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Effect of Exposure to Quran Recitation on Cell Viability, Cell Migration, and *BCL2L12* Gene Expression of Human Prostate Adenocarcinoma Cell Line in Culture

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Abstract

Background and Objectives: Prostate cancer is the third most important cause of cancer deaths and one of the most common cancers in the world. Given the limited knowledge on environmental sounds and their effects, the important role of sounds is neglected in every culture across the world. The aim of this study was to investigate the impact of *Quran* recitation on prostate cancer cell line (PC-3) and compare it with the effects of cisplatin.

Methods: The effects of cisplatin and Quran recitation on the viability and migration capacity of PC-3 cells were investigated by evaluating *BCL2L12*. In addition, mRNAs were assessed by real-time polymerase chain reaction (RT-PCR). Gene expressions of *BCL2L12* and *TBP* were investigated as target and internal control genes, respectively.

Results: The results of the in vitro assay showed a considerable inhibitory effect of *Quran* recitation on the proliferation and migration of PC-3 cells. Furthermore, the significant inhibitory effects of cisplatin on the proliferation and migration of PC-3 cells exposed to *Quran* recitation was more than that of the PC-3 cells only treated with cisplatin. Results of quantitative real time PCR demonstrated that the gene expression of *BCL2L12* was significantly down regulated in PC-3 cells treated by cisplatin and those exposed to both cisplatin and *Quran* recitation.

Conclusion: This was the first report about the direct effects of *Quran* recitation on non-auditory cells in culture. The results of this study suggested that *Quran* recitation could alter cell proliferation via the down regulation of *BCL2L12* and migration of PC-3 cells.

Keywords: Quran Recitation, Prostate Cancer, Apoptosis, Cell Viability, BCL2L12.

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Introduction

One of the most frequent non-dermatologic malignancies is prostate cancer (1). Different therapeutic methods, such as new treatment options, early diagnosis, and routine screening, have positive and important effects on the survival rate of prostate cancer patients (2). Based on the statistics, in 2012, about one million men were diagnosed with prostate cancer worldwide, 70% of whom were in developed countries. The incidence of prostate cancer in Iran is about 10 per 100,000 individuals (3). It is estimated that about 43% of male cancer survivors in the United States are survivors of prostate cancer, and the rate of 5-year survival across different stages is 99.7% (4).

When sound travels through a medium, it can cause turbulence in it. Sound is defined as the compression of a waveform at a specific frequency that passes through air or materials (5). A number of studies have divided sound into three groups: 1) infrasound (below 20 Hz); 2) audible sound (20-20,000 Hz), and 3) ultrasound (above 20 kHz) (6). In the field of medicine, the use of ultrasound is well-known in the diagnosis and treatment of medical problems. Sound can also create mechanical stress and directly affect cells. The disturbance created by sound via the mechanism of particle interaction is probably the cause of such an effect (7).

On the other hand, music is an integral part of many cultures worldwide; however, its various effects on human life are not widely known yet. Previous studies have shown that music may be useful in the alleviation of stress, medical care, and nociception in cancer and burn patients, as well as some patients undergoing surgical procedures. However, the main mechanisms through which these effects occur are still unidentified (8,9).

Since the beginning of the 21st century, different studies have demonstrated that the response to sound, in general, and to music, in particular, is complex and might not be fully ascribed to emotion, given that cell types other than auditory hair cells can also respond directly to audible sound (6). In a study, sound was used to improve cell growth in plants (10). Other researchers have also confirmed the positive effects of sound on human beings. Human cells are well reacted to sound, and sound can resonate through human somatic and auditory cells (6,8). The approach of using sound for therapeutic purposes can introduce further non-invasive treatment options to patients.

In Islam, Quran is the main source of principles that acts as a leader for Muslims and offers guidelines to solve social problems and improve human lives (11). Quran recitation has been already used as a treatment method in some Islamic countries (12,13). A previous study indicated that junior high school students who recited Quran regularly had less anxiety, depression, and stress as compared to other groups (14). Furthermore, other research demonstrated that those who listen to Quran regularly have a lower degree of anxiety, compared to those who do not listen to this holy book recitation (15).

It appears that studies investigating the response of human body cells to Quran recitation are limited. Therefore, the aim of this study was to evaluate the effect of Quran recitation alone and along with cisplatin on the viability, motility, and *BCL2L12* (BCL2-like 12) gene expression of PC-3 cells. This study was conducted to understand the effects of acoustic vibrations in the form of Quran recitation on human cells in culture.

Methods

Cell Line and Cell Culture: PC-3 cells were obtained from the National Cell Bank of Iran in Tehran. These cells were incubated in RPMI 1640 supplemented with 10% fetal bovine serum (FBS) and 100 U/mL antibiotics (all from Gibco, Scotland) at 37°C with 95% O2 and 5% CO2 in an incubator. The experiments were done overnight after seeding in order to ensure adherence of the cells. Each experiment was repeated at least three times (16).

Treatment with Quran Recitation: The cells were exposed to Quran recitation using four speakers symmetrically surrounding the 96-well plates for 2 h. To this end, the recitation of Surah Al-Fatiha by the recorded voice of Sheikh Abdul Basit was used for 70 times. A small platform was used so that the speakers were not in contact with the floor. As a control group, the cells had exposure to silent speakers to ensure the deletion of possible magnetic field or background noise produced by the speakers. Pressure levels of sound were maintained at 90 dB (6).

MTT Assay: The effects of sounds on PC-3 cells were defined by an MTT assay (Sigma, USA). A total of l04 cells were exposed to Quran recitation for 2 h. However, l04 cells were treated with different concentrations of cisplatin for 48 h. Furthermore, l04 cells treated with IC50 concentration of cisplatin for 48 h were exposed to Quran recitation for 2 hours. Then, the cells were washed twice with culture medium, and MTT (0.5 mg/ml PBS) was incorporated to each well and incubated at 37°C for 4 h. Formazan was dissolved by isopropanol incorporation, and the absorbance was read at 540 nm using a microplate scanning spectrophotometer (ELISA Reader, OrganonTeknika, Netherlands) (17).

Cell Migration: A CytoSelect Cell Migration Assay Kit (Cell Biolabs, USA) was applied particularly to measure the effect of Quran recitation and cisplatin on the migration of PC-3 cells. The assay was conducted according to the manufacturer's guidelines. The 5×105 cells were suspended in 100 µl of RPMI without FBS and added to the upper chamber. In addition, 150 µl of culture medium with FBS was added to the bottom chamber and

incubated at 37°C for 18 h. Migratory cells passed through the membrane pores in response to the chemoattractant. Furthermore, these migratory cells were separated from the membrane (18).

Total RNA Extraction and Complementary DNA Synthesis: RNA was extracted using RNX solution. High-quality RNAs were chosen for complementary DNA (cDNA) synthesis. The cDNA synthesis was performed using RevertAidTM First Strand cDNA Synthesis Kit (Fermentas, USA).

Primers and Real-time Polymerase Chain Reaction: The sequence of forward and reverse primers for the PCR BCL2L12 gene was 5'-CAGCTACTCCAGACTTCTATGCTTTG-3' and 5'- CAGTATGGCTTCCTTCTCTGTCG-3', respectively. Moreover , for housekeeping gene, namely TBP (TATA-binding protein), forward and reverse primer sequences were 5'-AATCATGAGGATAAGAGAGCCACG-3' and 5'-AGTCTGGACTGTTCTTCACTCTTGG-3', respectively (19).

Power SYBR Green PCR Master Mix (Applied Biosystems, Warrington, UK) was employed to perform quantitative PCR on the Rotor-Gene 6000 (Corbett Research, Australia). The thermal-cycling conditions of the PCR included 10 min at 95°C (one repeat) as primary denaturation and hot-start enzyme activation, followed by 40 cycles at 95°C for 15 sec and 60°C for 1 min.

In this case, each complete amplification stage was followed by a melting stage at 95°C, 60°C, and 95°C for 15, 30, and 15 sec, respectively. The PCR amplification was performed in 25 µl reaction containing 12.5 µl Power SYBR Green PCR Master Mix (2x), 1 µl forward primer (0.4 µM), 1 µl reverse primer (0.4 µM), 5 µl first strand cDNA (100 ng), and 5.5 µl double-distilled water. In the current study, *BCL2L12* and TBP primers designed in previous research (18) were used.

Calculation of gene expression was performed using the comparative threshold cycle (Ct). The mean threshold cycle (mCt) was obtained from triplicate amplifications during the exponential phase. Then, mCt value of the reference gene (TBP) was subtracted from the mCt value of the target gene (BCL2L12) to obtain ΔCt and ΔΔCt values of each sample that were calculated from the corresponding Ct values as follows:

ΔΔCt=[Ct target - mCt reference] (treated sample) - [mCt target - mCt reference] (untreated sample)

Finally, BCL2L12 gene expression/tbp gene expression ratio was computed by the ratio formula 2-ΔΔCt(20).

Statistical Analysis: The data were analyzed in SPSS software (version 16, SPSS Inc., USA). Each test was repeated at least three times. The data were expressed as mean and standard error, and analyzed using one-way ANOVA with Dunnett's post hoc test for comparison. In conclusion, the P-values and expression proportion related to the tested genes were calculated using the Relative Expression Software Tool (REST). P-value less than 0.05 was considered statistically significant.

Result

Cisplatin and Quran Recitation Cytotoxicity on PC-3 Cells : In this study, the cytotoxicity of cisplatin was determined by treating PC-3 cells with various concentrations of cisplatin (0-100 µM) for 48 h followed by an MTT assay. the total cell numbers were decreased by a lower dose of cisplatin at 2.5 µM (13.38%; $P<0.05$) and a higher dose of cisplatin at 100 µM (98.13%; $P<0.001$), compared to the control group (Figure 1a). Moreover, the cytotoxic activity of cisplatin increased with the elevation of cisplatin concentration. The half maximal inhibitory concentration (IC50) of cisplatin was computed at 7.4 µM.

The percentage of surviving PC-3 cells that were exposed to Quran recitation for 2 h was 91.8% ($P<0.05$; Figure 1b). Figure 1c illustrates that the percentage of live cells significantly diminished 48 h after cisplatin treatment at 7.4 µM concentrations and exposure to Quran recitation (43.8%; $P<0.001$).

Inhibitory Effect of Cisplatin and Quranic Recitation on PC-3 Cell Migration: The obtained results of applying CytoSelect Cell Migration Assay Kit showed that cisplatin significantly reduced the migration of PC-3 cells. Compared to the control group, different

concentrations of cisplatin (2.5, 5, 10, 25, 50, and 100 µM) after 48 h resulted in the reduction of PC-3 cells migration by 7.25%, 11.27%, 16.56% , 20.6%, 27.57%, and 34.93% (P<0.05), respectively (Figure 1a).

The percentage of PC-3 cell migration exposed to Quran recitation for 2 h was 95.4% (P<0.05; Figure 1b). Figure 1c shows that Quran recitation significantly diminished the migration of PC-3 cells that were treated with 7.4 µM concentration of cisplatin (18.6%; P<0.001).

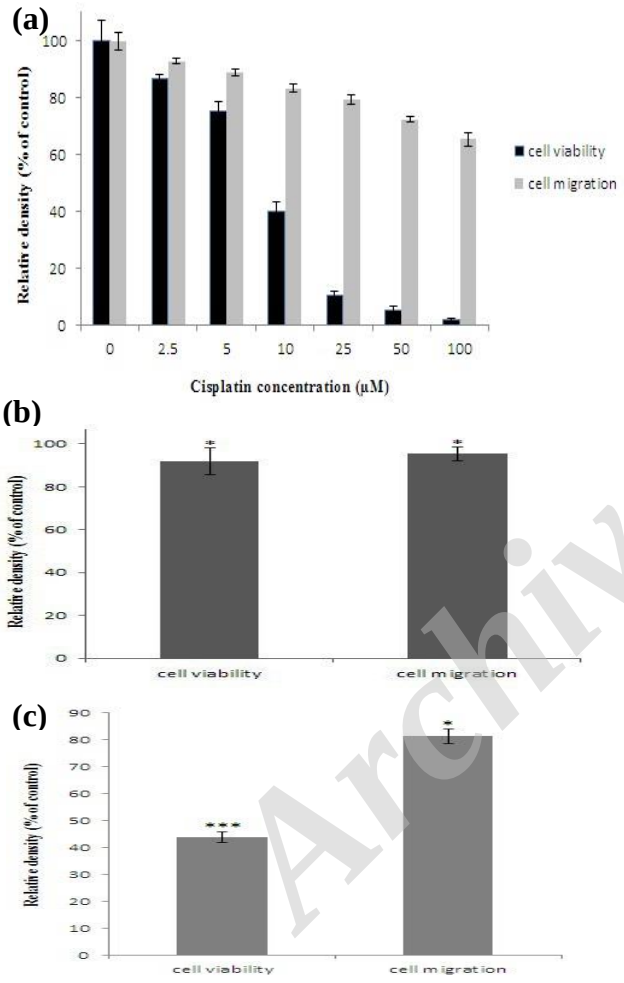

Figure 1. a) Comparison of dose-dependent effects of cisplatin on the viability and migration of the PC-3 cells (results are expressed as a percentage of inhibition rate, compared to control, and mean±standard deviation from at least three independent experiments (P<0.05), b) effects of exposure to Quran recitation for two hours on the viability and migration of the PC-3 cells (data are expressed as the mean±SD of at least three independent experiments), c) effects of exposure to Quran recitation for two hours on the viability and migration of PC-3 cells treated with 7.4 µM concentrations of cisplatin for 48 hours (data are expressed as the mean±SD of at least three independent experiments); asterisk indicates that treated group is statistically significant (*P<0.05, **P<0.01, *** P<0.001) from the control group.

Mitigatory Effect of Cisplatin and Quran Recitation on BCL2L12 mRNA Level: Exposure to cisplatin at IC50 concentrations for 48 h led to a significant decline in the mRNA level of BCL2L12 by a factor of 0.30, compared to the control group (P<0.001). Similarly, the PC-3 cells exposure to Quran recitation for 2 h significantly decreased the mRNA level of BCL2L12 by a factor of 0.48 (P<0.001). The ratio was calculated as 0.21 (P<0.001) for PC-3 cells that were treated with 7.4 µM concentration of cisplatin and also exposed to Quran recitation for 2 h. The P-values and expression ratio related to the genes were computed by the REST, and the investigated genes were normalized against TBP (Figure 2).

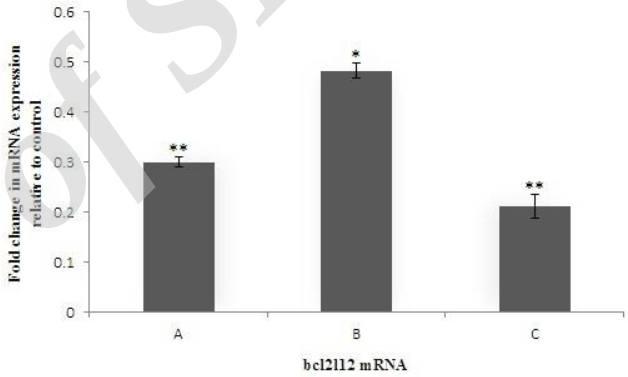

Figure 2. Inhibitory effects of cisplatin (A), *Quran* recitation (B), and cisplatin with *Quran* recitation on the expression of *BCL2L12* mRNA (all data were normalized against TBP, by REST software. Bars represent fold differences of the mean of normalized expression mean±SD. Significance was set at *P<0.05, **P<0.01, ***P<0.001.)

Discussion

The use of sound as an alternative and supplementary agent for therapeutic purposes has gained attention in recent years. This type of treatment is widely used as an alternative to clinical medicine in order to improve the health level of human life (21). Due to the fact that water (that acts as a good conductor for sound) forms about 70% of the body, sound can easily pass through the human body. The interaction between cells and sound has become a very challenging topic to be explored. Responses of human body cells to sound are a very complicated process (6).

Many studies stated that the response to sound not only may be limited to emotion, but

can also affect the very small structures of living mechanisms like cells (8). This hypothesis relates the in vitro direct effects of sound on non-auditory cells to mechanical stress, which is reasonable since sound is a mechanical vibration that can cause mechanical stress (8).

Previous studies suggested that cellular responses to sound depend on different factors, such as the intrinsic characteristics of cellular type and nature of sound (22). Therefore, it seems that the use of appropriate sound can improve the human healing process. Meanwhile, Aggio et al. (2012) added that sound can reduce biomass production in the yeast cells (23). Another report showed that audible sound can alter the proliferation and protein activity of cells. It was also suggested that sound healing was able to alter the cell cycle, morphology, and functional parameter in non-auditory human cells (4).

The use of Quran recitation as a technique in sound healing approaches seems to be promising. Quran recitation as a natural sound can have unique qualities that are capable of healing the cancerous cells. The findings of the current study are in line with those of other authors who showed that Quran recitation can have positive effects on the cancerous cells. This idea is supported by a study, which showed that the recitation of Quran as an alternative therapy can lead to the improvement of chronic diseases (14).

Tumiran et al. (2013) suggested that Quran recitation is a complementary sound healing technique that provides better outcomes as compared to routine sound healing (24). In another study, it was demonstrated that reciting or listening to Quran verses can have a promising effect on human cells (25). Quran recitation also has a fluent style, powerful expression, and relaxing sound. It has an inner tendency toward rhythm and harmony and can influence the audience (26). Babamohamadi et al. demonstrated that reciting the Holy Quran to a sick person will have considerable healing effects (12).

Tumiran et al. (2013) confirmed several deep points regarding healing with Quran recitation. It seems that the sound of Quran recitation can provide a deep spiritual energy to both audience and reciter. The healing process using Quran recitation can affect listeners even if they do not understand the meaning of the verses (24).

To the best of our knowledge, this study was the first attempt investigating the restrictive effects of Quran recitation, and the viability and migration capacities of PC-3 prostate cancer cells through examining BCL2L12 mRNA levels. The significant inhibitory effects of cisplatin on the proliferation and migration of PC-3 cells exposed to Quran recitation was more than that on PC-3 cells that were treated with cisplatin alone. Furthermore, these results were observed with regard to the expression of *BCL2L12*. Therefore, there was a reduction in the expression of this gene in PC-3 cells that were exposed to Quran recitation.

However, downregulation in the BCL2L12 gene was greater in PC-3 cells that were treated with cisplatin. Several studies have demonstrated that audible sound waves can alter cell proliferation. Jones et al. showed that human gingival fibroblasts that have been exposed to 261 Hz sound changed the rate of cell proliferation according to the time and amplitude of exposition (27). Therefore, it is not surprising that in this study, we observed decreased cell proliferation induced by Quran recitation.

Cisplatin is an important antineoplastic drug that can display a significant clinical activity against a wide variety of solid tumors. Although cisplatin has a widespread clinical use as an anticancer medication, molecular mechanisms leading to cell death after cisplatin treatment are not well-understood. However, cisplatin-DNA crosslink may induce programmed cell death (19).

This study showed that cisplatin treatment resulted in the downregulation of *BCL2L12* and was the main cellular event. BCL-2 family members, consisting of pro-apoptotic and anti-apoptotic genes, are the key regulators of DNA damage-induced apoptosis. Although *BCL2L12* is certainly involved in apoptosis, its anti-apoptotic or pro-apoptotic role still remains controversial depending on the cellular context.

A high expression of *BCL2L12* mRNA has been confirmed in many cancers.

Conclusion

Since the current study was the first report on the direct effects of Quran recitation on non-auditory cells in culture, more studies are needed to achieve further comprehension of these phenomena. Results of this investigation strongly suggested that Quran recitation could alter cell proliferation and migration in human PC-3 cells. Moreover, it was shown that *BCL2L12* was downregulated in PC-3 prostate adenocarcinoma cell lines that were exposed to Quran recitation. Our results revealed that Quran recitation can also induce apoptosis in these tumor cells through the downregulation of *BCL2L12* expression. Considering the type, frequency, range, and level of sound used during the cell culture, the effect of sound on human cells may be different. These results warrant further investigations in animal tumor models.

Conflict of interest

The author declares no conflict of interest.

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References

1. Gerber L, Bañez LL, Freedland SJ. Defining and treating high-risk prostate cancer: can we do better?. Eur Urol. 2010;58(1):8-9.
2. Kangmennaang J, Luginaah I. The influences of health insurance and access to information on prostate cancer screening among men in Dominican Republic. J Cancer Epidemiol. 2016;2016:7284303
3. Hassanipour S, Fathalipour M, Salehiniya H. The incidence of prostate cancer in Iran: a systematic review and meta-analysis. Prostate Int. 2018;6(2):41-45.
4. Sundararajan S, Vogelzang N. Chemotherapy in the Treatment of Prostate Cancer--The Past, the Present, and the Future. Am J Hematol Oncol. 2014;10(6):14-21.
5. Sarvaiya N, Kothari V. Effect of audible sound in form of music on microbial growth and production of certain important metabolites. Microbiol. 2015;84(2):227-35.
6. dos Reis Lestard N, Valente RC, Lopes AG, Capella MA. Direct effects of music in non-auditory cells in culture. Noise Health. 2013;15(66):307-14.
7. Miller DL, Smith NB, Bailey MR, Czarnota GJ, Hynynen K, Makin IRS, et al. Overview of therapeutic ultrasound applications and safety considerations. J Ultrasound Med. 2012;31(4):623-34.
8. Lestard NR, Capella MA. Exposure to Music Alters Cell Viability and Cell Motility of Human Nonauditory Cells in Culture. J Evid Based Integr Med. 2016;2016: 6849473.
9. Linnemann A, Wenzel M, Grammes J, Kubiak T, Nater UM. Music listening and stress in daily life—a matter of timing. Int J Behav Med. 2018;25(2):223-30.
10. Hassanien RH, Hou T-z, Li Y-f, Li B-m. Advances in effects of sound waves on plants. J Integr Agric. 2014;13(2):335-48.
11. Salarvand S, Pournia Y. Perception of medical university members from nutritional health in the Quran. Iran Red Crescent Med J. 2014;16(4):e10846.
12. Babamohamadi H, Sotodehasl N, Koenig HG, Jahani C, Ghorbani R. The effect of Holy Qur'an recitation on anxiety in hemodialysis patients: A randomized clinical trial. J Relig Health. 2015;54(5):1921-30.
13. Rafique R, Anjum A, Raheem SS. Efficacy of Surah Al-Rehman in Managing Depression in Muslim Women. J Relig Health. 2017:1-11.
14. Darabinia M, Gorji AMH, Afzali MA. The effect of the Quran recitation on mental health of the Iranian medical staff. J Nurs Educ Pract. 2017;7(11):30.
15. Pouralkhas S, Rajabi S, Pishgar A. Investigating the Rate of Quran Reciting by Persian Language and Literature Students in Comparison with Students of Other Fields and Its Effect on Depression, Anxiety and Stress. J Lang Teach Res. 2012;3(5):1004-8.
16. Mokhtari MJ, Akbarzadeh A, Hashemi M, Javadi G, Mahdian R, Ghasemi S, et al. Cisplatin Induces Up-Regulation of KAI1, a Metastasis Suppressor Gene, in MCF-7 Breast Cancer Cell Line. Trop J Pharm Res. 2012;11(4):523-9.
17. Koohpeima F, Mokhtari MJ, Doozandeh M, Jowkar Z, Yazdanshenas F. Comparison of Cytotoxicity of New Nanohybrid Composite, Giomer, Glass Ionomer and Silver Reinforced Glass Ionomer using Human Gingival Fibroblast Cell Line. J Clin Pediatr Dent. 2017;41(5):368-73.
18. Mokhtari MJ, Koohpeima F, Mohammadi H. A comparison inhibitory effects of cisplatin and MNPs-PEG-cisplatin on the adhesion capacity of bone metastatic breast cancer. Chem Biol Drug Des. 2017;90(4):618-28.
19. Taghavi MS, Akbarzadeh A, Mahdian R, Azadmanesh K, Javadi G. Cisplatin downregulates BCL2L12, a novel apoptosis-related gene, in glioblastoma cells. In Vitro Cell Dev Biol Anim. 2013;49(6):465-72.

20. Rao X, Huang X, Zhou Z, Lin X. An improvement of the 2ˆ (–delta delta CT) method for quantitative real-time polymerase chain reaction data analysis. Biostat Bioinforma Biomath. 2013;3(3):71.
21. Mahjoob M, Nejati J, Hosseini A, Bakhshani NM. The effect of Holy Quran voice on mental health. J Relig Health. 2016;55(1):38-42.
22. Ventura C. Fashioning cellular rhythms with magnetic energ y and sound vibration: a new perspective for regenerative medicine. CellR4. 2014;2(2): e839.
23. Aggio RBM, Obolonkin V, Villas-Bôas SG. Sonic vibration affects the metabolism of yeast cells growing in liquid culture: a metabolomic study. Metabolomics. 2012;8(4):670-8.
24. Tumiran MA, Mohamad SP, Saat RM, Yusoff MYZM, Rahman NNA, Adli DSH. Addressing sleep disorder of autistic children with Qur'anic sound therapy. Health. 2013;5(08):73-9.
25. Gunawan TS, Kartiwi M, editors. On the characteristics of various quranic recitation for lossless audio coding application. Computer and Communication Engineering (ICCCE), 2016 International Conference on; 2016.
26. Zulkurnaini NA, Kadir RSSA, Murat ZH, Isa RM, editors. The comparison between listening to al-Quran and listening to classical music on the brainwave signal for the alpha band. Intelligent Systems, Modelling and Simulation (ISMS), 2012 Third International Conference on; 2012.
27. Jones H, Feth L, Rumpf D, Hefti A, Mariotti A. Acoustic energy affects human gingival fibroblast proliferation but leaves protein production unchanged. J Clin Periodontol. 2000;27(11):832-8.