Original Article / Özgün Araştırma

Meandros Med Dent J
doi:10.4274/meandros.galenos.2023.70973
Meandros Med Dent J 2023;24(4):303-308

Antioxidant Effects of Styrax Liquidus on DMBA-exposed Rat Tongue Tissues

Styrax Liquidus'un DMBA Uygulanmış Rat Dil Dokuları Üzerindeki Antioksidan Etkileri

Dilara Nur Şengün[1], İnci Rana Karaca[2], Hasan Serdar Öztürk[3]

[1]Ankara University Faculty of Dentistry, Department of Oral and Maxillofacial Surgery, Ankara, Turkey
[2]Gazi University Faculty of Dentistry, Department of Oral and Maxillofacial Surgery, Ankara, Turkey
[3]Ankara University Faculty of Medicine, Department Of Medical Biochemistry, Ankara, Turkey

Abstract

Objective: Natural products with antioxidant components are believed to have a strong potential for the prevention of cancer and some degenerative diseases. Liquidambar orientalis Miller (Styrax Liquidus) has strong *in vitro* antioxidant activity. The purpose of this study was to evaluate the effects of Styrax Liquidus on antioxidant defense mechanisms in 7,12-dimethylbenz(a)anthracene (DMBA)-applied rat tongue tissue.

Materials and Methods: Wistar rats (n=30) were randomly divided into control, DMBA, DMBA + SL, and SL groups. The control group was treated with liquid paraffin only, the DMBA group was treated with 0.5% DMBA, DMBA and Styrax Liquidus were applied to the DMBA + SL group, and only Styrax Liquidus was applied to the SL group. All applications were made to the oral mucosa. Sixteen weeks later, the tongue tissue of all animals were removed. Superoxide dismutase, catalase, glutathione peroxidase enzyme activities, and malondialdehyde and total antioxidant status levels were measured.

Results: All parameters were significantly lower in the SL + DMBA group. Antioxidant enzyme activities and oxidative stress parameters were lowered in the SL + DMBA and SL groups. SL + DMBA application is believed to have an inhibitory effect on the antioxidant enzymes measured in this study; however, the decrease in malondialdehyde levels (lipid peroxidation marker) highlights the antioxidant effect of Styrax Liquidus in 7,12-dimethylbenz(a)anthracene-exposed rat tongue tissues.

Conclusion: Styrax Liquidus exhibited *in vivo* antioxidant activity in an oral cancer model. Further research may be useful in understanding the exact mechanisms underlying this effect.

Keywords: Antioxidant, 7,12-Dimethylbenzanthracene, Liquidambar, oral cancer, oxidant, Styrax

Öz

Amaç: Antioksidan içerikli doğal ürünlerin kanser ve bazı dejeneratif hastalıkların önlenmesinde güçlü bir potansiyele sahip olduğuna inanılmaktadır. Liquidambar orientalis Miller'ın (Styrax Liquidus) güçlü *in vitro* antioksidan aktiviteye sahip olduğu bilinmektedir. Bu çalışmada, Styrax Liquidus'un 7,12-dimetilbenz(a)antrasen (DMBA) uygulanan rat dil dokularında antioksidan savunma mekanizmaları üzerindeki etkilerinin değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntemler: Çalışmaya dahil edilen otuz adet Wistar türü rat rastgele kontrol, DMBA, DMBA + SL ve SL gruplarına ayrılmıştır. Kontrol grubuna sıvı parafin, DMBA grubuna %0,5'lik DMBA, DMBA + SL grubuna DMBA ve Styrax Liquidus, SL grubuna ise yalnızca Styrax Liquidus uygulanmıştır. Tüm uygulamalar oral mukozaya yapılmış olup, deney süresinin sonunda tüm hayvanların dil dokuları alınmıştır. Dokulardaki süperoksit dismutaz, katalaz, glutatyon peroksidaz enzim aktiviteleri ve malondialdehit ve total antioksidan durum düzeyleri ölçülmüştür.

Bulgular: SL + DMBA grubunda tüm parametreler anlamlı derecede düşük bulunmuştur. SL + DMBA ve SL gruplarında antioksidan enzim aktiviteleri ve oksidatif stres parametreleri diğer gruplara kıyasla daha az olarak bulunmuştur. Styrax Liquidus ve DMBA uygulamasının bu çalışmada ölçülen antioksidan enzimler üzerinde inhibitör etkisi olduğu düşünülmüştür; ancak, lipid peroksidasyon

Address for Correspondence/Yazışma Adresi: Dilara Nur Şengün, Ankara University Faculty of Dentistry, Department of Oral and Maxillofacial Surgery, Ankara, Turkey
Phone: +90 506 504 07 06 **E-mail:** dnsengun@ankara.edu.tr
ORCID ID: orcid.org/0000-0002-6452-1580

Received/Geliş Tarihi: 24.08.2023
Accepted/Kabul Tarihi: 29.09.2023

Copyright© 2023 The Author. Published by Galenos Publishing House on behalf of Aydın Adnan Menderes University, Faculty of Medicine and Faculty of Dentistry. This is an open access article under the Creative Commons AttributionNonCommercial 4.0 International (CC BY-NC 4.0) License.

belirteci olan malondialdehit düzeylerindeki azalma, Styrax Liquidus'un DMBA'ya maruz kalan rat dil dokuları üzerindeki antioksidan etkisinin altını çizmektedir.

Sonuç: Styrax Liquidus bu çalışmada oral kanser modelinde *in vivo* antioksidan aktivite sergilemiştir. Bu etkinin mekanizmasının anlaşılması için başka çalışmalara da ihtiyaç vardır.

Anahtar Kelimeler: Antioksidan, 7,12-Dimetilbenzanthrasen, Liquidambar, oral kanser, oksidan, Styrax

Introduction

Reactive oxygen species (ROS) are a highly reactive group of molecules produced mostly in the mitochondria (electron transport chain) during the respiratory functions of a cell. These molecules include hydroxyl and superoxide radicals and stable molecules such as H_2O_2 (1). In healthy cells, ROS levels are balanced through various mechanisms and detoxification processes. However; if the redox balance is somehow disrupted, this causes oxidative stress and may have pathological consequences such as diabetes mellitus, atherosclerosis and cancer (2).

Cancer is serious disease that has very high mortality rates around the world (3). Oral cancer, including lip and oral cavity, salivary gland, oropharynx, nasopharynx and hypopharynx cancers, is the 7th most commonly encountered type of cancer worldwide (4). Oral squamous cell carcinomas (OSCC) include more than 90% of oral cancers (5). Oral cancers have high mortality and morbidity rates, treatment is costly, and it does not always have such good prognosis (6,7).

Chemoprevention is a novel and promising method that has been subject to numerous studies investigating the anticancer effects of plants and plant-derived chemicals (8). Many plants used in chemoprevention studies have a variety of pharmacological activities such as antioxidant, anticancer, antitumor and/or cytotoxic properties (9-12). Chemopreventive plants or plant-derived chemicals may inhibit the initiation phase or revert the promotion phase of the carcinogenesis process. They also have the potential to inhibit the progression of premalignant lesions to malignant stages (13).

L. orientalis Mill. is an herbaceous plant mostly found in several regions of Southeast Asia and the Mediterranean region. L. orientalis Mill. trees exudate a resinous balsam from the wounded parts of their trunk called Styrax Liquidus. It has been reported to possess antiseptic (14), antimicrobial (15), antibacterial (16,17), antiulcerogenic (18), antiviral (19), antifungal (20), antihypertensive (21), anticonvulsant (22), antioxidant (23) and antimutagenic (24) properties. Moreover, it has been used in different cultures as a phytotherapeutic agent for skin (wounds, cuts, burns, psoriasis and other skin diseases), stomach (ulcers, stomach ache) and respiratory diseases (cough, asthma and bronchitis) (25). Also, it has been shown to have neuroprotective effects in cerebrovascular diseases (26). The major components of Styrax Liquidus are cinnamic esters (especially cinnamic acid), styrene and vanillin. Cinnamic acid is known for its antimicrobial and antioxidant properties (20,22). The aim of this study was to evaluate the effects of Styrax Liquidus on antioxidant defense mechanisms in tongue tissues of rats that have been locally exposed to 7,12-dimethylbenz(a)anthracene (DMBA).

Materials and Methods

This study was approved by the Gazi University Animal Experiments Local Ethics Committee (approval number: G.Ü.ET-17.018, date of approval: 02.03.2017).

Plant Material

The crude balsam of L. orientalis Mill. was obtained from the provincial directorate of Ministry of Agriculture and Forestry in Muğla province of Turkey. 100 mg pure Styrax Liquidus was dissolved in 1 mL ethanol (99.9%) (100 mg/mL). The solution was diluted with ethanol to procure 50 mg/mL concentration. Then it was mixed with glycerin with a 1:1 (v/v) ethanol-glycerin ratio to achieve 10 mg/mL concentration. The solution was kept in dark-colored bottles at +4 ˚C.

Carcinogenic Material

DMBA (Sigma-Aldrich, Milwaukee, WI, USA) is a potent carcinogen used in oral cancer animal models, which induces oxidative stress and eventually results in precancerous and cancerous lesions histopathologically and morphologically similar to human oral precancerous and cancerous lesions (10,27). The DMBA used in this study was prepared with liquid paraffin (0.5%, w/v), according to previous research protocols (28,29). The solution was kept in opaque bottles at 27 ˚C.

Animals and Experimental Design

Male Wistar rats (n=30) were randomly divided into four groups (Control, DMBA, SL + DMBA, SL). The control group consisted of 6 rats, while 8 rats were assigned to other groups. Liquid paraffin, 0.5% DMBA dissolved in liquid paraffin and L. orientalis Mill. extract was applied to the oral mucosa of the animals with a no 4 paint brush. Control group was treated with liquid paraffin thrice a week (Monday, Wednesday, and Friday). DMBA group was painted with 0.5% DMBA frequently as the control group. SL + DMBA group received 10 mg/mL L. orientalis Mill. extract application twice (Tuesday, Thursday), and was treated with 0.5% DMBA thrice a week. SL group was applied L. orientalis Mill. extract twice a week (Tuesday, Thursday). Animals were kept under controlled conditions. Animals

were provided with rat chow and water. After 16 weeks of applications, all animals were sacrificed and tongue tissues of all animals were excised as a whole. The samples were kept at -80 ˚C until homogenization.

Biochemical Analysis

Tongue tissue samples were prepared and analyzed using the same methods as previously by the authors (30). Catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) enzyme activities and malondialdehyde (MDA) and total antioxidant status (TAS) levels measured in all samples.

Statistical Analysis

SPSS 11.5 software was used for statistics. Mean ± standard deviation and median (minimum-maximum) were used to express normally and not normally distributed values, respectively. ANOVA was used if the values were normally distributed and Kruskall-Wallis test, if not. Post-hoc Tukey test was used for binary comparisons after ANOVA and Bonferroni adjusted Mann-Whitney U test was used after Kruskall-Wallis test. P<0.05 were considered as statistically significant.

Results

All enzyme activities were significantly lower in Styrax Liquidus applied groups in comparison to the DMBA group (p<0.05) (Table 1, Figures 1-3). SL+DMBA and SL groups also revealed significantly lower values compared to the Control group considering CAT and SOD levels (p<0.05) (Table 1, Figures 1, 2). MDA levels were statistically lower with regard to the DMBA group, in SL + DMBA and SL groups (p<0.05) (Table 1, Figure 4). TAS levels were significantly decreased in the SL + DMBA group with regard to control and DMBA groups (p<0.05) (Table 1, Figure 5).

Discussion

This study was conducted to evaluate the impact of Styrax Liquidus on the oxidant/antioxidant system in tongue tissues of DMBA-exposed rats. DMBA is a potent carcinogen that exhibits its effects mainly through chronic inflammation, ROS production and oxidative DNA damage (9,27,31). It is widely used in experimental OSCC models because it induces lesions very similar to human OSCC regarding histological, morphological and invasive properties (11). Carcinogenesis is a multi-stage process with initiation, promotion and progression phases.

Table 1. CAT (IU/mg), SOD (U/mg), GSH-Px (mIU/mg) enzyme activities and MDA (nmol/mg) and TAS (µmol Trolox eq/L) values of the tongue tissue samples, descriptive statistics and multiple comparisons

	CAT Median (min-max)	SOD (mean ± SD)	GSH-Px (mean ± SD)	MDA (mean ± SD)	TAS (mean ± SD)
Control (n=6)	12.17 (11.04-23.04)	3.96±0.84	36.83±12.91	1.42±0.53	0.27±0.04
DMBA (n=8)	12.47 (10.77-16.89)	3.30±0.30	32.13±2.10	1.18±0.15	0.23±0.03
SL + DMBA (n=8)	7.17 (5.40-9.18)	2.27±0.14	23.50±5.95	0.88±0.10	0.17±0.02
SL (n=8)	7.13 (6.09-8.77)	2.47±0.20	28.87±2.10	0.97±0.04	0.23±0.07
Kruskall-Wallis test/ANOVA					
Multiple comparison (p-values)	0.000	0.000	0.007	0.002	0.002
Bonferroni adjusted Mann-Whitney U test/Tukey test (p-values)					
Control vs. DMBA	1.0	0.338	0.813	0.717	0.342
Control vs. SL + DMBA	0.010	0.015	0.180	0.173	0.001
Control vs. SL	0.006	0.025	0.501	0.270	0.265
DMBA vs. SL + DMBA	0.007	0.000	0.017	0.002	0.042
DMBA vs. SL	0.004	0.000	0.035	0.020	0.998
SL + DMBA vs. SL	1.0	0.151	0.147	0.173	0.062

CAT: Catalase, SOD: Superoxide dismutase, GSH-Px: Glutathione peroxidase, MDA: Malondialdehyde, TAS: Total antioxidant status, min-max: Minimum-maximum, DMBA: 7,12-dimethylbenz(a)anthracene, SD: Standard deviation

There is no single element associated with cancer formation. However, DNA oxidation is considered the most important factor due to involvement of oxidative stress in all stages of carcinogenesis (6).

L. orientalis Mill. has numerous pharmacological properties including antimutagenic, genotoxic, cytotoxic and antioxidant effects (23,24,32). Recently, in other studies its biological effects on cancer cell lines through cytotoxicity, apoptosis and autophagy has been investigated (32-34). Atmaca et al. (32) has shown that Styrax Liquidus inhibits viability of prostate cancer cell lines through induction of autophagy by inhibition of various signaling pathways. Cetinkaya et al. (34) used the aerial parts of the plant to obtain an extract and found that the extract showed anticancer activity on colorectal cancer cell lines through apoptotic pathways, Lastly, Baloglu et al. (33) used L. orientalis oil on breast, lung and prostate cell lines which revealed antitumor effect on all cancer lines but that it had the most cytotoxic effect on the breast cancer cell lines. All studies were done in vitro on cancer cell lines; however, the present study was carried out *in vivo*. In this study, the in vivo antioxidant effect of Styrax Liquidus was investigated on DMBA-exposed rat tongue tissues.

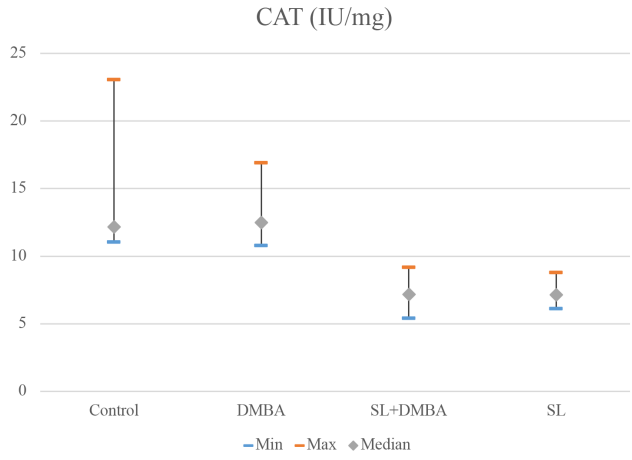

Figure 1. Descriptive statistics of the CAT (IU/mg) values
CAT: Catalase, DMBA: 7,12-dimethylbenz(a)anthracene

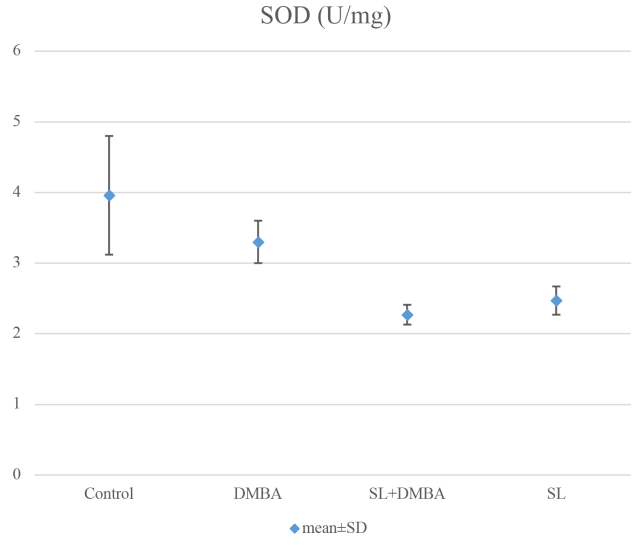

Figure 2. Descriptive statistics of the SOD (U/mg) values
SOD: Superoxide dismutase, DMBA: 7,12-dimethylbenz(a) anthracene, SD: Standard deviation

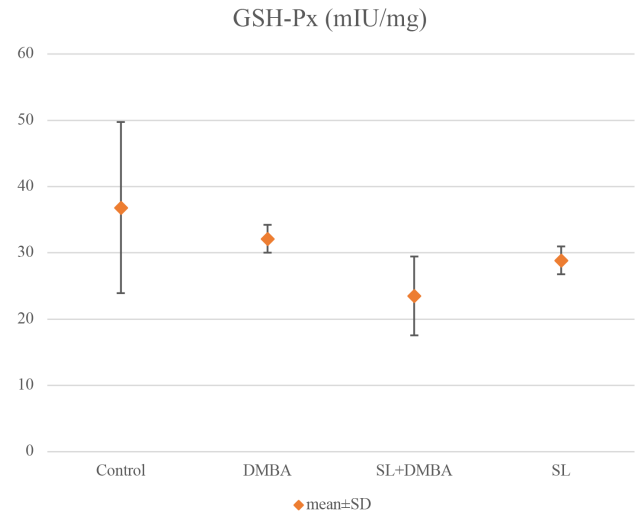

Figure 3. Descriptive statistics of the GSH-Px (mIU/mg) values
GSH-Px: Glutathione peroxidase, DMBA: 7,12-dimethylbenz(a) anthracene

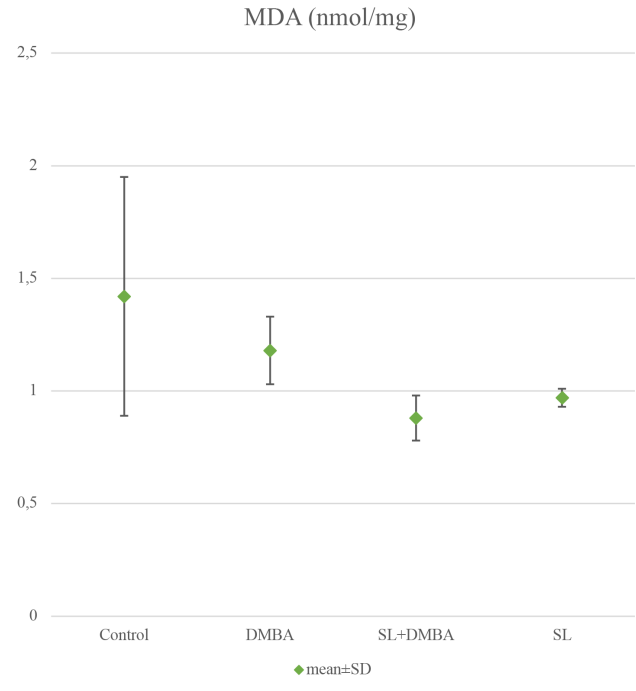

Figure 4. Descriptive statistics of the MDA (nmol/mg) values
MDA: Malondialdehyde, DMBA: 7,12-dimethylbenz(a)anthracene, SD: Standard deviation

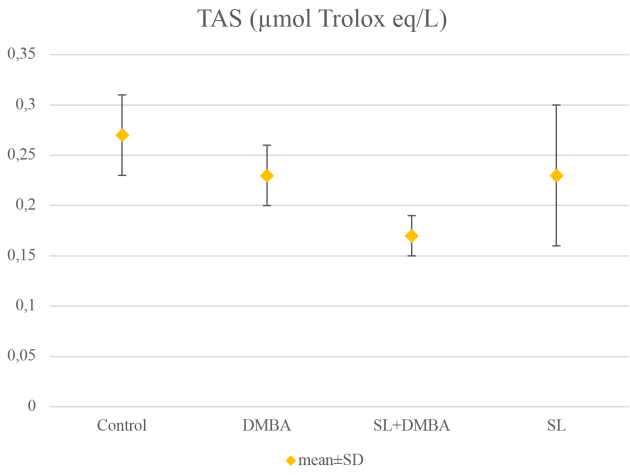

Figure 5. Descriptive statistics of the TAS (µmol Trolox eq/L) values
TAS: Total antioxidant status, DMBA: 7,12-dimethylbenz(a) anthracene

CAT, SOD and GSH-Px enzyme activities were lowest in SL + DMBA group. This is believed to have happened because of an interaction between Styrax Liquidus and DMBA, which may have produced a by-product that has the potential to disrupt these enzymes' activities. More studies are needed to further enlighten this mechanism. TAS levels were also significantly decreased in the corresponding group. This decrease was interpreted to be the result of lowered antioxidant enzyme activities (CAT, SOD, and GSH-Px). TAS levels express the residual free radical scavenging capacity after ROS neutralization (35). The decrease in TAS levels is supportive of the assumption that the antioxidant enzyme activities were disrupted in the SL + DMBA group.

In the SL group, all enzyme activities were significantly lower compared to the DMBA and control groups. This decrease was believed to be a result of the antioxidant effect of Styrax Liquidus. The antioxidant effect of Styrax Liquidus as an exogenous antioxidant might have prevented oxidative stress formation; thus, suppressing the need for endogenous antioxidant enzyme production and activity.

MDA is a marker of lipid peroxidation that is considered indicative of oxidation or oxidative stress. As lipid peroxidation in a tissue is increased consequently MDA levels increase (6,36). In the present study, MDA levels were significantly lower in Styrax Liquidus-applied groups compared to other groups. In the SL + DMBA group, the MDA levels were thought to have decreased as a consequence of the radical scavenging activity of Styrax Liquidus. Lower MDA levels in the S group show Styrax Liquidus does not cause any oxidative damage to healthy tissues; on the contrary, it acts as an exogenous antioxidant and lowers the oxidative stress.

Conclusion

Considering the results obtained in this study, Styrax Liquidus has revealed *in vivo* antioxidant efficacy in DMBA-exposed rat tongue tissues. Especially the decreased MDA levels in the SL + DMBA group is an important data pointing out the antioxidant effect. The fact that a decrease in MDA level was achieved regardless of the suppressed antioxidant enzyme activities is believed to be caused by the antioxidant components of Styrax Liquidus. Styrax Liquidus may be a promising candidate for further research regarding its mechanisms of action against oxidative stress and cancer.

Acknowledgements

We acknowledge Prof. Aysel Uğur and Assoc. Prof. Nurdan Saraç for their contributions about extract preparation.

Ethics

Ethics Committee Approval: This study was approved by the Gazi University Animal Experiments Local Ethics Committee (approval number: G.Ü.ET-17.018, date of approval: 02.03.2017).

Informed Consent: Informed consent is not required.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: D.N.Ş., Concept: İ.R.K., H.S.Ö., Design: İ.R.K., H.S.Ö., Data Collection or Processing: D.N.Ş., Analysis or Interpretation: H.S.Ö., Literature Search: D.N.Ş., Writing: D.N.Ş., İ.R.K., H.S.Ö.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: This study was funded by Gazi University, Projects of Scientific Investigation Unit (Grant number: 03/2017-19).

References

1. Perillo B, Di Donato M, Pezone A, Di Zazzo E, Giovannelli P, Galasso G, et al. ROS in cancer therapy: the bright side of the moon. Exp Mol Med 2020; 52: 192-203.
2. Aggarwal V, Tuli HS, Varol A, Thakral F, Yerer MB, Sak K, et al. Role of Reactive Oxygen Species in Cancer Progression: Molecular Mechanisms and Recent Advancements. Biomolecules 2019; 9: 735.
3. World Health Organization. Cancer Key Facts 2022. https://www.who.int/news-room/fact-sheets/detail/cancer.
4. Global Cancer Observatory. Cancer Fact Sheets 2020. https://gco.iarc.fr/today/fact-sheets-cancers.
5. Abati S, Bramati C, Bondi S, Lissoni A, Trimarchi M. Oral Cancer and Precancer: A Narrative Review on the Relevance of Early Diagnosis. Int J Environ Res Public Health 2020; 17: 9160.
6. Scrobota I, Bolfa P, Filip AG, Catoi C, Alb C, Pop O, et al. Natural chemopreventive alternatives in oral cancer chemoprevention. J Physiol Pharmacol 2016; 67: 161-72.

7. Chau L, Jabara JT, Lai W, Svider PF, Warner BM, Lin HS, et al. Topical agents for oral cancer chemoprevention: A systematic review of the literature. Oral Oncol 2017; 67: 153-9.
8. Li Y, Zheng Y, Wang H. Anticancer activity of Vicenin-2 against 7,12 dimethylbenz[a]anthracene-induced buccal pouch carcinoma in hamsters. J Biochem Mol Toxicol 2021; 35: e22673.
9. Suresh K, Manoharan S, Vijayaanand MA, Sugunadevi G. Chemopreventive and antioxidant efficacy of (6)-paradol in 7,12-dimethylbenz(a)anthracene induced hamster buccal pouch carcinogenesis. Pharmacol Rep 2010; 62: 1178-85.
10. Manoharan S, Sindhu G, Vinothkumar V, Kowsalya R. Berberine prevents 7,12-dimethylbenz[a]anthracene-induced hamster buccal pouch carcinogenesis: a biochemical approach. Eur J Cancer Prev 2012; 21: 182-92.
11. Manoharan S, Vasanthaselvan M, Silvan S, Baskaran N, Kumar Singh A, Vinoth Kumar V. Carnosic acid: a potent chemopreventive agent against oral carcinogenesis. Chem Biol Interact 2010; 188: 616-22.
12. Gonçalves Vde P, Ortega AA, Guimarães MR, Curylofo FA, Rossa Junior C, Ribeiro DA, et al. Chemopreventive activity of systemically administered curcumin on oral cancer in the 4-nitroquinoline 1-oxide model. J Cell Biochem 2015; 116: 787-96.
13. Surh YJ. Cancer chemoprevention with dietary phytochemicals. Nat Rev Cancer 2003; 3: 768-80.
14. Okmen G, Turkcan O, Ceylan O, Gork G. The antimicrobial activity of Liquidambar orientalis mill. against food pathogens and antioxidant capacity of leaf extracts. Afr J Tradit Complement Altern Med 2014; 11: 28-32.
15. Guzman JD. Natural cinnamic acids, synthetic derivatives and hybrids with antimicrobial activity. Molecules 2014; 19: 19292-349.
16. Sağdiç O, Ozkan G, Ozcan M, Ozçelik S. A study on inhibitory effects of Siğla tree (Liquidambar orientalis Mill. var. orientalis) storax against several bacteria. Phytother Res 2005; 19: 549-51.
17. Karadeniz B, Ulker Z, Alpsoy L. Genotoxic and cytotoxic effects of storax in vitro. Toxicol Ind Health 2013; 29: 181-6.
18. Gurbuz I, Yesilada E, Demirci B, Sezik E, Demirci F, Baser KH. Characterization of volatiles and anti-ulcerogenic effect of Turkish sweetgum balsam (Styrax liquidus). J Ethnopharmacol 2013; 148: 332-6.
19. Yesilada E, Gürbüz I, Bedir E, Tatli I, Khan IA. Isolation of anti-ulcerogenic sesquiterpene lactones from Centaurea solstitialis L. ssp. solstitialis through bioassay-guided fractionation procedures in rats. J Ethnopharmacol 2004; 95: 213-9.
20. Lingbeck JM, O'Bryan CA, Martin EM, Adams JP, Crandall PG. Sweetgum: An ancient source of beneficial compounds with modern benefits. Pharmacogn Rev 2015; 9: 1-11.
21. Charehsaz M, Reis R, Helvacioglu S, Sipahi H, Guzelmeric E, Acar ET, et al. Safety evaluation of styrax liquidus from the viewpoint of genotoxicity and mutagenicity. J Ethnopharmacol 2016; 194: 506-12.
22. Guo J, Duan JA, Tang Y, Li Y. Sedative and anticonvulsant activities of styrax after oral and intranasal administration in mice. Pharm Biol 2011; 49: 1034-8.
23. Suzek H, Celik I, Dogan A, Yildirim S. Protective effect and antioxidant role of sweetgum (Liquidambar orientalis) oil against carbon tetrachloride-induced hepatotoxicity and oxidative stress in rats. Pharm Biol 2016; 54: 451-7.
24. Saraç N, Şen B. Antioxidant, mutagenic, antimutagenic activities, and phenolic compounds of Liquidambar orientalis Mill. var. orientalis. Industrial Crops and Products 2014; 53: 60-4.
25. Gürdal B, Kültür S. An ethnobotanical study of medicinal plants in Marmaris (Muğla, Turkey). J Ethnopharmacol 2013; 146: 113-26.
26. Zhang M, Ma Y, Chai L, Mao H, Zhang J, Fan X. Storax Protected Oxygen-Glucose Deprivation/Reoxygenation Induced Primary Astrocyte Injury by Inhibiting NF-κB Activation in vitro. Front Pharmacol 2019; 9: 1527.
27. Baskaran N, Manoharan S, Karthikeyan S, Prabhakar MM. Chemopreventive potential of coumarin in 7, 12-dimethylbenz[a] anthracene induced hamster buccal pouch carcinogenesis. Asian Pac J Cancer Prev 2012; 13: 5273-9.
28. Nagini S, Kowshik J. The Hamster Buccal Pouch Model of Oral Carcinogenesis. Methods Mol Biol 2016; 1422: 341-50.
29. Babukumar S, Vinothkumar V, Velu P, Ramachandhiran D, Ramados Nirmal M. Molecular effects of hesperetin, a citrus flavanone on7,12-dimethylbenz(a)anthracene induced buccal pouch squamous cell carcinoma in golden Syrian hamsters. Arch Physiol Biochem 2017; 123: 265-78.
30. Şengün DN, Demirci G, Karaca İR. Antioxidant Efficacy of Hypericum Perforatum L. on 7,12-Dimethylbenzanthracene-Applied Rat Tongue Tissues. Archives of Current Medical Research 2021; 2: 33-7.
31. Alias LM, Manoharan S, Vellaichamy L, Balakrishnan S, Ramachandran CR. Protective effect of ferulic acid on 7,12-dimethylbenz[a]anthracene-induced skin carcinogenesis in Swiss albino mice. Exp Toxicol Pathol 2009; 61: 205-14.
32. Atmaca H, Camli Pulat C, Cittan M. Liquidambar orientalis Mill. gum extract induces autophagy via PI3K/Akt/mTOR signaling pathway in prostate cancer cells. Int J Environ Health Res 2022; 32: 1011-9.
33. Cengiz Baloglu M, Yildiz Ozer L, Pirci B, Zengin G, Ibrahim Uba A, Celik Altunoglu Y. Evaluation of the Potential Therapeutic Properties of Liquidambar orientalis Oil. Chem Biodivers 2023; 20: e202300291.
34. Çetinkaya S, Çınar Ayan İ, Süntar İ, Dursun HG. The Phytochemical Profile and Biological Activity of Liquidambar orientalis Mill. var. orientalis via NF-κB and Apoptotic Pathways in Human Colorectal Cancer. Nutr Cancer 2022; 74: 1457-73.
35. Strycharz-Dudziak M, Kiełczykowska M, Drop B, Świątek Ł, Kliszczewska E, Musik I, et al. Total Antioxidant Status (TAS), Superoxide Dismutase (SOD), and Glutathione Peroxidase (GPx) in Oropharyngeal Cancer Associated with EBV Infection. Oxid Med Cell Longev 2019; 2019: 5832410.
36. Kolanjiappan K, Ramachandran CR, Manoharan S. Biochemical changes in tumor tissues of oral cancer patients. Clin Biochem 2003; 36: 61-5.