Published online 2022 January 20 **Original Article**

A Quantitative Study on the Protective Effect of Resveratrol against Bisphenol-A-Induced Oral Mucosa and Tongue Toxicity in Male Rats: A Stereological Study

Ebrahim Rahmani Moghadam[1], Elham Nadimi[2], Afsoon Bordbar[3], Nazanin Ayareh[4], Ehsan Bordbar[5] and Hossein Bordbar[6,*]

[1]Department of Anatomical Sciences, Medical School, Shiraz University of Medical Sciences, Shiraz, Iran
[2]Histomorphometry and Stereology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
[3]Doctor of Dental Medicine, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran
[4]Student Research Committee, Shiraz University of Medical Sciences, Shiraz, Iran
[5]Department of Biomedical Engineering, Shiraz University, Shiraz, Iran
[6]Histomorphometry and Stereology Research Center, Department of Anatomical Sciences, Medical School, Shiraz University of Medical Sciences, Shiraz, Iran

*** *Corresponding author:*** Hossein Bordbar, Histomorphometry and Stereology Research Center, Department of Anatomical Sciences, Medical School, Shiraz University of Medical Sciences, Shiraz, Iran. Tel/Fax: +987132304372; Email: bordbarh@sums.ac.ir

Received 2021 April 04; **Revised** 2021 October 25; **Accepted** 2021 November 05.

Abstract

Background: Bisphenol-A (BPA) is one of the hazardous chemicals, which is extensively used. BPA can cause oxidative stress. Resveratrol (RES) is a natural polyphenol that possesses several health benefits, such as antioxidant effects.
Objectives: This study aimed to investigate the protective effects of RES against BPA-induced oral mucosa and tongue toxicity.
Methods: A total of five groups of Sprague-Dawley male rats (n=30) were used in this study. Group 1 was the control group, and group 2 received 50 mg /kg BPA by gavage. Group 3 was given 100 mg/kg of RES, and group 4 received 50 mg/kg BPA plus 100 mg/kg RES. In group 5, the sham group, the volume of the injectable drug received from olive oil was given as gavage for eight weeks. Following that, the paraffinized samples were sectioned in 5µm thickness to estimate the volume.
Results: The results showed that BPA had a different effect on tissues. Moreover, the total volume of lamina propria, mucosal glands, the total glands of the tongue, volumetric density of the epithelium, and glands of the oral mucosa increased in the BPA group, while in the BPA+RES group, these structures reduced, compared to the BPA group. In addition, the total volume of the epithelium of the tongue decreased in the BPA group, whereas this structure increased in the BPA+RES group, compared to the BPA group.
Conclusion: BPA has different effects on the oral mucosa and tongue. These effects can exert influence on the normal function of the cells in these areas. RES, with its antioxidant properties, had a protective effect on these structures against the BPA.

Keywords: Bisphenol-A, Oral mucosa, Resveratrol, Tongue, Toxicity

1. Background

Humans and wildlife are negatively affected by hazardous chemicals and increasing environmental pollution (1). Bisphenol-A, 2,2-(4,4-dihydroxy phenyl, BPA) is one of the widely used chemicals which is extensively used in polycarbonate bottles, resins, plastics, fungicides, heat resistant materials, and the epoxy layer of canned foods (2-4). Application of BPA is on the rise, such that each year, more than 2.2 million tons of these products are manufactured worldwide (5). BPA-based coatings are forbidden in the US since BPA can leach to food and water under normal conditions (4, 6, 7). It has been indicated that BPA can be released in human tissues, such as the hair (5, 8), and it could be accumulated in the human body (1). BPA is a biologic toxin that can be released, independent of their pH temperature, into food, air, saliva, and blood (9); moreover, it has been reported to act as a hepatotoxic agent that leads to elevated levels of alanine transaminase, aspartate transaminase, and lactate dehydrogenase, as well as defects in the liver morphology (5, 10). Furthermore, it can cause oxidative stress, inflammation, endocrine disturbance, and genome/transcriptome modifications in different tissues, such as the brain, liver, and kidney (4, 7, 11-13). Folia et al. showed that oral exposure to BPA in the rat disrupts the thirst and buccal homeostasis and raises questions about the salivary gland secretions (14). In another study, Seki et al. reported that oral epithelial cells exposed to BPA showed a significant gene expression alteration. In these cells, several genes related to the acceleration of cell death processes were significantly up-regulated, indicating the detrimental effect of BPA on cell survival (15). Natural antioxidants, such as vitamins and polyphenols, play an essential role in preventing diseases associated with oxidative stress and inflammation (16).

Resveratrol (RES) is one of the natural polyphenols commonly found in various plants, especially in grapes (17). RES possesses several health benefits, including antioxidant (18, 19), anticarcinogenic (20), antibacterial (21), antifungal (22), antiviral (23), cardioprotective (24), hepatoprotective (18), and neuroprotective (25) effects. Although the exact mechanism of the action of RES is not well-understood, it is supposed that RES exerts its protective effect mainly through free radical scavenging and up-regulation of antioxidant genes, such as heme oxygenase-1 (26, 27). There is evidence

indicating the protective effects of RES against BPA-induced toxicity. It has been shown that RES can reverse the BPA-induced metabolic syndrome and endothelial dysfunction by regulating insulin signaling and antioxidant properties (28, 29). Furthermore, liver damage caused by BPA was attenuated by the effect of RES on the extent of oxidative status and improved enzymatic/non-enzymatic antioxidant defense system (30). In addition, research suggests that RES can increase endogenous antioxidant biosynthesis, inhibit membrane lipids peroxidation, decrease interleukins and TNF-α, and increase endothelial nitric oxide (eNOS) expression, followed by increasing blood supplying the damaged tissues (31-33). Despite extensive studies on the toxicity of BPA on various tissues, there is no research about the stereology parameters of oral mucosa and tongue toxicity as part of the gastrointestinal tract by BPA.

2. Objectives

Protective agents seem urgent regarding the increasing use of BPA and its serious harmful effects on biological systems. Therefore, this study aimed to investigate the possible protective effect of RES against BPA-induced oral mucosa and tongue toxicity.

3. Methods

3.1. Animals

A total of 30 male Sprague Dawley rats weighing 200-250 g were purchased from the comparative and experimental medical center of Shiraz University of Medical Sciences, Shiraz, Iran (SUMS). Animals were kept in the standard conditions (ambient light: 12:12 light-dark cycle) and free access to water and food were provided to them for eight weeks to maintain their environmental compatibility. The research was performed with the approval and based on the guidelines of the Animal Care and Ethics Committee (IR.SUMS.REC.1398.392) of the SUMS, Shiraz, Iran.

3.2. Methods of drug administration for all groups

The rats were randomly divided into five groups of six animals per group. Group 1 received distilled water as control, and group 2 received 50 mg /kg BPA. Furthermore, group 3 was given 100 mg/kg of RES, and group 4 received 50 mg/kg BPA plus 100 mg/kg RES. In group 5, the sham group, the volume of injectable drugs taken from olive oil (BPA and RES solvent) was given as oral gavage. This process was performed for the groups for eight weeks (34).

3.3. Tissue preparation

The mucosal tissue of the oral cavity with the tongue was removed for histological and stereological studies. The tissue preparation steps were performed according to the usual method. After that, the paraffinized tongue and oral mucosa were sectioned in 5µm thickness (H&E staining) for volume estimation.

3.4. Sampling of the tongue

To determine the shrinkage and volume density of the tongue structures, isotropic uniform random sections were used in this study. For this determination, the tongue was randomly placed on the φ clock. After selecting a random number from 1 to 10, an opposite cut was made along with the selected number, which caused two pieces of the tongue. The first piece was then located on the θ clock along with its previous cut surface on the 0-0 axis. Following that, a random number was selected again, and a similar cut was made along with the selected number. The other piece that caused from the cut made on the φ clock was placed on the θ clock steeply so that its cut surface overlay the 0-0 axis. The slab thickness was chosen to guarantee that a total of 8-12 slabs would be obtained (Figure 1 B, C). To estimate the shrinkage, a tissue cylinder was punched out from the tongue and slab by a trocar (diameter 3 mm), and the trocar radius was considered the “area” before (πr2). After tissue processing, the area was calculated using a microscope (40× magnification).

With the following formula, the number of wrinkles related to the tongue of each rat was calculated:

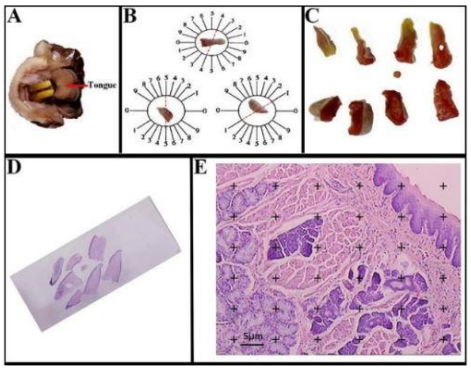

Figure 1. A summary of stereological techniques: A. The tongue was separated from other parts of the mouth. B. To obtain isotropic uniform random sections of the tongue according to the orientation method, the tongue was placed on an equally divided circle and sectioned into two parts in a random direction (here 4). Other parts of the tongue were sectioned in the new random directions (here 5 and 1) after being placed on the cosine-weighted divided circle. C. The slabs were collected, and a circle was punched out. D. A-stained section on one microscopic slide. E. Point-counting method to estimate the volume density on the H&E staining sections. (Scale bar: 5µm)

Volume Shrinkage=1- {(area after/area before)1.5

3.5. Sampling of the oral mucosa

To determine the density of the structures of the oral mucosa, vertical uniform sections (VUR) were needed, and VUR sampling (VURS) was used in this study. The oral mucosa was laid parallel to the horizontal plane and then cut vertically in a random manner. The resulting vertical sections were located on a transverse plane that was parallel to the horizontal plane. The object had to be positioned in the same direction (0°) as the previously detected internal marker. The VUR sections were obtained by uniformly, randomly, and systematically rotating a knife (Figure 2) (35)

3.6. Estimation of the volume density

The stereological counting equipment consisted of a Nikon E-200 microscope (Nikon, Japan) was connected to a computer, and the image of each section was assessed at a final magnification of 152×. According to the point-counting method and using the stereological software (Stereo Lite, SUMS, Shiraz, Iran), the stereological probe (point grid) (1E, 2E) was superimposed on the images of the tissue sections viewed on the monitor. Briefly, each section of the microscopic fields of view was systematically sampled in a random manner (fixed equal distances in x- and y-axes) on each histological slide. The right upper corner of each cross point was considered a point, and the volume density (VV) of the epithelium and glands of the oral mucosa and epithelium, lamina propria, total glands, serous glands, and mucous glands of the tongue were estimated using the point-counting method and the following formula: Vv=ΣP structure/ΣP reference,

where Vv is the volume density and ΣP signifies the total number of points composed on the structure and the reference (36).

3.7. Estimation of the total volume of the tongue

The tongue was cleaned, and its total volume was estimated using the Scherle method (37). Briefly, a jar filled with isotonic saline was placed on a scale and weighed. Subsequently, the tongue was suspended by thin cotton in the jar, and the tongue volume was estimated by the immersion method. Afterward, the tongue "V (tongue)" was isolated and weighed, and the volume of the tongue was estimated. In the end, the final volume was calculated using the following formula:

V final tongue = V primary× (1-Volume Shrinkage)

Ultimately, the total volume associated with each component of the tongue was estimated through the multiplication of the volume density by the total volume per animal (34):

V structure=V final tongue ×Vv structure

3.8. Statistical analysis

The data were analyzed through one-way ANOVA, and a p-value less than 0.05 was considered statistically significant. The statistical analyses were performed in SPSS software (version 15), and the chart was drawn in GraphPad Prism, Version 8.1 (IBM, Armonk, NY, USA program).

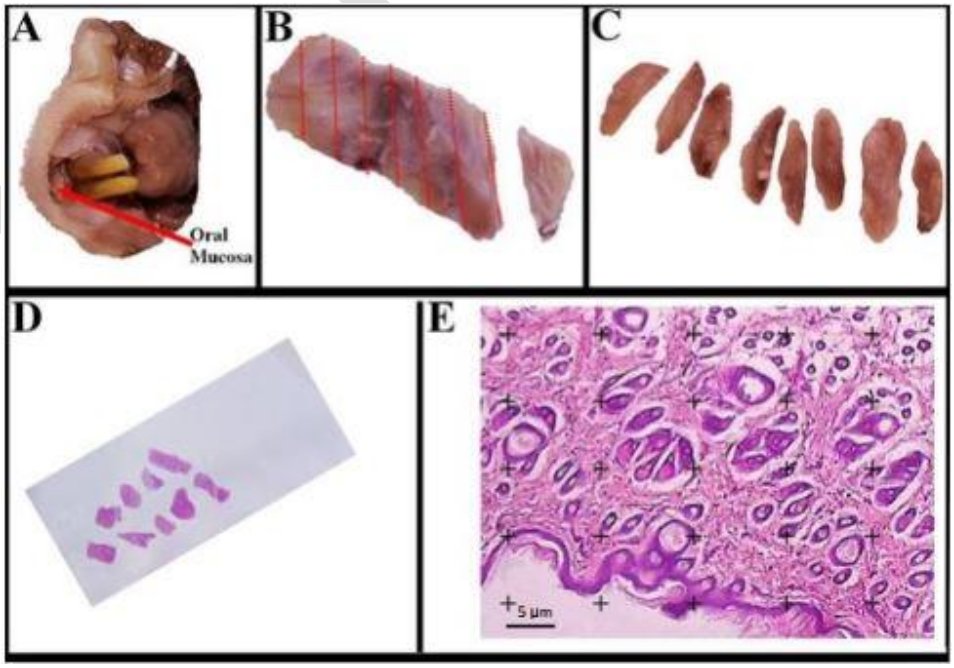

Figure 2. A summary of the stereological techniques. A. The oral mucosa was separated from other parts of the mouth. B. To obtain vertical uniform sections of the oral mucosa. C. The slabs were collected for blocking. D. A stained section on one microscopic slide. E. Point-counting method to estimate the volume density on the H&E staining sections. (Scale bar: 5µm)

4. Results

4.1. Total volume of the tongue structures

The results showed that BPA had different effects on the total volume of the epithelium, lamina propria, total glands, serous glands, mucous glands of the tongue, volume density of epithelium, and glands of the oral mucosa. Moreover, RES prevented these changes and acted as a protective agent against changes in BPA-induced toxicity.

The results of the study on the tongue showed that the total volume of the epithelium decreased significantly in the BPA group, compared to the control and sham groups (P<0.001), while in the BPA+RES group, compared to the BPA group, the total volume of the tongue epithelium increased significantly (P<0.05) (Figure 2A). The results regarding the volume of the lamina propria showed that the volume of lamina propria in the BPA group increased significantly, compared to the control group (P<0.05). However, in the BPA+ RES group, the volume of lamina propria reduced significantly, compared to the BPA group (P<0.05) (Figure 2B).

The study results on the total volume of the tongue total glands showed a significant increase in the BPA group, compared to the control and sham groups (P<0.01). However, the total gland volume decreased significantly in the BPA+RES group, compared to the BPA group (P<0.01). Furthermore, the total volume of the serous glands decreased in the BPA group, compared to the control group; however, the difference was not statistically significant (P>0.05). In the BPA+RES group, the serous gland volume increased significantly, compared to the BPA group (P<0.05). Based on the results of the study, the total volume of the mucosal glands increased in the BPA group, compared to the control and sham groups (P<0.05). The total volume of the mucosal glands in

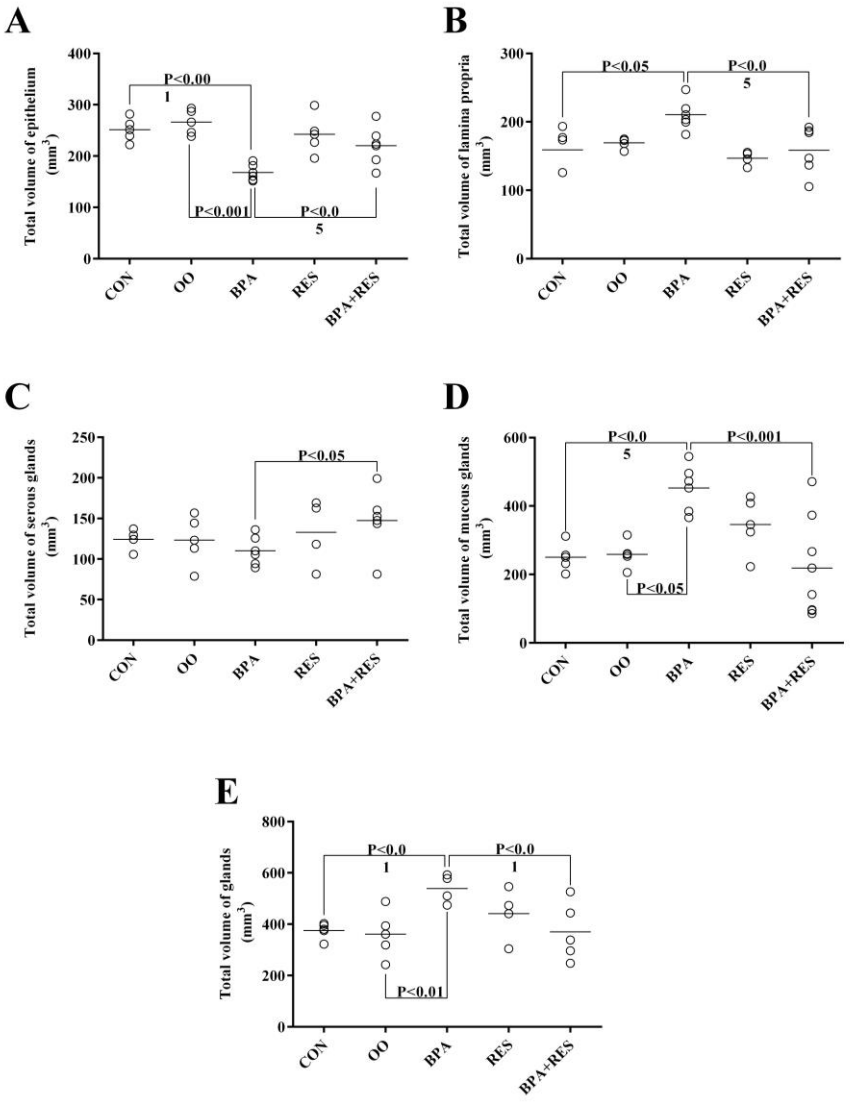

Figure 3. Dot plots showing stereological findings in the tongue structures in different groups. The total volume of A. epithelium B. lamina propria C. serous glands D. mucous glands E. total glands. The lines over each dot plot represent mean±SEM (a standard deviation of the mean). (CON: Control / OO: Olive Oil / BPA: Bisphenol A / RES: Resveratrol)

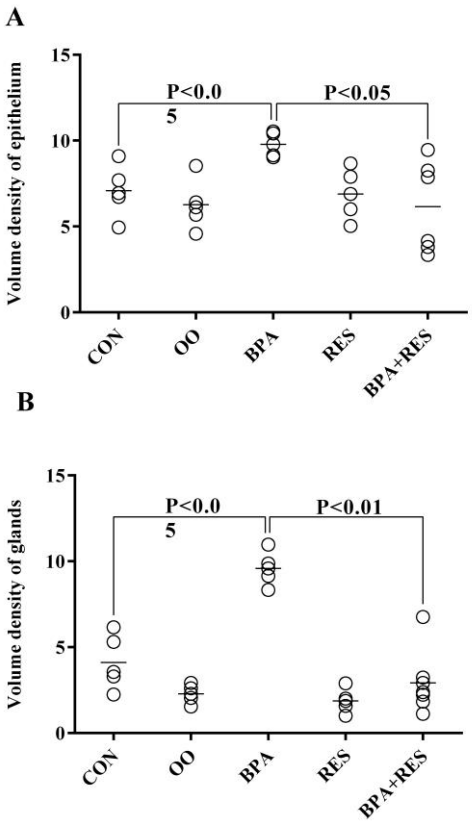

Figure 4. Dot plots showing stereological findings in the oral mucosa structures in different groups. The volume density of A. epithelium B. total glands. The lines over each dot plot represent mean±SEM (a standard deviation of the mean). (CON: Control / OO: Olive Oil / BPA: Bisphenol A / RES: Resveratrol)

the BPA+RES group decreased, compared to the BPA group (P<0.05) (Figure 2).

4.2. Volume density structures of the oral mucosa

The results showed that the volumetric density of the epithelium and glands increased significantly in the BPA group, compared to the control group (P<0.05). In the BPA+RES group, the volume density of the epithelium and glands reduced significantly, compared to the BPA group (P<0.05).

5. Discussion

This study shows that BPA has systemic toxic effects on the stereology parameters of the tongue and oral mucosa as part of the gastrointestinal tract. This is the first stereology study on the toxicity induced by BPA on oral toxicity, and other studies have confirmed the cellular and molecular oral toxicity of BPA (14, 15). Various animal studies, in vitro cellular investigations, and molecular assessment, could demonstrate the beneficial effects of RES on modeling health-related physiological processes and pharmacological activities (38). In this regard, its protective effects against oxidative and inflammatory reactions, as well as carcinogen processes, have been recently shown (39). Furthermore, its preventive effects on atherosclerotic pathways by inhibiting the production of reactive oxygen species (ROS) have been recently indicated as the main components of the stress oxidative process (40). Additionally, mediated by its anti-carcinogenic properties, RES has an effective anti-mutagenic effect by suppressing the tumor promoter-induced cell proliferation and regulating tumor cell apoptosis phenomenon (41). In this regard, the protective effects of this agent on arresting the tongue squamous carcinoma cells have also been revealed (42). Based on such evidence, it was first hypothesized that applying RES might protect the oral mucosa and tongue against the toxicity induced by BPA. The first evidence related to the cellular protective role of RES is that it has been recently shown that RES can significantly reduce ROS production, cellular apoptosis reaction, as well as caspase 3 and caspase 9 enzymes activation, all induced by BPA on the cellular levels (43). In other words, one of the main protective mechanisms of RES to neutralize BPA toxicity was demonstrated to be the deactivation of oxidative stress. As the second protective role, RES can modify mitochondrial membrane depolarization, and therefore, regulate the mitochondrial function, thereby increasing the viability of the normal cells in different tissues (44). Considering the toxic damaging effects of BPA via the activation of stress oxidative pathways, it seems that its main protective effects on the cellular toxicity induced by this compound can be mediated by the antioxidant effects of RES (45). Along with the antioxidant properties of RES, cell regenerative effects of this agent via its vasodilatory effect have been demonstrated as well (46). In this regard, RES can increase the expression of eNOS, leading to improved vascular nitric oxide responses, induced endothelial-dependent vasorelaxation, as well as decreased superoxide production in the vessels that all lead to tissue regeneration (47).

The key finding of the present study was that the use of RES could lead to a significant increase in the total volume of the tongue epithelium and normalize the oral serous glands that were disturbed by BPA induction. Although the protective effects of RES on the malignant cells of the tongue had been previously assessed (42), the present study was the first that investigated its beneficial effects on regenerating the buccal and tongue cells damaged by BPA. As indicated previously by Mikami et al. (48), RES could significantly inhibit cell growth, enhance chemo- and radio-sensitivity, and block the cancer invasion of the head and neck squamous cell carcinoma cells by the up regulation of the regenerating gene (REG) Ⅲ expression. Since REG expression is significantly associated with the progression of oral and gastrointestinal inflammatory damages, it is possible

that down-regulation of REG expression in such tissues by RES could be other mechanisms explaining the protective effects of RES.

As another mechanism, Quagliariello et al. (49) showed that BPA could stimulate lipid peroxidation. Since one of the main protective effects of RES is based on inhibiting the membrane lipid peroxidation, it seems that such effects can also be considered another main regenerative role of RES on the damaged buccal and tongue cells by BPA induction. Moreover, the effects of BPA on the activation of inflammatory cascade by the induction of producing and secreting inflammatory cytokines, such as TNF-α or IL-6, have been also demonstrated (50). In this regard, due to inhibiting effects of RES on such pro-inflammatory biomarkers, such effects can also be suggested as the protective role of RES on the oral tissues damaged by BPA.

In summary, different cellular and molecular pathways can be described as the arms of RES to generate its protective roles in the regeneration of the oral and buccal cells induced by toxic agents, such as BPA that includes reducing free radicals and ROS, as well as increasing endogenous antioxidant biosynthesis, 2) inhibiting membrane lipids peroxidation, 3) decreasing intracellular pro-inflammatory mediators, such as interleukins and TNF-α, 4) increasing eNOS expression and NO synthesis in vascular beds (followed by increasing blood supplying the damaged tissues), and 5) preventing free radical-mediated damage through SIRT1 pathway activation (31-33, 51, 52). Finally, due to the carcinogenic effects of BPA, it seems that RES can deal with this carcinogenic effect by inducing apoptosis and inhibiting proliferation in the tumor cells, which was not considered in the present study.

6. Conclusion

In conclusion, BPA has different effects on the oral mucosa and tongue. These effects can influence the normal function of the cells in these areas. RES, with its antioxidant properties, has a protective effect on these structures against the BPA.

Acknowledgments

The authors would like to thank the Student Research Committee of Shiraz University of Medical Sciences, Shiraz, Iran, and Dr. Laleh Khojasteh for editorial assistance.

Footnotes

Authors' Contribution: None
Conflict of Interests: The authors report no conflicts of interest.
Ethical Approval: All animal procedures were approved by the local Ethics Committee of Shiraz University of Medical Sciences, Shiraz, Iran (IR.SUMS.REC.1398.392).
Funding/Support: The present study was financially supported by Shiraz University of Medical Sciences, Shiraz, Iran (No. 97-01-21-18338).
Financial Disclosure: None
Informed consent: None

References

1. Poormoosavi SM, Najafzadehvarzi H, Behmanesh MA, Amirgholami R. Protective effects of Asparagus officinalis extract against Bisphenol A- induced toxicity in Wistar rats. *Toxicol Rep*. 2018;**5**:427-33. doi: 10.1016/j.toxrep.2018.02.010
2. Dükkancı M. Sono-photo-Fenton oxidation of bisphenol-A over a LaFeO3 perovskite catalyst. *Ultra Sonoch*. 2018;**40**:110-6. doi: 10.1016/j.ultsonch.2017.04.040
3. Gules O, Yildiz M, Naseer Z, Tatar M. Effects of folic acid on testicular toxicity induced by bisphenol-A in male Wistar rats. *Biot Histo*. 2019;**94**(1):26-35. doi: 10.1080/10520295.2018.1493222
4. Zhu X, Wu G, Xing Y, Wang C, Yuan X, Li B. Evaluation of single and combined toxicity of bisphenol A and its analogues using a highly-sensitive micro-biosensor. *J Hazard Mater*. 2019; **381**:120908. doi: 10.1016/j.jhazmat.2019.120908
5. Mahdavinia M, Alizadeh S, Raesi Vanani A, Dehghani MA, Shirani M, Alipour M, et al. Effects of quercetin on bisphenol A-induced mitochondrial toxicity in rat liver. *Ir J Basic Med Sci*. 2019;**22**(5):499-505. PMCID: PMC6556511 doi: 10.22038/ijbms.2019.32486.7952
6. Braniste V, Jouault A, Gaultier E, Polizzi A, Buisson-Brenac C, Leveque M, et al. Impact of oral bisphenol A at reference doses on intestinal barrier function and sex differences after perinatal exposure in rats. *Proce Nat Acad Sci*. 2010;**107**(1):448-53. PMCID: PMC2806743 doi: 10.1073/pnas.0907697107
7. Rameshrad M, Imenshahidi M, Razavi BM, Iranshahi M, Hosseinzadeh H. Bisphenol A vascular toxicity: Protective effect of Vitis vinifera (grape) seed extract and Resveratrol. *Phytother Res*. 2018;**32**(12):2396-407. doi: 10.1002/ptr.6175
8. Tzatzarakis MN, Vakonaki E, Kavvalakis MP, Barmpas M, Kokkinakis EN, Xenos K, et al. Biomonitoring of bisphenol A in hair of Greek population. *Chemosphere*. 2015;**118**:336-41. doi: 10.1016/j.chemosphere.2014.10.044
9. Acconcia F, Pallottini V, Marino M. Molecular mechanisms of action of BPA. *Dose Res*. 2015;**13**(4):1559325815610582. PMCID: PMC4679188 doi: 10.1177/1559325815610582
10. Tong X, Li Y, Zhang F, Chen X, Zhao Y, Hu B, et al. Adsorption of 17beta-estradiol onto humic-mineral complexes and effects of temperature, pH, and bisphenol A on the adsorption process. *Environ Pollut*. 2019;**254**(PtA):112924. doi: 10.1016/j.envpol.2019.07.092
11. Kourouma A, Quan C, Duan P, Qi S, Yu T, Wang Y, et al. Bisphenol A induces apoptosis in liver cells through induction of ROS. *Adva Toxic*. 2015;**2015**. doi: 10.1155/2015/901983
12. Morgan AM, El-Ballal SS, El-Bialy BE, El-Borai NB. Studies on the potential protective effect of cinnamon against bisphenol A-and octylphenol-induced oxidative stress in male albino rats. *Toxicology reports*. 2014;**1**:92-101. PMCID: PMC5598475 doi: 10.1016/j.toxrep.2014.04.003
13. Han C, Hong Y-C. Bisphenol A, hypertension, and cardiovascular diseases: epidemiological, laboratory, and clinical trial evidence. *Cur Hypert Reps*. 2016;**18**(2):11-22. doi: 10.1007/s11906-015-0617-2
14. Folia M, Boudalia S, Ménétrier F, Decocq L, Pasquis B, Schneider C, et al. Oral homeostasis disruption by medical plasticizer component bisphenol A in adult male rats. *Laryng*. 2013;**123**(6):1405-10. doi: 10.1002/lary.23791
15. Seki K, Koshi R, Sugano N, Masutani S, Yoshinuma N, Ito K. Microarray Analysis of Bisphenol A-induced Changes in Gene Expression in Human Oral Epithelial Cells. *Acta Bioch Bioph Sinica*. 2007;**39**(11):879-84. doi: 10.1111/j.1745-

7270.2007.00351.x
16. Bradamante S, Barenghi L, Villa A. Cardiovascular protective effects of resveratrol. *Cardio Dru Rev*. 2004;**22**(3):169-88. doi: 10.1111/j.1527-3466.2004.tb00139.x
17. Liu F-C, Tsai H-I, Yu H-P. Organ-protective effects of red wine extract, Resveratrol, in oxidative stress-mediated reperfusion injury. *Oxida Med Cell Long*. 2015;**2015**:56834. PMCID: PMC4487914 doi: 10.1155/2015/568634
18. Jia R, Li Y, Cao L, Du J, Zheng T, Qian H, et al. Antioxidative, anti-inflammatory and hepatoprotective effects of Resveratrol on oxidative stress-induced liver damage in tilapia (Oreochromis niloticus). Comparative Biochemistry and Physiology Part - C: *Toxic Pharma*. 2019;**215**:56-66. doi: 10.1016/j.cbpc.2018.10.002
19. Jia E, Yan Y, Zhou M, Li X, Jiang G, Liu W, et al. Combined effects of dietary quercetin and Resveratrol on growth performance, antioxidant capability and innate immunity of blunt snout bream (Megalobrama amblycephala). *Anim Feed Sci Tech*. 2019;**256**:19-25. https://doi.org/10.1016/j.anifeedsci.2019.114268
20. Han Z, Yang Q, Liu B, Wu J, Li Y, Yang C, et al. MicroRNA-622 functions as a tumor suppressor by targeting K-Ras and enhancing the anticarcinogenic effect of Resveratrol. *Carcinog*. 2012;**33**(1):131-9. doi: 10.1093/carcin/bgr226
21. Singh D, Mendonsa R, Koli M, Subramanian M, Nayak SK. Antibacterial activity of Resveratrol structural analogues: A mechanistic evaluation of the structure-activity relationship. *Toxi App Pharma*. 2019;**367**:23-32. doi: 10.1016/j.taap.2019.01.025
22. Vestergaard M, Ingmer H. Antibacterial and antifungal properties of resveratrol. *Int J Antiml Ag*. 2019;**53**(6):716-23. doi: 10.1016/j.ijantimicag.2019.02.015
23. Zhao X, Tong W, Song X, Jia R, Li L, Zou Y, et al. Antiviral effect of Resveratrol in piglets infected with virulent pseudorabies virus. *Viruses*. 2018;**10**(9). PMCID: PMC6164078 doi: 10.3390/v10090457
24. Riba A, Deres L, Sumegi B, Toth K, Szabados E, Halmosi R. Cardioprotective effect of Resveratrol in a postinfarction heart failure model. *Oxid Med Cell Long*. 2017;21-32. doi: 10.1155/2017/6819281
25. Lin KL, Lin KJ, Wang PW, Chuang JH, Lin HY, Chen SD, et al. Resveratrol provides neuroprotective effects through modulation of mitochondrial dynamics and ERK1/2 regulated autophagy. *Free Radic Res*. 2018;**52**(11-12):1371-86. doi: 10.1080/10715762.2018.1489128
26. Sticozzi C, Belmonte G, Cervellati F, Muresan XM, Pessina F, Lim Y, et al. Resveratrol protects SR-B1 levels in keratinocytes exposed to cigarette smoke. *Free Radic Res*. 2014;**69**:50-7. PMCID: PMC3967961 doi: 10.1016/j.freeradbiomed.2014.01.007
27. Cai Y-J, Fang J-G, Ma L-P, Yang L, Liu Z-L. Inhibition of free radical-induced peroxidation of rat liver microsomes by Resveratrol and its analogues. *Bioch Bioph Acta (BBA)-Mole Disease*. 2003;**1637**(1):31-8. doi: 10.1016/s0925-4439(02)00174-6
28. Rameshrad M, Razavi BM, Imenshahidi M, Hosseinzadeh H. Vitis vinifera (grape) seed extract and Resveratrol alleviate bisphenol-A-induced metabolic syndrome: Biochemical and molecular evidences. *Phyto Res*. 2019;**33**(3):832-44. doi: 10.1002/ptr.6276
29. Rameshrad M, Imenshahidi M, Razavi BM, Iranshahi M, Hosseinzadeh H. Bisphenol A vascular toxicity: Protective effect of Vitis vinifera (grape) seed extract and Resveratrol. *Phyto Res*. 2018;**32**(12):2396-407. doi: 10.1002/ptr.6175
30. Hamza RZ, Abu-El-Zahab HS, Al-Juaid NS. Hepatoprotective and antioxidant role of Resveratrol on bisphenol a - Induced toxicity in rats. *Toxi Int*. 2017;**24**(3):292-300. https://www.informaticsjournals.com/index.php/toxi/article/view/21106
31. Biswas P, Dellanoce C, Vezzoli A, Mrakic-Sposta S, Malnati M, Beretta A, et al. Antioxidant Activity with Increased Endogenous Levels of Vitamin C, E and A Following Dietary Supplementation with a Combination of Glutathione and Resveratrol Precursors. *Nutrients*. 2020;**12**(11):3224. PMCID: PMC7690269 doi: 10.3390/nu12113224
32. Li C-Y, Zhao Y-H, Hao H-S, Wang H-Y, Huang J-M, Yan C-L, et al. Resveratrol significantly improves the fertilisation capacity of bovine sex-sorted semen by inhibiting apoptosis and lipid peroxidation. *Sci Rep*. 2018;**8**(1):1-10. PMCID: PMC5954094 doi: 10.1038/s41598-018-25687-z
33. Haghighatdoost F, Hariri M. Can Resveratrol supplement change inflammatory mediators? A systematic review and meta-analysis on randomized clinical trials. *Euro J Clin Nutr*. 2019;**73**(3):345-55. doi: 10.1038/s41430-018-0253-4
34. Bordbar H, Soleymani A, Nadimi E, Yahyavi S-S, Fazelian-Dehkordi K. A Quantitative Study on the Protective Effects of Resveratrol against Bisphenol A-induced Hepatotoxicity in Rats: A Stereological Study. *Iran J Med Sci*. 2020;**46**(3):218-227. PMCID: PMC8163701 doi: 10.30476/ijms.2020.83308.1233
35. Unal B, Kara A, Aksak S, Unal D. A stereological assessment method for estimating the surface area of cycloids. *Eurasian J Med*. 2010;**42**(2):66-73. PMCID: PMC4261337 doi: 10.5152/eajm.2010.20
36. Karbalay-Doust S, Noorafshan A. Stereological study of the effects of nandrolone decanoate on the mouse liver. *Micron*. 2009;**40**(4):471-5. doi: 10.1016/j.micron.2008.12.006
37. Scherle W. A simple method for volumetry of organs in quantitative stereology. *Mikroskopie*. 1970;**26**:57-60. PMID: 5530651
38. Zhang H, Zhang J, Ungvari Z, Zhang C. Resveratrol improves endothelial function: role of TNFα and vascular oxidative stress. *Arteriosc Thromb Vasc Biology*. 2009;**29**(8):1164-71. PMCID: PMC2761384 doi: 10.1161/ATVBAHA.109.187146
39. Guthrie AR, Chow HHS, Martinez JA. Effects of Resveratrol on drug-and carcinogen-metabolizing enzymes, implications for cancer prevention. *Pharmacol Res Perspect*. 2017;**5**(1):e00294. PMCID: PMC5461649 doi: 10.1002/prp2.294
40. Zhou L, Long J, Sun Y, Chen W, Qiu R, Yuan D. Resveratrol ameliorates atherosclerosis induced by high-fat diet and LPS in ApoE−/− mice and inhibits the activation of CD4+ T cells. *Nutr Metab (Lond)*. 2020;**17**:1-12. PMCID: PMC7251691 doi: 10.1186/s12986-020-00461-z
41. Polonio N, Rocha C, Clemente E. Trans-resveratrol concentrations and antimutagenic potential of juice from the grape cultivars Vênus, BRS Violeta and Isabel. *Gene Molec Res*. 2014;**13**(1):1152-9. doi: 10.4238/2014.February.25.1
42. Kim JY, Cho KH, Lee HY. Effect of Resveratrol on oral cancer cell invasion induced by lysophosphatidic acid. *J Den Hyg Sci*. 2018;**18**(3):188-93. doi: 10.17135/jdhs.2018.18.3.188
43. Çiğ B, Yildizhan K. Resveratrol diminishes bisphenol A-induced oxidative stress through TRPM2 channel in the mouse kidney cortical collecting duct cells. *J Rece Sig Transd*. 2020;**1**:1-14. doi: 10.1080/10799893.2020.1769657
44. Yener A, Mustafa N. Resveratrol attenuates hypoxia-induced neuronal cell death, inflammation and mitochondrial oxidative stress by modulation of TRPM2 channel. *Sci Rep*. 2020;**10**(1):11-25. https://doi.org/10.1038/s41598-020-63577-5
45. Xia N, Daiber A, Förstermann U, Li H. Antioxidant effects of Resveratrol in the cardiovascular system. *Br J Pharmacol*. 2017;**174**(12):1633-46. PMCID: PMC5446570 doi: 10.1111/bph.13492
46. Gordish KL, Beierwaltes WH. Resveratrol induces acute endothelium-dependent renal vasodilation mediated through nitric oxide and reactive oxygen species scavenging. *Am J Physiol Renal Physiol*. 2014;**306**(5):F542-F50. PMCID: PMC3949032 doi: 10.1152/ajprenal.00437.2013
47. Babich H, Reisbaum A, Zuckerbraun H. In vitro response of human gingival epithelial SG cells to Resveratrol. *Toxicol Lett*. 2000;**114**(1-3):143-53. doi: 10.1016/s0378-4274(99)00288-x
48. Mikami S, Ota I, Masui T, Itaya‐Hironaka A, Shobatake R, Okamoto H, et al. Effect of Resveratrol on cancer progression through the REG Ⅲ expression pathway in head and neck cancer cells. *Int J Oncol*. 2016;**49**(4):1553-60. doi: 10.3892/ijo.2016.3664
49. Quagliariello V, Coppola C, Mita D, Piscopo G, Iaffaioli R, Botti G, et al. Low doses of Bisphenol A have pro-inflammatory and pro-oxidant effects, stimulate lipid peroxidation and increase the cardiotoxicity of Doxorubicin in cardiomyoblasts. *Envir Toxic Pharmy*. 2019;**69**:1-8. doi: 10.1016/j.etap.2019.03.006
50. Lee J, Lim K-T. Expression of TNF-α and IL-6 in HMC-1 cells treated with bisphenol A is attenuated by plant-originating glycoprotein (75 kDa) by blocking p38 MAPK. *Nau Schmie Arch*

Pharma. 2010;**382**(1):51-61. doi: 10.1007/s00210-010-0527-4
51. Wu Y, Pang Y, Wei W, Shao A, Deng C, Li X, et al. Resveratrol protects retinal ganglion cell axons through regulation of the SIRT1-JNK pathway. *Exp Eye Res*. 2020;**200**:108249. doi: 10.1016/j.exer.2020.108249
52. Csiszar A, Labinskyy N, Pinto JT, Ballabh P, Zhang H, Losonczy G, et al. Resveratrol induces mitochondrial biogenesis in endothelial cells. *Am J Physiol Heart Circ Physiol.* 2009;**297**(1):H13-H20. PMCID: PMC2711732 doi: 10.1152/ajpheart.00368.2009