WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 1, 1809-1819. Research Article ISSN 2277– 7105

ROSEMARY EXTRACT ATTENUATES APOPTOTIC EFFECT OF ASPARTAME IN LIVER OF MALE RATS

Manar Salah[1], Samiha M. Abd El Dayem[1], Mahmoud M. Arafa[2] and Nawal Zakaria Haggag[1*]

[1]Zoology Department, Faculty of Women for Arts, Science and Education, Ain Shams University, 11757, Cairo, Egypt.

[2]Chemistry Department, Animal Health Research Institute, Cairo, Egypt.

Article Received on
22 Nov. 2019,

Revised on 12 Dec. 2019,
Accepted on 01 Jan. 2020,

DOI: 10.20959/wjpr20201-16584

***Corresponding Author**
Nawal Zakaria Haggag
Zoology Department, Faculty of Women for Arts, Science and Education, Ain Shams University, 11757, Cairo, Egypt.

ABSTRACT

Impaired apoptosis plays a central role in the development of diseases such as cancer or autoimmune diseases. Low calorie and non-weight-bearing nutritional replacements as aspartame can cause cellular toxicity via inflammatory and apoptotic pathway which finally may lead to severe cellular effects with incidence of cancer. On other side, natural antioxidants are receiving strong attention as effective alternatives to protect humans against any harmful effects produced from synthetic substances. Consequently, in this study aqueous extract of rosemary (*R. officinalis*), has been used as a natural antioxidant to evaluate its role against aspartame-induced apoptosis in rat liver. Daily oral doses were used at 125 mg/kg.b.wt for rosemary against 250 mg/kg.b.wt for aspartame. Rosemary extract which was co-administered, pre-administered or post-administered of aspartame showed a powerful capacity to improve apoptotic proteins expressions; caspase-3 and Bax, and anti-apoptotic protein, Bcl2 along with anti-inflammatory role represented by increase of IL-10 expression. Thus, rosemary extract has anti-apoptotic and anti-inflammatory role against aspartame toxicity.

KEYWORDS: Apoptosis, Rosemary, IL-10, Liver, Aspartame.

INTRODUCTION

Programmed cell death (PCD; apoptosis) is a biological cell death manner which is critical for development, homoeostasis as well as stress responses.[1] Dead cells produced by

apoptosis are engulfed by macrophages and the body generates the same number of died cells to maintain its homeostasis.[2]

The apoptotic pathway is organized by the Bcl-2 (B-cell lymphoma-2) family of proteins, which contains both pro-apoptotic and anti-apoptotic members that control the decision between cell life and death.[3] However the imbalance between apoptotic and anti-apoptotic proteins leads to irreversible towards cellular death.[4] Thus, impaired apoptosis plays a direct role in the development of diseases as cancer or autoimmune diseases, and limits the efficacy of conventional cytotoxic therapies.[5]

As people are progressively interested in physical health and fitness, so low calorie and non-weight-bearing nutritional replacements such as aspartame have been wanted.[6,7] Unfortunately, aspartame produces severe cellular toxicity via inflammatory and apoptotic pathway, and consumption of aspartame may lead to severe cellular effects with incidence of cancer and it was recommended that using of aspartame should be taken under medical supervision along with natural antioxidants.[6]

However, natural antioxidants such as those used from herbs and spices has been considered as an optimistic approach as effective alternatives to protect humans from any possible harmful effects afterward synthetic substances[8], as well as they provide extra benefits due to the incidence of the bioactive compounds.[8,9] In this regard, natural extracts of the *Lamiaceae* family, such as rosemary (*R. officinalis*), have been studied for its bioactive properties and ability to reduce destructive effects of free radicals.[10]

The effects demonstrated by rosemary include its ability to attenuate asthma, cataract, atherosclerosis, renal colic, peptic ulcer, hepatotoxicity, inflammatory diseases, neuro-protective, depressive behavior and others.[9]

In the first part of this study, authors focused on the capacity of rosemary extract on the scavenger of oxidative stress caused by aspartame in rat liver. However, the current study tries to give more lights on the cellular toxicological effects of aspartame and scoped on the role of rosemary extract in the attenuation of apoptotic features in liver tissue of male rats.

MATERIALS AND METHODS

Experimental animals

Male albino rats (120-140 g) were obtained from the National Research Center (Cairo). All rats were housed at a temperature of (24±2°C) and (12 h light/dark cycles). Standard laboratory diet was provided with water and food to the animals. Rats were left to acclimatization for two weeks before the experiment. Also, animals cared for according to the guidelines for animal experiments which were approved by Ethical Committee of Ain Shams University, Cairo, Egypt.

Chemicals

Aspartame (C14H18N2O5): Was purchased from Sigma Chemical Company (Louis, Mo, USA).

Rosemary extract

Rosemary leaves (were purchased from a local supermarket (Cairo, Egypt). Extraction was done according to Botsoglou et al.[11] and as mentioned in our previous study.[12] In brief, the extract was prepared by refluxing rosemary leaves with bi-distilled H_2O for 36 hours (12 hours × 3). Water was evaporated and the powder was re-dissolved in bi-distilled water before administration.

Experimental design

Animals were distributed into 6 groups (n=10 rats/group): The 1st group is the control (Con) left with no treatment. The 2nd group is (Rose), rats were treated with oral dose of rosemary extract (125 mg/ kg b.wt, daily) for two months.[13] The 3rd group is (Asp), rats administrated oral dose of aspartame (250 mg/ kg b.wt, in distilled water, daily) for two months.[14] The 4th group is (Rose + Asp) was co-treated with rosemary and aspartame (as the same previous corresponding doses, route of administration and period). The 5th group is (Rose then Asp), rats were given rosemary for two hours then aspartame for two months. The 6th group is (Asp then Rose) was given aspartame for two months then rosemary for two months. After the experimental period, rats were sacrificed after ether inhalation. Tissue samples from liver were dissected, washed and frozen for the experimental analysis.

PCR analysis

Caspase-3, Bax, Bcl2 and IL-10 gene expressions in liver tissue were measured using QRT-PCR (Quantitative real time polymerase chain reaction) method. Briefly, liver tissues of all

rats were used for RNA extraction by using Qiagen tissue extraction kit (Qiagen, USA). Total RNA was used for cDNA conversion according to manufacturer's instructions by using high capacity cDNA reverse transcription kit, Fermentas, USA.[17] β-actin gene was used as a reference. SYBR Green was used for monitor a double stranded synthesis of DNA. The amplifications were completed by using (40 cycles of denaturation at 95 C^o/ 15 and annealing and extension at 60 C^o /60 seconds). Amplification and analysis were done by using step One Plus real time thermal cycler (Applied Biosystems, Life technology, USA).[17] The following primers were used.

Gene	Forward primer (5′---3′)	Reverse primer (3′---5)
β-actin	TCCTCCTGAGCGCAAGTACTCT	GCTCAGTAACAGTCCGCCTAGAA[15]
Caspase-3	AGTTGGACCCACCTTGTGAG	AGTCTGCAGCTCCTCCACAT[15]
Bax	CACCAGCTCTGAACAGATCATGA	TCAGCCCATCTTCTTCCAGATGGT[15]
Bcl2	CACCCCTGGCATCTTCTCCTT	AGCGTCTTCAGAGACAGCCAG[15]
IL-10	CACAAAGCAGCCTTGCAGAA	AGAGCAGGCAGGATAGCAGTG[16]

RESULTS AND DISCUSSION

Caspases (cysteine-aspartic proteases or cysteine-dependent aspartate-directed proteases) such as caspase-3 are a family of cysteine proteases that play an essential role in apoptosis.[18] Bcl-2 is a key regulator of apoptosis, promotes cell survival either by inhibiting factors that activate caspases or regulating apoptosis by antagonizing the formation of heterodimers with other Bcl-2 family members.[19] Bax (Bcl-2 associated X) protein was the first identified pro-apoptotic member of the Bcl-2 protein family, on the other hand, binds to the anti-apoptotic Bcl-2 protein and thus acts by antagonizing the function of Bcl-2 to inhibit apoptosis.[20]

In the current study the administration of ASP (250 mg/kg) for two months is considered as a remarkable stressor of apoptosis in rat liver. Where pro-apoptotic markers represented by gene expressions of caspase-3 and Bax protein increased markedly ($p< 0.001$) in the liver of ASP group comparing to control with changes of (342% (4.4 folds) and 193% (2.9 folds)) respectively. While anti-apoptotic marker represented by gene expression of Bcl-2 decreased with 0.27 folds (-72.2%), $p< 0.001$. Table1.

It was proved that, aspartame is a kay factor to induce apoptosis; where aspartame up-regulated Bax and caspase-3 and down-regulated Bcl-2, suggesting an activation of cellular apoptosis.[21]

On view of the first part of this study and based on the previous investigations, the apoptotic effect of aspartame is due to increased formation of formaldehyde which is produced by the oxidation of methanol.[12, 22] Thus, the apoptotic induction of aspartame is mainly due to its action as a stressor for free radicals and their harmful effects on cellular and organillar membranes. Where reactive oxygen species and loss of mitochondrial membrane permeability are accompanying with the performance of the apoptosis pathways; extrinsic death receptor and intrinsic mitochondrial pathway.[6, 22] Moreover, the generation of free radicals induced mitochondrial-dependent apoptosis via activation of BAX protein which is trans-located from cytosol to mitochondria and increasing permeability leading to cytochrome release and activation of caspases.[23]

On other side, rosemary administration caused improvement in apoptotic and anti-apoptotic markers. Where caspase-3 and Bax gene expressions showed marked decreases after all treatments with rosemary as comparing to ASP group, $p< 0.001$; caspase-3 decreased with changes of (-32.9%, Rose +Asp, -43.5%, Rose then Asp and -61 %, Asp then Rose) from ASP. Also, Bax decreased with changes of (-32%), (-37%) and (-46.7%), respectively from ASP. On the other hand, after rosemary treatment, Bcl-2 expressions increased as comparing to ASP group ($p<0.05$, Rose +Asp, $p<0.01$, Rose then Asp, and $p<0.001$, Asp then Rose) with changes of (134.7%), (186.9%) and (208.6%), respectively (Table1 and Figure 1).

These data reflects the anti-apoptotic capability of rosemary extract. This is in accordance with the previous studies which documented that rosemary extract has anti-apoptotic capacity.[24] The first part of this study, proved the strong anti-oxidative capacity of rosemary extract in liver tissue against aspartame.[12] SO, the attenuated effects of rosemary on apoptosis might be mediated through the large amounts of phenolic and flavonoid compounds and to the antioxidant, anti-inflammatory or free radical-scavenging properties of this plant.[25] Also, the antioxidant molecules from rosemary not only act as free radical scavengers but also play a role in regulating the activity and/or expression of certain enzymatic systems associated in appropriate physiological processes like apoptosis and intracellular signal transduction.[26]

More to the point, several studies described crosstalk between inflammatory mediators and apoptosis.[27, 28] According to the results which obtained in our previous study;[12], aspartame increased TNF-α level which is one of the important factors that affect apoptotic action by aspartame, once gets activated by the oxidative stress TNF-α releases and binds to TNF

receptor-1 which leads to the initiation of cleaving pro-caspase-8 to caspase-8 that activates caspase-3 and initiates apoptosis.

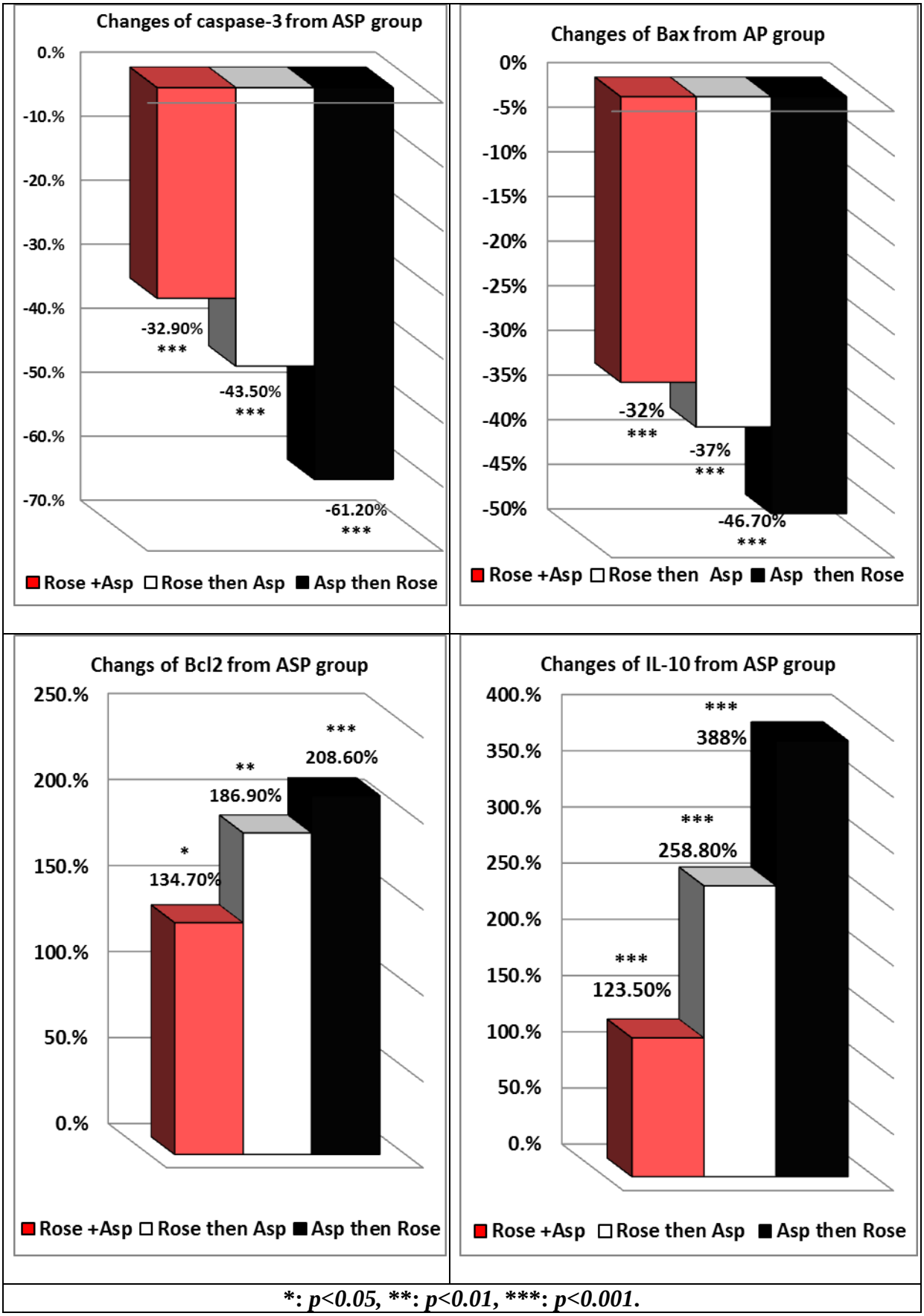
On opposite side, IL-10 is considered as inhibitory factor; it has ability to inhibit production of pro-inflammatory cytokines as TNF-α.[29] Additionally, IL-10 is considered to be the essential immunosuppressive cytokine, and its capability to resolve inflammation, autoimmune disorders, inflammatory diseases, and cancer through regulation of innate immunity, and promotion of tissue repairing mechanisms.[30]

Therefore, the elevation of IL-10 by rosemary in this study confirmed the marked anti-inflammatory activity of this nature plant. Recent articles reviewed that rosemary targets several deregulated pathways related with cancer, inflammation and apoptotic related proteins.[8, 9] Moreover, carnosic acid and carnosol in rosemary have powerful synergistic activities on anti-inflammation and anti-cancer.[10]

Table 1: Effects of rosemary (Rose) on the apoptotic and anti-apoptotic markers as well as IL-10 in liver of aspartame (Asp)-treated rats.

Parameters / Group	Caspase-3 Relative exp.	Bax Relative exp.	Bcl2 Relative exp.	IL-10 Relative exp.
Con	1.09±0.05	1.00±0.03	0.83±0.15	1.10±0.02
Rose	1.18±0.06	1.04±0.02	0.93±0.08	1.06±0.05
	N.S	N.S	N.S	N.S
Asp	4.82±0.08	2.93±0.04	0.23±0.13	0.17±0.04
	a***	a***	a***	a***
Rose +Asp	3.23±0.04	1.99±0.03	0.54±0.07	0.38±0.02
	b***	b***	b*	b***
Rose then Asp	2.72±0.03	1.84±0.02	0.66±0.05	0.61±0.01
	b***	b***	b**	b***
Asp then Rose	1.87±0.11	1.56±0.03	0.71±0.08	0.83±0.05
	b***	b***	b***	b***

The results are presented as Mean ± SE (n=10). N.S= non- significant comparing to control (Con).
a: Significant change from Con. b: Significant change from Asp.
*: *p<0.05*, **: *p<0.01*, ***: *p<0.001*.



*: p<0.05, **: p<0.01, ***: p<0.001.*

Figure (1): Changes of apoptotic and anti-apoptotic markers as well as IL-10 in liver of male rats after rosemary treatments comparing to ASP.

CONCLUSION AND RECOMMENDATION

On sight of the results obtained in the current study, it can be concluded that the leaf extract of *R. officinalis* reduces the apoptotic effect of aspartame via the scavenger capacity for free radicals and anti-inflammatory effect in liver tissue of rats. Therefore, it may be recommended to include rosemary in healthy lifestyle.

ACKNOWLEDGEMENT

Authors acknowledge staff members of the Department of Biochemistry and Toxins & Nutritional Deficiencies, Animal Health Research Institute, Cairo, Egypt. for their providing the required facilities in the experimental analysis**.** This research did not receive any specific grant from funding agencies in public, commercial, or not-for-profit sectors**.**

REFERENCES

1. Rogers C, Erkes DA, Nardone A, Aplin AE, Fernandes-Alnemri T, Alnemri ES. Gasdermin pores permeabilize mitochondria to augment caspase-3 activation during apoptosis and inflammasome activation. Nature communications, 2019; 10(1): 1689.
2. Nagata S. Apoptosis and clearance of apoptotic cells. Annual review of immunology, 2018; 36: 489-517.
3. Singh R, Letai A, Sarosiek K. Regulation of apoptosis in health and disease: The balancing act of Bcl-2 family proteins. Nature Reviews Molecular Cell Biology, 2019; 20(3): 175-193.
4. Knight T, Luedtke D, Edwards H, Taub JW, Ge Y. A delicate balance-the Bcl-2 family and its role in apoptosis, oncogenesis, and cancer therapeutics. Biochemical pharmacology, 2019; 162: 250-261.
5. Adams JM, Cory S. The Bcl-2 arbiters of apoptosis and their growing role as cancer targets. Cell death and differentiation, 2018; 25(1): 27.
6. Otman S, Bin-Jumah M. Histopathological effect of aspartame on liver and kidney of mice. International Journal of Pharmacology, 2019; 15(3): 336-342.
7. Johnson RK, Lichtenstein AH, Anderson CA, Carson JA, Després J-P, Hu FB, et al. Low-calorie sweetened beverages and cardiometabolic health: A science advisory from the american heart association. Circulation, 2018; 138(9): e126-e140.
8. de Oliveira VS, Ferreira FS, Cople MCR, Labre TdS, Augusta IM, Gamallo OD, et al. Use of natural antioxidants in the inhibition of cholesterol oxidation: A review. Comprehensive Reviews in Food Science and Food Safety, 2018; 17(6): 1465-1483.

9. de Oliveira JR, Camargo SEA, de Oliveira LD. *Rosmarinus officinalis l.*(rosemary) as therapeutic and prophylactic agent. Journal of biomedical science, 2019; 26(1): 5.
10. Nieto G, Ros G, Castillo J. Antioxidant and antimicrobial properties of rosemary (*rosmarinus officinalis, l.*): A review. Medicines, 2018; 5(3): 1-13.
11. Botsoglou N, Taitzoglou I, Zervos I, Botsoglou E, Tsantarliotou M, Chatzopoulou P. Potential of long-term dietary administration of rosemary in improving the antioxidant status of rat tissues following carbon tetrachloride intoxication. Food and chemical toxicology, 2010; 48(3): 944-950.
12. Salah M, Abd-El Dayem SM, Arafa MM, Haggag N.Z. Hepatoprotective effect of rosemary extract against aspartame-induced liver toxicity in male rats. European Journal of Biomedical and Pharmaceutical Sciences, 2019; 6(7): 11-19.
13. Abdella EM, Ahmed R. Suppression of doxorubicin apoptotic, histopathologic, mutagenic and oxidative stress effects in male mice bone marrow and testis tissues by aqueous rosemary leaves extract. Iranian Journal of Cancer Prevention, 2012; 2(1): 35-49.
14. Christian B, McConnaughey K, Bethea E, Brantley S, Coffey A, Hammond L, et al. Chronic aspartame affects T-maze performance, brain cholinergic receptors and Na^{+}, K^{+}-ATPase in rats. Pharmacology biochemistry and behavior, 2004; 78(1): 121-127.
15. Lebda MA, Sadek, K. M., & El-Sayed, Y. S. Aspartame and soft drink-mediated neurotoxicity in rats: Implication of oxidative stress, apoptotic signaling pathways, electrolytes and hormonal levels. Metabolic brain disease, 2017; 32(5): 1639-1647.
16. Khalil A, Omran H, Alsheikh F. Balance of pro-and anti-inflammatory cytokines in livers of high fat diet rats exposed to fractionated gamma irradiation. BMC research notes, 2018; 11(1): 741.
17. El Asmar MF, Atta HM, Mahfouz S, Fouad HH, Roshdy NK, Rashed LA, et al. Efficacy of mesenchymal stem cells in suppression of hepatocarcinorigenesis in rats: Possible role of wnt signaling. Journal of Experimental & Clinical Cancer Research, 2011; 30(1): 49.
18. Renema P, Stevens T. Pseudomonas aeruginosa exoenzyme y induces caspase-3/7 release independent of apoptosis in pulmonary microvascular endothelial cells. A55 pedal to the metal: New insights into endothelial responses to lung injury: American Thoracic Society, 2019; A1981-A1981.
19. Zhang Y, Yang X, Ge X, Zhang F. Puerarin attenuates neurological deficits via Bcl-2/Bax/cleaved caspase-3 and SIRT3/SOD2 apoptotic pathways in subarachnoid hemorrhage mice. Biomedicine & Pharmacotherapy, 2019; 109: 726-733.

20. Xu X-F, Cheng R-B, Zhang X-J, Gao R-L. Total saponins in rubus parvifolius l. Induce lymphoma cells apoptosis through upregulated Bax/Fas and downregulated Bcl-2 in vivo and in vitro. Traditional Medicine Research, 2019; 4(2): 99-108.
21. Qu D, Jiang M, Huang D, Zhang H, Feng L, Chen Y, et al. Synergistic effects of the enhancements to mitochondrial ROS, p53 activation and apoptosis generated by aspartame and potassium sorbate in HepG2 cells. Molecules, 2019; 24(3): 457.
22. Allam WA, Mahmoud SF, Mahmoud AS. Protective effect of ascorbic acid and N-acetyl cysteine in aspartame induced nephrotoxicity in albino rats. The Egyptian Journal of Forensic Sciences and Applied Toxicology, 2019; 19(1): 107-128.
23. Nematollahi H, Haddadi G, Jorat M. The effect of vitamin C on apoptosis and Bax/Bcl-2 proteins ratio in peripheral blood lymphocytes of patients during cardiac interventional procedures. Journal of Biomedical Physics and Engineering. 2019. https://doi.org/10.31661/jbpe.v0i0.917.
24. Salvi V, Gianello V, Tiberio L, Sozzani S, Bosisio D. Cytokine targeting by mirnas in autoimmune diseases. Frontiers in immunology, 2019; 1: 15. DOI: 10.3389/fimmu.2019.00015.
25. Rasoulian B, Hajializadeh Z, Esmaeili-Mahani S, Rashidipour M, Fatemi I, Kaeidi A. Neuroprotective and antinociceptive effects of rosemary (rosmarinus officinalis l.) extract in rats with painful diabetic neuropathy. The Journal of Physiological Sciences, 2019; 69(1): 57-64.
26. Hamed H, Boulila S, Ghrab F, Kallel R, Boudawara T, El Feki A. The preventive effect of aqueous extract of rosemary (rosmarinus officinalis) leaves against the nephrotoxicity of carbon tetrachloride in mice. Archives of physiology and biochemistry, 2018; 1-8. DOI: 10.1080/13813455.2018.1508236
27. Sakai K, Nozaki Y, Murao Y, Yano T, Ri J, Niki K, et al. Protective effect and mechanism of IL-10 on renal ischemia–reperfusion injury. Laboratory Investigation, 2019; 99(5): 671-683.
28. Duvigneau JC, Luís A, Gorman AM, Samali A, Kaltenecker D, Moriggl R, et al. Crosstalk between inflammatory mediators and endoplasmic reticulum stress in liver diseases. Cytokine, 2019; 124: 154577. https://doi.org/10.1016/j.cyto.2018.10.018.
29. Saiki P, Kawano Y, Nakajima Y, Van Griensven LJ, Miyazaki K. Novel and stable dual-color IL-6 and IL-10 reporters derived from RAW 264.7 for anti-inflammation screening of natural products. International journal of molecular sciences, 2019; 20(18): 4620.

30. Wang X, Wong K, Ouyang W, Rutz S. Targeting IL-10 family cytokines for the treatment of human diseases. Cold Spring Harbor perspectives in biology, 2019; 11(2): a028548.