Inflammation, Vol. 42, No. 5, October 2019 (© 2019)
DOI: 10.1007/s10753-019-01023-z

ORIGINAL ARTICLE

Anti-histaminic Effects of Resveratrol and Silymarin on Human Gingival Fibroblasts

Amir Farzanegan,[1] Mohammad Shokuhian,[2] Soudeh Jafari,[3] Fatemeh Sadeghi Shirazi,[2] and Minoo Shahidi[2,4]

Abstract—Periodontitis as a chronic inflammatory disease leads to the destruction of the supportive tissues of affected teeth. Crosstalk between periodontitis and the host immune system plays a crucial role in the pathogenesis of this disease. Since polyphenol components such as silymarin and resveratrol have anti-bacterial and anti-inflammatory effects on periodontal tissues, the purpose of this study was to investigate the anti-histaminic effects of silymarin and resveratrol on human gingival fibroblasts (HGFs). HGFs were treated with a concentration of silymarin or resveratrol (100 μg/ml) and a combination of these two polyphenols (50/100 or 100/200 μg/ml silymarin/resveratrol). The effect of silymarin and resveratrol on cell viability was assessed by MTT assay. Also, HGFs were treated with silymarin and/or resveratrol and were stimulated by histamine. The levels of interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor alpha (TNF-α), and tissue plasminogen activator 1 (TPA-1) were assessed by enzyme-linked immunosorbent assay (ELISA). After treatment with silymarin, the viability of fibroblast cells significantly increased, whereas treatment with resveratrol and combinations of these flavonoids (silymarin 50 μg/ml and resveratrol 100 μg/ml) did not have any significant effect on cell viability after 24 h.

Highlights

- Inhibition of inflammatory effects of histamine by resveratrol and silymarin.
- Anti-histaminic effects of the combination of resveratrol and silymarin in periodontitis.
- Invigorating anti-histaminic response of human gingival fibroblasts via resveratrol and silymarin.

[1] Research Center for Prevention of Oral and Dental Diseases, Baqiyatallah University of Medical Sciences, Tehran, Iran
[2] Department of Hematology & Blood Banking, School of Allied Medical Sciences, Iran University of Medical Sciences, Tehran, 14155-5983, Iran
[3] Department of Oral Medicine, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[4] To whom correspondence should be addressed at Department of Hematology & Blood Banking, School of Allied Medical Sciences, Iran University of Medical Sciences, Tehran, 14155-5983, Iran. E-mail: shahidi.m@iums.ac.ir; shahidi.minoo@gmail.com

Treatment with 100/200 μg/ml silymarin/resveratrol significantly decreased the cell viability after 48 h. Resveratrol inhibited histamine-induced IL-6 secretion by HGFs significantly, whereas silymarin showed significant effect on TNF-α. A blend of silymarin and resveratrol displayed more valuable results. In conclusion, combination of resveratrol and silymarin could significantly inhibit inflammatory effects of histamine on cultured HGFs by reduction of IL-6, IL-8, TPA-1, and TNF-α.

KEY WORDS: resveratrol; silymarin; human gingival fibroblasts; histamine.

INTRODUCTION

Periodontitis is one of the most common chronic human diseases caused by microorganisms (gram-negative bacteria) and it can be accompanied with the destruction of gingival connective tissue and alveolar bone (as a supporting tissue for teeth) [1, 2]. Crosstalk between periodontopathogens forming the biofilm (as a primary etiological factor) with host's immune responses can play an important role in the pathogenesis of periodontitis [1]. Lipopolysaccharide (LPS) production by periodontopathogens can be associated with the production of several inflammatory mediators, including interleukin-8 (IL-8), IL-6, tumor necrosis factor alpha (TNF-α), prostaglandins, serotonin, and histamine through stimulating gingival fibroblasts that is the major constituent of gingival connective tissue [1, 3]. Increase of inflammatory markers in periodontitis can be associated with symptoms such as bleeding and swelling of the gingiva, bad breath, and tooth loss [1]. Histamine as a mediator of immune responses and inflammatory mediator is released by several cells, including mast cells. It can also be produced by cells containing histamine-forming enzyme and histidine decarboxylase (HDC) [3, 4]. Production of HDC can be induced by various inflammatory stimuli, including LPS, IL-8, IL-6, and TNF-α in various organs or tissues [4]. Therefore, an increase in production of HDC can be associated with enlarged histamine production. Additionally, inflammatory markers such as IL-1 and TNF-α can play a major role in increasing levels of tissue plasminogen activator 1 (TPA-1) in human gingival fibroblasts (HGFs) [5–7]. Subsequently, TPA-1 can induce expression of more pro-inflammatory chemokines by activating the nuclear factor-kappa B (NF-κB) pathway [8]. Therefore, an increase in TPA-1 levels can contribute to the destruction of pulp and periapical tissues through the rise of the pro-inflammatory chemokines and dysregulation of proteolysis [5].

Entirely, histamine along with inflammatory cytokines and bacterial components like LPS may play a role in pathogenesis of periodontitis. Growing evidence about the contribution of histamine in the development of periodontitis has been published and some of them suggested that therapeutic administration of antihistamines could improve symptoms of this disease [4, 9]. The effect of histamine as a stimulator of salivary secretion in submandibular gland (SMG) has been reported, and histamine could also prevent radiation-induced damage to SGM which is one of the main salivary glands [10]. Histamine has important effects on HGFs including promoting synthesis of cyclooxygenase 2 (COX2), and secretion of prostaglandin E2 (PGE2), increasing the expression of Toll-like receptors (TLR) 2 and 4, and increasing production of IL-8; consequently, histamine has a crucial role in activating and amplifying inflammatory responses of gingival fibroblasts. Histamine could also be considered as a predictive index in the outcome of periodontitis [4, 9, 11]. There are some traditional therapeutic modalities for periodontitis including surgical periodontal therapy and local or systemic antimicrobial therapy [1, 12]. However, due to the existence of some factors like antibiotic resistance, the use of natural compounds as moderators of host inflammatory response can be helpful in treating inflammation and tissue destruction. Polyphenols are one of the herbal origins of natural compounds that act as anti-bacterial and anti-inflammatory agents for periodontal tissues through powerful antioxidant activity and modulation of host inflammatory responses [1]. Resveratrol is one of the natural nonflavonoid polyphenolic phytoalexin compounds found in grapes, mulberries, peanuts, and red wine [13, 14]. Studies have reported that resveratrol can play a role in suppressing inflammation by reducing levels of IL-1β, IL-10, IL-6, IL-8, prostaglandin E synthases-1, TNF-α, nitric oxide synthase (iNOS), and COX2 *via* the downregulation of the p38 MAPK signaling and inhibition of the transcription function of NF-κB [2, 14, 15]. Silymarin, a component of the polyphenolic flavonolignans, extracted from seeds of milk thistle contains about 70–80% and 20–30%

polymeric and oxidized polyphenolic compounds [16, 17]. This polyphenol plays different roles including anti-carcinogenic, immunomodulatory, hepatoprotective, regenerative, and antioxidant as reported by various studies [17, 18]. This flavonoid can perform its anti-inflammatory action through suppression of NF-κB, which regulates gene products, including COX-2, PGE2, histamine, and inflammatory cytokines [2, 16, 19]. Considering some therapeutic features of resveratrol and silymarin, we aimed to investigate their anti-histaminic roles by examining their effects on the secretion of inflammatory cytokines by histamine-induced HGFs.

MATERIALS AND METHODS

Materials

We provided human gingival fibroblast cell line (HGF 3 - PI 53 NCBI code C502) from Pasteur Institute, Tehran, Iran. Silymarin, resveratrol, 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), and histamine were purchased from Sigma (Seelze, Germany). Enzyme-linked immunosorbent assay (ELISA) kits (IL-6, IL-8, TPA-1, and TNF-α) were bought from Abcam, UK. All reagents for cell culture were purchased from GIBCO Life Technologies (Paisley, UK).

Cell Culture and Treatment

Cells were cultured in Dulbecco modified Eagle's medium (DMEM) by adding 10% fetal bovine serum (FBS), 100 U/ml penicillin G sodium, 100 g/ml streptomycin sulfate, and L-glutamine, at 37 °C in a humidified 5% CO_2 atmosphere. Serial concentrations (50, 100, and 200 μg/ml) of silymarin or resveratrol were prepared by dissolving in 100% dimethyl sulfoxide (DMSO, Sigma). To investigate the combined effect of silymarin and resveratrol, cells were treated with 50 μg/ml of silymarin and 100 μg/ml of resveratrol or 100 μg/ml of silymarin and 200 μg/ml of resveratrol as obtained by MTT test.

Cell Viability Assay

Cell growth assessment was studied by MTT test. Human gingival fibroblast cells were seeded at 5×10^3 cells per 200 μl of medium in 96-well plates, and cultured for 1 day at 37 °C. Afterward, the cells were treated with different concentrations of silymarin, resveratrol, or a combination of both for 24 h and 48 h in the presence of histamine (10 μg/ml). MTT test was also carried out to detect cell viability for histamine with regard to previous study [20]. After adding 20 μl MTT (5 mg/ml) to each well of the cells, they were incubated for an extra 3 h. The supernatants were removed and 100 μl of DMSO was added to each well for solubilizing of Formosan violet crystals afterward. The absorbance was measured at 570-nm wavelength by spectrophotometry, subsequently.

ELISA

HGFs were seeded at 5×10^4 cells in four-well plates with 2% FBS and 1% penicillin-streptomycin. The cells were incubated at 37 °C and 5% CO_2 in air. After 1 day, HGFs were stimulated with histamine (10 μg/ml) immediately before treatment with different concentrations of silymarin and/or resveratrol, their combinations, and medium alone for 24 h. The culture medium was collected and stored at − 80 and IL-6, IL-8, TPA-1, and TNF-α levels were measured with ELISA kits according to the manufacturer's instructions (Abcam, UK). After 24-h incubation, the absorbance was measured at 586 nm using a colorimetric micro plate reader (Bio-Rad, Hercules, CA, USA).

Statistical Analysis

Excel software was used for data collection from at least three independent experiments. The distribution of the groups evaluated by a one-way analysis of variance, the post hoc Tukey's test, and Dunnett's test as analyzed by GraphPad Prism 5. Values of less than 0.05 were considered statistically significant.

RESULTS

MTT Cell Viability Assay

MTT test was evaluated in 24 and 48 h after treatment of HGF cells with 100 μg/ml silymarin, 100 μg/ml resveratrol, and combination of both with concentrations of 50/100 and 100/200 μg/ml of silymarin/resveratrol in the presence of histamine (10 μg/ml) in order to measure the cytotoxic doses of silymarin and resveratrol. There was a significant increase in the survival of gingival fibroblast cells at 100 μg/ml concentrations of silymarin in comparison with controls in 24 h ($P = 0.002$), while the survival of gingival fibroblasts cells did not change significantly with resveratrol treatment in 24 and 48 h. According to the last achieved results, separate analyses were performed to recognize the perfect concentration for the survival of cells with combinations of resveratrol and silymarin. The cells

showed a significant reduction in viability with a combination of 100 μg/ml silymarin and 200 μg/ml resveratrol in 48 h ($P = 0.015$, Fig. 1). Different concentrations of histamine alone were also selected as previously reported for MTT test [20]. However, no effect on cell proliferation was found from 10^0 to 10^2 μg/ml (data not shown).

Effect of Histamine on IL-6 and IL-8 Secretion in HGFs

After treatment with histamine (10 μg/ml for 24 h), the secretion of IL-6 and IL-8 significantly increased in comparison with the controls ($P = 0.04$ and $P = 0.003$, respectively). Resveratrol, at a concentration of 100 μg/ml, significantly reduced the secretion of IL-6 in the supernatant of the histamine-induced fibroblasts ($P = 0.008$), whereas the effect of silymarin was not significant at 100 μg/ml concentration compared with histamine alone. However, the two selected compounds of a 50 μg/ml silymarin and 100 μg/ml resveratrol and a 100 μg/ml silymarin and 200 μg/ml resveratrol could reduce IL-6 release, which was significant in the former combination, when compared with histamine alone ($P = 0.013$; Fig. 2a). No significant effects of 100 μg/ml concentrations of resveratrol and silymarin on IL-8 levels were found in the present study. Though, two different combinations (50 μg/ml silymarin/100 μg/ml resveratrol or 100 μg/ml silymarin/200 μg/ml resveratrol) showed significant effects on IL-8 secretion of the histamine-induced fibroblasts as compared with histamine alone ($P = 0.0034$ and $P = 0.0021$; Fig. 2b).

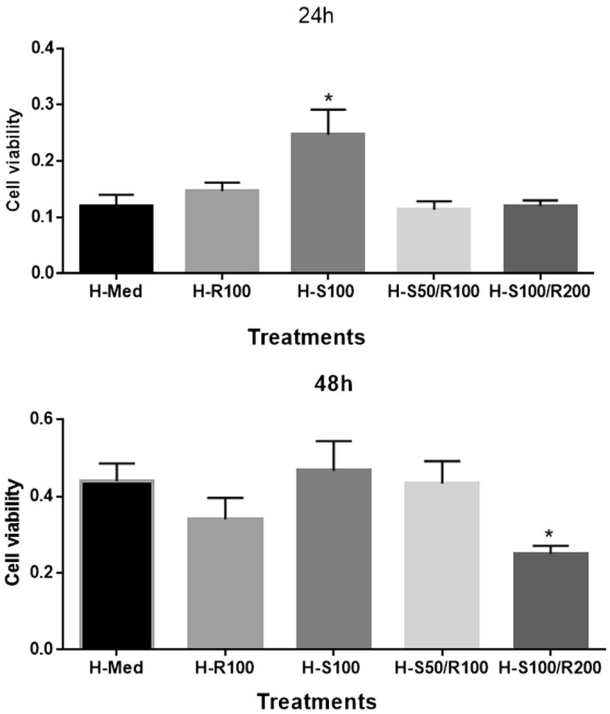

Fig. 1. Cell viability test. Various treatments of HGFs with silymarin (100 μg/ml), resveratrol (100 μg/ml), and their combinations (50 μg/ml silymarin with 100 μg/ml resveratrol, and 100 μg/ml silymarin with 200 μg/ml resveratrol) compared with the controls in 24 and 48 h. As it is presented, silymarin (100 μg/ml) enhanced the cell proliferation after 24 h and the combination of 100 μg/ml silymarin and 200 μg/ml resveratrol reduced cell viability after 48 h. R, resveratrol; S, silymarin; H, histamine. Data are presented as mean ± SD of the three separate experiments, analyzed by GraphPad Prism software *via* ANOVA and Student's *t* test.

Effect of Histamine on TPA-1 and TNF-α Secretion in HGFs

Although the effect of silymarin at 100 μg/ml concentration on dropping the secretion of TPA-1 was more than resveratrol, it was not statistically significant. Conversely, significant effects of their blend were found in the present study. However, the combination of 100 μg/ml silymarin and 200 μg/ml resveratrol had a more premium effect on TPA-1 secretion when compared with histamine alone ($P = 0.01$ and $P = 0.04$ respectively; Fig. 3a). Concentration of secreted TNF-α remarkably dropped after treatment with silymarin at 100 μg/ml concentration ($P = 0.032$), whereas 100 μg/ml resveratrol had no significant effect on the levels of TNF-α in the supernatant. Though, the lessening of TNF-α after treatment with compound of 50 μg/ml silymarin and 100 μg/ml resveratrol was significant ($P = 0.02$). Unexpectedly, combination of 100 μg/ml silymarin and 200 μg/ml resveratrol did not show an important fall of TNF-α in the supernatant when compared with control (histamine alone; Fig. 3b). Resveratrol alone had significant effect on the reduction of IL-6, but this effect was not significant on IL-8 secretion, while silymarin alone had a significant effect on the reduction of TNF-α. Though, selected combinations of silymarin and resveratrol at both concentrations could affect IL-8 and TPA-1 secretions significantly. In the case of IL-6 and TNF-α, the blend of 100 μg/ml silymarin with 200 μg/ml resveratrol had no significant effect, while the combination of 50 μg/ml silymarin/100 μg/ml resveratrol displayed a significant anti-inflammatory effect.

DISCUSSION

Periodontal disease is a biofilm-induced chronic inflammatory disease that contributes to the destruction of the supportive tissues of affected teeth. It starts from

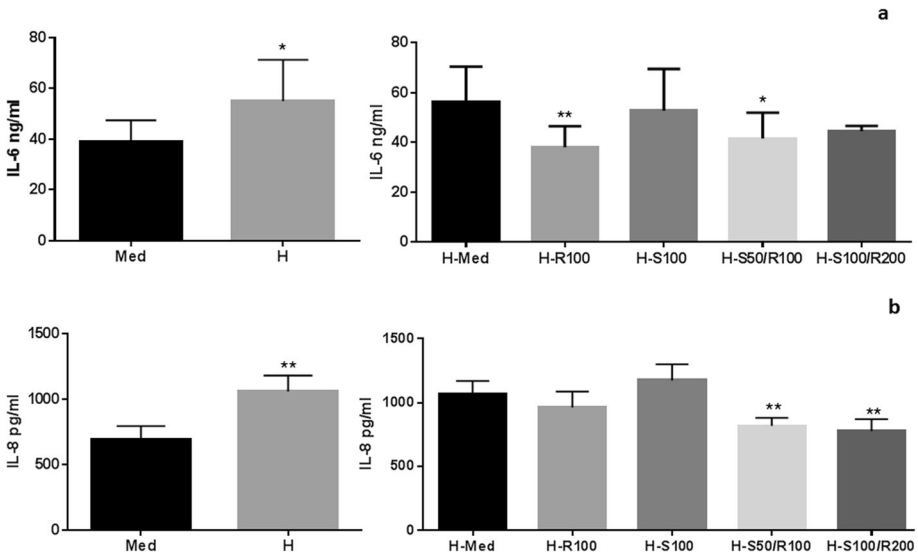

Fig. 2. The effects of silymarin and resveratrol on IL-6 and IL-8. The histogram shows no significant effect of silymarin on IL-6 at 100 μg/ml concentration, whereas resveratrol decreased the levels of this cytokine at 100 μg/ml. The effects of the first combinations (50 μg/ml S/100 μg/ml R) showed significant reduction on IL-6 secretion (**a**). As the histogram shows, no significant effect of silymarin and resveratrol alone on secretion of IL-8 was found. The effects of two different combinations (50 μg/ml S/100 μg/ml R or 100 μg/ml S/200 μg/ml R) showed significant decline in IL-8 levels (**b**). R, resveratrol; S, silymarin; H, histamine. Individual data are presented with mean + SD of at least three independent experiments using ELISA method and analyzed *via* ANOVA and Student's *t* test. $*P < 0.05$ and $**P < 0.01$, when compared with the controls.

chronic exposure to oral bacteria, but it is the host response that finally leads to the deterioration of the periodontium [21]. According to the recent studies, the host response not only has an effect on susceptibility to disease but also determines the extent and severity of the disease. In this regard, investigation of inflammatory cytokines could be a valuable diagnostic and prognostic tool. Previously, we found increased salivary levels of IL-6 as a diagnostic and therapeutic target in oral lichen planus, an idiopathic inflammatory condition, cases [22]. LPS-inducing periodontitis microorganisms stimulate host immune response [1, 23]. In fact, LPS stimulates expression of several inflammatory cytokines such as IL-6, IL-8, and TNF-α by activating the NF-κB and mitogen-activated protein kinases (MAPK)/AP-1 signaling pathways [24]. These inflammatory cytokines can stimulate the production of histamine through HDC. It has been demonstrated that an increase in LPS-induced HDC activity could be associated with survival of infective bacteria by histamine activity [3, 4]. On the other hand, binding of histamine to the histamine receptor 1, on the surface of the gingival fibroblasts, may increase the expression of IL-8, IL-6, COX2, and PGE2 through activating of phospholipase C/MAPK/NF-κB [4, 25]. It has been demonstrated that through increasing the expression of TLR2 and TLR4 in HGFs, histamine could modulate the innate immune response. Further, there is a direct association between histamine and vascular inflammation by its well-recognized vasoactive function [25, 26]. Evaluation of the effect of resveratrol on the production of histamine demonstrated that it could conduct both inhibition and degranulation of mast cells (MCs). MCs have a crucial role in activation of the acquired immune response and stimulating infiltration of leukocytes as central cells in the inflammatory processes like periodontitis, where the count and density of these cells have been higher compared with healthy subjects [27–29]. It has also been shown that neutrophils produce histamine and this amount has been higher in neutrophils from patients with periodontitis than from healthy participants [30]. Totally, histamine along with inflammatory cytokines and bacterial components like LPS plays role in pathogenesis of periodontitis synergistically. Additionally, LPS and these inflammatory cytokines can induce human pulp and gingival fibroblast to increase the expression of the TPA-1. This could cause tissue degradation during pulpal and periapical lesions [5]. Studies have also shown that TPA-1 can increase the inflammatory cytokine levels by activating the NF-κB pathway [8]. In contrast, it has been shown that host modulatory therapy (HMT) can ameliorate the damage, downregulate the destructive aspects of the host response, or upregulate the protective or regenerative responses [23]. Hence, numerous dietary compounds have been introduced as anti-inflammatory agents;

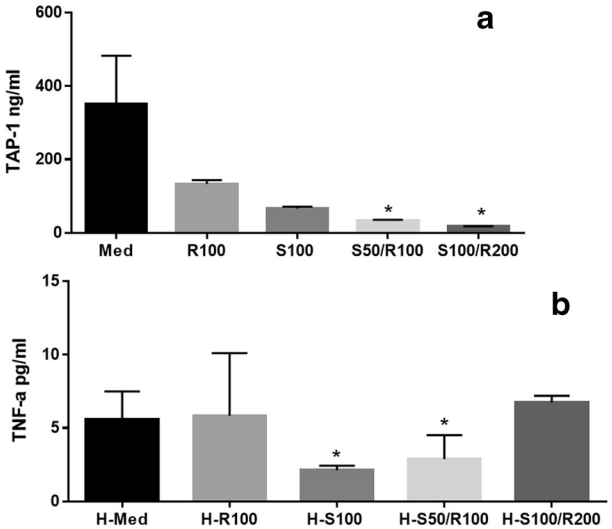

Fig. 3. The effects of silymarin and resveratrol on TPA-1 and TNF-α. Resveratrol and sylymarin did not show significant effect on the histamine release induced of TPA-1 from the HGF cells separately. The effect of two combinations of 50 μg/ml S/100 μg/ml R and 100 μg/ml S/200 μg/ml R on TPA-1 release was significant (**a**). The histogram exhibits a significant effect of silymarin at 100 μg/ml concentration on TNF-α, but this effect was not significant for resveratrol at 100 μg/ml. The combination of 50 μg/ml S/100 μg/ml R had a significant effect on TNF-α secretion while this effect was not significant for combination of 100 μg/ml S/200 μg/ml R (**b**). R, resveratrol; S, silymarin; H, histamine. Individual data are presented with mean + SD of at least three independent experiments using ELISA method and analyzed *via* ANOVA and Student's *t* test. $^*P < 0.05$, when compared with the controls.

we have investigated the anti-histaminic effects of silymarin and resveratrol on HGFs *in vitro* [31, 32]. In this study, we examined different concentrations of silymarin and resveratrol or combination of both in order to find an effective concentration in which they have an anti-histaminic effect while maintaining cell viability. Similar to the previous study, the results indicate that silymarin has a significant impact on cell viability [2]. Even et al. in a similar study showed that silymarin can reduce the viability of biofilm-forming cells [33]. However, our results showed that the cell viability effect of silymarin was more prominent at 100 μg/ml concentrations in 24 and 48 h. Furthermore, applying mixtures of silymarin and resveratrol exhibited that cell viability increased significantly with the combination of 50 μg/ml silymarin and 100 μg/ml resveratrol in 48 h. Nevertheless, no proliferative effect was found after 24 h. Besides, the viability of gingival fibroblast cells did not change significantly with resveratrol treatment in 24 and 48 h. Moreover, we investigated the anti-histaminic effects of resveratrol and silymarin on HGFs by determination of some inflammatory markers. After treatment of the HGF cells with histamine, a considerable rise of IL-8, IL-6, and TNF-α levels was found in the supernatant. Similarly, Kohda F et al. reported that histamine amplified the production of IL-6 and IL-8 in human keratinocyte [34]. Correspondingly, in a previous study, histamine showed a crucial role in magnifying inflammatory responses of gingival fibroblasts and considered as a predictive index in the outcome of periodontitis [9]. Furthermore, we found that silymarin alone, at the concentration of 100 μg/ml, had a remarkable effect on the reduction of TNF-α. This synchronizes with a recent study of silymarin effect on the liver, representing blocking of p38 and JNK phosphorylation and activation effect of this flavonoid [35]. Though, resveratrol alone at the same concentration had a significant effect on the fall in the level of IL-6. The results of the present study are nearly consistent with those of our previous study on the LPS-treated HGF cells, demonstrating a greater effect of resveratrol on IL-6 when compared with silymarin [2]. Shirley et al., also, demonstrated that 100 μg/ml resveratrol strongly reduced secretion of IL-6, while this effect was not significant for TNF-α secretion, as conducted on mast cells [36]. Likewise, resveratrol had reversed the induced levels of IL-6 in a previous study [37]. Correspondingly, silymarin was separately assessed in a study by Fordham et al. on LPS-induced cells which resulted in a significant reduction in the level of TNF-α. Studies, however, have been shown different effects of silymarin on various types of cells. Though, the differences between the results could be due to different types of the cells which have been used in various studies. Combination of 200 μg/ml resveratrol and 100 μg/ml silymarin led to a significant decline in the secretion of IL-8 and TPA-1. However, the lower concentration of resveratrol/silymarin combination (100 μg/ml and 50 μg/ml) demonstrated a considerable drop in the secretion of all detected factors (IL-8, IL-6, TPA-1, and TNF-α), indicating a greater influence of this combination. Meanwhile, histamine and all of detected factors in the present study are involved in angiogenic process [38, 39]; the blend of resveratrol and silymarin might also be considered as an anti-aniogenic component. The present study though confirms the reported data indicating a significant role of resveratrol and silymarin in suppressing inflammation and empowers the anti-histaminic effect of resveratrol in combination with silymarin, which is novel. Nevertheless, further investigations would be constructive for better understanding of the principal mechanisms of this effect.

Our findings indicate that resveratrol and silymarin play an important role in invigorating the HGF cell response over the stimulation by histamine, which totally advocates their

anti-inflammatory effects. The combination of resveratrol with silymarin showed a greater effect on the inflammatory and angiogenic cytokines; however, the concentrations of these compounds are crucial in determining their effect on histamine-induced HGFs. The viability of the cells could be enhanced by applying silymarin either alone or in combination with resveratrol. These results suggest that combination of resveratrol induces stronger anti-inflammatory effect than with silymarin or resveratrol alone in histamine-induced HGFs. Moreover, the presence of silymarin may also keep the viability of the cells which could be a helpful therapeutic tool. These results may contribute to the development of the HMT strategies and novel therapeutic approaches for inflammations like periodontitis.

ACKNOWLEDGMENTS

We wish to thank all our colleagues in the Research and Enterprise Committee and the School of Allied Medicine, Iran University of Medical Science.

AUTHOR'S CONTRIBUTION

M.Sh. as a director of the project has conceived the manuscript and revised it. A.F. and M.Sh. wrote the manuscript. M.Sh., S.J., and F.S.Sh. provided clinical data and information. A.F. and M.Sh. performed the ELISA and MTT tests, respectively.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest. The authors declare that they have no conflict of interest.

REFERENCES

1. Palaska, I., E. Papathanasiou, and T.C. Theoharides. 2013. Use of polyphenols in periodontal inflammation. *European Journal of Pharmacology* 720 (1–3): 77–83. https://doi.org/10.1016/j.ejphar.2013.10.047.
2. Shahidi, M., F. Vaziri, A. Haerian, A. Farzanegan, S. Jafari, R. Sharifi, and F.S. Shirazi. 2017. Proliferative and anti-inflammatory effects of resveratrol and silymarin on human gingival fibroblasts: a view to the future. *Journal of Dentistry (Tehran, Iran)* 14 (4): 203.
3. Shoji, N., A. Yoshida, Z. Yu, Y. Endo, and T. Sasano. 2006. Lipopolysaccharide stimulates histamine-forming enzyme (histidine decarboxylase) activity in murine dental pulp and gingiva. *Archives of Oral Biology* 51 (10): 856–860. https://doi.org/10.1016/j.archoralbio.2006.04.004.
4. Minami, T., T. Kuroishi, A. Ozawa, H. Shimauchi, Y. Endo, and S. Sugawara. 2007. Histamine amplifies immune response of gingival fibroblasts. *Journal of Dental Research* 86 (11): 1083–1088. https://doi.org/10.1177/154405910708601112.
5. Chang, Y.-C., S.-F. Yang, F.-M. Huang, K.-W. Tai, and Y.-S. Hsieh. 2003. Induction of tissue plasminogen activator gene expression by proinflammatory cytokines in human pulp and gingival fibroblasts. *Journal of Endodontics* 29 (2): 114–117. https://doi.org/10.1097/00004770-200302000-00007.
6. Toyman, U., G. Tüter, B. Kurtiş, E. Kıvrak, Ş. Bozkurt, A. Yücel, and M. Serdar. 2015. Evaluation of gingival crevicular fluid levels of tissue plasminogen activator, plasminogen activator inhibitor 2, matrix metalloproteinase-3 and interleukin 1-β in patients with different periodontal diseases. *Journal of Periodontal Research* 50 (1): 44–51. https://doi.org/10.1111/jre.12179.
7. Xiao, Y., C.L. Bunn, and P.M. Bartold. 2001. Effect of lipopolysaccharide from periodontal pathogens on the production of tissue plasminogen activator and plasminogen activator inhibitor 2 by human gingival fibroblasts. *Journal of Periodontal Research* 36 (1): 25–31. https://doi.org/10.1034/j.1600-0765.2001.00608.x.
8. Lin, L., and K. Hu. 2014. Tissue plasminogen activator and inflammation: from phenotype to signaling mechanisms. *American journal of clinical and experimental immunology* 3 (1): 30.
9. Venza, M., M. Visalli, M. Cucinotta, D. Cicciù, P. Passi, and D. Teti. 2006. Salivary histamine level as a predictor of periodontal disease in type 2 diabetic and non-diabetic subjects. *Journal of Periodontology* 77 (9): 1564–1571.
10. Medina, V.A., J.P. Prestifilippo, M. Croci, E. Carabajal, R.M. Bergoc, J.C. Elverdin, and E.S. Rivera. 2011. Histamine prevents functional and morphological alterations of submandibular glands induced by ionising radiation. *International Journal of Radiation Biology* 87 (3): 284–292. https://doi.org/10.3109/09553002.2010.533247.
11. Talreja, J., M.H. Kabir, M.B. Filla, D.J. Stechschulte, and K.N. Dileepan. 2004. Histamine induces Toll-like receptor 2 and 4 expression in endothelial cells and enhances sensitivity to Gram-positive and Gram-negative bacterial cell wall components. *Immunology* 113 (2): 224–233.
12. Feghali, K., M. Feldman, V.D. La, J. Santos, and D. Grenier. 2011. Cranberry proanthocyanidins: natural weapons against periodontal diseases. *Journal of Agricultural and Food Chemistry* 60 (23): 5728–5735. https://doi.org/10.1021/jf203304v.
13. Tsai, H.-Y., C.-T. Ho, and Y.-K. Chen. 2017. Biological actions and molecular effects of resveratrol, pterostilbene, and 3′-hydroxypterostilbene. *Journal of Food and Drug Analysis* 25 (1): 134–147. https://doi.org/10.1016/j.jfda.2016.07.004.
14. Alarcon De La Lastra, C., and I. Villegas. 2005. Resveratrol as an anti-inflammatory and anti-aging agent: mechanisms and clinical implications. *Molecular Nutrition & Food Research* 49 (5): 405–430. https://doi.org/10.1002/mnfr.200500022.
15. Chin, Y.T., G.Y. Cheng, Y.J. Shih, C.Y. Lin, S.J. Lin, H.Y. Lai, J. Whang-Peng, H.C. Chiu, S.Y. Lee, and E. Fu. 2017. Therapeutic applications of resveratrol and its derivatives on periodontitis. *Annals of the New York Academy of Sciences* 1403 (1): 101–108. https://doi.org/10.1111/nyas.13433.
16. Esmaeil, N., S.B. Anaraki, M. Gharagozloo, and B. Moayedi. 2017. Silymarin impacts on immune system as an immunomodulator: one key for many locks. *International Immunopharmacology* 50: 194–201. https://doi.org/10.1016/j.intimp.2017.06.030.
17. Křen, V., and D. Walterova. 2005. Silybin and silymarin–new effects and applications. *Biomed papers* 149 (1): 29–41.

18. Shahidi, M., R. Sharifi, F. Sadeghi-Shirazi, and F. Parhizkary. 2018. The generation of hematopoietic progenitor cells from endothelial cells by silymarin. *Clinical Laboratory* 64 (11): 180534. https://doi.org/10.7754/Clin.Lab.2018.180534.
19. Fordham, J.B., A. Raza Naqvi, and S. Nares. 2014. Leukocyte production of inflammatory mediators is inhibited by the antioxidants phloretin, silymarin, hesperetin, and resveratrol. *Mediators of Inflammation* 2014: 938712. https://doi.org/10.1155/2014/938712.
20. Hatamochi, A., H. Ueki, C. Mauch, and T. Krieg. 1991. Effect of histamine on collagen and collagen m-RNA production in human skin fibroblasts. *Journal of Dermatological Science* 2 (6): 407–412.
21. Hajishengallis, G. 2014. Immunomicrobial pathogenesis of periodontitis: keystones, pathobionts, and host response. *Trends in Immunology* 35 (1): 3–11. https://doi.org/10.1016/j.it.2013.09.001.
22. Shahidi, M., S. Jafari, M. Barati, M. Mahdipour, and M.S. Gholami. 2017. Predictive value of salivary microRNA-320a, vascular endothelial growth factor receptor 2, CRP and IL-6 in oral lichen planus progression. *Inflammopharmacology* 25 (5): 577–583. https://doi.org/10.1007/s10787-017-0352-1.
23. Anarthe, R., A. Mani, and P. Marawar. 2013. Host modulatory therapy: a novel approach in the periodontal therapy. *ISOR Journal of Pharmacy* 3: 9–13.
24. Tipton, D., J. Babu, and M.K. Dabbous. 2013. Effects of cranberry components on human aggressive periodontitis gingival fibroblasts. *Journal of Periodontal Research* 48 (4): 433–442. https://doi.org/10.1111/jre.12023.
25. Gutiérrez-Venegas, G., S. Cruz-Arrieta, M. Villeda-Navarro, and J.A. Méndez-Mejía. 2011. Histamine promotes the expression of receptors TLR2 and TLR4 and amplifies sensitivity to lipopolysaccharide and lipoteichoic acid treatment in human gingival fibroblasts. *Cell Biology International* 35 (10): 1009–1017. https://doi.org/10.1042/CBI20100624.
26. Tan, X., S. Essengue, J. Talreja, J. Reese, D.J. Stechschulte, and K.N. Dileepan. 2007. Histamine directly and synergistically with lipopolysaccharide stimulates cyclooxygenase-2 expression and prostaglandin I2 and E2 production in human coronary artery endothelial cells. *The Journal of Immunology* 179 (11): 7899–7906. https://doi.org/10.4049/jimmunol.179.11.7899.
27. Catalli, A., C. MacDonald, P. Pundir, and M. Kulka. 2012. Inhibitory effects of resveratrol on human mast cell degranulation, cytokine, chemokine and leukotriene release. *Open Journal of Immunology* 2 (4): 187–194. https://doi.org/10.4236/oji.2012.24022.
28. Sheethal, H., H. Kn, T. Smitha, and K. Chauhan. 2018. Role of mast cells in inflammatory and reactive pathologies of pulp, periapical area and periodontium. *Journal of oral and maxillofacial pathology: JOMFP* 22 (1): 92–97.
29. Rathod, S., A. Raj, and I. Wanikar. 2018. Quantitative analysis of mast cell count and density in chronic periodontal disease. *Journal of Indian Society of Periodontology* 22 (2): 107–111.
30. Ribas, D., M.C. Fernández-Carranco, N. Hajji, P. Bobadilla, and J. Monteseirín. 2018. Eosinophil cationic protein and histamine production by neutrophils from patients with periodontitis. *Journal of Periodontology* 89 (2): 228–234. https://doi.org/10.1902/jop.2017.160679.
31. Zeinali, M., S.A. Rezaee, and H. Hosseinzadeh. 2017. An overview on immunoregulatory and anti-inflammatory properties of chrysin and flavonoids substances. *Biomedicine & Pharmacotherapy* 92: 998–1009. https://doi.org/10.1016/j.biopha.2017.06.003.
32. Patil, R.H., R. Babu, M.N. Kumar, K.K. Kumar, S.M. Hegde, R. Nagesh, G.T. Ramesh, and S.C. Sharma. 2016. Anti-inflammatory effect of apigenin on LPS-induced pro-inflammatory mediators and AP-1 factors in human lung epithelial cells. *Inflammation* 39 (1): 138–147.
33. Evren, E., and E. Yurtcu. 2015. In vitro effects on biofilm viability and antibacterial and antiadherent activities of silymarin. *Folia Microbiologica* 60 (4): 351–356.
34. Kohda, F., T. Koga, H. Uchi, K. Urabe, and M. Furue. 2002. Histamine-induced IL-6 and IL-8 production are differentially modulated by IFN-γ and IL-4 in human keratinocytes. *Journal of Dermatological Science* 28 (1): 34–41.
35. Wang, L., Q.-H. Huang, Y.-X. Li, Y.-F. Huang, J.-H. Xie, L.-Q. Xu, Y.-X. Dou, Z.-R. Su, H.-F. Zeng, and J.-N. Chen. 2018. Protective effects of silymarin on triptolide-induced acute hepatotoxicity in rats. *Molecular Medicine Reports* 17 (1): 789–800.
36. Shirley, D., C. McHale, and G. Gomez. 2016. Resveratrol preferentially inhibits IgE-dependent PGD2 biosynthesis but enhances TNF production from human skin mast cells. *Biochimica et Biophysica Acta (BBA) - General Subjects* 1860 (4): 678–685.
37. Mbimba, T., P. Awale, D. Bhatia, W.J. Geldenhuys, A.S. Darvesh, R.T. Carroll, and A. Bishayee. 2012. Alteration of hepatic proinflammatory cytokines is involved in the resveratrol-mediated chemoprevention of chemically-induced hepatocarcinogenesis. *Current Pharmaceutical Biotechnology* 13 (1): 229–234.
38. Qin, L., D. Zhao, J. Xu, X. Ren, E.F. Terwilliger, S. Parangi, J. Lawler, H.F. Dvorak, and H. Zeng. 2013. The vascular permeabilizing factors histamine and serotonin induce angiogenesis through TR3/Nur77 and subsequently truncate it through thrombospondin-1. *Blood* 121 (11): 2154–2164. https://doi.org/10.1182/blood-2012-07-443903.
39. Stassi, A.F., M.E. Baravalle, E.M. Belotti, F. Rey, N.C. Gareis, P.U. Díaz, F.M. Rodríguez, C. Leiva, H.H. Ortega, and N.R. Salvetti. 2017. Altered expression of cytokines IL-1α, IL-6, IL-8 and TNF-α in bovine follicular persistence. *Theriogenology* 97: 104–112. https://doi.org/10.1016/j.theriogenology.2017.04.033.