, it was found in Papua, in the eastern part of Indonesia. However, it is also found in Malaysia and some tropical countries. Only ants from the *Ochetellus* species colonize Sarang Semut plants. The only two species of Sarang Semut that are colonized by these ants are *Myrmecodia* (45 species) and *Hypoiphytum* (26 species). In ethnic medicine, *M. pendans* is consumed as a tea after boiling in water; it is believed to be a remedy for nausea, hypertension, and some cancers. The people of Papua consume *M. pendans* mixed into porridge and as a boiled drink to enhance body immunity.[3]

Several articles have proposed the medicinal value of *M. pendans.* The basis for its efficacy is its active substance content, which includes polyphenols, flavonoids, tannins, tocopherols and glycosides.[4] A chemical screening test of the ant nest plant (*M. pendans*) revealed that it contains flavonoids and tannins. Many bioactivities of flavonoids have been reported, including carcinogen inactivation, antiproliferation, cell cycle inhibition, induction of apoptosis, differentiation, and inhibition of angiogenesis.

Allegedly, the antitumor effect of *M. pendans* is owing to its flavonoid content. Flavanoids, which are theoretically proven to block the receptors of growth factors, inhibit mitogen activated protein kinase on the receptor tyrosine kinase signaling pathways (RTKs). This leads to cell cycle disruption.[4]

Apoptosis is a highly regulated cellular death process. It occurs as part of the cell response to damage and stress, as well as during normal cell development and morphogenesis.[5] Conversely, necrosis is pathological cell death owing to ischemia, and chemical, physical, and mechanical factors. A balanced apoptosis and cellular division process results in controlled cellular homeostasis and does not cause abnormal proliferation. Apoptosis is preferred over necrosis because apoptosis does not cause inflammation to its surrounding cells in contrast to necrosis. Cells possess genes that differ substantially in their structure and function. Therefore, these genes have varying expression patterns. All biological functions including cell death are regulated following the central dogma, which begins at the genes.[6] Apoptosis is an important cell function, which relies on the orderly activation of special apoptosis-related genes. Previously reported apoptosis-related genes include Bcl-2 and Bax. Bcl-2 is an antiapoptotic gene (prosurvival effector), whereas Bax is a proapoptotic effector.

Genes belonging to the Bcl-2 family regulate apoptosis via the mitochondrial pathway by controlling the integrity of the outer membrane of the mitochondria. The interaction of the Bcl-2 gene with other members of the Bcl-2 family regulates complex interactions that negatively affect the release of proapoptotic proteins. In contrast, Bax functions in forming pores or associating with the outer mitochondrial membrane to induce mitochondrial permeability. As a result, proteins inducing cell death are released into the cell.[5]

18sRNA is a stable housekeeping gene that is widely used as a control in qRT-PCR analyses because of its invariant expression across tissues, cells, and experimental treatments.[7,8] In the present study, the expression of Bcl-2 and Bax in human oral squamous cell carcinoma HSC-3 cell line was examined.

In conclusion, *M. pendans* might be promoted as a new anticancer remedy because it targets the intrinsic apoptotic pathway by imparting a physiologic death process specifically on tumor cells. Further studies on the effect of *M. pendans* on normal cells should be established to rule out the cytotoxic effect.

Methods

Preparation of aqueous plant extract. *M. pendans* plant was acquired from Bintuni, West Papua, Indonesia. No specific botanical specifications were acknowledged. Aqueous extraction methods were performed on the plant sample as follows: first, the hypocotyl of the plant was cleaned, cut and air-dried for seven days. Next, the dried sample was boiled and filtered; the filtrate was cooled down to -80 °C before being freeze dried to remove remaining moisture in the sample. The resulting crude extract powder was stored at 4 °C for the next part of the experiments.

For *M. pendans* aqueous extract preparation, up to 200 mg of the crude extract was weighed and 1 mL of distilled water was added. Thus, using this ratio, 0.125 mL from this was added to 9.875 mL of growth medium to make a total of 10 mL of 2.5 mg/mL of *M. pendans* concentration; 0.250 mL of this extract was added to 9.750 mL of growth medium to make a total of 10 mL of 5.0 mg/mL of *M. pendans* concentration. Further, 0.010 mL of this extract was added to 9.990 mL of growth medium to make a total of 10 mL of 7.5 mg/mL of *M. pendans* concentration.

Cell cycle analysis. The progression of the cell cycle was determined using flow cytometric analysis after staining with propidium iodide (PI). Briefly, cells were plated into 10 cm Petri dishes and cultured until they reached 80% confluence. Similar to the MTT assay, the cells were treated with different concentrations of *M. pendans* extract and incubated for 24 and 48 h. Positive control cells (treated with doxorubicin) were also included. All adherent and floating cells were then collected, washed with cold phosphate buffer saline (PBS) three times, and centrifuged at 2000 rpm for 5 min (4°C). Cells were then transferred into a bigger collection tube and washed once again with cold PBS. Subsequently, the cells were fixed with 500 mL of fixing solution containing 75% ethanol and 25% PBS for 15 min at room temperature. Cells were stained with 300 µL of PI/RNAse (1 mg/mL PI containing 10 mg/mL RNAse) solution. Samples were analyzed using a Guava easyCyte HT System flow cytometer with the Guava Cell Cycle version 2.5.6 application. The cell cycle distributions were expressed as the percentage of cells in the G0/G1, S, and G2/M phases.

Cell culture and sample preparation for qRT-PCR analysis. HSC-3 cells were kindly provided by Associate Professor Dr. Masa Aki-Ikeda, Tokyo Medical and Dental University, Tokyo, Japan. The cells were cultured in Dulbecco's Modified Eagle's Medium, DMEM (Gibco, USA), supplemented with 10% fetal bovine serum and penicillin–streptomycin (Nascalai Tesque, Japan). The HSC-3 cells were grown in 10-cm Petri dishes at 37 °C in a humidified atmosphere (5% CO_2). The cells were trypsinized using trypsin (Nascalai Tesque, Japan) in PBS (Sigma, Germany) and passaged twice a week. The subconfluent cultured cells (70%–80%) were treated with 0.005 mg/mL of doxorubicin

hydrochloride (Toronto Research Chemicals Inc., Canada) as the positive control and 2.5 and 5.0 mg/mL of *M. pendans* aqueous extract for 24 and 48 h.

Total RNA extraction. The total RNA Mini Kit was acquired from Geneaid (Taiwan). RNA extraction was performed following the manufacturer's protocol.

Cell harvesting and cell lysis. After treatment with the *M. pendans* extract and controls, the cell culture medium was removed from the plate. The cells were washed with PBS to remove any traces of culture medium; 1 mL of TRIzol® solution was added to lyse the cells. Then, the cells were scraped off the plate and transferred to a 15-mL centrifuge tube. The cell lysate was incubated at room temperature for 5 min. Next, the cells were centrifuged for 5 min at 300 ×g, followed by addition of RB buffer and β-mercaptoethanol after removal of the supernatant. The cells were incubated at room temperature for 5 min.

RNA binding. Then, 70% ethanol was prepared in ddH_20 (RNase and DNase-free) and added to the cells. The mixture was shaken thoroughly and pipetted to dissociate the precipitated cells. The mixture was then transferred into 2-mL collection tubes through the RB column and centrifuged for 1 min at 16,000 ×g.

RNA wash and elution. W1 Buffer was added into the RB column. The mixture was centrifuged once at 14,000–16,000 ×g for 30 s. Then, Wash Buffer with ethanol was added into the RB column and centrifuged twice at 14,000–16,000 ×g for 30 s. The flow-through was discarded, and RNA was centrifuged at 14,000–16,000 ×g for 3 min to dry the column matrix. RNase-free water was added into the column matrix and left for at least 1 min to ensure the RNase-free water was absorbed. Then the column matrix was centrifuged at 14-16,000 ×g for 1 min to elute the purified RNA.

Quantitative real-time (RT) polymerase chain reaction analysis. Quantitative RT-PCR analysis was conducted in two parts: preparation of the cDNA via reverse transcription of the RNA sample, followed by analysis using qRT-PCR assay.

cDNA synthesis. cDNA synthesis was conducted using ReverTra Ace® qPCR RT Master Mix Kit (Toyobo, Japan) based on manufacturer's instructions. A total of 8 µl of solution consisting of 4× DN Master Mix, RNA template, and nuclease-free water was prepared. After incubation for 5 min, 5× Master Mix was added to the solution to a total of 10 µl of reverse transcription solution. Then, the solution was incubated at 37 °C for 15 min and heated at 98 °C for 5 min to obtain cDNA.

Quantitative analysis using RT polymerase chain reaction (QRT-PCR). qRT-PCR analysis using SensiFAST SYBR master mix (Bioline, London UK) was conducted according to the manufacturer's instructions. A qRT-PCR reaction mixture consisting of SensiFAST SYBR master mix, primers mix, cDNA and water was prepared (Table 1). Each sample was prepared in triplicate with total volume of 20 µl. The selected apoptotic gene primers (Bax and Bcl-2) were ordered accordingly. 18sRNA was used as an internal control. The primers used in the gene expression assay are listed in Table 2.

Table 1. qRT-PCR reaction under optimal amplification condition

Gene	Predenaturation	Denaturation	Annealing	Extension	Cycling (times)
Bax	95 °C, 1 min	95 °C, 5 s	60 °C, 7 s	72 °C, 10 s	50
Bcl-2	95 °C, 1 min	95 °C, 5 s	63 °C, 10 s	72 °C, 10 s	50
18sRNA	95 °C, 1 min	95 °C, 5 s	58 °C, 10 s	72 °C, 10 s	50

Table 2. Applied primer sequences for qRT-PCR

Gene		Primers	References
Bax	Forward	5 GAGAGGTCTTTTTCCGAGTGG	Kim *et al*. (2015)[9]
	Reverse	3 CCTTGAGCACCAGTTTGCTG	
Bcl-2	Forward	5 CTGCACCTGACGCCCTTCACC	Kim *et al*. (2013)[10]
	Reverse	3 CACATGACCCCACCGAACTCAAAGA	
18sRNA	Forward	5 GCTTAATTTGACTCAACACGGGA	Li *et al*. (2013)[11]
	Reverse	3 AGCTATCAATCTGTCAATCCTGTC	

PCR results were obtained through quantitative gene expression analysis of targeted mRNA in the treated HSC-3 cells. The expression level of mRNA in the sample was normalized to the 18sRNA housekeeping gene, which was used as the internal control. Data were normalized to the internal control and analyzed by a direct conventional method.

Statistical analysis. Various concentrations of *M. pendans* aqueous extract were used to treat cultured HSC-3 cells. qRT-PCR was performed to analyze the gene expression. The experimental samples were compared against the control (untreated) using one-way ANOVA with Dunnett post hoc test, using SPSS 23.0, IBM software. Results were reported as mean values (standard error of means = SEM).

Results

Flow cytometry analysis of Sub-G1 phase. To investigate the apoptotic effect of aqueous *M. pendans* or Sarang Semut plant extracts on HSC-3 oral cancer cell lines, flow cytometry analysis, using PI as a staining agent. Figure 1 shows the graph of the sub-G_1 population (apoptotic HSC-3 cell lines) percentage after being treated with different concentrations of aqueous *M. pendans* extracts (2.5 and 5 mg/mL) for 24 and 48 h. Doxorubicin, an anticancer drug, served as the positive control in this study. The results revealed that *M. pendans* extracts increase the percentage of apoptotic cells of the HSC-3 cells line at 24 and 48 h. We noticed that the sub-G1 phase populations were higher at 24 h than at 48 h. However, the inductions were lesser, as shown by doxorubicine. We did not observe any differences of the sub-G1 populations between 2.5 and 5 mg/mL of extract.

Quantitative gene expression analysis. In the present study, we investigated the apoptotic effect of the *M. pendans* aqueous extract on the human oral carcinoma (HSC-3) cell line. The gene expression of the apoptotic markers Bax and Bcl-2 were analyzed by qRT-PCR (Figure 1 & Figure 2).

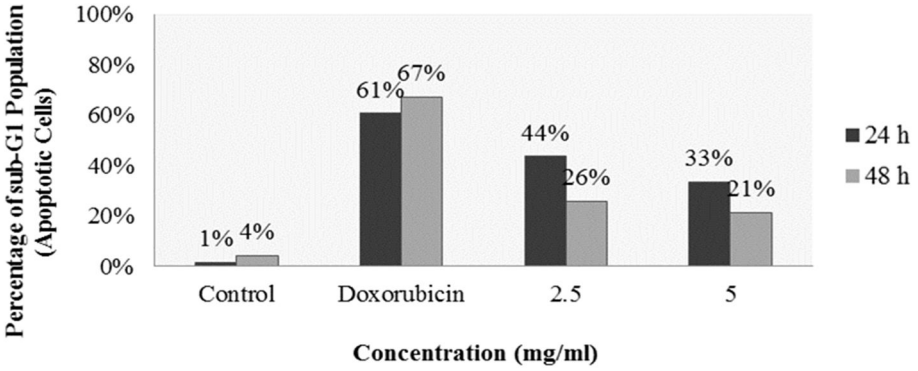

Figure 1. Percentages of sub-G1 phase after HSC-3 cell lines being treated with 2.5 and 5.0 mg/mL of *M. pendans* aqueous extract. Higher percentages of the sub-G1 population at 24 and 48 h were at concentrations of 2.5 and 5.0 mg/mL, respectively

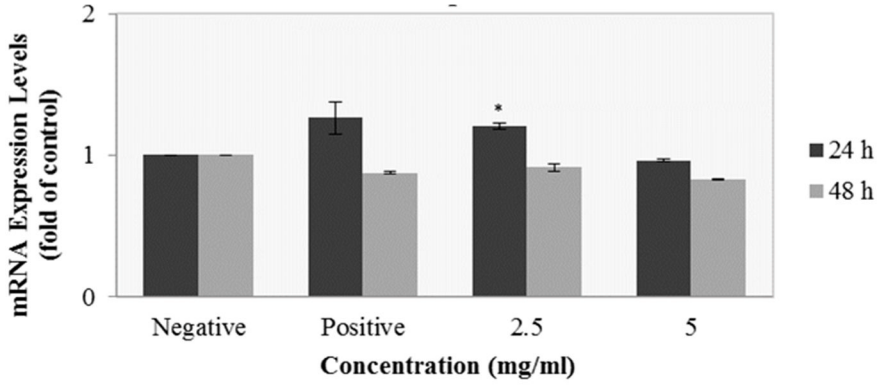

Figure 2. Relative mRNA expressions of Bax in HSC-3 cells after 24 and 48 h in control, treated control, and extract-treated groups. HSC-3 cells were treated with 2.5 and 5.0 mg/mL *M. pendans* aqueous extract. At 24 h, the HSC-3 cells treated with 2.5 mg/mL of *M. pendans* extract showed significant Bax upregulation. However, there were no change in Bax expression in all groups at 48 h. Values are shown as mean; error bars indicate standard error. * $p < 0.05$

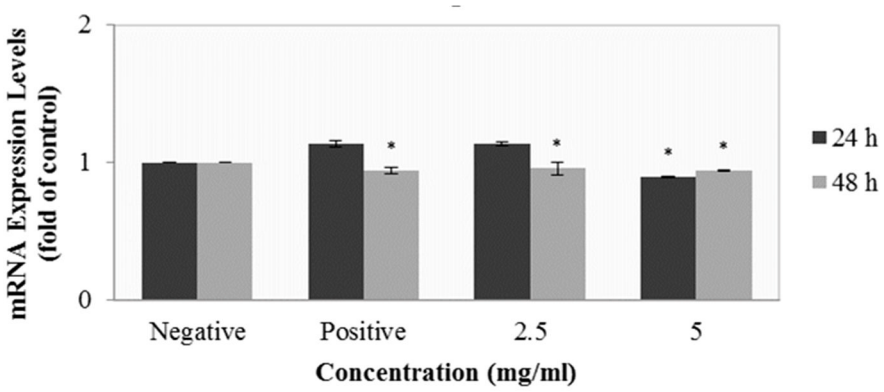

Figure 3. Relative mRNA expression of Bcl-2 in HSC-3 cells at 24 and 48 h in control, treated control, and compound-treated groups. HSC-3 cells were treated with 2.5 and 5.0 mg/mL of aqueous extract of *M. pendans*. At 24 h, downregulation of Bcl-2 was significant in the 5 mg/mL-treated group. Bcl-2 showed significant downregulation at all concentration at 48 h after treatment. Values are shown as mean; error bars indicate standard error. * $p < 0.05$

Discussion

Apoptosis or cell death is a tightly controlled process initiated via one of the following two pathways: the death receptor (extrinsic) pathway and the mitochondrial (intrinsic) pathway. The activation of genes related to apoptosis results in the induction or repression of apoptosis in cancer cells. Positive activation is regulated by proapoptotic factors, such as the apoptotic promoter Bax, whereas negative activation is regulated by antiapoptotic factors, such as the apoptotic inhibitor Bcl-2. These two major proteins are controlled by the guardian of the genome or the tumor suppressor gene p53. Any mutation or defect of and subfunctional p53 would cause elevated levels of Bcl-2 protein and reduced levels of Bax in several tissues. This may permit a population of genetically damaged cells to escape the normal process of apoptotic deletion.[12]

The expression of Bax and Bcl-2 has previously been ad with induction and repression of apoptosis, respectively, in oral cancer cell lines.[13,14] The results of this study demonstrate that expression of Bax and Bcl-2 is key to the induction of apoptosis in HSC-3 cells. Any alteration in the expression, especially overexpression of Bcl-2, has been found in histological studies of epithelial dysplasia, in early carcinogenesis of oral cancer.[15] As an antiapoptotic regulator, Bcl-2 influences disease progression because it increases the survival rate of neoplastic cells, allowing new genetic mutations to occur and granting a higher resistance to chemotherapy and radiotherapy.

In the current study, flow cytometry with PI staining was used on HSC-3 cell lines to further explore the apoptotic anticancer properties of *M. pendans* in a selected dose- and time-dependent manner. We observed the induction of apoptosis by both concentrations of *M. pendans* extract. However, it was relatively no change between these two concentrations. In this study, we limited the concentration of *M. pendans* to 5 mg/mL only because the maximum effective concentration reported in a previous study involving the use of the MTT assay was 5 mg/mL. At 24 h, we noticed the induction of apoptosis as similar to previous studies[9] but could not rule out the possibility of cell loss at 48 h.

M. pendans significantly affected Bax expression in HSC-3 cells. At 24 h, 2.5 mg/mL of *M. pendans* showed upregulation of Bax expression, whereas 5 mg/mL of *M. pendans* showed no change in Bax expression as observed in Figure 2. However, there was no change in Bax expression in any group at 48 h as seen in Figure 2. The downregulation of Bax expression might be owing to mRNA degradation because the processes of translation and the general degradation of mRNA are tightly linked.[16]

Bcl-2 proteins are one of the most prominent antiapoptotic proteins expressed in oral squamous cell carcinoma. In this study, the downregulation of Bcl-2 was significantly observed in the 5 mg/mL-treated group at 24 h. Bcl-2 showed significant downregulation at all concentrations at 48 h after treatment as interpreted from Figure 3. These results were in line with the results of flow cytometry (Figure 1).

Conclusions

Our results demonstrate that *M. pendans* has interesting apoptotic-inducing activities on human oral squamous carcinoma (HSC-3) cell line. *M. pendans* regulated apoptosis via two pathways, the induction and repression of apoptosis by Bax (proapoptotic marker) and Bcl-2

(antiapoptotic marker). The results obtained are in agreement with the results of flow cytometry.

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Conflict of Interest Statement

We declare no conflict of interest associated with this study.

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References

1. Rodriguez AE. Characterization of CAL 27 and HSC-3 cell lines. DPAGT1 gene expression and association with oral squamous cell carcinoma genesis and metastasis [dissertations]. London, USA: Boston University; 2016.
2. López-Verdín S, Lavalle-Carrasco J, Carreón-Burciaga RG, Serafín-Higuera N, Molina-Frechero N, González-González R, Bologna-Molina R. Molecular markers of anticancer drug resistance in head and neck squamous cell carcinoma: A literature review. *Cancers*. 2018;10:1–15.
3. Soeksmanto MA. Subroto PS. Anticancer activity tests for extracts of Sarang Semut plant (M. pendans) to HeLa and MCm-B2 cells. *Pak J Biol Sci*. 2010;13:148–51.
4. Achmad H, Armyn SA, Singgih MF. Anti-cancer activity and anti-proliferation ant nests flavonoid fraction test (Myrmecodia pendans) human tongue cancer cells in Sp-C1. *IOSR J Dent Med Sci.* 2014;13;1–5.
5. Nikoletopoulou V, Markaki M, Palikaras K, Tavernarakis N. Crosstalk between apoptosis, necrosis and autophagy. *Biochim Biophys Acta-Mol Cell Res.* 2013;1833:3448–59.
6. Jones DL, Brewster RC, Phillips R. Promoter architecture dictates cell-to-cell variability in gene expression. *Sci*. 2014;346:1533–6.
7. Lemma S, Avnet S, Salerno M, Chano T, Baldini N. Identification and validation of housekeeping genes for gene expression analysis of cancer stem cells. *PloS one.* 2016;11:e0149481.
8. Kuchipudi SV, Tellabati M, Nelli, RK, White GA, Perez BB, Sebastian S, Chang KC. 18S rRNA is a reliable normalisation gene for real time PCR based on influenza virus infected cells. *Virol J* ,2012;9:230.
9. Kim C, Lee JH, Kim SH, Sethi G, Ahn KS. Artesunate suppresses tumor growth and induces apoptosis through the modulation of multiple oncogenic cascades in a chronic myeloid leukemia xenograft mouse model. *Oncotarget*. 2015;6:4020–35.
10. Kim JA, Kim Y, Kwon BM, Han DC. The natural compound cantharidin induces cancer cell death through inhibition of heat shock protein 70 (HSP70) and BCL-associated athanogene domain 3 (BAG3) Expression by blocking heat shock factor 1 (HSF1) binding to promoters. *J Biol Chem*. 2013;288:28713–26.
11. Li X, Chen YT, Josson S, Mukhopadhyay NK, Kim J, Freeman MR, Huang WC. MicroRNA-185 and 342 inhibit tumorigenicity and induce apoptosis through blockade of the SREBP metabolic pathway in prostate cancer cells. *PloS one*. 2013;8:e70987.
12. Li X, Miao X, Wang H, Xu Z, Li B. The tissue dependent interactions between p53 and Bcl-2 in vivo. *Oncotarget*. 2015;6:35699–709.
13. Arumugam J, Jeddy N, Ramamurthy A, Thangavelu R. The expression of Bcl-2 in oral squamous cell carcinoma—A review. *J Orofac Sci*. 2017;9:71–4.
14. Coutinho-Camillo CM, Lourenço SV, Puga RD, Damascena AS, Teshima THN, Kowalski LP, Soares FA. Profile of apoptotic proteins in oral squamous cell carcinoma: A cluster analysis of 171 cases. *Appl Cancer Res*. 2017;37:2.
15. Pallavi N, Nalabolu G, Hiremath S. Bcl-2 and c-Myc expression in oral dysplasia and oral squamous cell carcinoma: An immunohistochemical study to assess tumor progression. *J Oral MaxilloFac Pathol JOMFP*. 2018;22:325–31.
16. Huch S, Nissan T. *Interrelations between translation and general mRNA degradation in yeast.* Wiley interdisciplinary reviews. RNA. 2014;5:747–63.