Websites: http://www.lifesciencesite.com http://www.sciencepub.net

Emails: editor@sciencepub.net sciencepub@gmail.com



Dose-response modeling using MTT assay: a short review

Safora Nikzad^{1*}, Milad Baradaran-Ghahfarokhi², Parto Nasri³

Department of Medical Physics, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.
^{2.} Department of Medical Physics, Isfahan University of Medical Sciences, Isfahan, Iran
^{3.} School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
<u>Safora2000n@yahoo.com</u>

Abstract: MTT assay is widely used to determine the response of cells to ionizing radiation. This method has several advantages such as relative low cost, short time to assess the samples and the possibility of acquiring the results semi-automatically. However, for this method, there are some restrictions/limitations and some important factors that should be considered. This paper is focused to discuss a standard procedure in using MTT assay for calculating the cell survival after irradiation.

[Nikzad S, Baradaran-Ghahfarokhi M, Nasri P. **Dose-response modeling using MTT assay: a short review**. *Life Sci J* 2021;18(6):41-47] (ISSN:1097-8135). <u>http://www.lifesciencesite.com.</u> <u>6</u>.doi:<u>10.7537/marslsj180621.06</u>.

Keywords: MTT assay, dose-response, ionizing radiation

1. Introduction

MTT assay is widely used to determine the survival curve of cells to ionizing radiation (1-6). In this method a short period of time is needed after irradiation to count the colonies and calculate the survival (6-13).

In 3-(4, 5-dimethylthiazol-2-yl)-2, 5diphenyltetrazoliumbromide (MTT) assay, viable cells reduce the yellow color of the MTT liquid to purple formazan crystals (13-19). The amount of the formazan crystals is proportional to the metabolic activity, and the crystals are produced by mitochondrial enzyme succinate dehydrogenase which is produced by live cells (3, 13-16, 20-22).

The most important point in using MTT assay instead of the clonogenic assay is that, there is no difference between the morphologic end point caused by various factors affecting either the metabolic activity or the inactivation of the cells (15). Moerover, using this method has some advantages such as: a short time taken to assess the samples, possibility of assessing large number of samples simultaneously, possibility of acquiring the results objectively and semi-automatically using an instrument, high reproducibility, lower number of the cells required, possibility of assessing cells growth rate, possibility of using it on several types of the cell cultures (monolayer cells, spheroids, and clones), and low cost (13-16, 23, 24). But in using this method truly some important points should be considered. In this paper, the standard method of using the MTT assays is described.

1.1. MTT assay

The 3-(4, 5-dimethylthiazol-2-yl)-2, 5diphenyltetrazoliumbromide (MTT) assay is the procedure of using yellow color of the MTT liquid to purple formazan crystals (19, 28-30). The amount of the formazan crystals is proportional to the metabolic activity, and the crystals are produced by mitochondrial enzyme succinate dehydrogenase which is produced by live cells (3, 4, 13-15, 23, 25, 31, 32). It has been shown that, the cells with poisoned mitochondria are able to produce the same amount of formazan when compared to the cells with normal mitochondria (3, 25, 33-35).

To perform the MTT assay the 96-well plates are used for every experimental condition. The MTT-medium is prepared from 5 mg/1cc MTT in phosphate-buffered saline which is filtered and kept in the dark at 4 °C. The MTT solution at appropriate concentrations (10 μ l MTT solution in each 100 μ l media) should added to each well and the plates should then incubated at 37°C for 4 hours. Following the incubation, the remaining MTT solution has to remove and 100 λ of DMSO added to each well to dissolve the formazan crystals. The plates have to shake for 5 minutes on a plate shaker to ensure adequate solubility. Absorbance readings of each well performed at a single suitable wavelength using a multi scan plate reader (3, 13, 15, 16, 36, 37).

Although MTT assay is simpler and more rapid than the clonogenic assay, but some notifications should be mentioned because this simple test may impress by some different factors, which are divided in two parts (3, 15, 38) that affect the spectrum of the produced formazan and amount of formazan produced per cell.

Therefore some important agents may help to have better results:

- 1. Using the proper mediums that could reduce the amount of light absorption in DMSO medium, which crystals will solved in.
- 2. Determining the best time to do the MTT test after irradiation of each cell line.
- 3. The spectrum of formazan, produced by cells, dissolved in DMSO is susceptible to pH. The peak shifts to a lower wavelength due to the addition of HC1 and color shift from purple-red to brown-red. Such spectrum shifts, will result in lower absorbance values, and can be overcame by adding a base such as NaOH to the solution.
- 4. A peak shift to a lower wavelength as observed after the addition of HC1 was also found with a formazan/DMSO which contained a residue of MTT-medium. To avoid this shift, it is essential to remove the MTT-medium as completely as possible before the addition of DMSO.
- 5. A spectrum shift may also occur as a result of the quality of the used DMSO. To remove this effect, the same DMSO medium should be used for all the samples, and, some free cell wells should be used which have tested with MTT and DMSO medium like the cell wells. Optical density (OD) of this wells have to subtract from the OD of the other wells (3, 15, 38).
- 6. When the number of cells increased and the culture medium went to acidic, culture medium of cells should be renewed every 2 or 3 days and also before adding the MTT medium.
- 7. The best incubating time for different cell lines is different and should be determine but almost the best time for much cells is 4 hour (15).

1.2. Calculating the survival fraction

Different methods have been proposed to calculate the cell survival by different researches (13, 16). In this section these methods are explained and compared. In the research done by Kim et al. in 1993, the MTT test was used to determine the survival curve of hepatoma cells and claimed to be an appropriate method for determining the cell survival

and dose-response curves due to the linear relationship between the cell numbers (<5000) and the MTT readings estimated by the following formula in which the test wells are those irradiated to ionizing radiation:

Survival fraction

$=\frac{\text{mean OD in test wells}-\text{mean OD in cell free wells}}{\text{mean OD in control wells}-\text{mean OD in cell free wells}}$ (1)

A linear relationship between the OD and live cells has confirmed in this study. The authors have done the MTT test for a period of up to 9-11 days post irradiation for various cells and concluded that when about 5000 cells are used in each well, the 7th day is an appropriate day for determining the cell survival curve (16).

But in other research that has been done by Price et al. in 1990, it has emphasized that, there is a non-linear relationship between the MTT reading for higher numbers (>20000) (3). However, the important conclusion made regarding this matter/limitation of the MTT test was that when a large number of cells (>20000) are used in the wells, a calibration curve is required to be calculated, but there will be no need for such calibration if the cell numbers is lower due to the linear relationship existed between the MTT readings and the cell numbers. This claim has also been proved by other researchers (31).

In addition, it has also pointed out that comparing simply the cell numbers of experimental groups at a given day (post irradiation) with that of the control group did not correctly provide the cell survival (3, 39, 40). This has been attributed to the fact that radiation causes a delay in the re-growth of the cells. As in the clonogenic test the cells ability to make even small clones is taken into account for calculating the growth rate, it is recommended that for the MTT test the survival rate of the irradiated cells should be measured continuously for several days post irradiation in order to determine the precise period in which the cells get back to their exponential growth phase and become similar to that of the controls (40-47). Thereafter, the cell survival should be calculated from the displacement of the irradiated cell growth from that of the controls when their growth curves become parallel to that of the controls. They have done this approach for the RT112 cell line and claimed that the relevant cell survival determined from the MTT assay had been in a good agreement with that of their clonogenic test (14).

In other investigation by Sieuwerts et al. in 1995, the application of the MTT assay for determining cells growth characteristics and survivals has been investigated in which, apart from the source of errors, the relationship between the samples cell numbers and ODs has been discussed (47-51). These authors have also proved that when the number of cells is low, there will be a linear relationship between the cell numbers and relevant ODs measured in MTT test (15).

In the other research by Buch et al. in 2012, the authors have determined and compared the cell survival curves using both of the MTT and clonogenic tests in an attempt to replace the clonogenic assay test with the MTT. They have pointed out that, in many studies that only the MTT test has been used, for determining cell survivals, some important characteristics and parameters such as: the cell doubling time, and the delay induced in irradiated cells and their growth behavior have been ignored (51-55). Therefore, they have proposed an appropriate method for determining the survival curves in which these confounding parameters are taken into account. For this purpose, they have used consequential MTT tests with several samples in which various numbers of cells have been used in each well and the numbers of clones have been counted for a period of up to 9 days post irradiation.

It was indicated that a more precise equation that has proposed for deriving the cell survival curve from the MTT test has been led to the results in good agreement with that of the clonogenic assay, especially with the clonogenic test with plating before irradiation (3, 4, 14, 41-43).

Then, the irradiated groups' delay time has been calculated based on the time differences of their growth curve with that of the controls at the exponential region of the curves and finally their survival fraction has been defined from the following equation:

SF

$$=2^{-(\frac{t_{delay}}{t_{doubling time}})}$$
(2)

In which the t_{delay} is the amount of the time required to pass in the exponential region of the irradiated cells to reach the same survival rate of the control group and $t_{doubling time}$ is the time period in which the number of the cells becomes double.

Following the above studies an investigation has done by our group in 2013 (55-61), to compare the formulas used in previous studies. The multiple MTT assay tests performed for several days after irradiation and the relevant data calculated. The doubling time of the two cancerous cell lines obtained from the growing curve of the control as well as the treatment groups exposed to different levels of ionizing radiation (61-64). In addition, by using the growing curves, the time delay, doubling time, and survival fraction of the two cancerous cell lines determined. using the methods and mathematical formulas proposed by Kim et al. and Buch et al. (13, 16). The results showed that, if the first common MTT assay based method of Kim et al is used; the test should be carried out, up to about 124 hours after the irradiation of the cell lines for the radiation dose levels below 4 Gy. But, when the second method is used, all the points drawn after various irradiation times as well as different dose levels (even high doses) are reliable and also independent from the conditions/limitations of the first method (64-71). Hence, the second MTT assay based method could be recommended to be used for drawing the survival curves of different cell lines instead of the clonogenic assay method. Even though, it seems to be more complicated and time consuming to implement and get all the required points compared to the other commonly used method (44, 71-78).

2. Conclusions

The MTT assay is a sensitive and accurate method and some researchers have used this method, considering its' several advantages such as relative low cost, short time to assess the samples and the possibility of acquiring the results semiautomatically. However, for this method, there are some restrictions/limitations and some important factors that should be considered further.

Corresponding Author:

Safora Nikzad Department of Medical Physics Faculty of Medical Sciences Tarbiat Modares University Tehran, Iran. E-mail: Safora2000n@yahoo.com

References

- 1. Hamburger AW, Salmon SE. Primary bioassay of human tumor stem cells. Science 1977;197:461-3.
- Salmon SE, Hamburger AW, Soehnlen B, Durie BG, Alberts DS, Moon TE. Quantitation of differential sensitivity of human-tumor stem cells to anticancer drugs. N Engl J Med 1978;298(24):1321-7.
- 3. Carmichael J, DeGraff WG, Gazdar AF, Minna JD, Mitchell JB. Evaluation of a

tetrazolium-based semi automated calorimetric assay: assessment of radiosensitivity. Cancer Res 1987;47(4):943-6.

- Alavian-Ghavanini A, Baradaran-Ghahfarokhi M. Comment on: MTT assay instead of the clonogenic assay in measuring the response of cells to ionizing radiation. J Radiobiol. 2014;1(1):15-16 (doi: 10.15171/jrb.2014.04).
- Ali Alavian-Ghavanini, Aminollah Bahaodini, Ehsan Salimi. The effect of whole body gamma irradiation on nitric oxide pathway of rat's aorta. J Radiobiol. 2014;1(1):9-13 (doi: 10.15171/jrb.2014.03).
- Baradaran-Ghahfarokhi M. Normal tissue complication probability modeling of radiation-induced bladder complications. J Radiobiol. 2014;1(1):19-20 (doi: 10.15171/jrb.2014.06).
- Williams JR, Zhang Y, Zhou H, Gridley DS, Koch CJ, Slater JM, Little JB. Overview of radiosensitivity of human tumor cells to lowdose-rate irradiation. Int J Radiat Oncol Biol Phys 2008;72(3):909-17.
- Eble MJ, Hensley FW, Flentje M, Schlotz A, Wannenmacher M. A modified computerassisted colorimetric microtitre assay (MTT) to assess in vitro radiosensitivity of V79, CaSki, HeLa and WiDr cells. Int J Radiat Biol 1994;65(2):193-201.
- 9. Kratzke RA, Kramer BS. Evaluation of in vitro chemosensitivity using human lung cancer cell lines. J Cell Biochem Suppl 1996;24:160-4.
- Foldbjerg R, Dang DA, Autrup H. Cytotoxicity and genotoxicity of silver nanoparticles in the human lung cancer cell line. Arch Toxicol 2010;85(7):743-50.
- 11. Fattahi-asl J, Baradaran-Ghahfarokhi M, Karbalae M, Baradaran-Ghahfarokhi HR, Haghighizadeh MH. Diagnostic performance of the human serum ferritin level decreased due to mobile phone exposure. J Res Med Sci. 2013;18(1):84.
- Hematti S, Baradaran-Ghahfarokhi M, Khajooei-Fard R, Mohammadi-Bertiani Z. Spiritual Well-Being for Increasing Life Expectancy in Palliative Radiotherapy Patients: A Questionnaire-Based Study. J Relig Health. 2014. (DOI: 10.1007/s10943-014-9872-9)
- Mosleh-Shirazi MA, Hadad K, Faghihi R, Baradaran-Ghahfarokhi M, Naghshnezhad Z, Meigooni AS. EchoSeed Model 6733 Iodine-

125 brachytherapy source: improved dosimetric characterization using the MCNP5 Monte Carlo code. Med Phys. 2012;39(8):4653-9.

- Nasri H. Journal of Radiobiology: A need of the hour. J Radiobiol. 2014;1(1):1-2 (DOI: 10.15171/jrb.2014.01).
- 15. Sieuwerts AM, Klijn JG, Peters HA, Foekens JA. The MTT Tetrazolium Salt Assay Scrutinized:How to Use this Assay Reliably to Measure Metabolic Activity of Cell Cultures in vitro for the Assessment of Growth Characteristics, IC50-Values and Cell Survival. Eur J Clin Chem Clin Biochem 1995;33:813-23.
- 16. Safora Nikzad, Bijan Hashemi. MTT assay instead of the clonogenic assay in measuring the response of cells to ionizing radiation. J Radiobiol. 2014;1(1):3-8 (doi: 10.15171/jrb.2014.02).
- Shahbazi-Gahrouei D. Radiobiological modeling in radiation oncology. J Radiobiol. 2014;1(1):17-18 (doi: 10.15171/jrb.2014.05).
- Shahbazi-Gahrouei D, Baradaran-Ghahfarokhi M. Investigation of patient dose from common radiology examinations in Isfahan, Iran. Adv Biomed Res. 2012;1:11.
- Shahbazi-Gahrouei D, Baradaran-Ghahfarokhi M. Assessment of entrance surface dose and health risk from common radiology examinations in Iran. Radiat Prot Dosimetry. 2013;154(3):308-13.
- Shahbazi-Gahrouei D, Karbalae M, Moradi HA, Baradaran-Ghahfarokhi M. Health effects of living near mobile phone base transceiver station (BTS) antennae: a report from Isfahan, Iran. Electromagn Biol Med. 2013. (DOI:10.3109/15368378.2013.801352)
- 21. Shahbazi-Gahrouei D, Mortazavi SM, Nasri H, Baradaran A, Baradaran-Ghahfarokhi M, Baradaran-Ghahfarokhi HR. Mobile phone radiation interferes laboratory immunoenzymometric assays: Example chorionic gonadotropin assays. Pathophysiology. 2012;19(1):43-7.
- 22. Shokrani P, Baradaran-Ghahfarokhi M, Zadeh MK. A novel approach in electron beam radiation therapy of lips carcinoma: a Monte Carlo study. Med Phys. 2013;40(4):041720.
- 23. Plumb JA, Milroy R, Kaye SB. Characterisation of a tetrazolium based chemosensitivity assay suitable for nonadherent small-cell lung cancer cell lines. Br J Cancer 1988; 58: 231-239.

- Henriksson E, Kjellén E, Wahlberg P, Wennerberg J, Kjellström JH. Differences in estimates of cisplatin-induced cell kill in vitro between colorimetric and cell count/colony assaysi. In Vitro cell Dev Biol 2006;42(10):320-3.
- 25. Romijn JC, Verkoelen CF, Schroeder FH. Application of the MTT assay to human prostate cancer cell lines in vitro: establishment of test conditions and assessment of hormone-stimulated growth and drug-induced cytqstatic and cytotoxic effects. Prostate 1988;12(1):99-110.
- 26. Hall E. Radiobiology for the Radiologist: Philadelphia: J.B. Lippincott Company; 1994.
- 27. Joiner M, Kogel AVD. Basic Clinical Radiobiology: Hodder Arnold; 2009.
- Hansen J, Bross P. A cellular viability assay to monitor drug toxicity. Methods Mol Biol 2010;748:303-311.
- 29. Roehm NW, Rodgers GH, Hatfield SM, Glasebrook AL. An improved colorimetric assay for cell proliferation and viability utilizing the tetrazolium salt XTT. J Immunol methods 1991;142(2):257-65.
- Gerlier D, Thomasset N. Use of MTT colorimetric assay to measure cell activation. J Immunol methods 1986;94:57-63.
- 31. Twentyman PR, Luscombe M . A study of some variables on a tetrazolium dye (MTT) based assay for cell growth and chemosensitivity. BrJ Cancer 1987;56(3):279-85.
- Azria D, Larbouret C, Cunat S, Ozsahin M, Gourgou S, Martineau P, Evans DB, Romieu G, Pujol P, Pèlegrin A. Letrozole sensitizes breast cancer cells to ionizing radiation. Breast Cancer Res 2005;7(1):156-63.
- 33. Denizot F, Lang R. Rapid colorimetrie assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. J Immunol Methods 1986;89(2):271-7.
- 34. Alley MC, Scudiero DA, Monks A, Hursey ML, Czerwinski MJ, Fine DL, Abbott BJ, Mayo JG, Shoemaker RH, Boyd MR. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. Cancer Res 1988;48(3):589-601.
- 35. Cole SP. Rapid chemosensitivity testing of human lung tumor cells using the MTT assay. Cancer Chemother Pharmacol 1986;17(3):259-63.

- Campling BG, Pym J, Baker HM, Cole SP, Lam YM. chemosensitivity testing of small cell lung cancer using the MTT assay Br J Cancer 1991;63(1):75-83.
- 37. Mitsudomi T, Kaneko S, Tateishi M, Yano T, Ishida T, Kohnoe S, Maehara Y, Sugimachi K. Chemosensitivity testing of human lung cancer tissues using the sussinate dehydrogenase inhibitation test. Anticancer Res 1990;10(4):987-90.
- Plumb JA, Milroy R, Kaye SB. Effects of the pH dependence of 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl-tetrazolium bromideformazan absorption on chemosensitivity determined by a novel tetrazolium-based assay. cancer Res 1989;49(16):44359-40.
- 39. Tolmach LJ. Growth patterns in X-irradiated HeLa cells. Ann NY AcadSci 1961;95:743-57.
- Elkind MM, Sutton H. Radiation response of mammalian cells grown in culture I.Repair of X-ray damage in surviving chinese hamester cells. Radiat Res 2012;178(2):8-26.
- 41. Stratford IJ, Stephens MA.The differential hypoxicity of bioreductive agents determined in vitro by the MTT assay Int J Radial Oncol Biol Phys 1989;16(4):973-6.
- Kawada K, Yonei T, Ueoka H, Kiura K, Tabata M, Takigawa N, Harada M, Tanimoto M. Comparison of chemosensitivity tests:clonogenic versus MTT assay. Acta Med Okayama 2002;56(3):129-34.
- Perez RP, Godwin AK, Handel LM, Hamilton TC. A comparison of clonogenic microtetrazolium and sulforhodamine B assays for determination of ciplatin cytotoxicity in human ovarian carcinoma cell lines. Eur J cancer 1993;3:395-9.
- 44. Abdi MR, Rezaee Ebrahim Saraee KH, Rezvani-Fard M KJ, Baradaran-Ghahfarokhi M. The evaluation of trace elements of interest in kidney disease in commonly consumed greenhouse vegetables in Isfahan, Iran: preliminary results. J Renal Inj Prev. 2014;3(2):51-53.
- 45. Ardalan MR. Parathyroid carcinoma in hemodialysis patients; it should not be diagnosed as a thyroid nodule. J Parathyr Dis. 2013;1(2):25-26.
- 46. Ardalan MR, Nasri H. Acute kidney injury; the focus of world kidney day in 2013. J Nephropharmacol. 2013;2(2):15-16.
- 47. Ardalan MR, Sanadgol H, Nasri H, Baradaran A, Tamadon MR, Rafieian-Kopaei R. Vitamin D therapy in diabetic kidney disease; current

- 48. Asgari A. Herbal medicines and kidney; friends or foes? J Nephropharmacol. 2014;3(1):5-6.
- 49. Ashrafi F, Haghshenas S, Nematbakhsh M, Nasri H, Talebi A, Eshraghi-Jazi F, et al. The role of magnesium supplementation in cisplatin-induced nephrotoxicity in a rat model: No nephroprotectant effect. Int J Prev Med. 2012;3(9):637-43.
- 50. Baradaran A. Antiphospholipid syndromeassociated nephropathy; a nephropathy needs classification. J Nephropharmacol. 2012;1(1):9-11.
- 51. Baradaran A. Primary hyperparathyroidism and kidney; recent findings. J Parathyr Dis. 2014;2(1):5-6.
- Baradaran A, Behradmanesh S, Nasri H. Association of body mass index and serum vitamin D level in healthy Iranian adolescents. Endokrynologia Polska. 2012;63(1):29-33.
- 53. Baradaran A, Nasri H. Correlation of serum magnesium with serum parathormone levels in patients on regular hemodialysis. Saudi Journal of Kidney Diseases and Transplantation. 2006;17(3):344-50.
- Baradaran-Ghahfarokhi M. Radiation-induced kidney injury. J Renal Inj Prev. 2012;1(2):49-50.
- 55. Gheissari A. Acute kidney injury and renal angina. J Renal Inj Prev. 2013;2(2):33-34.
- 56. Gheshlaghi F. Toxic renal injury at a glance. J Renal Inj Prev. 2012;1(1):15-16.
- 57. Haghighi M, Nematbakhsh M, Talebi A, Nasri H, Ashrafi F, Roshanaei K, et al. The role of angiotensin II receptor 1 (AT1) blockade in cisplatin-induced nephrotoxicity in rats: Gender-related differences. Renal Failure. 2012;34(8):1046-51.
- 58. Hajian S. Renoprotective effects of green tea. . J Nephropharmacol. 2013;2(2):21-22.
- Hajivandi A, Amiri M. World diabetes day: diabetes mellitus and nephrology. J Nephropharmacol. 2013;2(2):31-32.
- Hajivandi A, Amiri M. World Kidney Day 2014: Kidney disease and elderly. J Parathyr Dis. 2014;2(1):3-4.
- 61. Karimifar M. Thrombotic thrombocytopenic purpura treated with rituximab in systemic lupus erythematosus. J Renal Inj Prev. 2012;1(2):53-54.

- 62. Mardani S, Nasri P, Tavakoli M. Contrast induced nephropathy; recent findings. J Nephropharmacol. 2013;2(2):27-30.
- 63. Nasri H. Serum C-reactive protein (CRP) in association with various nutritional parameters in maintenance hemodialysis patients. Bratislavské Lekárske Listy. 2005;106(12):390-95.
- 64. Nasri H. Atypical presentations of the sarcoidosis with kidney involvement. J Renal Inj Prev. 2012;1(2):51-52.
- 65. Nasri H. Cisplatin therapy and the problem of gender-related nephrotoxicity. J Nephropharmacol. 2013;2(2):13-14.
- 66. Nasri H. The awareness of chronic kidney disease and aging; the focus of world kidney day in 2014. J Nephropharmacol. 2014;3(1):1-2.
- 67. Nasri H, Mubarak M. Renal injury due to vitamin D intoxication; a case of dispensing error. J Ren Inj Prev. 2013;2:85-87.
- 68. Nasri H, Mubarak M. Renal injury due to vitamin D intoxication; a case of dispensing error. J Renal Inj Prev. 2013;2:85-87.
- 69. Nasri H, Rafieian-Kopaei M. Association of serum vitamin D level with age in individuals with normal renal function. J Nephropharmacol. 2012;1(1):7-9.
- Nasri H, Baradaran A. Correlation of serum magnesium with dyslipidemia in maintenance hemodialysis patients. Acta medica (Hradec Kralove) / Universitas Carolina, Facultas Medica Hradec Kralove. 2004;47 (4):263-65.
- Nasri H, Baradaran A. The influence of serum 25-hydroxy vitamin D levels on Helicobacter Pylori Infections in patients with end-stage renal failure on regular hemodialysis. Saudi journal of kidney diseases and transplantation. 2007;18 (2):215-19.
- Nasri H, Baradaran H-R. Lipids in association with serum magnesium in diabetes mellitus patients. Bratislava Medical Journal. 2008;109(7):302-06.
- 73. Nasri H, Nematbakhsh M, Ghobadi S, Ansari R, Shahinfard N, Rafieian-Kopaei M. Preventive and curative effects of ginger extract against histopathologic changes of gentamicin-induced tubular toxicity in rats. Int J Prev Med. 2013;4(3):316-21.
- 74. Nasri H, Yazdani M. The relationship between serum LDL-cholesterol, HDL-cholesterol and systolic blood pressure in patients with type 2 diabetes. Kardiologia Polska. 2006;64(12):1364-68.

- 75. Nematbakhsh M, Ashrafi F, Safari T, Talebi A, Nasri H, Mortazavi M, et al. Administration of vitamin E and losartan as prophylaxes in cisplatin-induced nephrotoxicity model in rats. J Nephrol. 2012;25(3):410-17.
- 76. Nematbakhsh M, Pezeshki Z, Moaeidi B-A, Eshraghi-Jazi F, Talebi A, Nasri H, et al. Protective role of silymarin and deferoxamine against iron dextran-induced renal iron deposition in male rats. Int J Prev Med. 2013;4(3):286-92.

2/11/2021

- 77. Rouhi H, Ganji F, Nasri H. Effects of ginger on the improvement of asthma [The evaluation of its' treatmental effects]. Pakistan Journal of Nutrition. 2006;5(4):373-76.
- 78. Shirani M, Davoudian A, Sharifi A. Retroperitoneal fibrosis associated with propranolol: a case report; is corticosteroid administration necessary after ureterolysis? J Renal Inj Prev. 2013;2(2):67-69.