UNIVERSIDADE ESTADUAL DE MONTES CLAROS

Karina Marini Aguiar

Potencial antineoplásico de antioxidantes e ação adjuvante do ácido gálico à radiação ionizante no carcinoma epidermoide de boca: uma revisão sistemática e estudo *in vitro*

Montes Claros

2020

Karina Marini Aguiar

Potencial antineoplásico de antioxidantes e ação adjuvante do ácido gálico à radiação ionizante no carcinoma epidermoide de boca: uma revisão sistemática e estudo *in vitro*

Tese de Doutorado apresentado ao Programa de Pós-graduação em Ciências em Saúde da Universidade Estadual de Montes Claros-Unimontes, como parte das exigências para a obtenção do título de Doutora em Ciências da Saúde.

Área de Concentração: Mecanismos e Aspectos Clínicos das Doenças

Orientadora: Profa. Dra. Lucyana Conceição Farias

Coorientador: Prof. Dr. André Luiz Sena Guimarães

Montes Claros

2020

A282p

Aguiar, Karina Marini.
Potencial antineoplásico de antioxidantes e ação adjuvante do ácido gálico à radiação ionizante no carcinoma epidermoide de boca [manuscrito] : uma revisão sistemática e estudo *in vitro* / Karina Marini Aguiar. – Montes Claros, 2020.
96 f. : il.

Inclui Bibliografia.
Tese (Doutorado) - Universidade Estadual de Montes Claros - Unimontes, Programa de Pós-Graduação em Ciências da Saúde /PPGCS, 2020.

Orientadora: Profa. Dra. Lucyana Conceição Farias.
Coorientador: Prof. Dr. André Luiz Sena Guimarães.

1. Carcinoma epidermoide de boca. 2. Antioxidantes. 3. Ácido gálico. 4. Radioterapia. 5. Radiação ionizante terapêutica. I. Farias, Lucyana Conceição. II. Guimarães, André Luiz Sena. III. Universidade Estadual de Montes Claros. IV. Título. V. Título: Uma revisão sistemática *in vitro.*

Catalogação Biblioteca Central Professor Antônio Jorge

UNIVERSIDADE ESTADUAL DE MONTES CLAROS-UNIMONTES

Reitor: Antônio Avilmar Souza

Vice-reitora: Ilva Ruas de Abreu

Pró-reitora de Pesquisa: Profª. Clarice Diniz Alvarenga Corsato

Coordenadoria de Acompanhamento de Projetos: Vigílio Mesquita Gomes

Coordenadoria de Iniciação Científica: Sônia Ribeiro Arrudas

Coordenadoria de Inovação Tecnológica: Dario Alves de Oliveira

Pró-reitor de Pós-Graduação: André Luiz Sena Guimarães

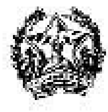
Coordenadoria de Pós-Graduação *Lato sensu*: Marcos Flávio D'Ângelo

Coordenadoria de Pós-Graduação *Stricto sensu*: Marcelo Perim Baldo

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE

Coordenador: Prof. Dr. Alfredo Maurício Batista de Paula

Subcoordenadora: Profa. Dra. Marise Fagundes Silveira



UNIVERSIDADE ESTADUAL DE MONTES CLAROS
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE

DOUTORANDA: KARINA MARINI AGUIAR

TÍTULO DO TRABALHO: "Potencial antineoplásico de antioxidantes e ação adjuvante do ácido gálico à radiação ionizante no carcinoma epidermoide de boca: uma revisão sistemática e estudo *in vitro*".

ÁREA DE CONCENTRAÇÃO: Mecanismos e Aspectos Clínicos da Doença.

LINHA DE PESQUISA: Clínica, Diagnóstico e Terapêutica das Doenças.

BANCA (TITULARES)	**ASSINATURAS**
PROFª. DRª. LUCYANA CONCEIÇÃO FARIAS - ORIENTADOR	Lucyana Conceição Farias
PROF. DR. ANDRÉ LUIZ SENA GUIMARÃES	
PROFª DRª. ALESSANDRA REJANE ERICCSON DE OLIVEIRA XAVIER	Alessandra Rejane Ericcson de Oliveira Xavier
PROF. DR. LEANDRO DE FREITAS TELES	Leandro de Freitas Teles
PROFª. DRª. ANA PAULA VENUTO MOURA	[illegible]
PROF. DR. OTÁVIO CARDOSO FILHO	[illegible]

BANCA (SUPLENTES)	**ASSINATURAS**
PROF. DR SÉRGIO HENRIQUE SOUSA SANTOS	
PROFª. DRª. CARLA SILVANA DE OLIVEIRA E SILVA	

[x] **APROVADA** [ ] **REPROVADA**

Hospital Universitário Clemente Farias – HUCF
http://www.unimontes.br / ppgcs@unimontes.br
Telefone: (0xx38) 3224-8372 / Fax: (0xx38) 3224-8372
Av. Cula Mangabeira, 562, Santo Expedito, Montes Claros – MG, Brasil – Cep: 39401-001

RESUMO

O Carcinoma epidermoide de boca (CEB) é uma doença comumente relacionada com alterações genéticas, epigenéticas e a fatores de risco, como o tabagismo crônico e etilismo, sendo responsável por mais de 90% de todas as neoplasias malignas orais. Em indivíduos acometidos pelo CEB, a radioterapia é uma estratégia terapêutica importante para promover a morte das células neoplásicas e controlar a progressão da doença. Apesar dos avanços obtidos nesta modalidade de tratamento, observam-se, ainda, quadros de radiorresistência, podendo levar um prognóstico desfavorável. Por isso, é crucial realizar pesquisas com foco no desenvolvimento de terapêuticas complementares, visando favorecer a eficácia da radiação ionizante. As substâncias derivadas de plantas são excelentes fontes de descoberta e desenvolvimento de novos agentes antineoplásicos. O ácido gálico (AG) é um potente antioxidante, derivado de plantas do cerrado brasileiro que pode inibir o desenvolvimento de neoplasias através de vários mecanismos, tais como inibição da metástase, supressão da angiogênese, indução de apoptose, inibição da proliferação, migração e da invasão. Com base no envolvimento de espécies reativas de oxigênio (EROs) na patogênese do câncer, bem como o de boca, os antioxidantes têm sido alvo de pesquisas científicas, investigando o uso dessas substâncias como moduladores de EROs na prevenção ou terapêutica do câncer de boca ou, ao contrário, como indutores da geração de EROs promovendo estresse oxidativo e morte de células de CEB. Portanto, este trabalho apresenta duas vertentes de estudo. A primeira teve como objetivo elaborar uma revisão sistemática da literatura sobre o potencial antienoplásico de substâncias antioxidantes no carcinoma epidermoide de boca. O objetivo da segunda vertente foi investigar ação adjuvante do AG sobre o efeito terapêutico da radiação ionizante em células de CEB. A revisão sistemática do primeiro estudo envolveu a busca de estudos *in vitro* e clínicos relacionados ao objetivo proposto. O estudo foi registrado na Plataforma Internacional Prospero (CRD42018107206) e seguiu os critérios de busca da Plataforma PRISMA para revisões sistemáticas e meta-análises, utilizando as bases de dados PubMed, Scopus, Web of Science e EBSCO. Foram identificadas 260 publicações em todas as bases de dados, sendo elegíveis 32 artigos para a leitura do texto completo. As principais fontes antioxidantes com potencial terapêutico no CEB identificadas neste estudo foram Curcumina, Quercetina, Resveratrol, Licochalcones, α-mangostin, Shikonin, Pterostilbene, 2,4-bis(p-hydroxyphenyl)-2-butenal, β-lapachone and 3-iodine derivates, Selenium,

Butein, Fisetin, Oridonin, Betulinic acid, Thymol, Verbacoside, Xanthorrhizol, e derivados da Dihydrocaffeic acid amide. Várias destas substâncias são polifenois, terpenoides ou flavonoides. Diferentes extratos de plantas e diluições de mel também demonstraram possuir propriedades antioxidantes e exercer efeitos antineoplásicos, incluindo extrato metanólico de *Gracilaria tenuistipitata,* extrato metanólico de *Pergularia daemia*, extrato etanólico de semente de *Acacia catechu,* extrato alcoólico de *Salvia miltiorrhiza*, extrato de *Areca nut*, extrato de *Duchesnea indica*, extrato de *Hibiscos sabdariffa* e mel *Tualang*. Todas essas substâncias apresentaram efeitos biológicos nas células OSCC, controlando a proliferação ou levando à morte celular. Em segundo momento deste estudo, foram realizados ensaios *in vitro* de proliferação, morte celular, e níveis de espécies reativas de oxigênio (EROs). Células imortalizadas de CEB, SCC-9, e queratinócitos normais HaCaT, utilizados como controle, foram tratadas com o AG na concentração de 10 µg/ml, seguida da exposição a 2, 4 e 6 Gy de irradiação, em um acelerador linear. O AG reduziu significativamente o número de células viáveis em diferentes doses de radiação e potencializou a morte celular radio-induzida nas células de CEB, mas não nas células HaCaT. Em concordância a esses achados, houve um aumento do acúmulo intracelular de EROs nas células SCC-9 e uma redução nas HaCaT. Assim o AG exerceu um efeito potencial como adjuvante da radiação ionizante terapêutica no controle comportamental das células de CEB, além de atuar como um protetor dos queratinócitos normais. Novos estudos *in vivo* são necessários para entender melhor o papel do AG e de outros antioxidantes como estratégia terapêutica promissora contra o câncer de boca.

Palavras-chave: Carcinoma epidermoide de boca, antioxidantes, ácido gálico, radioterapia, radiação ionizante terapêutica.

ABSTRACT

Oral squamous cell carcinoma (OSCC) is a disease commonly related to genetic and epigenetic changes, and to risk factors such as chronic smoking and alcoholism, being responsible for more than 90% of all oral malignancies. In individuals affected by OSCC, radiotherapy is an important therapeutic strategy to promote the death of neoplastic cells and to control the progression of the disease. Despite the advances obtained in this treatment modality, there are still cases of radioresistance, which can lead to an unfavorable prognosis. Therefore, it is crucial to conduct research focusing on the development of complementary therapies, aiming to favor the effectiveness of ionizing radiation. Plant-derived substances are excellent sources of discovery and development of new antineoplastic agents. Gallic acid (GA) is a potent antioxidant, derived from plants in the Brazilian cerrado, which can inhibit the development of neoplasms through various mechanisms such as inhibition of metastasis, suppression of angiogenesis, induction of apoptosis, inhibition of proliferation, migration and invasion. Based on the involvement of reactive oxygen species (ROS) in the pathogenesis of oral cancer, antioxidants have been the target of scientific research, investigating the use of these substances as ROS modulators in preventing oral cancer or, conversely, as inducers of the generation of ROS, to promote oxidative stress and death of OSCC cells. This work presents two aspects of study: the first aimed to review the literature on antineoplastic potential of antioxidant substances in oral squamous cell carcinoma. The second was to investigate the adjuvant action of GA on the therapeutic effect of ionizing radiation in OSCC cells. The systematic review of the first study involved the search for *in vitro* and clinical studies related to the proposed objective. The study was registered on the International Platform Prospero (CRD42018107206) and followed the search criteria of the PRISMA Platform for systematic reviews and meta-analyzes, using PubMed, Scopus, Web of Science and EBSCO databases, 260 publications were identified, with 32 articles eligible for full-text reading. The main antioxidant sources identified in this study with therapeutic potential in OSCC were Curcumin, Quercetin, Resveratrol, Licochalcones, α-mangostin, Shikonin, Pterostilbene, 2,4-bis (p-hydroxyphenyl) -2-butenal, β-lapachone and 3- iodine derivates, Selenium, Butein, Fisetin, Oridonin, Betulinic acid, Thymol, Verbacoside, Xanthorrhizol, and derivatives of Dihydrocaffeic acid amide. Several of these substances are polyphenols, terpenoids or flavonoids. Different plant extracts and dilutions of honey have also been

shown to have antioxidant properties and exert antineoplastic effects, including methanol extract from *Gracilaria tenuistipitata*, methanol extract from *Pergularia daemia*, ethanol extract from *Acacia catechu* seed, alcohol extract from *Salvia miltiorrhiza*, extract from Areca nut, *Duchesnea indica* extract, *Hibiscus sabdariffa* extract and Tualang honey. All of these substances had biological effects on OSCC cells, controlling proliferation or leading to cell death. In the second part of this study, *in vitro* assays of proliferation, cell death, and levels of reactive oxygen species (ROS) were performed. Immortalized cells of CEB, SCC-9, and normal HaCaT keratinocytes, used as controls, were treated with GA at a concentration of 10 µg/ml, followed by exposure to 2, 4 and 6Gy of irradiation, in a linear accelerator. GA significantly reduced the number of viable cells at different doses of radiation and potentiated radio-induced cell death in OSCC cells, but not in HaCaT cells. In agreement with these findings, there was an increase in the intracellular accumulation of ROS in SCC-9 cells and a reduction in HaCaT. Thus, GA exerted a potential effect as an adjunct to therapeutic ionizing radiation in the behavioral control of OSCC cells, in addition to acting as a protector of normal keratinocytes. Further *in vivo* studies are needed to better understand the role of GA and other antioxidants as a promising therapeutic strategy against oral cancer.

Keywords: Oral squamous cell carcinoma, antioxidants, gallic acid, radiotherapy, therapeutic ionizing radiation.

LISTA DE ABREVIATURAS E SIGLAS

CEB	Carcinoma Epidermoide de Boca
AG	Ácido Gálico
EROs	Espécies Reativas de Oxigênio
RT	Radioterapia

SUMÁRIO

1 INTRODUÇÃO.. 10

2 OBJETIVOS.. 14

2.1 Objetivo geral.. 14

2.2 Objetivos Específicos.. 14

3 PRODUTOS.. 15

3.1 Produto 1: Antineoplastic potential of antioxidant substances in oral squamous cell carcinoma: a systematic review.. 16

3.2 Produto 2: Gallic acid enhances the effect of therapeutic ionizing radiation in oral squamous carcinoma cells …..…….. 65

3.3 Produto 3: Pitch para divulgação online dos resultados da tese................................ 81

4 CONSIDERAÇÕES FINAIS.. 82

REFERÊNCIAS.. 83

ANEXO A - Normas para publicação no periódico Free Radical Biology & Medicine.. 86

ANEXO B - Normas para publicação no periódico Journal of Oral Pathology & Medicine... 87

ANEXO C - Aprovação da pesquisa pelo Comitê de Ética em Pesquisa/Unimontes 88

ANEXO D - Registro da Revisão Sistemática na Plataforma PROSPERO 92

1 INTRODUÇÃO

O carcinoma epidermoide de boca (CEB), também denominado carcinoma de células escamosas ou carcinoma espinocelular, é um grave problema de saúde pública em diversos países do mundo, sendo responsável por mais de 90% de todas as neoplasias bucais malignas (1, 2). Acomete, com maior frequência, indivíduos do sexo masculino, com idade superior a 60 anos, associado a hábitos tabagista e/ou etilista, agentes biológicos como vírus, bem como alterações genéticas e/ou epigenéticas em oncogenes e genes supressores de tumor (3-5). Estimativas apontam que cerca de 350 mil novos casos de câncer de boca são diagnosticados a cada ano, causando mais de 170.000 mortes no mundo (6-8).

No Brasil, o câncer é uma das principais causas de morte e a distribuição dos novos casos de câncer bucal é heterogênea, com a maior concentração de casos nas regiões Sudeste e Sul do país. Estima-se que no triênio 2020-2022 o número de novos casos em homens será de 11.180 e de 4.010 em mulheres (9).

A carcinogênese de boca é um processo complexo, de desenvolvimento de distúrbios potencialmente malignos, onde as células adquirem e acumulam uma série de alterações genéticas ou epigenéticas, que levam a desregulação do ciclo celular e proliferação descontrolada de células, que possibilitam a transformação dessas células biologicamente normais em células funcionalmente alteradas (10-12). Essa transformação pode ser provocada por alterações epigenéticas e citogenéticas que afetam a progressão do ciclo celular, mecanismos de reparo do DNA, diferenciação e apoptose, que podem ser causadas por mutações aleatórias, pela exposição a qualquer um ou mais de uma variedade de fatores biológicos, químicos ou agentes carcinogênicos fisiológicos ou por erros nos processos de reparo do DNA (13).

O processo de progressão do CEB pode ser representado por uma sucessão de modificações no material genético, que acarreta alterações teciduais particulares, como: hiperplasia epitelial, displasia epitelial, carcinoma *in situ*, carcinoma invasivo e metástase. A hiperplasia epitelial é caracterizada por uma hiperproliferação celular, no entanto as células encontram-se morfologicamente normais, mas com alterações genéticas e moleculares. Por outro lado, no epitélio displásico, as células de uma determinado estrato celular tendem a apresentar alterações morfológicas e estruturais, como pleomorfismo celular e nuclear, hipercromatismo nuclear e mitoses aberrantes, características estas

também evidenciadas no carcinoma *in situ*. Nesse último estágio, todos os estratos celulares apresentam tais alterações morfológicas e as células encontram-se em um estágio altamente proliferativo e de desorganização de sua arquitetura tecidual; contudo, continuam restritas ao epitélio. No carcinoma epidermoide invasivo, as células neoplásicas já apresentam um rompimento da membrana basal, iniciando o processo de invasão tecidual. Finalmente, o processo de metástase ocorre quando as células neoplásicas atingem órgãos à distância, por meio da disseminação vascular ou linfática (10, 14).

O estresse oxidativo e o acúmulo de espécies reativas de oxigênio (ERO) desempenham um papel importante na carcinogênese de boca. Os níveis aumentados de malondialdeído e óxido nítrico revelaram que o aumento do estresse oxidativo em conjunto com a redução dos mecanismos de defesa antioxidante em pacientes com CEB podem estar envolvidos na progressão do câncer. A detecção de EROs e níveis de enzimas antioxidantes pode ser um marcador valioso no prognóstico do câncer e para melhorar estratégias terapêuticas no câncer bucal (15). De maneira geral, no processo da carcinogênese, as EROs são capazes de induzir a peroxidação lipídica, danos ao DNA e afetar as vias de transdução de sinal de genes supressores de tumor (16). Nesse contexto, antioxidantes são substâncias capazes de exercer efeitos protetores no metabolismo celular por sua capacidade de sequestrador de EROs, inibindo os danos no DNA oxidativo induzido por EROs e, portanto, reduzindo o risco de câncer, promovendo assim, a morte celular (17-19).

O reconhecimento precoce do CEB é um fator que pode conferir melhores taxas de sobrevida e, também, está associado a uma menor morbidade, além de requerer intervenções cirúrgicas menos mutiladoras. Por isso, pesquisas têm sido direcionadas para a prevenção e detecção precoce do câncer de boca, para que a taxa de sobrevivência aumente e contribua para diminuir a prevalência da doença e melhorar a sobrevida. O grande estímulo para isso está no fato da taxa de sobrevida de cinco anos estar associada ao estágio em que a doença é diagnosticada (20).

Como modalidade terapêutica, a cirurgia é o principal método de escolha para o tratamento de pacientes acometidos pela doença (21). Porém, a radioterapia (RT), assim como a quimioterapia podem ser adotadas como estratégias adjuvantes. A RT desempenha um papel importante no controle do crescimento neoplásico em muitos pacientes com essa enfermidade, especialmente quando o indivíduo acometido não apresenta condições

clínicas para ser submetido à cirurgia ou não aceita as possíveis mutilações faciais que a intervenção cirúrgica pode acarretar (22). Essa modalidade terapêutica é atualmente o tratamento adjuvante padrão para o CEB. Os pacientes com CEB avançado necessitam de RT adjuvante, sendo ela pré ou pós-operatória (23), sabendo-se que a RT pré-operatória aumenta o risco de complicações na cirurgia. A RT pré-operatória foi apresentada como uma estratégia que não traz vantagens consideráveis para a sobrevida do paciente, além de dificultar a realização da cirurgia em um intervalo menor do que seis semanas após o término da RT (23).

Os avanços técnicos conquistados na RT têm reduzido os efeitos colaterais agudos e crônicos relacionados à terapia, principalmente no desenvolvimento de melhoria na compreensão da dose de radiação e da resposta da dose-volume (24, 25). Porém, apesar dos avanços obtidos nesta modalidade de tratamento, os tumores podem recorrer dentro do campo irradiado devido à ineficiência da terapia, levando a um mau prognóstico (25, 26). Por isso, é crucial a realização de pesquisas com foco no desenvolvimento de tratamentos alternativos para aumentar a eficácia da radiação ionizante. Uma abordagem promissora para aumentar a eficácia da radioterapia em pacientes com CEB é a descoberta e uso de drogas que favoreçam a radiossensibilidade de células neoplásicas, a fim de melhorar as taxas de resposta à RT.

A busca de novas terapias para o tratamento do câncer deve buscar um equilíbrio entre a capacidade dos pacientes em tolerar os efeitos colaterais do tratamento e a toxicidade potencial que pode ocorrer pós-tratamento (27).

Os produtos derivados de plantas são excelentes fontes de descoberta e desenvolvimento de novos agentes antineoplásicos desde os anos 60, com as primeiras pesquisas sobre epipodofilotoxina e seus derivados como agentes citotóxicos, com o estudo de alcalóides de vinca, vinblastina, vincristina, epipodofilotoxina e taxanos, que são fontes naturais de fármacos com atividade contra o câncer (28).

Tem sido apontado que o tratamento com substâncias específicas derivadas de plantas pode levar a efeitos colaterais de menor intensidade do que drogas sintéticas (29). Estudos com extratos de plantas destacaram os efeitos citotóxicos em linhagens celulares de diferentes tipos de câncer, como mama, esôfago, estômago, pulmão e outros (29-31). Portanto, a busca por derivados de compostos naturais para o tratamento de câncer, especialmente do CEB, é uma estratégia promissora.

A ação de antioxidantes sobre a modulação de mecanismos da carcinogênese é um foco de interesse de pesquisas científicas (18). Os estudos revelaram resultados relevantes sobre as potencialidades terapêuticas de antioxidantes contra câncer, por exemplo, a ingestão de vitaminas antioxidantes e alguns fitoquímicos estão inversamente relacionados ao risco de câncer, podendo inibir o crescimento celular e induzir seletivamente a morte em células neoplásicas (32).

O ácido gálico (ácido 3,4,5-trihidroxibenzóico, AG), um composto antioxidante de ocorrência natural em plantas (33-36). Ele exerce propriedades medicinais como antibacteriana, antiviral, anti-inflamatória e antitumoral (37). Estudos mostraram, também, que o ácido gálico exerceu atividade no metalolismo das células como forma de prevenção e tratamento de doenças metabólicas associadas a obesidade (38). E especificamente, pode inibir o desenvolvimento de neoplasias através de vários mecanismos, tais como a inibição da metástase (39); a supressão da angiogênese (40); a indução de apoptose e/ou necrose (41); a inibição da viabilidade celular, proliferação, invasão e formação de novos vasos (42); e a inibição da migração e da invasão (37).

Estudos funcionais revelaram que o ácido gálico apresenta ação antineoplásica em células de câncer be boca, promovendo redução da população de células, morte por apoptose e controle dos processos de migração e invasão (43-46). No entanto, ainda não foi investigada a possibilidade de atuação do ácido gálico como substância adjuvante ao efeito terapêutico da radiação ionizante no carcinoma epidermoide de boca. Assim, a combinação de radioterapia com extratos naturais, tais como o ácido gálico, é uma abordagem interessante para novas investigações terapêuticas para o CEB, visando o controle e/ou progressão da doença.

2 OBJETIVOS

2.1 Objetivo Geral

Analisar o potencial antineoplásico de antioxidantes e ação adjuvante do ácido gálico à radiação ionizante no carcinoma epidermoide de boca

5.2 Objetivos Específicos

- Realizar uma revisão sistemática da literatura sobre o potencial antineoplásico de substâncias antioxidantes no carcinoma epidermoide de boca

- Avaliar o efeito do ácido gálico associado à radiação ionizante terapêutica sobre o comportamento neoplásico e estresse oxidativo em células de carcinoma epidermoide de boca.

3 PRODUTOS

3.1 Produto 1:

- Artigo científico: "Antineoplastic potential of antioxidant substances in oral squamous cell carcinoma: a systematic review". Formatado para submissão segundo as normas do periódico "Free Radical Biology & Medicine".

3.2 Produto 2:

- Artigo científico: "Gallic acid enhances the effect of therapeutic ionizing radiation in oral squamous carcinoma cells". Formatado para submissão segundo as normas do periódico "Journal of Oral Pathology & Medicine".

3.3 Produto 3:

- *Pitch* para divulgação online dos resultados da tese: Foi elaborado um vídeo de curta duração para divulgação online dos resultados do estudo, direcionado à população geral. Este produto será apresentado à banca, para posterior divulgação online. Após a avaliação do *Pitch* pela banca avaliadora, será incluído aqui o link para acesso e disponibilização online do vídeo.

3.1 Produto 1

Antineoplastic potential of antioxidant substances in oral squamous cell carcinoma: a systematic review

Karina Marini Aguiar[1]
Angeliny Tamiarana Lima Tabosa[1]
Renata Sousa Leite[1]
Tamara Mayrink Alcântara[1]
Renato Sobral Monteiro-Junior[1]
André Luiz Sena Guimarães[1,2#]
Lucyana Conceição Farias[1,2#]

[1]Postgraduate Program in Health Sciences, Universidade Estadual de Montes Claros, Minas Gerais, Brazil
[2]Department of Dentistry, Universidade Estadual de Montes Claros, Minas Gerais, Brazil
# These authors have contributed equally to this work.

*Corresponding author:
Lucyana Conceição Farias
Universidade Estadual de Montes Claros
Hospital Universitário Clemente de Faria
Laboratório de Pesquisa em Saúde
Avenida Cula Mangabeira, 562
Montes Claros, Minas Gerais, Brasil
Zip code: 39401-001
Phone: +55 38 32248327
E-mail: lucyanacfarias@gmail.com

Abstract

Despite the advances in therapeutic strategies, oral squamous cell carcinoma (OSCC still represents a serious worldwide public health problem. Based on the involvement of reactive oxygen species (ROS) in oral cancer pathogenesis, the antioxidants have been targets of new scientific researches, investigating the use of these substances as ROS modulators to the prevention of oral cancer, or contrary as inducers of ROS generation to promote exacerbated oxidative stress and death of OSCC cells. Thus, this review aimed to describe the therapeutic potential of antioxidant substances in human oral squamous cell carcinoma. The study was registered in the Prospero database and followed the standards set by the Preferred Reporting Items for Systematic Reviews and Meta-analyses. The quality of *in vitro* studies was assessed using the ToxRTool platform, and for human studies, we adopted the parameters of "The Cochrane Collaboration's tool" when applicable. Eligible studies were selected in international databases PubMed, Web of Science, Scopus, and Ebsco, using inclusion and exclusion criteria. A total of 260 publications were identified in all databases, being obtained the full-text of 32 eligible studies for a full reading. The antioxidant sources with therapeutic potential on OSCC identified in this study were Curcumin, Quercetin, Resveratrol, Licochalcones, α-mangostin, Shikonin, Pterostilbene, 2,4-bis(p-hydroxyphenyl)-2-butenal, β-lapachone and 3-iodine derivates, selenium, Butein, Fisetin, Oridonin, Betulinic acid, Thymol, Verbacoside, Xanthorrhizol, and Derivatives of Dihydrocaffeic acid amide. Several of these are polyphenols, terpenoids or flavonoids. Different plant extracts and honey dilutions reported to have antioxidant propriety and antineoplastic effects were identified in this systematic research, including Methanolic extract of *Gracilaria tenuistipitata, Pergularia daemia* methanolic extract, *Acacia catechu* ethanolic seed extract*, Salvia miltiorrhiza* alcohol extract, *Areca nut* extract, *Duchesnea indica* extract, *Hibiscos sabdariffa* extract, and *Tualang* honey. All these substances and extracts showed biologic effects on OSCC cells, mainly controlling cell proliferation or leading to cancer cell death. Thus, antioxidant substances played a relevant antineoplastic role in OSCC cells, being pointed out as promising therapeutic strategies for future clinical research, focusing on preventing and treatment for oral cancer patients.

Introduction

Head and neck cancer represents an overall public health problem with a poor outcome in advanced stages, and oral squamous cell carcinoma (OSCC) is the most common type in the oral cavity, making it the sixth most common human cancer in the world. It affects approximately 4.5 million people and 2.15 million deaths each year. This high incidence is mainly due to the risk factors like the use of tobacco, alcohol, and *Human Papillomavirus* infection(1-4). Approximately 40% of cases of head and neck cancer are diagnosed as oral cancer leading to 177,384 deaths per year. Over 90% of patients with oral cancer represent oral squamous cell carcinoma(5).

In addition to surgical removal of the tumor, radiotherapy is one of the main adjuvant methods for the treatment of oral cancer patients, being used as a single modality in 67.1% of oral cancer cases, promoting the cancer cell death by stimulating apoptosis(6). Despite the great progress in recent years regarding anticancer treatments, it has been reported a 5-year survival rate for OSCC patients, not improving since. The main anticancer drugs can be highly toxic and their effects can be unbearable for patients, resulting in discontinuation of treatment. Thus, it is necessary to investigate new antineoplastic therapeutic options, leading to tumor cell death and cell growth control to improve the success rate of cancer treatments(7, 8).

The increased oxidative stress and changes in antioxidant defense systems have been related to oral cancer pathogenesis(9). Oxidative stress and accumulation of reactive oxygen species (ROS) play an important role in carcinogenesis by inducing lipid peroxidation, DNA damages and thus affecting signal transduction pathways of tumor-suppressing genes(10). In this context, antioxidants are substances able to exerts protective effects on cellular metabolism by its ability ROS scavenger, inhibiting the ROS-induced oxidative DNA damage and therefore reducing the risk for cancer. These substances, according to the treatment concentrations, also have been shown to plays antineoplastic functions in several cancer types, preventing the oxidative stress-induced DNA damage or promoting cancer cell death(11-13). For example, curcumin was highly effective in inhibiting the epithelial dysplasia stage of chemically-induced oral cancer, by suppressing nuclear factor kappa B and cyclooxygenase-2 expression(14). However, the effects of other antioxidant substances in oral squamous cell carcinoma is not well understood. So, this study aimed to develop a systematic review of the literature on the therapeutic potential of antioxidant substances in oral squamous cell carcinoma.

Material and methods

The PICOS tool was used to design the systematic Search by parameters Population, Intervention, Comparison, Outcomes, and Study type[15]. This systematic review of the literature followed the standards set by the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA)[16] and was registered and approved in an international database of systematic reviews PROSPERO, under Protocol CRD42018107206.

Eligibility Criteria of the Studies

In order, as searching criteria, we adopted as eligible the randomized controlled trials and observational studies (cohort, case-control, and descriptive), and original *in vitro* studies that investigated the therapeutic potential of antioxidant substances in human oral squamous cell carcinoma. Animal model studies were not eligible, once the systematic reviews are registered in the PROSPERO database according to the investigated category, as animal or human. We considered the published studies until November 2019 in the English language. Duplicated papers found in the databases were excluded.

Resources

The data-collection occurred from July 2018 to November 2019 and the papers were retrieved by online search on databases PubMed, Web of Science, Scopus and Ebsco.

Search Strategy

Initially, descriptors were selected through the Medical Subject Headings (MeSH) and common terms from literature. Furthermore, booleans AND/OR were added in the search strategy. The term combinations used were [“epidermoid carcinoma of oral cavity” OR “squamous cell carcinoma of oral cavity” OR “oral squamous cell carcinoma” OR “oral epidermoid carcinoma” OR “squamous cell carcinoma of mouth” OR “epidermoid carcinoma of mouth”] AND [“antioxidant” OR “antioxidant effect” OR “antioxidative” OR “anti-oxidative” OR "free radical scavenger" OR "reactive oxygen species damage"] AND [“antineoplastic” OR “therapy” OR “treatment” OR “therapeutic”]. All of these terms were identified by reading the title or abstract of the articles. This procedure aimed not only to filter the results but also to cross the main terms to obtain the maximum possible studies.

Exclusion Criteria

Studies wrote in another language than English, that is not related to the purpose of this study, review article, case report, letter to the editor, conference papers, theoretical studies (bioinformatics) were excluded. In addition, studies that assessed substances without antioxidant effect, endogenous antioxidants, and other cancer types were not included.

Study selection

First, we performed an inter-rater calibration by *Kappa* statistic for articles selection, as previously described[17]. For the selection and analysis of studies, after obtained inter-rater calibration, two reviewers (ATLT and KMA) selected the articles in the four databases through reading the titles and abstracts. Disagreements were resolved by consensus, where each reviewer presented his reasons for the initial choice, and when necessary, a third evaluator (LCF) was consulted. After completing this first stage, the full text of the selected articles was read to carry out a new selection, data collection, and evaluation of studies.

Data Extraction and Strategy for data synthesis

Data were extracted and recorded including study results and methodological data using a standardized data collection form. The data was synthesized as a systematic narrative synthesis of the findings from the included studies, covering the therapeutic effect of antioxidant substances in OSCC patients or human OSCC cell lines. We provided summaries about the antioxidant agent, the antineoplastic effect of that, treatment dosage and frequency, and treatment type antioxidant alone, or antioxidant plus other treatment protocol for OSCC, such as chemo-or radiotherapy, and the main outcomes.

Quality assessment

To the quality assessment of original *in vitro* studies, we used ToxRTool[18]. The ToxRTool provides criteria, guidance and a spreadsheet for evaluation of the quality of toxicological studies,[18] consisting of an 18-points checklist, among them the methodological aspects and result data. The spreadsheet is available in URL https://ec.europa.eu/jrc/en/scientific-tool/toxrtool-toxicological-data-reliability assessment-tool. It tool computes a score, categorizing the studies as following: 15–18 (reliable

without restrictions), 11–14 points (reliable with possible restrictions), and <11 (not reliable). To assess the risk of bias for human studies, the parameters of the "Cochrane Collaboration's tool" were adopted when applicable[19].

Results

Inter-rater agreement

In order to obtain the inter-rater calibration to article selection, we performed the *Kappa* statistic. The Cohen's k was run to determine the agreement level between the two evaluators for selecting studies for inclusion in the systematic review. There was an almost perfect inter-rater agreement ($K=0.87; p<0.000$).

Characteristics of included studies and Quality assessment

Based on the selected descriptors, a total of 260 citations were retrieved from the literature search. After exclusion of articles that did not meet inclusion criteria and those duplicated in the databases; 33 studies were eligible for the full-text reading. As one study was not available online, we obtained the full text for eligible 32 articles, including only one human study[20] and 31 *in vitro* studies using human oral cancer cells[6, 21-50].

Detailed descriptions of the search flow are shown in Figure 1. *In vitro* studies and human studies that investigated the therapeutic potential of antioxidant substances in oral squamous cell carcinoma are summarized in Table 1 and Figure 2. The randomized controlled trials related to the purpose and criteria of the systematic review were not identified in the literature search. The only human study identified in this systematic review was an intervention study in humans, using control and treatment groups.

According to the ToxRTool, from the 31 eligible *in vitro* articles, all were considered "reliable without restrictions" (scoring over 15 points), showed in Table 2.

The Cochrane Collaboration's tool was not fully used to assess the single human study selected, because it was not a randomized clinical trial[20]. So, to minimize the risk of bias, we considered as parameters the outcome data addressed and follow-up to verify the antioxidant effect at the short- or long-term in oral cancer patients. Although this study has revealed an adjuvant therapeutic role of selenium to radiotherapy, we analyzed it as showing a risk of bias to strongly suggest the therapeutic effect of selenium in this study since tumor size regression and follow-up were not investigated, but only the improving the blood levels of therapeutic response markers.

Characteristics and effects of antioxidants on oral squamous cell carcinoma

Phytochemicals containing phenolic compounds, saponins, flavonoids, tannins, terpenoids, and alkaloids have been widely studied due to their antioxidant properties. Most of these compounds are found in teas, grapes, red wine, nuts, and other natural products(51). The main sources of polyphenols with therapeutic potential in OSCC presented in this study were Curcumin(6, 26, 34, 37), Verbacoside(50), Resveratrol(39), Thymol(36), α-Mangostin(32). The flavonoids include Butein(35), Fisetin(44), Licochalcones B(28), C(42) , D(46) , H(41) , Quercetin(30) , and terpenoids substances, such as Betulinic acid(47) , Oridonin(43) , Xanthorrhizol(38) .

Different plant extracts and honey dilutions reported to have antioxidant propriety and antineoplastic effects were also identified in this systematic research, including methanolic extract of *Gracilaria tenuistipitata*(22), *Pergularia daemia*(24) methanolic extract, *Acacia catechu* ethanolic seed extract(21), *Salvia miltiorrhiza*(48) alcohol extract, *Areca nut*(45) extract, *Duchesnea indica*(49) extract, *Hibiscos sabdariffa*(25) extract, and *Tualang*(23) honey.

The summary of the effect of antioxidants on OSCC cells and oral cancer patients showed in selected articles is below described.

Selenium

Selenium is an essential element in the antioxidant defense system, reproduction, DNA synthesis, muscle function, and cancer prevention. It is present as organic and/or inorganic forms, among them selenomethionine and selenocysteine, and selenite (SeO3−2), selenide (Se2−), selenate (SeO4−2), selenium element (Se)(52). The natural sources of selenium can accumulate quantities of this element up to several thousand parts per million when grown on seleniferous soil. Such plants include members of the genera *Astragalus, Haplopappas, Machaeiantheia,* and *Stanleya*, which are called "selenium accumulators" or "selenium indicators" because their presence indicates a seleniferous soil(53). Interestingly, selenium does not appear to be a metabolic essential for higher plants, except for the accumulator species, so that its biological functions are most clearly expressed by animals consuming plant material, directly or indirectly(53).

The selenium forms were shown to exhibit toxicity against cancer cells, showing greater toxicity in malignant cells than benign cells. The anticancer action mechanism of

selenite is based on the induction of apoptosis by producing superoxide radicals especially in or adjacent to mitochondria(54). A meta-analysis study showed that selenium deficiency can be a cancer-promoting factor; especially in lung cancer, the high selenium serum levels selenium may have some protective effect(55).

The selenium was able to act as an adjuvant to radiotherapy in oral carcinoma patients, improving the blood levels of therapeutic response markers (5'Nucleotidase, Pseudo choline esterase/PschE, Leucine amino peptidase/LAP, γ-Glutamyl transpeptidase/γ-GTP, Lactate dehydrogenase/LDH, Asparate transaminase/SGOT, Alanine transaminase/SGPT, Acid phosphatase/ACP, Alkaline phosphatase/ALP, Creatinine phosphokinase/CPK), compared to disease-free groups treated with radiotherapy alone. However, the study did not provide evident data on tumor size regression, follow-up or improvement in the patient's clinical condition. The improvement in the trace element levels and reducing in the levels of serum biomarkers in selenium supplemented patients was attributed to the membrane stabilizing promoted by selenium, proving their antioxidative/anticarcinogenic impact on biochemical indices assessed(20).

Acacia catechu

Acacia catechu Willd (*Fabaceae*), commonly known as catechu, cachou and black cutch, is an important medicinal plant, commonly cultivated in Asia. Several phytochemicals have been isolated and characterized from it, which include 4-hydroxybenzoic acid, kaempferol, quercetin, 3,4,7-trihydroxy-3,5-dimethoxyflavone, catechin, rutin, isorhamnetin, epicatechin, afzelechin, epiafzelechin, mesquitol, opioglonin, aromadendrin, and phenol. These active compounds have been implicated by their myriad biological effects. *A. catechu* has been studied for its hepatoprotective, antipyretic, antidiarrheal, hypoglycemic, anti-inflammatory, immunomodulatory, antinociceptive, antimicrobial, free radical scavenging and antioxidant activity(56).

A. catechu extract induced the dose-dependent inhibition of cell proliferation of oral cancer cell line SCC25, and also increased the apoptosis marker expression, such as cytochrome c, caspase-8, and caspase-9(21) .

Gracilaria tenuistipiatata

Marine natural products provide abundant resources for antitumor drug discovery(57). Many *Gracilaria* vegetable species are well established to be potential

sources to search in natural medicine due to their antibacterial, antiviral, antifungal, antihypertensive, cytotoxic, spermicidal, embryotoxic and anti-inflammatory effects. The *Gracilaria tenuistipiatata* water extract can reduce oxidative damage to hydrogen peroxide-induced DNA[(57)]. In the study by Chi-Chen Yeh *et al*[(22)], Ca9-22 cells derived from gingiva carcinoma treated with *Graciliaria tenuispitata* methanol extract (MEGT) showed a significantly reduced cell viability in a dose-response manner. In agreement with this result, the percentages of G1 phase were significantly increased at the concentration of 0.25mg/mL MEGT, leading to G1 arrest. The induction of apoptosis and an increase in the generation of reactive oxygen species (ROS) were also caused by methanol extract of *Graciliaria tenuispitata*. That is, the MEGT induced a potent anticancer effect through induction of apoptosis, DNA damage, and oxidative stress pathways can be a potential substance to therapeutic investigation in OSCC.

***Tualang* honey**

Tualang Honey (TH) is a honey produced in the multi-floral jungle, by the species *Apis dorsata* bee, which builds its hives on top of *Tualang* trees (*Kompassia excelsa*) in Malaysia's rainforests. The TH exhibits antimicrobial, anti-inflammatory, antioxidant and antidiabetic effects, and it has been shown to have anticancer effects against OSCC, human osteosarcoma cell line HOS, human breast cancer cell line and cervical cancer cell line[(58)].

Ghashm *et al*. (2010)[(23)] used OSCC and HOS cell lines to evaluate the antiproliferative activity of Malaysian *Tualang* honey. Cells treated with 2% and 10% *Tualang* honey for 24, 48, and 72 hours had their morphology altered, typical of what is seen in apoptosis. It was noted that 50% of honey concentration was required to inhibit cell growth of OSCC and HOS cells. Maximum inhibition of cell growth greater than 80% was obtained at 15% of both cell lines.

Pergularia daemia

Pergularia daemia (Forsk) (Asclepiadaceae family) is a laticiferous twiner that grows on the warmer plains of the Indian regions. It has pharmacologic properties, such as anthelmintic, laxative, antipyretic and expectorant, as well as to treat childhood diarrhea, malaria, and intermittent fevers. It is an excellent antifertility, antidiabetic, and it has hepatoprotective, anti-inflammatory effects[(59)].

There is little scientific research regarding its anti-cancer effect. In the study by Mirunalini *et al*. (2014)[24], the antiproliferative effect of *Pergularia daemia* methanolic extract (PMDE) on human oral cancer KB cell line was investigated, demonstrating that it at lower concentrations was unable to inhibit cell growth, only at higher treatment concentrations showed a significant increase in cytotoxic effect on KB cells. It was also observed that maximum concentrations of treatment led to the ROS generation in 95% of cells analyzed. Analysis by fluorimetric spectrometry showed that membrane potential was reduced in PDME-treated cells, as well as apoptotic morphological characteristics, DNA fragments, increased levels of lipid peroxidation, changes in enzymatic antioxidant activity and decreased GSH levels in the KB cells.

Hibiscus sabdariffa

Hibiscus sabdariffa L. (*Roselle*), a plant of *Malvaceae* family widely cultivated in many countries, is rich in polyphenols, such as anthocyanins, polysaccharides, and organic acids. It has high pharmacological potential in the treatment of diseases like hyperlipidemia, hypertension, inflammatory diseases of kidney and liver, and prevention of degenerative diseases associated with oxidative stress[60].

Owing to the antioxidant role, research about the antitumoral therapeutic potential has been aiming of interest[61]. The cytostatic effect of *Hibiscus* extract on cancer cells growth of multiple myeloma and OSCC was demonstrated, being its effects dependent-time and dose. Furthermore, it was able to reduce neoplastic cell motility and invasiveness. In normal keratinocytes cells HaCat and HEK292, *Hibiscus* extract did not change cell viability. The therapeutic potential of Hibiscus on these cancer cells was associated with MAPKs ERK1/2 modulation and p38 activation[25]. The upregulation of ERK1/2 proteins was related to the cancer cell proliferation[62], whereas p38 inactivation can be associated with blocking of cell cycle and apoptosis by neoplastic cells[63]. So, it is suggested that Hibiscus can contain substances with a chemopreventive role against tumor reoccurrence alone or in combination therapy[25].

Curcumin

It is a polyphenolic pigment derived from rhizomes of Curcuma longa plant, commonly used to flavor foods. It has antiallergic and antirheumatic properties, acting in respiratory and liver diseases. The antioxidant, antimicrobial and antitumor activities also

have been identified in curcumin[14, 26]. This systematic review appointed an article associating this substance with the reduction of viability and OSCC cell death[26]. The curcumin treatment led to apoptosis and autophagy pathways. High expression of LC3-II, dependent- dose and time, was observed. A curcumin-induced intracellular ROS generation can be responsible for autophagy in OSCC cells[26]. It was also assessed the nanoemulsions (NE) formulation by combining curcumin and 5-FU to improve *in vitro* antineoplastic activity against OSCC cells, SCC090, and cells of human hypo-pharynx squamous carcinoma, SCC152. The 5-FUNE/Cur-NE/5-FU-Cur-NE formulations decreased the level of viability of cells, for time-concentration-dependent. Additionally, the OSCC cells treated with 5-FUNE/Cur-NE/5-FU-Cur-NE formulations showed up-regulation in expression protein, p53, p21, and Bax proteins, and down-regulation of Bcl2 protein in both cells with time-dose-dependent manner up to 48 h. The 5-FU and Cur in nanoparticle systems shown their efficacy to deliver improved anticancer activity, against oral cancer[6].

Due to the important role played by sub-population of cancer stem cells (CSCs) in cancer pathogenesis, metastasis, and drug resistance, the Curcumin effect was investigated on cell proliferation, the orosphere formation and miRNA-21 expression in HPV16+ve/-ve oral cancer stem cells. Curcumin treatment promoted significant differential inhibition in CSC proliferation, the orosphere formation and miRNA-21 expression in a dose-dependent manner; the effect was highly pronounced in HPV positive CSCs. The effect of curcumin between HPV+ve and HPV–ve cells indicated relatively a stronger cytotoxic effect on UD-SCC2 HPV+ve SP cells (IC50-36.21μM) when compared to UPCI: SCC84 HPV–ve (IC50-45.12μM)/UPCI: SCC131 SP cells (IC50-46.56μM). The downregulation of miR-21 expression upon curcumin treatment in both HPV+ve/HPV–ve CSCs but with higher effects in HPV+ve CSCs[34].

The curcumin was also shown to play an adjuvant effect to cisplatin on PE/CA-PJ15 human oral squamous carcinoma, enhancing cisplatin cytotoxicity and apoptotic activation. The dose-dependent proapoptotic effect of curcumin was identified in both mono- and combinatorial chemotherapy. Its effects were associated with inhibiting pSTAT3 signaling and inhibiting of Nrf-2 nuclear translocation, providing chemosensitization to cisplatin[37].

Shikonin

Shikonin, a natural compound of red naphthoquinone, is a major component of Zicao (purple gromwell). Zicao is a traditional Chinese herbal medicine, whose extracts have been shown to have multiple pharmacological activities, including anti-inflammatory, antioxidant, anti-virus, antimicrobial, and anticancer. In addition, shikonin can play therapeutic action on many other pathologies or disorders, such as inhibiting hypertrophic scar formation, reducing hepatic fibrosis, inhibiting pulmonary hypertension, protecting hypoxia and reoxygenation damage in heart and brain, protecting β-amyloid-induced neurotoxicity, improving spinal cord injury[(64, 65)].

In Tca-8113 cell line derived from squamous cell carcinoma of the human tongue, the shikonin treatment inhibited the neoplastic cell growth and induced the cell death, leading to the DNA fragmentation and activation of apoptotic proteins caspase-3, caspase-8, and caspase-9. This therapeutic potential of this antioxidant substance can be associated with inactivation of the NF-κB signaling and activation of caspases[(27)].

Chalcones: Licochalcone B, C, D, and H

The chalcones are precursor substances to flavonoids and isoflavonoids of yellow-orange coloration. Licochalcone B, a chalconoid compounds present in the root of *Glycyrrhiza uralensis Fisch*, is recognized by its several health benefits, such as cardioprotection, antioxidant effect, which can reduce inflammation, cell migration, angiogenesis, and tumorigenesis, inducing arrest of the cell cycle and apoptosis of various cancer cells[(66, 67)]. In OSCC cells, this substance caused reducing the cell viability and led to the cell cycle arrest, high ROS production and loss of mitochondrial membrane potential[(28)].

Despite the anticancer role of different licochalcones, there are few reports on licochalcone C (LCC) in oral cancer. A study investigated the effect of LCC isolated from Gl*ycyrrhiza inflata* roots on human tongue squamous carcinoma cell line HSC4. LCC inhibited cell viability, mitochondrial function, anchorage-independent growth, and induced apoptosis in a dose-dependent manner, being such effects associated with suppression of JAK2/STAT3 pathway and induction of ROS generation in OSCC cells[(42)].

The anticancer effects of other forms of Licochalcones on OSCC were investigated. The Licochalcone D (LCD) inhibited the kinase activity of JAK2 blocking the JAK2/STAT3 pathway, reduced the soft agar colony formation, and the proliferation of

HN22 and HSC4 cells. Furthermore, LCD induced apoptosis through changes in mitochondrial membrane potential, reactive oxygen species generation, upregulation of death receptor CHOP, DR4, DR5 and increase of expression levels of proapoptotic proteins Bad and Bax. LCD treatment promoted Cyto C release from mitochondria and its transfer to the cytosol, and also a dose-dependent increase in Apaf 1, cleaved PARP, and lowered caspase 3 expression. These results were corroborated in the xenograft study, wherein LCD significantly inhibited HN22 tumor growth, and suppressed p JAK2 and p STAT3 expression and induced cleaved caspase 3 expression(46).

Licochalcone H is a chalconoid isolated from the root of *Glycyrrhiza inflate*; it has several pharmacological properties, such as anti-inflammatory, antioxidant, antitumor, and antineoplastic effects. The LCH was able to reduce cell viability and colony-forming ability, and induced cell cycle arrest and apoptosis in OSCC cell, HSC2, and HSC3, through the suppression of Matrin3, a highly conserved nuclear matrix protein involved in DNA replication, apoptosis, transcription, translation, RNA processing, chromatin remodeling, and the stabilization of RNA and mRNA(41).

Pterostilbene

Pterostilbene is a natural antioxidant extracted from grapes, blueberries, and peanuts; it is considered one of the most powerful stilbene compounds. It has structural similarity to resveratrol, but it exhibits superior pharmacokinetic characteristics. Pterostilbene is reported to have therapeutic potential for many diseases and conditions, including cancer, dyslipidemia, aging, and inflammatory disorders. This substance can be effective in inhibiting breast cancer cell proliferation and reducing the expression of human telomerase reverse transcriptase(68).

High concentrations of Pterostilbene revealed an antineoplastic action on oral cancer cells, decreasing significantly cell viability and migration, by downregulation of MMP-2 and u-PA expression and reducing phosphorylation of Akt, ERK1/2, and JNK1/2. However, Pterostilbene was not cytotoxic to normal oral keratinocytes HOK. The inhibitory effects of Pterostilbene can be associated with the upregulation of tissue inhibitor of metalloproteinase-2, plasminogen activator inhibitor-1 and the downregulation of the transcription factors of NF-κB, SP-1, and CREB signaling pathways(29).

Quercetin

Quercetin (Qu) is one of the major natural flavonoids, described to exhibit anticancer potential due to its pro-apoptotic effect and to the inhibiting the progression of several human cancers cells, such as lung, prostate, liver, breast, colon, and cervical; these anticancer properties are exerted through various mechanisms that involve cellular signaling and the ability to inhibit enzymes responsible for the activation of carcinogens(69). In addition to the antitumor activities, including suppression of various tumor-related transcription factors, inhibition of protein kinase activities, down-regulation of gene expression associated with cycle progression cellular, angiogenesis, anti-apoptosis, and metastasis, it can address anticancer efficacy by regulating of growth, migration, and invasion in oral cancer cells(70, 71).

The Qu was able to decrease the survival rate of OSCC cells, leading to the increase of cell proportion in phase G1, and inducing apoptosis. The cell viability of human normal fibroblasts was not impaired. Furthermore, the Qu treatment significantly decreased cell migration capacity in a dose-dependent manner, showing this substance as a potent therapeutic agent for OSCC(30).

2,4 bis (p-hydroxyphenyl)-2- butenal- HPB242

Maillard reaction between amino acids and reducing sugars, producing products such as glucose tyrosine, has been studied more extensively in food. It can also occur in animals during the formation of advanced glycation end products. Maillard reaction products (MRPs) are known to have antioxidant, antimutagenic and anticarcinogenic activities(72). The MPR (E)-2,4-bis(p-hydroxyphenyl)-2-butenal (HPB242) induces antioxidant and anti-inflammatory effects through inhibition of NF-κB and STAT3(73).

So, little is known about other biological effects of MRPs. It was investigated whether fructose tyrosine MRP, and HPB242 can modulate cell cycle progression and protein specificity(3) repression, and thus induce apoptosis of OSCC cells(31). Sp is a transcription factor and universally expressed in all mammalian cells and it plays a significant role in several biological processes, such as cell cycle regulation, apoptosis, and angiogenesis. In addition, the Sp1 is highly expressed in different types of cancer, affecting the breast, thyroid, liver, pancreas, colorectal region, stomach, cervical region, and lung(31). In OSCC cells, the HPB242 treatment decreased the cell growth rate of HN22 and HSC4, as well as led to a decrease in the level of Sp-1 and inhibition of the expression of Sp1

downstream proteins, including p27, Cyclin D1, Mcl-1, and Survivin, in a dose-dependent manner. Furthermore, it was highlighted that HPB242 leads to apoptosis, through the increase Bax expression, reducing of Bid, and Bcl-xl, and activation of Caspase 3 and PARP. Thus, HPB242 can be an important target for therapeutic investigations for OSCC aiming for the improvement of clinical outcomes of patients(31).

α-Mangostin

Alpha-mangostin (αM) is a bioactive compound with antioxidant properties, that can be extracted from the *Garcinia mangostana* tree; is widely used in traditional Asian medicine(74). The αM is the most abundant xanthone in the mangosteen pericarp and shows anti-inflammatory, anti-apoptotic and antiproliferative properties in various types of human cancer cells(74, 75).

A potent anticancer effect of αM in oral squamous cells was verified. The α-mangostin treatment on the HSC-2, HSC-3, and HSC-4 cell lines were effective in a dose and time-dependent manner to decrease cell viability, to promote the cell cycle arrest in the G1 phase, beyond to induce morphological changes in cells, mitochondrial dysfunction, and cell death by via apoptosis intrinsic pathway. This substance did not change the morphology and viability of normal periodontal ligament fibroblasts(32).

β-lapachone and 3-iodine derivates

The β-lapachone (3.4-dihydro-2,2-dimethyl-2H-naphthol[1,2-b]pyran5,6-dione / β-lap) is a natural quinone derived from the bark of the purple Ipe (*Tabebuia avellanedae Lor, Bignoniaceae*)(76). This compound has biological activities, including anti-bacterial, anti-fungal, anti-inflammatory, anti-viral, anti-proliferative, and antioxidant(33, 77, 78). Furthermore, previous studies also have demonstrated that β-lap treatment induced cell death in different human cancers, like colon cancer, non-small cell Lung cancer, and melanoma (76, 79, 80).

The β-lap exerts a specific anti-tumor action through increased expression of NAD(P)H: quinone oxidoreductase 1 (NQO1) in cancer cells. The reduction of β-lap by NQO1 leads to futile redox cycle between the quinone and hydroquinone forms, with a concomitant depletion of NAD(P)H and NADH, and reactive oxygen species (ROS) generation, leading to the neoplastic cell death(81). So, the β-Lap has been shown as a potential anti-cancer drug(82) and also as an important sensitizing agent of human breast

cancer cells to ionizing radiation, increasing the generation of reactive oxygen species. The β-lap effectively improves the efficacy of radiation therapy in an NQO1-dependent manner. The combined treatment of ionizing radiation and β-lap synergistically promoted mitochondrial apoptotic cell death by inducing Endoplasmic Reticulum Stress, generation of ROS, and activation of the extracellular-regulated kinase and c-Jun N-terminal kinase (JNK)[(83)].

In OSCC cells, the β-lap and its 3-iodine derivatives (3-I-α-lapachone and 3-I-β-lapachone) played anticancer activities, promoting cell cycle arrest at G2/M phase, inter-nucleosomal DNA fragmentation and caspase- and ROS-mediated apoptosis, whose effects were more expressive at higher concentrations. Beyond, these compounds suppressed *in vivo* tumor growth, pointing them as new antitumor drug candidates. In contrast, treatment of normal human fibroblasts MRC5 and human normal keratinocytes HaCat with β-lap and its 3-iodine derivatives showed IC50 values heterogeneous between the tested substances and positive control doxorubicin[(33)].

Resveratrol

Resveratrol is a natural polyphenol produced in red grape skin, berries, and peanuts and food derived from them, such as wine. It exhibits anti-inflammatory, antioxidant, cardioprotective, and antitumor properties[(84)].

Resveratrol treatment was able to decrease cell viability in a time- and dose-dependent manner, and to promote apoptosis in CAL-27, SCC15, and SCC25 cells. The resveratrol-induced apoptosis was associated with an increase in the nuclear condensation, loss of mitochondrial membrane potential, and inducing of mitochondrial intrinsic pathway and caspase Cascade. The expression levels of apoptosis protein were modified by resveratrol; Bak and Bax were up-regulated, Bcl-2 and Bcl-xl were downregulated, Cytochrome c was released from mitochondria into the cytosol, Apoptotic protease activating factor 1 (Apaf-1) was increased and procaspase9, procasapase3, cleaved caspase3, and PARP. This antioxidant substance also decreased cell migration of CAL27 Cells, invasion by inhibiting of Epithelial-Mesenchymal Transition-inducing transcription factors, such as Snail and Slug Slug, Snail Smad2/3, and N-cadherin[(39)].

Thymol

Thymol is a polyphenol found in plants like thyme and oregano[85, 86]. It is a Transient Receptor Potential Ankyrin Subtype 1 channel, (TRPA1) agonist, and has antioxidant, anti-inflammatory, antifungal, antimicrobial, and properties. Cytotoxic role on human glioblastoma cells in vitro was reported, concomitantly to an increase in $Ca2+$ levels[87].

Studies investigating the therapeutic potential of thymol on OSCC cells are scarce in the literature. In this systematic review, we identified a study demonstrating thymol induced a concentration-dependent reduction in cell viability and led to long-lasting antiproliferative effects in several OSCC cell lines derived from primary tongue tumors, Cal27, SCC4, and SCC9. The thymol induced concentration-dependent depolarization of the mitochondria in both Cal27 and HeLa cells, generation of free radicals, and consequent apoptosis. Furthermore, the anticancer therapeutic potential of thymol occurred in the absence of TRP channel activation, as shown by calcium imaging studies using Cal27 and HeLa cells[36].

Xanthorrhizol

Xanthorrhizol (XNT) is a bisabolane-type sesquiterpenoid compound isolated from the essential oil of rhizomes of *Curcuma xanthorrhiza Roxb*. It has several biological activities, such as antioxidant, anticancer, antimicrobial, anti-inflammatory, antiplatelet, antihyperglycemic, hepatoprotective, nephroprotective, anti-estrogenic and estrogenic effects[88].

A study demonstrated that XNT decreased cell viability and induced apoptosis in SCC-15 from tongue OSCC cells in a concentration-dependent manner. The xanthorrhizol-induced apoptosis occurred through the caspase-independent pathway in SCC-15 cells, since xanthorrhizol-induced PARP degradation was not prevented by treatment with the caspase inhibitor Z-VAD-fmk. The XNT-induced apoptosis was associated with ROS-mediated p38 MAPK and JNK activation in SCC-15. In addition, XNT prevented chemical-induced oral carcinogenesis and increased the survival rate in the animal model study[38].

Fisetin

Fisetin (3,3-,4-,7-tetrahydroxyflavone) is a flavonoid with antioxidant, anti-inflammatory, and anticancer proprieties, being commonly found in fruits and vegetables, such as grapes, apples, cucumbers, strawberries, and onions. Fisetin is known to disrupt

Wnt, mTOR, and NF-κB signaling, promoting cell-cycle arrest and preventing the invasion of cancer cells[89].

In CAL-27 and Ca9-22 human oral squamous carcinoma cell lines, fisetin was able to reduce cell viability in a dose- and time-dependent manner, and also to promote apoptosis accompanied by morphological and biochemical changes. The cancer cells treated with fisetin showed condensed and fragmented nuclei and increased nuclear condensation ratio. In addition, this substance reduced the mitochondrial membrane potential and the proapoptotic proteins Bax and Bak, and increase caspase-3 and PARP in both cell lines. Beyond apoptosis, fisetin induced autophagy, which contrarily can play a role of pro-survival. However, it was demonstrated that inhibition of this process, as assayed with autophagy inhibitors, can enhance the sensitivity of OSCC cells to the fisetin-induced apoptosis[44].

Duchesnea indica

Duchesnea indica (Andr.) Focke, as used commonly plant in traditional Chinese medicine, has cytostatic properties as well as antioxidant and antimetastasis activities in different cancer cells[90].

The *D. indica* extract did not affect the cell viability of OSCC cells, SCC-9, SCC-14, and TW2.6. However, the extract revealed a potential antimetastatic role. It significantly attenuated motility, migration, and invasion of cancer cells by reducing the MMP-2 expression and activity in a dose-dependent manner, via the MEK/ERK signaling. The *D. indica* reduced the phosphorylation of both ERK1/2 and its upstream kinase[49].

Verbascoside

The Verbascoside (VB), a polyphenolic constituent of the *Tsoong* herb, exhibits anti-oxidative, anti-inflammatory and anticancer properties[91, 92]. Its therapeutic potential role in OSCC cells was few investigated.

In vitro, the VB inhibited cell viability, migration, and invasion, while promotes apoptosis in oral cancer cells, HN4 and HN6. It impaired OSCC tumor cell growth and metastasis through suppression of NF-κB related signaling activation; expression of IκBa was increased and the activation of NF-κB was reduced. In addition, VB inhibited growth and lung metastasis of implanted tumor cells in the xenograft OSCC mouse model, by impairment of MMP-9 expression via suppression of NF-κB activation[50].

Oridonin

Oridonin (Ordn) is diterpenoid isolated from herb *Rabdosia* rubescens, commonly used in traditional Chinese medicine. It is known to exert antioxidant, inflammatory, anti-pro-apoptotic, anti-bacterial, neurological and anticancer effects[(93)].

We identified a study revealing Ordn markedly suppressed the viability and colony-forming ability of both the oral cancer cell lines, HN22 and HSC4, in a dose and time-dependent manner, resulting in the cell cycle arrest in the sub-G1 phase. Ordn also induced the apoptosis, as shown by the increase in the early and late apoptotic population high multi-caspase activity (caspase-1, -3, -4, -5, -6, -7, -8 and -9), and the amount of cytochrome C increased in the cytoplasm of cancer cells. Moreover, Ordn-induced apoptosis was related to the ROS generation, loss of mitochondrial membrane potential, phosphorylation of JNK and p38 mitogen-activated protein kinase (MAPK), and enhanced cleavage of PARP[(43)].

Areca nut

Areca nut is a seed from the *pinang* plant (*Areca catechu Linn*; *areca*, *Palmaceae*) widely used in Indian and Malaysia. Although *Areca nut* has been associated with a high-risk of oral cancer-inducing[(94)], it has antioxidant, antidiabetic, antidepressant, antimicrobial, anti-inflammatory, hepatoprotective, and antiaging effects[(95)].

Interestingly, a study searched in this systematic review showed that *Areca nut* was able to induce apoptosis and caspase-3 activity in oral cancer cell lines, HSC-2 and HSC-3, pointing it as a potential anticancer agent against oral cancer[(45)].

Salvia miltiorrhiza

Salvia miltiorrhiza is a commonly widely used herbal in Asia. Danshensu, a substance isolated from *Salvia miltiorrhiza* root, can exert a therapeutic role in cardiovascular diseases and cerebral lesions. Its cardioprotective effects are mainly related to the antioxidant and anti-apoptosis proprieties[(96)]. The bioactive polyphenols present in Salvia, like Salvianolic acid A & B, were reported to have anti-cancer and anti-inflammatory activities, by promoting apoptosis and inhibiting cell cycle and metastasis[(97)].

In OSCC cell lines, HSC-3 and OC-2, *Salvia miltiorrhiza* alcohol extract exhibited significantly antiproliferative effects dose- and time-dependent and activated the caspase-3 apoptotic pathway by decreases in both XIAP and survivin expression, inhibitor

proteins of apoptosis, but not by regulating the Bcl-2-triggered mitochondrial via. The tumor xenograft growth with HSC-3 showed *Salvia* alcohol extract significantly reduced tumors [(48)].

Butein

Butein, a tetrahydroxychalcone and potent flavonoid, was firstly isolated from *Toxicodendron verniciflluum*, a traditional plant of Chinese medicine. It exhibits antioxidant, anti-inflammatory, anti-hypertensive, and anti-tumor activities[(98)].

The effect of Butein on the viability, apoptosis, migration, and invasion was assessed in OSCC cells, SAS and KB. It exhibited a strong radical-scavenging effect, as demonstrated by DPPH assay. Interestingly, at high concentrations, the Butein promoted a significant anti-proliferative, cytotoxic, anti-invasive, and anti-migratory effects on cancer cells, suppressing the expression of NF-κB regulated proteins, including COX-2, survivin, and MMP-9, which are involved with the regulation of several cancer-related pathways, such as survival, proliferation, invasion, and metastasis of OSCC cells[(35)].

Betulinic acid

Betulinic acid (BA) is a pentacyclic triterpenoid naturally occurring in several plants, being a is a principal constituent isolated from bark and bark extracts of *Betula* species. It has a wide range of biological and medicinal properties, including a potent antioxidant activity[(99)], anti-inflammatory, anthelmintic, antinociceptive, anti-HSV-1, and anti-cancer effects[(100)].

The BA on OSCC KB cell displayed a relevant antineoplastic and therapeutic role, inhibiting cell proliferation of dose-dependent form and promoting apoptosis, as shown by an increase in the activities of caspases 3 and 9, and high Bax expression and low Bcl-2 expression. BA markedly increased the cell population in the G0/G1 phase and decreases the S phase cell number, leading to the G0/G1 cell cycle arrest. The ROS generation was significantly increased by BA, and also ROS increased the dose-dependently p53 expression in KB cells and implanted tumors. Moreover, the phosphorylation of STAT3 was optimized by BA[(47)].

Derivatives of Dihydrocaffeic acid amide

The dihydro-caffeic acid is a potent antioxidant and novel antioxidants derivatives of dihydrocaffeic acid amide were synthesized and also demonstrated this propriety(101).

A study investigated the effect of four antioxidants on normal human tissues and on OSCC cells (CAL-27, UMSCC1, and UMSCC47) and, additionally, it was used human breast adenocarcinoma MCF-7 cells. Among the antioxidants, it was assayed the trolox trihydroxybenzoate, trolox 3,5-dimethoxy-4-hydroxycinnamate, and derivatives of dihydrocaffeic acid amide, N-decyl-N-(3-methoxy-4-hydroxybenzyl)-3-(3,4-dihydroxyphenyl) propanamide and N-decyl-N-(3,5-dimethoxy-4-hydroxybenzyl)-3-(3,4-dihydroxyphenyl) propanamide. More relevantly, the propanamide derivatives of dihydrocaffeic acid amide presented therapeutic effects more promising. The main results demonstrated apoptosis induction and an increase in the levels of cleaved caspase-3 in the cancer cell lines. The levels of γH2AX were also upregulated. The antioxidants do not appear to be toxic to normal cells(40).

Discussion

Laboratory and epidemiological researches have highlighted the key role of oxidative stress and reactive oxygen species in the carcinogenesis pathways, through lipid peroxidation, degradation of polyunsaturated fatty acids, causing cross-linking in lipids, proteins, and nucleic acids[11, 21]. In this context, increased intracellular ROS level or inhibiting of antioxidant systems may favor cellular and molecular changes inducing carcinogenesis. To protect cells of ROS-induced DNA damage, several endogenous antioxidant systems, such as superoxide dismutase, glutathione peroxidase, catalase, and glutathione contrary acts to the deleterious effects of ROS, inhibiting increased oxidative stress[102, 103].

Antioxidant substances have been shown in the literature to play a protective role against carcinogenesis, inhibiting lipid peroxidation and other free radical-mediated processes, through its biological function as radical scavengers[11, 104]. For example, the Phloretin, a natural dihydrochalcone found in apples, when conjugated with chitosan nanoparticles, acted as a potent antioxidant and anticancer-preventive agent in DMBA-induced oral cancer. It enhanced the cellular antioxidant mechanism by quenching the carcinogen-induced-free radicals[104].

On the other hand, studies have shown an important therapeutic role of antioxidant substances in several cancer types[105, 106], promoting mitochondrial deregulations and tumor cell death. These substances according to the treatment concentrations can become prooxidants leading to the generating of superoxide anion radicals and induction of apoptosis in cancer cells. Thymoquinone is a plant-derived substance considered as an antioxidant at lower treatment concentrations; however, it at higher concentrations was able to act as a prooxidant in prostate cancer cells, mobilizing and reducing endogenous copper to ROS generation and cytotoxicity induction[12].

In this systematic review, almost all studies searching antineoplastic potential of antioxidants on human oral cancer cells investigated phytochemical extracts or plant-derived compounds, such as *extract of Acacia catechu*[21], *Gracilaria tenuistipiatata*[22], *Tualang honey*[23], *Pergularia daemia*[24], *Hibiscus sabdariffa*[25], curcumin[6, 26, 34, 37], *Shikonin*[27], *Licochalcones*[28, 41, 42, 46], *Pterostilbene*[29], Quercetin[30], *α-Mangostin*[32], *β-lapachone and 3-iodine derivates*[33], *Salvia miltiorrhiza* alcohol extract[48], *Areca nut* extract[45], *Duchesnea indica* extract[49], Resveratrol[39], Butein[35], Fisetin[44], Oridonin[43], Betulinic acid[47], Thymol[36], Verbacoside[50], and Xanthorrhizol[38]. Other studies used

antioxidant chemical substances, like selenium[20], and HPB242[31] and derivatives of Dihydrocaffeic acid amide[40].

When we assessed the quality of *in vitro* studies through the ToxRTool criteria, all studies were classified as a high score, being "reliable without restrictions". Considering the quality of the studies selected in this review, we can highlight the antioxidant substances herein described have a potential role in the treatment of OSCC. Moreover, we identified a scarcity of human studies investigating this purpose; only one study assessed the therapeutic potential of selenium in oral cancer patients, showing promising results[20].

It is important to emphasize that the literature search allowed us to identify other antioxidants that exerted antineoplastic action on oral cancer cells, such as gallic acid[107], and grape seed extract, rich in antioxidants[108]. However, these studies were not included in the list of selected articles, as they did not present the descriptors defined in this systematic review and any other term related to antioxidants in OSCC cells. So, we considered that the precise definition of keywords in published abstracts is extremely important to the initial stage of article selection in a systematic review.

All antioxidants of *in vitro* studies analyzed in systematic review inhibited the neoplastic behavior of OSCC cells, for example, promoting the reduction of cell viability[21-33] and migration[25, 29, 30, 33], and also an increase of cell death[22-24, 26-28, 30-33].

The inhibitory effect played by antioxidants on the neoplastic behavior of OSCC cells was shown to be dependent-dose and time manner, especially the studies investigating *Acacia catechu*, *Gracilaria tenuistipitata*, *Tualang* honey, *Pergularia daemia*, *Hibiscus sabdariffa*, Curcumin, Shikonin, Licochalcone B, Pterostilbene, Quercetin, 2,4-bis(p-hydroxyphenyl)-2-butenal, α-mangostin, and β-lapachone and 3-iodine derivates[21-33]. It is known that high-dose antioxidant substances can act as a pro-oxidant in cancer cells, leading to ROS generation and cancer cell death[12]. Interestingly, low doses may have no inhibitory effect on cell proliferation. In a study using Grape seed extract rich in polyphenols (GSE), at low concentrations (1-10 μg/ml), it maintained the cell viability around 91%. In contrast, a significantly concentration-dependent decrease in cell viability was observed at high GSE concentrations (50, 100, 200 and 400 μg/ml)[108]. Since *in vitro* studies have shown that antioxidants may favor the death of oral cancer cells, it is also necessary to know their effects on normal cells. In other words, would the high doses of antioxidants that exert an antineoplastic therapeutic effect on OSCC cells be harmful to normal cells or would promote the improvement of patients' clinical conditions

and reduce the adverse effects of chemo-or radiotherapy due to its ability ROS scavenger? This should be considered since it was shown that antioxidant therapies may alleviate the adverse effects of chemotherapy[109] but can antagonize its antitumor effects by reducing oxidative damage[51, 110].

Some of the studies selected in this review also investigated the effect of antioxidants on the viability of normal cell lines such as skin keratinocytes[25, 33], oral keratinocytes[29], and fibroblasts[30, 32, 33]. Contrarily to their effects on cancer cells, antioxidant substances or extracts did not compromise the viability of normal cells. However, a divergent result was identified in the treatment of normal human fibroblasts MRC5 and human normal keratinocytes HaCat with β-lapachone and its 3-iodine derivatives; the IC50 showed heterogenous values between the tested substances and positive control doxorubicin[33].

Phytochemicals are one of the major sources of anticancer drugs. These compounds are isolated from different kinds of fruits, vegetables, beverages, and edible plants. The derived-plant polyphenols, including flavonoids, catechin, hesperetin, flavones, quercetin, phenolic acids, ellagic acid, lignans, stilbenes, among other represent substances, which proprieties can be used to development of new drugs to prevention and treatment of cancer[106]. From the 32 articles included in this systematic review, 29 studies[21-30, 32-50] searched the plant extracts or compounds rich in polyphenols with therapeutic potential on OSCC cells, highlighting Curcumin[6, 26, 34, 37], Verbacoside[50], Resveratrol[39], Thymol[36], α-Mangostin[32]. The flavonoids include Butein[35], Fisetin[44], Licochalcones (B, C, D, H)[28, 41, 42, 46], Quercetin[30], and terpenoids substances, such as Betulinic acid[47], Oridonin[43], Xanthorrhizol[38] were also identified to play antineoplastic effect.

The natural dietary polyphenol compounds can promote anti-cancer effects through the inhibiting of cancer cell-activating signaling pathways, activating DNA repair mechanism, stimulating the apoptosis increasing caspases expression, and the inducing antioxidant action (antioxidant enzymes e.g. GSH, GST, and GPxn)[105].

Among the selected studies, several molecular pathways involved in the therapeutic potential of antioxidants on oral cancer cells were investigated. In particular, pathways promoting cell death, like apoptosis activation[21, 26, 27, 30-32], increase of ROS generation[22, 24, 26, 28, 33] and autophagy was shown to mediate the antineoplastic effects of antioxidants[26]. The cell cycle arrest in the G1 phase[22, 28, 30, 32] or G2/M phase[33], and modulation of cell cycle regulating proteins, such as inhibition of p27, Cyclin D1, Mcl-1,

and Survivin[31] was also associated with antioxidant effects on neoplastic behavior. Some metastasis-related genes were regulated by antioxidant substance, as the downregulation of MMP-2 and upregulation of tissue inhibitor of metalloproteinase-2[29]. Another signaling via and molecular regulations was also targeted by antioxidants to plays its antineoplastic role, like the reducing phosphorylation of Akt, ERK1/2, and JNK1/2, downregulation of the transcription factors NF-κB, SP-1, and CREB[29], MAPKs ERK1/2 modulation and p38 activation[25, 38] and the inhibiting of Epithelial-Mesenchymal Transition-inducing transcription factors, such as Snail and Slug Slug, Snail Smad2/3, and N-cadherin[39].

We have identified a dearth of clinical studies that assessed the therapeutic effect of antioxidants in oral cancer patients. According to the search criteria, only a single human study was selected which indicated that treatment with radiotherapy-adjuvant selenium led to a reduction in therapeutic response markers, but this study did not objectively investigate whether selenium can promote the reduction of tumor size. We emphasize this substance as an interesting and promising target for more consistent clinical research[20].

Taking together all the analyzes of the selected studies, antioxidant substances can control the behavior of oral cancer cells in *in vitro* study models. To better verify its therapeutic effect in humans, randomized clinical trials should be performed to understand whether antioxidants alone or by combination therapies are able to control the progression of oral squamous cell carcinoma, or if they decrease the deleterious effects of chemo-or radiotherapy or improve the nutritional status of the oral cancer patients.

References

1. D'Souza G, Kreimer AR, Viscidi R, Pawlita M, Fakhry C, Koch WM, et al. Case-control study of human papillomavirus and oropharyngeal cancer. N Engl J Med. 2007;356(19):1944-56.
2. Hinther A, Nakoneshny SC, Dort JC, Chandarana SP, Matthews TW. Historical compliance rates for providing postoperative radiotherapy in oral cavity squamous cell carcinoma. Head & neck. 2016;38(10):1525-9.
3. Karnov KKS, Gronhoj C, Jensen DH, Wessel I, Charabi BW, Specht L, et al. Increasing incidence and survival in oral cancer: a nationwide Danish study from 1980 to 2014. Acta oncologica. 2017;56(9):1204-9.
4. Tapia JL, Goldberg LJ. The challenges of defining oral cancer: analysis of an ontological approach. Head and neck pathology. 2011;5(4):376-84.
5. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2018;68(6):394-424.
6. Srivastava S, Mohammad S, Pant AB, Mishra PR, Pandey G, Gupta S, et al. Co-delivery of 5-Fluorouracil and Curcumin Nanohybrid Formulations for Improved Chemotherapy Against Oral Squamous Cell Carcinoma. J Maxillofac Oral Surg. 2018;17(4):597-610.
7. Siddiqui RA, Harvey KA, Xu Z, Bammerlin EM, Walker C, Altenburg JD. Docosahexaenoic acid: a natural powerful adjuvant that improves efficacy for anticancer treatment with no adverse effects. Biofactors. 2011;37(6):399-412.
8. Camargo CQ, Brunetta HS, Nunes EA. Effects of cotreatment with omega-3 polyunsaturated fatty acids and anticancer agents on oxidative stress parameters: a systematic review of in vitro, animal, and human studies. Nutr Rev. 2018;76(10):765-77.
9. Huo W, Li ZM, Pan XY, Bao YM, An LJ. Antioxidant enzyme levels in pathogenesis of oral squamous cell carcinoma (OSCC). Drug Res (Stuttg). 2014;64(11):629-32.
10. Topdag S, Aslaner A, Tataroglu C, Ilce Z. Evaluation of antioxidant capacity in lung carcinoma. Indian Journal of Thoracic and Cardiovascular Surgery. 2005;21(4):269-71.
11. Anbuselvam C, Vijayavel K, Balasubramanian MP. Protective effect of Operculina turpethum against 7,12-dimethyl benz(a)anthracene induced oxidative stress with reference to breast cancer in experimental rats. Chem Biol Interact. 2007;168(3):229-36.
12. Zubair H, Khan HY, Sohail A, Azim S, Ullah MF, Ahmad A, et al. Redox cycling of endogenous copper by thymoquinone leads to ROS-mediated DNA breakage and consequent cell death: putative anticancer mechanism of antioxidants. Cell Death Dis. 2013;4:e660.
13. Prasad KN, Kumar A, Kochupillai V, Cole WC. High doses of multiple antioxidant vitamins: essential ingredients in improving the efficacy of standard cancer therapy. J Am Coll Nutr. 1999;18(1):13-25.
14. Maulina T, Hadikrishna I, Hardianto A, Sjamsudin E, Pontjo B, Yusuf HY. The therapeutic activity of curcumin through its anti-cancer potential on oral squamous cell carcinoma: A study on Sprague Dawley rat. SAGE Open Med. 2019;7:2050312119875982.
15. Methley AM, Campbell S, Chew-Graham C, McNally R, Cheraghi-Sohi S. PICO, PICOS and SPIDER: a comparison study of specificity and sensitivity in three search tools for qualitative systematic reviews. BMC Health Serv Res. 2014;14:579.
16. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, Ioannidis JP, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. PLoS Med. 2009;6(7):e1000100.
17. Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics. 1977;33(1):159-74.
18. Schneider K, Schwarz M, Burkholder I, Kopp-Schneider A, Edler L, Kinsner-Ovaskainen A, et al. "ToxRTool", a new tool to assess the reliability of toxicological data. Toxicol Lett. 2009;189(2):138-44.
19. Higgins JP, Altman DG, Gotzsche PC, Juni P, Moher D, Oxman AD, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ. 2011;343:d5928.

20. Elango S, Samuel S, Khashim Z, Subbiah U. Selenium Influences Trace Elements Homeostasis, Cancer Biomarkers in Squamous Cell Carcinoma Patients Administered with Cancerocidal Radiotherapy. Asian Pacific journal of cancer prevention : APJCP. 2018;19(7):1785-92.
21. Lakshmi T, Ezhilarasan D, Nagaich U, Vijayaragavan R. Acacia catechu Ethanolic Seed Extract Triggers Apoptosis of SCC-25 Cells. Pharmacogn Mag. 2017;13(Suppl 3):S405-S11.
22. Yeh CC, Yang JI, Lee JC, Tseng CN, Chan YC, Hseu YC, et al. Anti-proliferative effect of methanolic extract of Gracilaria tenuistipitata on oral cancer cells involves apoptosis, DNA damage, and oxidative stress. BMC complementary and alternative medicine. 2012;12:142.
23. Ghashm AA, Othman NH, Khattak MN, Ismail NM, Saini R. Antiproliferative effect of Tualang honey on oral squamous cell carcinoma and osteosarcoma cell lines. BMC complementary and alternative medicine. 2010;10:49.
24. Mirunalini S, Kandhan K, Vaithiyanathan V. Antiproliferative potential of pergularia daemia (forsk.) on human oral epidermoid carcinoma (kb) cells by inducing apoptosis and modifying oxidant antioxidant status. Asian Journal of Pharmaceutical and Clinical Research. 2014;7:89-95.
25. Malacrida A, Maggioni D, Cassetti A, Nicolini G, Cavaletti G, Miloso M. Antitumoral Effect of Hibiscus sabdariffa on Human Squamous Cell Carcinoma and Multiple Myeloma Cells. Nutrition and cancer. 2016;68(7):1161-70.
26. Kim JY, Cho TJ, Woo BH, Choi KU, Lee CH, Ryu MH, et al. Curcumin-induced autophagy contributes to the decreased survival of oral cancer cells. Arch Oral Biol. 2012;57(8):1018-25.
27. Min R, Tong J, Wenjun Y, Wenhu D, Xiaojian Z, Jiacai H, et al. Growth inhibition and induction of apoptosis in human oral squamous cell carcinoma Tca-8113 cell lines by Shikonin was partly through the inactivation of NF-kappaB pathway. Phytotherapy research : PTR. 2008;22(3):407-15.
28. Oh H, Yoon G, Shin JC, Park SM, Cho SS, Cho JH, et al. Licochalcone B induces apoptosis of human oral squamous cell carcinoma through the extrinsic- and intrinsic-signaling pathways. International journal of oncology. 2016;48(4):1749-57.
29. Lin CW, Chou YE, Chiou HL, Chen MK, Yang WE, Hsieh MJ, et al. Pterostilbene suppresses oral cancer cell invasion by inhibiting MMP-2 expression. Expert Opin Ther Targets. 2014;18(10):1109-20.
30. Chen SF, Nien S, Wu CH, Liu CL, Chang YC, Lin YS. Reappraisal of the anticancer efficacy of quercetin in oral cancer cells. J Chin Med Assoc. 2013;76(3):146-52.
31. Chae JI, Lee R, Cho J, Hong J, Shim JH. Specificity protein 1 is a novel target of 2,4-bis (p-hydroxyphenyl)-2-butenal for the suppression of human oral squamous cell carcinoma cell growth. J Biomed Sci. 2014;21:4.
32. Kwak HH, Kim IR, Kim HJ, Park BS, Yu SB. alpha-Mangostin Induces Apoptosis and Cell Cycle Arrest in Oral Squamous Cell Carcinoma Cell. Evidence-based complementary and alternative medicine : eCAM. 2016;2016:5352412.
33. Dias RB, de Araujo TBS, de Freitas RD, Rodrigues A, Sousa LP, Sales CBS, et al. beta-Lapachone and its iodine derivatives cause cell cycle arrest at G2/M phase and reactive oxygen species-mediated apoptosis in human oral squamous cell carcinoma cells. Free Radic Biol Med. 2018;126:87-100.
34. Bano N, Yadav M, Das BC. Differential Inhibitory Effects of Curcumin Between HPV+ve and HPV-ve Oral Cancer Stem Cells. Frontiers in oncology. 2018;8:412.
35. Bordoloi D, Monisha J, Roy NK, Padmavathi G, Banik K, Harsha C, et al. An Investigation on the Therapeutic Potential of Butein, A Tretrahydroxychalcone Against Human Oral Squamous Cell Carcinoma. Asian Pacific journal of cancer prevention : APJCP. 2019;20(11):3437-46.
36. De La Chapa JJ, Singha PK, Lee DR, Gonzales CB. Thymol inhibits oral squamous cell carcinoma growth via mitochondria-mediated apoptosis. Journal of oral pathology & medicine : official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology. 2018;47(7):674-82.

37. Fetoni AR, Paciello F, Mezzogori D, Rolesi R, Eramo SL, Paludetti G, et al. Molecular targets for anticancer redox chemotherapy and cisplatin-induced ototoxicity: the role of curcumin on pSTAT3 and Nrf-2 signalling. British journal of cancer. 2015;113(10):1434-44.
38. Kim JY, An JM, Chung WY, Park KK, Hwang JK, Kim du S, et al. Xanthorrhizol induces apoptosis through ROS-mediated MAPK activation in human oral squamous cell carcinoma cells and inhibits DMBA-induced oral carcinogenesis in hamsters. Phytotherapy research : PTR. 2013;27(4):493-8.
39. Kim SE, Shin SH, Lee JY, Kim CH, Chung IK, Kang HM, et al. Resveratrol Induces Mitochondrial Apoptosis and Inhibits Epithelial-Mesenchymal Transition in Oral Squamous Cell Carcinoma Cells. Nutrition and cancer. 2018;70(1):125-35.
40. Kovalchuk A, Aladedunye F, Rodriguez-Juarez R, Li D, Thomas J, Kovalchuk O, et al. Novel antioxidants are not toxic to normal tissues but effectively kill cancer cells. Cancer biology & therapy. 2013;14(10):907-15.
41. Nho SH, Yoon G, Seo JH, Oh HN, Cho SS, Kim H, et al. Licochalcone H induces the apoptosis of human oral squamous cell carcinoma cells via regulation of matrin 3. Oncology reports. 2019;41(1):333-40.
42. Oh HN, Seo JH, Lee MH, Kim C, Kim E, Yoon G, et al. Licochalcone C induced apoptosis in human oral squamous cell carcinoma cells by regulation of the JAK2/STAT3 signaling pathway. Journal of cellular biochemistry. 2018;119(12):10118-30.
43. Oh HN, Seo JH, Lee MH, Yoon G, Cho SS, Liu K, et al. Oridonin induces apoptosis in oral squamous cell carcinoma probably through the generation of reactive oxygen species and the p38/JNK MAPK pathway. International journal of oncology. 2018.
44. Park BS, Choi NE, Lee JH, Kang HM, Yu SB, Kim HJ, et al. Crosstalk between Fisetin-induced Apoptosis and Autophagy in Human Oral Squamous Cell Carcinoma. Journal of Cancer. 2019;10(1):138-46.
45. Sari LM, Subita GP, Auerkari EI. Areca nut extract demonstrated apoptosis-inducing mechanism by increased caspase-3 activities on oral squamous cell carcinoma. F1000Research. 2018;7:723.
46. Seo JH, Choi HW, Oh HN, Lee MH, Kim E, Yoon G, et al. Licochalcone D directly targets JAK2 to induced apoptosis in human oral squamous cell carcinoma. Journal of cellular physiology. 2019;234(2):1780-93.
47. Shen H, Liu L, Yang Y, Xun W, Wei K, Zeng G. Betulinic Acid Inhibits Cell Proliferation in Human Oral Squamous Cell Carcinoma via Modulating ROS-Regulated p53 Signaling. Oncology research. 2017;25(7):1141-52.
48. Wang WH, Hsuan KY, Chu LY, Lee CY, Tyan YC, Chen ZS, et al. Anticancer Effects of Salvia miltiorrhiza Alcohol Extract on Oral Squamous Carcinoma Cells. Evidence-based complementary and alternative medicine : eCAM. 2017;2017:5364010.
49. Yang WE, Ho YC, Tang CM, Hsieh YS, Chen PN, Lai CT, et al. Duchesnea indica extract attenuates oral cancer cells metastatic potential through the inhibition of the matrix metalloproteinase-2 activity by down-regulating the MEK/ERK pathway. Phytomedicine : international journal of phytotherapy and phytopharmacology. 2019;63:152960.
50. Zhang Y, Yuan Y, Wu H, Xie Z, Wu Y, Song X, et al. Effect of verbascoside on apoptosis and metastasis in human oral squamous cell carcinoma. International journal of cancer. 2018;143(4):980-91.
51. Yasueda A, Urushima H, Ito T. Efficacy and Interaction of Antioxidant Supplements as Adjuvant Therapy in Cancer Treatment: A Systematic Review. Integr Cancer Ther. 2016;15(1):17-39.
52. Mehdi Y, Hornick JL, Istasse L, Dufrasne I. Selenium in the environment, metabolism and involvement in body functions. Molecules. 2013;18(3):3292-311.
53. Oldfield JE. The two faces of selenium. J Nutr. 1987;117(12):2002-8.
54. Hosnedlova B, Kepinska M, Skalickova S, Fernandez C, Ruttkay-Nedecky B, Malevu TD, et al. A Summary of New Findings on the Biological Effects of Selenium in Selected Animal Species-A Critical Review. Int J Mol Sci. 2017;18(10).

55. Zhuo H, Smith AH, Steinmaus C. Selenium and lung cancer: a quantitative analysis of heterogeneity in the current epidemiological literature. Cancer Epidemiol Biomarkers Prev. 2004;13(5):771-8.
56. Lakshmi T, Ezhilarasan D, Vijayaragavan R, Bhullar SK, Rajendran R. Acacia catechu ethanolic bark extract induces apoptosis in human oral squamous carcinoma cells. J Adv Pharm Technol Res. 2017;8(4):143-9.
57. Yeh CC, Tseng CN, Yang JI, Huang HW, Fang Y, Tang JY, et al. Antiproliferation and induction of apoptosis in Ca9-22 oral cancer cells by ethanolic extract of Gracilaria tenuistipitata. Molecules. 2012;17(9):10916-27.
58. Ahmed S, Othman NH. The anti-cancer effects of Tualang honey in modulating breast carcinogenesis: an experimental animal study. BMC complementary and alternative medicine. 2017;17(1):208.
59. Vaithiyanathan V, Mirunalini S. Assessment of anticancer activity: A comparison of dose–response effect of ethyl acetate and methanolic extracts of Pergularia daemia (Forsk). Oral Science International. 2015;13.
60. Riaz G, Chopra R. A review on phytochemistry and therapeutic uses of Hibiscus sabdariffa L. Biomed Pharmacother. 2018;102:575-86.
61. Nguyen C, Baskaran K, Pupulin A, Ruvinov I, Zaitoon O, Grewal S, et al. Hibiscus flower extract selectively induces apoptosis in breast cancer cells and positively interacts with common chemotherapeutics. BMC complementary and alternative medicine. 2019;19(1):98.
62. De Luca A, Maiello MR, D'Alessio A, Pergameno M, Normanno N. The RAS/RAF/MEK/ERK and the PI3K/AKT signalling pathways: role in cancer pathogenesis and implications for therapeutic approaches. Expert Opin Ther Targets. 2012;16 Suppl 2:S17-27.
63. Koul HK, Pal M, Koul S. Role of p38 MAP Kinase Signal Transduction in Solid Tumors. Genes Cancer. 2013;4(9-10):342-59.
64. Wang F, Yao X, Zhang Y, Tang J. Synthesis, biological function and evaluation of Shikonin in cancer therapy. Fitoterapia. 2019;134:329-39.
65. Boulos JC, Rahama M, Hegazy MF, Efferth T. Shikonin derivatives for cancer prevention and therapy. Cancer Lett. 2019;459:248-67.
66. Wang J, Liao AM, Thakur K, Zhang JG, Huang JH, Wei ZJ. Licochalcone B Extracted from Glycyrrhiza uralensis Fisch Induces Apoptotic Effects in Human Hepatoma Cell HepG2. J Agric Food Chem. 2019;67(12):3341-53.
67. Kang TH, Yoon G, Kang IA, Oh HN, Chae JI, Shim JH. Natural Compound Licochalcone B Induced Extrinsic and Intrinsic Apoptosis in Human Skin Melanoma (A375) and Squamous Cell Carcinoma (A431) Cells. Phytotherapy research : PTR. 2017;31(12):1858-67.
68. Huang Y, Du J, Mi Y, Li T, Gong Y, Ouyang H, et al. Long Non-coding RNAs Contribute to the Inhibition of Proliferation and EMT by Pterostilbene in Human Breast Cancer. Frontiers in oncology. 2018;8:629.
69. Rauf A, Imran M, Khan IA, Ur-Rehman M, Gilani SA, Mehmood Z, et al. Anticancer potential of quercetin: A comprehensive review. Phytotherapy research : PTR. 2018;32(11):2109-30.
70. Zhao J, Fang Z, Zha Z, Sun Q, Wang H, Sun M, et al. Quercetin inhibits cell viability, migration and invasion by regulating miR-16/HOXA10 axis in oral cancer. European journal of pharmacology. 2019;847:11-8.
71. Wu H, Pan L, Gao C, Xu H, Li Y, Zhang L, et al. Quercetin Inhibits the Proliferation of Glycolysis-Addicted HCC Cells by Reducing Hexokinase 2 and Akt-mTOR Pathway. Molecules. 2019;24(10).
72. Ban JO, Kim DH, Lee HP, Hwang CJ, Shim JH, Kim DJ, et al. Anti-arthritis effects of (E)-2,4-bis(p-hydroxyphenyl)-2-butenal are mediated by inhibition of the STAT3 pathway. Br J Pharmacol. 2014;171(11):2900-12.
73. Lee YJ, Choi DY, Choi IS, Han JY, Jeong HS, Han SB, et al. Inhibitory effect of a tyrosine-fructose Maillard reaction product, 2,4-bis(p-hydroxyphenyl)-2-butenal on amyloid-beta generation and inflammatory reactions via inhibition of NF-kappaB and STAT3 activation in cultured astrocytes and microglial BV-2 cells. J Neuroinflammation. 2011;8:132.

74. Reyes-Fermin LM, Avila-Rojas SH, Aparicio-Trejo OE, Tapia E, Rivero I, Pedraza-Chaverri J. The Protective Effect of Alpha-Mangostin against Cisplatin-Induced Cell Death in LLC-PK1 Cells is Associated to Mitochondrial Function Preservation. Antioxidants (Basel). 2019;8(5).
75. Huang W, Liang Y, Ma X. Alpha-mangostin induces endoplasmic reticulum stress and autophagy which count against fatty acid synthase inhibition mediated apoptosis in human breast cancer cells. Cancer cell international. 2019;19:151.
76. Liu Y, Kwon T, Kim JS, Chandimali N, Jin YH, Gong YX, et al. Peroxiredoxin V Reduces beta-Lapachone-induced Apoptosis of Colon Cancer Cells. Anticancer Res. 2019;39(7):3677-86.
77. Park JS, Lee YY, Kim J, Seo H, Kim HS. beta-Lapachone increases phase II antioxidant enzyme expression via NQO1-AMPK/PI3K-Nrf2/ARE signaling in rat primary astrocytes. Free Radic Biol Med. 2016;97:168-78.
78. Sitonio MM, Carvalho Junior CH, Campos Ide A, Silva JB, Lima Mdo C, Goes AJ, et al. Anti-inflammatory and anti-arthritic activities of 3,4-dihydro-2,2-dimethyl-2H-naphthol[1,2-b]pyran-5,6-dione (beta-lapachone). Inflamm Res. 2013;62(1):107-13.
79. Jeon YJ, Bang W, Choi YH, Shim JH, Chae JI. Beta-Lapachone Suppresses Non-small Cell Lung Cancer Proliferation through the Regulation of Specificity Protein 1. Biol Pharm Bull. 2015;38(9):1302-8.
80. Bang W, Jeon YJ, Cho JH, Lee RH, Park SM, Shin JC, et al. beta-lapachone suppresses the proliferation of human malignant melanoma cells by targeting specificity protein 1. Oncology reports. 2016;35(2):1109-16.
81. Pink JJ, Planchon SM, Tagliarino C, Varnes ME, Siegel D, Boothman DA. NAD(P)H:Quinone oxidoreductase activity is the principal determinant of beta-lapachone cytotoxicity. J Biol Chem. 2000;275(8):5416-24.
82. Ahn KJ, Lee HS, Bai SK, Song CW. Enhancement of radiation effect using beta-lapachone and underlying mechanism. Radiat Oncol J. 2013;31(2):57-65.
83. Park MT, Song MJ, Lee H, Oh ET, Choi BH, Jeong SY, et al. beta-lapachone significantly increases the effect of ionizing radiation to cause mitochondrial apoptosis via JNK activation in cancer cells. PLoS One. 2011;6(10):e25976.
84. Tian B, Liu J. Resveratrol: a review of plant sources, synthesis, stability, modification and food application. Journal of the science of food and agriculture. 2019.
85. El Babili F, Bouajila J, Souchard JP, Bertrand C, Bellvert F, Fouraste I, et al. Oregano: chemical analysis and evaluation of its antimalarial, antioxidant, and cytotoxic activities. Journal of food science. 2011;76(3):C512-8.
86. Salehi B, Mishra AP, Shukla I, Sharifi-Rad M, Contreras MDM, Segura-Carretero A, et al. Thymol, thyme, and other plant sources: Health and potential uses. Phytotherapy research : PTR. 2018;32(9):1688-706.
87. Hsu SS, Lin KL, Chou CT, Chiang AJ, Liang WZ, Chang HT, et al. Effect of thymol on Ca2+ homeostasis and viability in human glioblastoma cells. European journal of pharmacology. 2011;670(1):85-91.
88. Oon SF, Nallappan M, Tee TT, Shohaimi S, Kassim NK, Sa'ariwijaya MS, et al. Xanthorrhizol: a review of its pharmacological activities and anticancer properties. Cancer cell international. 2015;15:100.
89. Kashyap D, Sharma A, Sak K, Tuli HS, Buttar HS, Bishayee A. Fisetin: A bioactive phytochemical with potential for cancer prevention and pharmacotherapy. Life sciences. 2018;194:75-87.
90. Xiang B, Yu X, Li B, Xiong Y, Long M, He Q. Characterization, antioxidant, and anticancer activities of a neutral polysaccharide from Duchesnea indica (Andr.) Focke. Journal of food biochemistry. 2019;43(7):e12899.
91. Jia WQ, Wang ZT, Zou MM, Lin JH, Li YH, Zhang L, et al. Verbascoside Inhibits Glioblastoma Cell Proliferation, Migration and Invasion While Promoting Apoptosis Through Upregulation of Protein Tyrosine Phosphatase SHP-1 and Inhibition of STAT3 Phosphorylation. Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology. 2018;47(5):1871-82.

92. Peerzada KJ, Faridi AH, Sharma L, Bhardwaj SC, Satti NK, Shashi B, et al. Acteoside-mediates chemoprevention of experimental liver carcinogenesis through STAT-3 regulated oxidative stress and apoptosis. Environmental toxicology. 2016;31(7):782-98.
93. Owona BA, Schluesener HJ. Molecular Insight in the Multifunctional Effects of Oridonin. Drugs in R&D. 2015;15(3):233-44.
94. Lee SS, Tsai CH, Yang SF, Ho YC, Chang YC. Hypoxia inducible factor-1alpha expression in areca quid chewing-associated oral squamous cell carcinomas. Oral diseases. 2010;16(7):696-701.
95. Peng W, Liu YJ, Wu N, Sun T, He XY, Gao YX, et al. Areca catechu L. (Arecaceae): a review of its traditional uses, botany, phytochemistry, pharmacology and toxicology. Journal of ethnopharmacology. 2015;164:340-56.
96. Zhang J, Zhang Q, Liu G, Zhang N. Therapeutic potentials and mechanisms of the Chinese traditional medicine Danshensu. European journal of pharmacology. 2019;864:172710.
97. Qin T, Rasul A, Sarfraz A, Sarfraz I, Hussain G, Anwar H, et al. Salvianolic acid A & B: potential cytotoxic polyphenols in battle against cancer via targeting multiple signaling pathways. International journal of biological sciences. 2019;15(10):2256-64.
98. Padmavathi G, Roy NK, Bordoloi D, Arfuso F, Mishra S, Sethi G, et al. Butein in health and disease: A comprehensive review. Phytomedicine : international journal of phytotherapy and phytopharmacology. 2017;25:118-27.
99. Peng J, Lv YC, He PP, Tang YY, Xie W, Liu XY, et al. Betulinic acid downregulates expression of oxidative stress-induced lipoprotein lipase via the PKC/ERK/c-Fos pathway in RAW264.7 macrophages. Biochimie. 2015;119:192-203.
100. Rastogi S, Pandey MM, Kumar Singh Rawat A. Medicinal plants of the genus Betula--traditional uses and a phytochemical-pharmacological review. Journal of ethnopharmacology. 2015;159:62-83.
101. Aladedunye F, Catel Y, Przybylski R. Novel caffeic acid amide antioxidants: Synthesis, radical scavenging activity and performance under storage and frying conditions. Food Chemistry. 2012;130(4):945-52.
102. Ray G, Batra S, Shukla NK, Deo S, Raina V, Ashok S, et al. Lipid peroxidation, free radical production and antioxidant status in breast cancer. Breast Cancer Res Treat. 2000;59(2):163-70.
103. Kilic N, Yavuz Taslipinar M, Guney Y, Tekin E, Onuk E. An investigation into the serum thioredoxin, superoxide dismutase, malondialdehyde, and advanced oxidation protein products in patients with breast cancer. Ann Surg Oncol. 2014;21(13):4139-43.
104. Mariadoss AVA, Vinayagam R, Xu B, Venkatachalam K, Sankaran V, Vijayakumar S, et al. Phloretin loaded chitosan nanoparticles enhance the antioxidants and apoptotic mechanisms in DMBA induced experimental carcinogenesis. Chem Biol Interact. 2019;308:11-9.
105. Iqbal J, Abbasi BA, Mahmood T, Kanwal S, Ali B, Shah SA, et al. Plant-derived anticancer agents: A green anticancer approach. Asian Pacific Journal of Tropical Biomedicine. 2017;7(12):1129-50.
106. Hazafa A, Rehman KU, Jahan N, Jabeen Z. The Role of Polyphenol (Flavonoids) Compounds in the Treatment of Cancer Cells. Nutrition and cancer. 2019:1-12.
107. Guimaraes TA, Farias LC, Fraga CA, Feltenberger JD, Melo GA, Coletta RD, et al. Evaluation of the antineoplastic activity of gallic acid in oral squamous cell carcinoma under hypoxic conditions. Anticancer Drugs. 2016;27(5):407-16.
108. Yen CY, Hou MF, Yang ZW, Tang JY, Li KT, Huang HW, et al. Concentration effects of grape seed extracts in anti-oral cancer cells involving differential apoptosis, oxidative stress, and DNA damage. BMC complementary and alternative medicine. 2015;15:94.
109. Suhail N, Bilal N, Khan HY, Hasan S, Sharma S, Khan F, et al. Effect of vitamins C and E on antioxidant status of breast-cancer patients undergoing chemotherapy. J Clin Pharm Ther. 2012;37(1):22-6.
110. Meyer F, Bairati I, Fortin A, Gelinas M, Nabid A, Brochet F, et al. Interaction between antioxidant vitamin supplementation and cigarette smoking during radiation therapy in relation to

long-term effects on recurrence and mortality: a randomized trial among head and neck cancer patients. International journal of cancer. 2008;122(7):1679-83.

Figures list

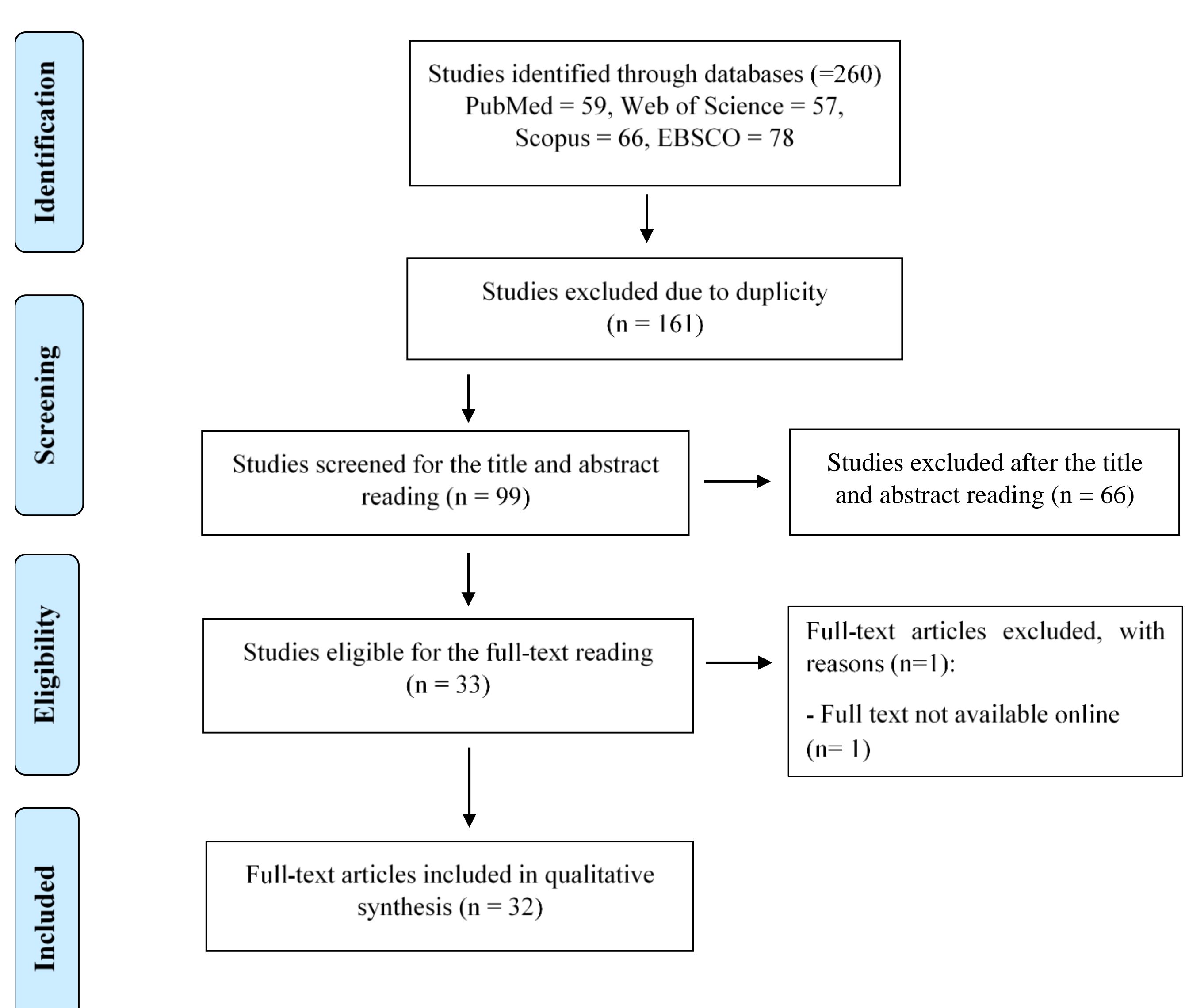
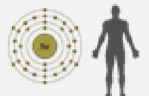


Figure 1: Flow diagram of the literature search process on therapeutic potential of antioxidant substances in oral squamous cell carcinoma, according to the PRISMA Statement

Human study

Selenium



Reducing the blood levels of therapeutic response markers

5'Nucleotidase - 5'ND; Pseudocholine esterase – PschE; Leucine aminopeptidase – LAP; γ-Glutamil transpeptidase - γ-GTP; Lactato desidrogenase – LDH; Aspartato transaminase – SGOT; Alanina transaminase - SGPT Fosfatase ácida – ACP; Fosfatase alcalina – ALP; Creatinina fosfoquinase - CPK

***In vitro* studies**

Plants Extract

Acacia catechu: ↓ Cell proliferation; ↑ Apoptosis

Areca nut: ↑ Apoptosis

Salvia miltiorrhiza: ↓ Cell proliferation; ↑ Apoptosis

Duchesnea indica: ↓ Motility; ↓ Migration; ↓ invasion

Gracilaria tenuistipitata: ↓ Cell proliferation; ↑ Apoptosis; ↑ Cycle arrest; ↑ ROS

Hibiscus sabdariffa: ↓ Motility; ↓ Cell proliferation; ↓ Invasion

Pergulária daemia: ↓ Cell proliferation; ↑ ROS; ↑ Apoptosis; ↓ Membrane potencial; ↑ Lipid peroxidation

Chalcones

Licochalcone B: ↓ Cell proliferation; ↑ ROS; ↑ Apoptosis; ↓ Membrane potencial

Licochalcone D: ↓ Cell proliferation; ↓ Colony formation; ↑ Apoptosis; ↑ ROS

Licochalcone C: ↓ Cell proliferation; ↑ Apoptosis; ↑ ROS

Licochalcone H: ↓ Cell proliferation; ↓ Colony formation; ↑ Cell cycle arrest; ↑ Apoptosis

Tualang honey

↓ Cell proliferation; ↑ Apoptosis

Flavonoids

Butein: ↓ Cell proliferation; ↓ Migration; ↓ Invasion; ↑ Apoptosis

Fisetin: ↓ Cell proliferation; ↓ Membrane potencial; ↑ Apoptosis; ↑ Autophagy

Quercetin: ↓ Cell proliferation; ↓ Migration; ↑ Apoptosis; ↑ Cell cycle arrest

Terpenoids

Betulinic Acid: ↓ Cell proliferation; ↑ Apoptosis; ↑ Cell cycle arrest; ↑ ROS

Oridonin: ↓ Cell proliferation; ↓ Colony formation; ↑ Apoptosis; ↑ Cell cycle arrest; ↑ ROS

Xanthorrhizol: ↓ Cell proliferation; ↑ Apoptosis; ↑ ROS

Polyphenols

Curcumin: ↓ Cell proliferation; ↑ Apoptosis; ↑ Autophagy; ↑ ROS

α- mangostin: ↓ Cell proliferation; ↑ Apoptosis; ↑ Cycle arrest

Thymol: ↓ Cell proliferation; ↑ Apoptosis

Resveratrol: ↓ Cell proliferation; ↑ Apoptosis; ↓ Migration; ↓ Invasion

Verbascoside: ↓ Cell proliferation; ↓ Invasion; ↓ Tumor cell growth; ↓ Metastasis; ↑ Apoptosis; ↓ Migration

Other substances

Shikonin: ↓ Cell proliferation; ↑ Apoptosis

β - lapachone: ↑ ROS; ↑ Apoptosis; ↑ Cell cycle arrest

Pterostilbene: ↓ Cell proliferation; ↓ Migration; ↑ Apoptosis

Derivatives of dihydrocaffeic acid amide: ↑ Apoptosis

HPB242: ↓ Cell proliferation; ↑ Apoptosis

Figure 2: Molecular mechanisms and clinical aspects associated with the therapeutic effect of antioxidants in oral squamous cell carcinoma in human and *in vitro* studies.

Table 1. Result summary on the therapeutic potential of antioxidant substances in oral squamous cell carcinoma by *in vitro* and Human studies.

References	Study nature (*In vitro* or Human study)*	Antioxidant substance or agent	Cell lines or Oral cancer patients	Dosage and frequency of treatment	Investigated assays	Main effects
Min *et al.,* 2008[27]	*In vitro*	Shikonin substance	Oral cancer cell line: Tca-8113	0, 10, 20, 30 and 40 µM for 24, 48 and 72 hours	MTT assay, electron microscopy, DNA fragmentation analysis, cell cycle analysis, annexin V staining, western blot analysis, electrophoretic mobility shift assay and caspase activity.	Shikonin treatment inhibited the growth of Tca8113 cells and induced apoptosis via the activation of caspase-8, -9 and -3.
Ghashm *et al*., 2010[23]	*In vitro*	Tualang honey	Oral cancer cell line: OSCC cells (CRL-1623, ATCC) Other cancer cell line: HOS	1% to 20% concentrations for 3, 6, 12, 24 and 48 hours for all concentrations	Morphological analysis, MTT assay, Non-peroxidase, acidity, and osmolar activity and Cell apoptosis assay by flow cytometry.	Tualang honey showed a promising antiproliferative and apoptotic effects on OSCC and HOS cell lines. Early apoptosis could be attributed, in part, to its ability to inhibit proliferation.
Kim *et al.,* 2012[26]	*In vitro*	Curcumin substance	Oral cancer cell line: YD10B	0–40 µM for different periods (0-24h)	MTT assay, apoptosis assay, MDC staining, detection and quantification of acidic vesicular organelles with acridine orange staining, western blotting, and analysis of reactive oxygen species (ROS) generation.	The curcumin induced autophagic cell death and suppress the proliferation of OSCC cells.

Kim *et al.,* 2012[38]	*In vitro*	Xanthorrhizol substance	Oral cancer cell lines: SCC-15	20, 40, 60, 80 and 100 µM for 12 and 24 hours	MTT assay, Hoechst staining, FITC-annexin V and propidium iodide doubling staining, Western blot analysis, Measurement of intracellular Ca2+ concentration and determination of ROS levels.	Xanthorrhizol decreased cell viability, induced apoptosis and decreased the level of full-length PARP in SCC-15 oral squamous cell carcinoma (OSCC) cells. The treatment elevated intracellular Ca2+ and ROS levels and may induce caspase-independent apoptosis through ROS-mediated p38 MAPK and JNK activation in SCC-15 OSCC cells and prevent chemical-induced oral carcinogenesis.
Yeh *et al.,* 2012[22]	*In vitro*	Methanolic extracts of *Gracilaria tenuistipitata*	Oral cancer cell line: Ca9-22	0.1, 0.25, 0.5 and 1 mg/ml for 24 hours	MTT assay, cell cycle, Flow cytometry-based detection of Annexin V staining, γ-H2AX staining, intracellular reactive oxygen species (ROS), glutathione (GSH) content and mitochondrial membrane potential.	Methanolic extracts of *Gracilaria tenuistipitata* induced antineoplastic effects thought the induction of apoptosis, DNA damage, and oxidative stress pathways.
Chen *et al.,* 2013[30]	*In vitro*	Quercetin substance	Oral cancer cell line: SCC25 Normal cell line: HGFs	25, 50 and 75 µM for 24 hours	MTT assay, clonogenic assay, cell cycle analysis, apoptosis analysis, migration analysis, invasion analysis, and western blotting analysis.	Quercetin treatment inhibited cell growth and invasion/migration of SCC-25 cells. The cellular and molecular mechanisms of the biopharmacological effects of Qu were mainly via cell cycle arrest and apoptosis.
Kovalchuk *et al.,* 2013[40]	*In vitro*	trolox trihydroxybenzoate; trolox 3,5-dimethoxy-4-	Oral cancer cell lines: CAL-27, UMSCC1, and UMSCC47	Treatment with antioxidant substances at 350 µg/mL for 2	RNA extraction and gene expression, protein extraction,	The examined antioxidants, mainly, the derivatives of dihydrocaffeic acid amide exerted an important cell-killing

		hydroxycinnamate and derivatives of dihydrocaffeic acid amide	Other cancer cell line: MCF-7 Normal human tissues: EpiOral and EpiGingival tissues	hours (for cell lines), and at 117 µg/mL for 2 hours (for tissues)	western blotting, and annexin V assay.	effect on cancer cells, including on chemotherapy-resistant breast cancer cells and OSCC cells. Furthermore, the antioxidants induced apoptosis and increased the levels of cleaved caspase-3. The antioxidants do not appear to be toxic to normal cells.
Chae *et al.,* 2014[31]	*In vitro*	*HPB242* Maillard reaction products	Oral cancer cell lines: HN22 and HSC4	5, 10, 15 and 20 µg/ml for 24 and 48 hours	Cell viability, DAPI staining, propidium iodide staining, RT-PCR, western blotting analysis and immunocytochemistry analysis.	OSCC cells were influenced by the chemotherapeutic effects of HPB242 that regulated Sp1 target proteins, resulting in apoptosis by the suppression of Sp1 levels in HN22 and HSC4 cells.
Lin *et al.,* 2014[29]	*In vitro*	Pterostilbene substance	Oral cancer cell line: SCC9 Normal cell line: HOK	Pterostilbene treatment at 5, 10, 20, 40, 80 µM for 24, 48 and 72 hours	MTT assay, clonogenic assays*, in vitro* wound closure, cell invasion, and migration assays, gelatin zymography, casein zymography, ELISA assay, RT-PCR, Western blot analysis, transfection and luciferase assays, and chromatin immunoprecipitation analysis.	Pterostilbene inhibited CREB, NF-kB and SP-1 expression and DNA-binding activities on MMP-2 and u-PA promoters; it reduced MMP-2 and u-PA expression and subsequently inhibited cell invasion and migration.
Mirunalini *et al.*, 2014[24]	*In vitro*	Methanolic extract of *Pergularia daemia*	Oral cancer cell line: KB	10, 20, 40, 80, 160, 320 µg/mL incubated at 24 hours	Clonogenic cell survival assay, analysis of reactive oxygen species (ROS) generation, mitochondrial	The polyphenolic constituents of *P. daemia* were responsible for anticancer activity on oral cancer cells due to an increased level of intracellular ROS, lipid peroxidation marker, DNA damage, apoptotic death, and cell cycle

					membrane potential, acridine orange/ethidium bromide staining, comet assay, and biochemical estimations.	arrest.
Fetoni *et al.,* 2015[37]	*In vitro*	Curcumin substance	Oral cancer cell lines: PE/CA-PJ15	0.5, 1.0, 3.37 and 6.75 mM for 24, 48 and 72 hours	Cell survival, pSTAT3, and Nrf-2 immunofluorescence, TUNEL assay, Western blot.	Curcumin attenuates cell survival and proliferation, by targeting pSTAT3 and Nrf-2 signaling pathways provide chemosensitization to cisplatin.
Kwak *et al.,* 2016[32]	*In vitro*	α-mangostin substance	Oral cancer cell lines: HSC2, HSC3, and HSC4 Normal cell line: PDLF	0–10 μM for 24 hours	Cell viability (MTT), Hoechst staining, flow cytometry analysis, immunofluorescent staining and western blot analysis.	The α-mangostin induced apoptosis in human OSCC cells and led to the dysregulation of mitochondrial function and cell cycle arrest.
Malacrida *et al.* 2016[25]	*In vitro*	*Hibiscus sabdariffa* total extract	Oral cancer cell line: SCC25 Other cancer cell line: RPMI 8226, U87-MG, Capan-1, Igrov-1, PC3, and H460 Normal cell lines: HaCat and HEK-293.	0.5, 1, 3 and 5 mg/mL for 24, 48 and 72 hours	MTT assay, trypan blue vital staining, scratch wound healing assay, Boyden chamber assay, and western blotting.	The results demonstrated the antitumoral properties of HS in *vitro*, as it proved to prevent both cell proliferation and invasiveness of OSCC cells through MAPKs modulation.
Oh *et al.,* 2016[28]	*In vitro*	Licochalcone B substance	Oral cancer cell lines: HN22 and HSC4	0, 10, 20 and 30 μM for 24 and 48 hours	Trypan blue staining, annexin V staining, DAPI staining, cell cycle analysis, western blotting analysis,	Lico B induced apoptosis in OSCC cells through the extrinsic pathway by upregulating the death receptor and also the intrinsic pathway by modulating

					mitochondrial membrane potential (MMP) detection assay, ROS measurement, multi-caspase assay.	Bcl-2 and IAP family members.
Lakshmi *et al*., 2017[21]	*In vitro*	Ethanol extract of *Acacia catechu* seed	Oral cancer cell line: SCC25	25 and 50 µg/mL for 24 hours	MTT assay, Acridine orange/ethidium bromide staining, propidium iodide staining, RT-PCR	Ethanol extract of *Acacia catechu* seed inhibited proliferation and triggers apoptosis of human oral squamous carcinoma cells in a dose-dependent manner.
Wang *et al*., 2017[48]	*In vitro*	Alcohol extract of Danshen (*Salvia miltiorrhiza*)	Oral cancer cell lines: HSC-3 Normal cell line: OC-2	Treatment with Alcohol extract of Danshen (*Salvia miltiorrhiza*) at 2, 5, 10, 25, or 50 *µ*g/mL for 24, 48 and 72 hours	DPPH assay, ABTS assays, cell viability analyses, caspase-3 activity assays, mitochondrial membrane potential, protein extraction, and Western blot analyses.	Danshen alcohol extracts significantly inhibited the proliferation of the human oral squamous carcinoma (OSCC) cell lines HSC-3 and OC-2. Furthermore, it activated the caspase-3 apoptosis executor by impeding members of the inhibitor of apoptosis (IAP) family, but not by regulating the Bcl2-triggered mitochondrial pathway in OSCC cells.
Bano *et al*., 2018[34]	*In vitro*	Curcumin substance	Oral cancer cell lines: HPV16+ve OSCC cell line, UD-SCC-2;HPV–ve OSCC lines, UPCI:SCC131 and UPCI:SCC84	Concentrations of curcumin (0-50µM) for 24 h	MTT assay, Western Blotting, Sphere formation assay, Analysis of SP and Non-SP Cancer Cells by FACS Quantitative Real-Time PCR	Curcumin treatment promoted significant differential inhibition in CSC proliferation, the orosphere formation and miRNA-21 expression in a dose-dependent manner; the effect was highly pronounced in HPV positive CSCs.
Chapa *et al.,* 2018[36]	*In vitro*	Thymol substance	Oral cancer cell lines: Cal27, SCC4, and SCC9 Other cancer cell lines: HeLa, H460,	200µM, 400µM, 600µM, 800µM, 1mM and 4.3 mM for 48 hours	Cell viability assay, clonogenic assay, calcium imaging,, mitochondrial membrane potential,	Thymol had significant, long-lasting antiproliferative effects in *vitro*. It induced a concentration-dependent reduction in cell viability in all OSCC cell lines and in all other cancer cell

			MDA -231and PC3 Normal cell line: CHO		Western blot.	lines tested.
Dias *et al*., 2018[33]	*In vitro*	*β-Lapachone and 3-iodine derivates substances*	Oral cancer cell lines: HSC3, SCC4, SCC9, SCC15, and SCC25 Normal cell line: HaCat, MRC5, and PBMCs	Treatment with *β-Lapachone and 3-iodine derivates at* 5, 10, or 20 μM for 12, 24, 48 and 72 hours	Cytotoxic activity assay, three-dimensional multicellular spheroid culture, trypan blue exclusion assay, internucleosomal DNA fragmentation, and cell cycle distribution, morphological analysis, annexin V/PI staining assay, flow cytometer, caspase-8 and -9 activation assays, ROS analysis, measurement of cellular superoxide anion levels, measurement of nitric oxide production, DNA intercalation assay and qPCR.	β-Lapachone promoted cell cycle arrest at G2/M phase, internucleosomal DNA fragmentation and caspase- and ROS-mediated apoptosis in OSCC cells.
Elango *et al*. 2018[20]	Human study	Selenium	Oral squamous cell carcinoma patients (Stage III)	An amount of 400 μg selenium (capsules)/day for 6 months. Supplemented orally selenium (capsule) after cessation of radiotherapy. The treatment schedule and selenium supplementation of the patients were carefully recorded and monitored.	Fluorometric method. Atomic absorption spectrometry. Estimation of Copper, Iron, Zinc, Sodium, Potassium, Calcium, Chloride. Biomarkers by ELISA, Immunobinding assay, Electro Immuno diffusion.	The selenium was able to act as an adjuvant to radiotherapy in oral carcinoma patients, improving the blood levels of therapeutic response markers

Kim *et al*., 2018[39]	*In vitro*	Resveratrol	Oral cancer cell lines: CAL-27, SCC15, and SCC25	Different concentrations of resveratrol (10-500 mM) for at least 24 h and up to 72 h	Cell Cytotoxicity Assay/MTT, Fluorescence Microscopy, Annexin V- FITC/PI Staining (Apoptosis assay), JC-1 Staining for Mitochondrial Membrane Potential, Western Blot Assay, Wound Healing Assay, Invasion Assay.	Resveratrol treatment caused in a time- and dose-dependent decrease in cell viability and increased the apoptosis in CAL-27, SCC15, and SCC25 cells. This antioxidant substance also decreased cell migration of CAL27 Cells, invasion by inhibiting of Epithelial-Mesenchymal Transition-inducing transcription factors, such as Snail and Slug Slug, Snail Smad2/3, and N-cadherin
OH *et al.,* 2018[43]	*In vitro*	Oridonin substance	Oral cancer cell lines: HN22 and HSC4	Oridonin treatment 1, 2, 4, 5, 7.5 and 10 µM for 24 and 48 hours	MTT assay, anchorage-indepent cell transformation assay; DAPI staining, cell cycle analysis, annexin V staining, ROS levels, measurement of MMP, Multi-caspase assay, and western blot analysis.	Oridonin suppressed oral cancer cell proliferation and soft agar colony formation, while it induced reactive oxygen species (ROS)-dependent apoptosis in a dose or time-dependent manner.
Oh et al., 2018[42]	*In vitro*	Licochalcone C substance	Oral cancer cell lines: HN22 and HSC4	0, 10, 20 and 30 µM for 24 and 48 hours	MTT assay, DAPI staining, Cell cycle assay, Annexin V/7-aminoactinomycin D staining, nchorage-independent cell growth assay, In vitro LCC-conjugated Sepharose 4B pull-down assay, Western blot analysis, In vitro Kinase assay,	LCC inhibited oral squamous cell carcinoma (OSCC) cell viability, mitochondrial function, and anchorage-independent growth in a dose-dependent manner. LCC induced the death receptor DR4 and DR5 expression levels with the generation of reactive oxygen species and the upregulation of CHOP protein expression.

					ATP and JAK2 competition assay, Molecular docking simulation, Molecular dynamics simulation, Reactive oxygen species assay, Mitochondrial membrane potential assay, Evaluation of cytochrome c release from mitochondrial and cytosolic fraction, Multi-caspase assay.	
Srivastava *et al*., 2018[6]	*In vitro*	Curcumin substance	Oral cancer cell lines and human hypo-pharynx squamous cell carcinoma, respectively SCC090, SCC152	Different concentrations (10, 20, 40, 80, 160, 320µg/ml) of 5-FU nanoemulsion, Cur nanoemulsion, and combined-loaded,5-FU-Cur nanoemulsion formulations in time interval 24, 48, 72, 96 h	MTT assay, cell uptake studies and Western Blot to protein expression of apoptosis (P53, P21, Bax, and Bcl2)	The 5-FU and Cur in nanoformulation proved their potency to deliver improved anticancer activity in OSCC cells. OSCC cells treated with 5-FUNE/Cur-NE/5-FU-Cur-NE formulations showed up-regulation in expression protein, p53, p21, and Bax proteins, and down-regulation of Bcl2 protein in both cells with time-dose-dependent manner.
Zhang *et al.,* 2018[50]	*In vitro*	Verbascoside substance	Oral cancer cell lines: HN4 and HN6	Verbacoside treatment 0 to 200 µM for 24 hours	Cell viability and proliferation assays, AO/EB staining assay, Flow cytometry analysis, transwell migration, and matrigel invasion assays, TUNEL assay, wound-healing assay, quantitative RT-PCR and Western blotting.	VB plays an essential role in decreasing the viability, migration, and invasion of HN4 and HN6 tumor cells while promoting apoptosis. VB effectively inhibited OSCC tumor cell growth and metastasis via suppression of IkB kinase complex (IKK)/NF-kBrelated signaling activation.

Bordoloi *et al.,* 2019[35]	*In vitro*	*Butein* substance	Oral cancer cell lines: SAS and KB	Butein treatment at 1, 10, 25, 50, and 100 µM) for 24, 48 and 72 hours	MTT assay, colony formation, PI/FACS, live and dead, scratch wound healing, matrigel invasion assays and Western blot analysis.	Butein exhibited potent anti-proliferative, cytotoxic, anti-migratory, and anti-invasive effects in OSCC cells. It suppressed the expression of NF-κB and NF-κB-regulated gene products such as COX-2, survivin, and MMP-9 which are involved in the regulation of different processes like proliferation, survival, invasion, and metastasis of OSCC cells.
Nho *et al*., 2019[41]	*In vitro*	Licochalcone H substance	Oral cancer cell line: HSC2, HSC3	Different concentrations of Licochalcone H (0-40 µM) for 24 and 48 h.	MTS assay, DAPI staining, Annexin V/propidium iodide (PI) staining, Cell cycle analysis, Western blot analysis, Pull-down assay, Anchorage-independent cell transformation assay, Matr3 small interfering RNA (siRNA), T-PCR analysis	Licochalcone H reduced cell viability and colony-forming and led to the cell cycle arrest and apoptosis in HSC2 and HSC3 cells through the suppression of Matr3.
Park *et al*., 2019[44]	*In vitro*	Fisetin substance	Oral cancer cell lines: CAL-27 and Ca9-22	Fisetin treatment 10 to 200 µM for 24 – 72 hours.	MTT assay, Hoechst staining, detection of mitochondrial membrane potential, detection of acidic vesicular organelles and Western blot analysis.	Fisetin induced apoptotic cell death in human tongue squamous cell line Ca9-22 could be enhanced by inhibition of autophagy. Thus, the autophagy process in fisetin treated OSCC might be presumed to play a role of pro-survival.

Sari; Subita; Auerkari, 2019[45]	*In vitro*	*Areca* nut extract	Oral cancer cell lines: HSC-2 and HSC-3	Treatment with *Areca* nut extract 629.50 µg/mL for HSC-2 cells and 164.06 µg/mL for HSC-3 cells incubated for 24 and 48 hours.	Analysis of apoptosis activity and analysis of caspase-3 activity.	Areca nut induced a significant increase in late apoptosis of HSC-2 cells and mostly occurred over 48 hours. The study also found that in HSC-3, there were significant increases in the percentage of cells in early apoptosis after 24 hours and late apoptosis at 48 hours. Caspase-3 activity increased after 24 and 48 hours of areca nut exposure in both cells.
Seo *et al*., 2019[46]	*In vitro*	Licochalcone D substance	Oral cancer cell line: HN22, HSC4	Various concentrations of Licochalcone D (0, 10, 20, and 30 µM) for 24 and 48 h	MTT assay, DAPI staining, Cell cycle analysis, Annexin V staining, In vitro and ex vivo pull-down assays, ATP and JAK2 competition assay, Western blot, Multi-caspase assay, In vitro kinase assay, Molecular docking stimulation, ROS measurements, Mitochondrial membrane potential detection assay, nude mouse xenograft, PET/CT imaging, and quantitative imaging analysis, Immunohistochemical analysis	Licochalcone D inhibited the colony formation, the proliferation, and the kinase activity of JAK2 of HN22 and HSC4 cells. Moreover, it also promoted mitochondrial apoptotic events such as altered mitochondrial membrane potential and reactive oxygen species production. LCD upregulated of death receptor CHOP, DR4, DR5, and increase of expression levels of proapoptotic proteins Bad and Bax in OSCC cells
Shen *et al.,* 2019[47]	*In vitro*	Betulinic Acid substance	Oral cancer cell lines:	Betulinic Acid treatment at 12.5, 25,	Determination of cell proliferation,	BA promoted ROS production, increased p53 expression and the transcription of

			KB	50 and 100 µM for 24–72 hours.	apoptosis, caspase 3 and caspase 9 activities, measurement of oxygen consumption rate, cell cycle measurement, determination of ROS Generation, quantitative Real-Time PCR, Western blot analysis, ChIP assays.	the proapoptotic regulator, and inhibited STAT3 signaling, resulting in the promotion of mitochondrial apoptosis, induction of cell cycle arrest, and final inhibition of cell proliferation. Taken together, the data demonstrated that ROS-p53 signaling was crucial for the BA-exhibited antitumor effect in OSCC.
Yang *et al.* , 2019[49]	*In vitro*	*Duchesnea indica* extract	Oral cancer cell lines: SCC-9, SCC-14, and TW2.6	Treatment with *Duchesnea indica* extract at 10, 20 and 40 µg/mL for 24 hours	MTT assay for cell viability, wound-healing assay, cell migration and invasion assays, gelatin zymography and Western blot analysis.	DIE significantly attenuated OSCC cells' motility, migration, and invasion by reducing the MMP-2 protein expression and MMP-2 activity in a dose-dependent manner. In addition, DIE reduced the phosphorylation of both ERK1/2 and its upstream kinase

*Assays and results of studies with *in vivo* approach was not considered in this study, as described in Material and Methods.

Table 2. Quality assessment of *in vitro* selected articles according to the ToxRTool.

References	Group I: test substance identification (points: 0-4)	Group II: test system characterization (points: 0-3)	Group III: Study design description (points: 0-6)	Group IV: Study results documentation (points: 0-3)	Group V: plausibility of study design and data (points: 0-2)	Total	Reliability categorization
Lakshmi *et al*. 2017	3	3	5	3	2	16	Reliable without restrictions
Yeh *et al*. 2012	*3*	3	5	3	2	16	Reliable without restrictions
Ghashm *et al*., 2010	*3*	3	5	3	2	16	Reliable without restrictions
Sankaran *et al*. 2014	*3*	3	6	3	2	17	Reliable without restrictions
Malacrida *et al*. 2016	*3*	3	5	3	2	16	Reliable without restrictions
Kim *et al*. 2012	*3*	3	6	3	2	17	Reliable without restrictions
Fetoni *et al*. 2015	4	3	6	3	2	18	Reliable without restrictions
Srivastava *et al*. 2018	4	3	6	3	2	18	Reliable without restrictions
Bano *et al*. 2018	3	3	5	3	2	16	Reliable without restrictions
Min *et al*. 2008	3	3	5	3	2	16	Reliable without restrictions
Oh *et al*. 2016	*4*	3	5	3	2	17	Reliable without restrictions
Oh *et al*. 2018	*3*	3	5	3	2	16	Reliable without

restrictions

Seo *et al*. 2019	*3*	3	5	3	2	16	Reliable without restrictions
Nho *et al*. 2019	*3*	3	5	3	2	16	Reliable without restrictions
Lin *et al*. 2014	*4*	3	5	3	2	17	Reliable without restrictions
Chen *et al*. 2013	3	3	5	3	2	16	Reliable without restrictions
Chae *et al*. 2014	*2*	3	5	3	2	15	Reliable without restrictions
Kwak *et al*. 2016	3	3	5	3	2	16	Reliable without restrictions
Dias *et al*. 2018	3	3	6	3	2	17	Reliable without restrictions
Kim *et al*.2018	4	3	5	3	2	17	Reliable without restrictions
Chapa *et al*. 2018	3	3	4	3	2	15	Reliable without restrictions
Kim *et al*. 2012	3	3	4	3	2	15	Reliable without restrictions
Park *et al*. 2019	3	3	5	3	2	16	Reliable without restrictions
Yang *et al*. 2019	3	3	5	3	2	16	Reliable without restrictions
Zhang *et al*. 2018	3	3	6	3	2	17	Reliable without restrictions
OH *et al*. 2018	3	3	5	3	2	16	Reliable without restrictions

Sari; Subita; Auerkari 2019	3	3	4	3	2	15	Reliable without restrictions
Wang *et al.* 2017	3	3	5	3	2	16	Reliable without restrictions
Bordoloi *et al.* 2019	3	3	5	3	2	16	Reliable without restrictions
Shen *et al.* 2019	3	3	5	3	2	16	Reliable without restrictions
Kovalchuk *et al.* 2013	3	3	5	3	2	16	Reliable without restrictions

3.2 Produto 2

Gallic acid enhances the effect of therapeutic ionizing radiation in oral squamous carcinoma cells

Karina Marini Aguiar[1]
Angeliny Tamiarana Lima Tabosa[1]
Rogério Gonçalves da Rocha[1]
Felipe Alberto Dantas Guimarães[1]
Renata Sousa Leite[1]
Amanda Maria Ribeiro Freitas[1]
Emisael Stênio Batista Gomes[1]
Sabrina Ferreira de Jesus[1]
André Luiz Sena Guimarães[1,2#]
Lucyana Conceição Farias[1,2#]

[1]Postgraduate Program in Health Sciences, Universidade Estadual de Montes Claros, Minas Gerais, Brazil
[2]Department of Dentistry, Universidade Estadual de Montes Claros, Minas Gerais, Brazil
# These authors have contributed equally to this work.

*Corresponding author:
Lucyana Conceição Farias
Universidade Estadual de Montes Claros
Hospital Universitário Clemente de Faria
Laboratório de Pesquisa em Saúde
Avenida Cula Mangabeira, 562
Montes Claros, Minas Gerais, Brasil
Zip code: 39401-001
Phone: +55 38 32248327
E-mail: lucyanacfarias@gmail.com

Abstract

Gallic acid (GA) is a potent antioxidant, derived from plants of the Brazilian Cerrado; it can inhibit the development of neoplasms through various mechanisms such as apoptosis induction, inhibition of proliferation, invasion, and metastasis of tumor cells inhibition, and angiogenesis suppression. Several pharmacological activities of this compound have been reported, including anti-inflammatory, anti-atherosclerotic, anticarcinogenic, antioxidant activities, as well as radioprotective activity. Thus, we investigated, through phenotypic and molecular analyses, the adjuvant action of GA on the therapeutic effect of ionizing radiation in oral squamous carcinoma cells (OSCC). The human OSCC-derived cell lines SCC-9 and normal control keratinocytes HaCat were treated with 10 µg/ml GA and exposed to 2Gy, 4Gy, and 6Gy of ionizing radiation. We performed the *in vitro* assays of cell proliferation, cell death by Acridine/Ethidium Bromide Orange and detection of reactive oxygen species (ROS) by carboxy-H2DCFDA. The GA was able to significantly reduce the number of viable cells at different radiation doses. It was also able to potentiate cell death induced by ionizing radiation in SCC-9 cells, but not in HaCaT cells, mainly at low doses of radiation. In the ROS analysis, a mechanism that explains cell death, there was an increase of ROS intracellular accumulation in SCC-9 and a reduction in HaCaT cells, revealing a potential effect of GA as an adjuvant to the radiotherapy. Our findings revealed a potential effect of GA as an adjuvant to therapeutic ionizing radiation on behavior control of OSCC cells, beyond to act as a protector of normal keratinocytes by reduction of radiation doses. New *in vivo* studies are necessary to better understand the GA role against oral cancer and its possible clinical applications.

Introduction

Oral cancer is one of the most common malignancies of the head and neck and a serious public health problem in several countries. Approximately 90% of oral cancer originates from squamous cells of the mucosa. [1] Worldwide, oral cavity cancer ranks sixth with less than 5 years of survival. [2] Each year, nearly 300,000 new cases are diagnosed for this type of oral cancer. [3, 4]

About 30% of them die as a result of distant metastases, as these cells spread rapidly or recur loco-regionally. [5, 6] It happens due to carcinogenic transformation in the mucosa of the oral lining commonly induced by tobacco or alcohol, which are well known as major risk factors. [6-9] Other factors are also responsible for causing the disease, such as *Human papillomavirus* infection, [10], vitamin deficiency, malnutrition as well as epigenetic and genetic factors. [11] Its highest prevalence is among young male adults under 40 years old, however, men over 60 years show to be the highest risk group. [12]

The main treatment options available to cease or eliminate the spread of oral cancer are surgery, radiotherapy, and chemotherapy. [13] The continuous improvement in diagnostic and treatment technologies has promoted an increase in the survival rate of Oral Squamous Cell Carcinoma (OSCC) patients, but there are still major challenges such as the non-specificity, non-selectivity and severe toxicity of drugs over time, affecting the post-treatment life of the patients. Despite the therapeutic effect of radio- and chemotherapy, deleterious side effects to normal tissues, adjacent to neoplastic tissue, drug resistance, and other toxic effects can affect the patient undergoing treatment due to cancer in the head and neck region. [14, 15]

The chemotherapy drugs act mainly inducing apoptosis in cancer cells. Some of the anticancer drugs available today are Epirubicin, cisplatin, 5-fluorouracil, doxorubicin, and cyclophosphamide. [16]

As these chemotherapeutic agents generally show dosage-dependent toxicity, it is necessary to seek alternative therapies concomitant with conventional treatment aiming to decrease the toxicity on normal cells, but improving its therapeutic effects against cancer. Over the years, phytochemicals have received a lot of attention due to their antineoplastic potential against some cancer types. The plant herbal and phenolic compounds have been shown to mediate apoptosis through mitochondrial events in cancer cells. [17-19]

The plant-derived compounds commonly have antioxidant activity, that can prevent damage induced by reactive oxygen species (ROS) and reactive nitrogen species. Studies have shown radioprotective effects of various natural phytochemicals in short-term in vitro tests such as lipid peroxidation, DNA damage, free radical scavenging, and antioxidant level assays, cell survival and micronucleus induction. (20) The antioxidants can exert a protective role preventing the initiation, promotion, and also the progression of oral cancer. In this sense, these substances can be important to new research to the development of anticancer drugs. (19)

According to the treatment concentrations, antioxidants also have been shown to plays antineoplastic functions in several cell lines of several cancer types. (21-23) They can become prooxidants promoting the generating of superoxide anion radicals and induction of apoptosis of cancer cells. (23) Gallic acid (GA) is a polyhydroxy phenol compound present in many dietary substances and naturally found in nuts, Sumac, hazel (Hamamelis virginiana), clove (Syzygium aromaticum), tealeaves, oak bark, sundew, and other plants. Edible foods like blackberry, hot chocolate, common nuts, Indian gooseberry, vinegar, wine, and white tea contain GA. (16) This is not toxic to mammals at pharmacological doses, being suggested the dose LD50 of GA of 5 g/kg body weight in rats. (20)

Several pharmacological activities of this compound have been reported, including anti-inflammatory, anti-atherosclerotic, anticarcinogenic, antioxidant activities, (24) as well as radioprotective activity. (20) Studies have also shown that GA induced cancer cell death by apoptosis. In particular, it has been described as an inducer of differentiation and programmed cell death in various tumor cell lines, such as HL-60RG, HeLa, dRLh-84, PLC/PRF/5, and KB cells. In addition, it interfered with different stages of tumor development and was also responsible for suppressing tumor angiogenesis and exerting an inhibitory effect on *in vitro* invasion. (24-28)

Although the anti-cancer properties of gallic acid have been demonstrated on OSCC cells (29, 30), its effects concomitant to other treatment modalities not yet explored in oral cancer. Thus, in this study, we hypothesized that GA could favor the therapeutic effect of ionizing radiation in OSCC cells. We investigated whether GA can interfere with the proliferation, cell death, and oxidative stress in OSCC cells exposed to ionizing radiation. Besides, its effects on non-neoplastic keratinocytes were also searched.

Methods

Cell lines, culture conditions

The human OSCC cell line SCC-9 (BCRJ Cat# 0196, RRID: CVCL_1685) was used in this study. For comparative analysis, we used HaCaT human normal keratinocytes. Both cell lines were maintained in Dulbecco's minimum essential media and Ham's F-12 (DEMEM/F12) (Gibco, Invitrogen, Carlsbad, CA), and supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin and 0.4µg/ml hydrocortisone (Gibco, Invitrogen, Carlsbad, CA). Cells were incubated at 37ºC in a humidified atmosphere containing 5% CO_2. During subculture, cells were grown in 75cm^2 culture flasks and detached by trypsinization. The experiments were done when they reached 70-80% confluency.

Cell treatment

Cells were treated with 10 µg/ml gallic acid (Sigma, St Louis, Missouri, USA) for 24hs as previous standardization. [(30)] After that, the cells were irradiated with different doses of radiations (0Gy, 2Gy, 4Gy, and 6Gy), using the Elekta Synergy linear accelerator (Atlanta, GA) to a field-source distance of 97.5 cm. Next incubated for 72hs, the phenotype analyses were performed. [(31)] In this study were adopted four groups for experimental: Group I: EtOH_vehicle (SCC-9/HaCaT GA-untreated + 0Gy), Group II: SCC-9/HaCaT GA-treated (10µg/ml) + 2Gy, Group III: SCC-9/HaCaT GA-treated (10µg/ml) + 4Gy, Group IV: OSCC-9 / HaCaT GA-treated (10µg/ml) + 6Gy.

Cell proliferation assay

Cell proliferation was determined by the trypan blue exclusion assay. The cells were plated at a density of 1x10^4 cell/well in a 12-well culture plate for groups treated with gallic acid as described earlier. Briefly, the cells were trypsinized and resuspended in complete medium, 90µL each sample was removed from the cell suspension, and 10µL trypan blue was added. Cell counting was measured using the Neubauer chamber [(32)], in proliferation data were obtained after treatment of the cells with GA, followed by irradiation.

Cell dead/viability assay

The SCC9 and HaCaT cells were plated at a density of 80,000 cells/well. After treatments, Acridine Orange/Ethidium Bromide (AO/EB) staining was performed in the equal volumes of cells and mixed with a solution contained Acridine Orange 100µg/ml (AO, Sigma, St. Louis, MO, USA) and Ethidium Bromide 100µg/ml (EB, Sigma, St. Louis, MO, USA) (1:1). The cells were examined by fluorescence light microscopy FSX100 (Olympus, Center Valley, PA, USA). The viable cells had green fluorescent color showed in intense of AO (Ex460-495, Em510-550, DM505 filter), and death cells had bright orange color was indicated with the intense EB staining (Ex360-370, Em420-460, DM400 filter). The percentage of dead cells was quantified by % of apoptotic cells (total number of dead cells/total number of cells counted] x 100).

Reactive oxygen species assay

Intracellular reactive oxygen species (ROS) production was determined 2',7'-dichlorofluorescin diacetate (H_2DCFDA; Invitrogen, Carlsbad, CA). After all experimental treatments as described above, the SCC-9 and HaCaT cells were incubated with 10µM of H2DCFDA for 30 min at 37ºC, washed twice with PBS buffer, and immediately photographed under a fluorescent microscope (Olympus, Center Valley, PA, USA). For quantification was using the ImageJ software (http://rsbweb.nih.gov/ij/).

Statistical analysis

The analyses were carried out using SPSS 20.0 software and Graph Prism. Shapiro-Wilk test was performed to evaluate data distribution. The data were analyzed according to the distribution normal using Two-way variance test (ANOVA), and the $p<0.05$ value was considered statistically significant in all cases. The study was approved by the local ethics committee of State University of Montes Claros/Brazil-Unimontes (nº 2.008.010/2017).

Results

Gallic Acid potentiated the therapeutic ionizing radiation by reduction of proliferative activity of OSCC and HaCaT cells

In order to examine the effects of GA on the inhibition of cell viability, proliferation assay was performed in SCC-9 and HaCaT cells treated with GA 10 µg/ml gallic acid and irradiated with different doses (0Gy, 2Gy, 4Gy, and 6Gy). The results are shown in Figure 1.

Initially, we observed that GA treatment without radiation dose exposure significantly reduced the neoplastic cell number, accordingly with a previous study. [30] Furthermore, GA enhanced the effect of radiation ionizing by reducing the number of viable cells when compared to the irradiated control at different doses. Statistical analysis revealed a significant interaction effect between irradiation and GA to reduce the proliferative behavior of cells.

The action of GA in HaCaT cells was similar to OSCC-9 cells. The GA controlled the proliferative activity of HaCaT cells synergistically to the effect of therapeutic ionizing radiation, reducing the number of viable cells. However, comparing the non-irradiated and GA-treated cells with the combined treatment groups, no potential synergism was observed, as the number of viable cells in the GA and irradiated groups showed no significant difference compared to the non-irradiated and treated with GA.

Gallic Acid promoted radio-induced cell death of OSCC cells but protected normal keratinocytes at low radiation dose

In order to investigate the cytotoxic effect of GA, after experimental treatments, the cell groups were stained with an AO/EB immunofluorescence solution to detect the number of cells death. As shown in figure 2, GA treatment resulted in a significant increase in the number of dead SCC-9 cells. Interestingly, the GA significantly reduced the percentage of dead cells HaCaT at a lower dose of radiation exposure. This GA effect on HaCaT cells was also observed in the cell group not irradiated. In this sense, it is important to highlight that GA exerted an interest adjuvant effect on ionizing radiation, especially at a lower dose of 2Gy, promoting the cell death of neoplastic cells and protecting normal HaCaT cells, but in larger doses, this was not possible.

Gallic Acid increased reactive oxygen species in OSCC cells but reduced it in normal keratinocytes

To test if GA-induced cell death was associated with stress oxidative, we measured intracellular ROS generation in OSCC-9 and HaCaT cells after treatments. The GA promoted a significant increase of ROS accumulation in SCC-9 cells at all radiation doses and also in the GA group not irradiated, as demonstrated in Fig. 3A. That is, GA at 10µg/mL exerted a synergistic effect on radiation, increasing the formation of ROS. However, it is believed a saturation of the synergistic effect in 6Gy because it did not give a significant difference in SCC-9 cells.

However, when we analyzed the ROS accumulation in HaCaT, in accordance with the decrease in cell death ratio, the GA treatment at different doses of irradiation impaired the formation of reactive oxygen species, reducing the cell number stained (Fig.3B). Probably, this is because in fact, the antioxidant effect on normal cells, as HaCaT, is expected to play protection against oxidative stress.

Discussion

The therapeutic ionizing radiation, despite its beneficial effect promoting control of neoplastic proliferation and metastasis, tumor size reduction, is known also to exert deleterious effects on the organism, especially in peritumoral tissues. [33] Thus, it is important to research new therapeutic approaches aiming the tumor reducing and lower damages to the normal cells. The present study proposes GA as a potential adjuvant substance to the ionizing radiation therapy on oral squamous carcinoma cells. Our findings suggested that GA treatment combined with lower doses of radiation can improve the deleterious effects of radiation since it was able to impair the neoplastic behavior and also to protect normal keratinocytes of cell death and oxidative stress.

Gallic acid is an antioxidant substance that has been reported to inhibit several cancer cell lines through different mechanisms, especially due to its antioxidant activity, inhibiting the ROS-induced DNA damage and therefore reducing the risk for cancer. [16] Antioxidants, according to the treatment concentrations, have been shown to plays antineoplastic functions in several cancer types, preventing oxidative stress-induced DNA damage or promoting cancer cell death. [21-23]

In the current study, GA treatment significantly inhibited the proliferation and promoted cell death of OSCC cells in different exposure doses of radiotherapy, potentializing its effects. Several other studies revealed a similar effect of GA on cell number reduction in different cancer cell lines. [24, 29, 34-37]; however synergic action of GA to the ionizing radiation was not yet investigated, especially on oral cancer cells.

An important finding revealed in this study is the possibility of exposing cells to lower radiation doses concomitantly to the GA treatment maintaining their antineoplastic effect. The GA potentiated oral cancer cells death induced by ionizing radiation, while in HaCaT cells, at lower 2Gy doses, the effect of GA was to protect cells from death, preserving the viability of normal keratinocytes.

To further confirm the mechanism of cell death related to GA treatment and radiation, the ROS assay identified that GA significantly intensified the radiation-induced oxidative stress in cancer cells, evidenced by the increase of ROS accumulation. The oxidative stress and ROS generation have long been associated with carcinogenesis pathways, through lipid peroxidation, degradation of polyunsaturated fatty acids, causing cross-linking in lipids, proteins, and nucleic acids. [21, 38]. However, oxidative

stress also associated with the inducting of neoplastic cell death promoted by several chemotherapy drugs. [39]

In this context, cancer cells often show high levels of ROS, but still preserve their viability; so it is necessary to maintain a balance between prooxidants and antioxidants. In order to protect cells of ROS-induced DNA damage, several endogenous antioxidant systems, such as superoxide dismutase, glutathione peroxidase, catalase, and glutathione contrary acts to the deleterious effects of ROS, inhibiting increased oxidative stress. [40, 41]

Despite the antioxidant role of GA, in our study, it was able to promote neoplastic cell death, according to the concomitant dose of radiation. GA is believed to play a prooxidative effect. Studies have reported that GA at low concentrations exerts a potent antioxidant effect that can prevent cell DNA damage; however, at high concentrations, it may induce cellular DNA damage. [42] This regulatory mechanism can be explained by the reduction of intracellular copper induced by high concentrations of antioxidants, which can promote cell death. Studies have shown that when compared to normal cells and neoplastic cells, the cancer cells have a high level of copper and may be more sensitive electron transfer with antioxidants to generate reactive oxygen species. Therefore, antioxidant-induced DNA damage in the presence of Cu (II) redox-active metal may be an important pathway through which neoplastic cells can be killed while normal cells survive. [43, 44]

In conclusion, GA exerts a promising and potent adjuvant role to ionizing radiation therapy on oral squamous cells to control neoplastic behavior, also enabling the reduction of radiation doses when thinking about its deleterious effects on normal keratinocytes.

References

1. Warnakulasuriya S. Living with oral cancer: epidemiology with particular reference to prevalence and life-style changes that influence survival. Oral oncology. 2010;46(6):407-10.
2. Macfarlane GJ, Boyle P, Evstifeeva TV, Robertson C, Scully C. Rising trends of oral cancer mortality among males worldwide: the return of an old public health problem. Cancer causes & control : CCC. 1994;5(3):259-65.
3. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. International journal of cancer. 2010;127(12):2893-917.
4. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA: a cancer journal for clinicians. 2015;65(2):87-108.
5. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA: a cancer journal for clinicians. 2015;65(1):5-29.
6. Ahmadi N, Gao K, Chia N, Kwon MS, Palme CE, Gupta R, et al. Association of PD-L1 expression in oral squamous cell carcinoma with smoking, sex, and p53 expression. Oral surgery, oral medicine, oral pathology and oral radiology. 2019;128(6):631-8.
7. Chaturvedi AK, Anderson WF, Lortet-Tieulent J, Curado MP, Ferlay J, Franceschi S, et al. Worldwide trends in incidence rates for oral cavity and oropharyngeal cancers. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2013;31(36):4550-9.
8. Chi AC, Day TA, Neville BW. Oral cavity and oropharyngeal squamous cell carcinoma--an update. CA: a cancer journal for clinicians. 2015;65(5):401-21.
9. Montero PH, Patel SG. Cancer of the oral cavity. Surgical oncology clinics of North America. 2015;24(3):491-508.
10. D'Souza G, Kreimer AR, Viscidi R, Pawlita M, Fakhry C, Koch WM, et al. Case-control study of human papillomavirus and oropharyngeal cancer. The New England journal of medicine. 2007;356(19):1944-56.
11. de Camargo Cancela M, Voti L, Guerra-Yi M, Chapuis F, Mazuir M, Curado MP. Oral cavity cancer in developed and in developing countries: population-based incidence. Head & neck. 2010;32(3):357-67.
12. Laronde DM, Hislop TG, Elwood JM, Rosin MP. Oral cancer: just the facts. Journal. 2008;74(3):269-72.
13. Huang YC, Lee PC, Wang JJ, Hsu YC. Anticancer Effect and Mechanism of Hydroxygenkwanin in Oral Squamous Cell Carcinoma. Frontiers in oncology. 2019;9:911.
14. Liu L, Chen J, Cai X, Yao Z, Huang J. Progress in targeted therapeutic drugs for oral squamous cell carcinoma. Surgical oncology. 2019;31:90-7.
15. Wang R, Xiao S, Niu Z. Anti-Cancer Activity of Aster Tataricus on Scc-9 Human Oral Squamous Carcinoma. African journal of traditional, complementary, and alternative medicines : AJTCAM. 2017;14(2):142-7.
16. Subramanian AP, John AA, Vellayappan MV, Balaji A, Jaganathan SK, Supriyanto E, et al. Gallic acid: prospects and molecular mechanisms of its anticancer activity. RSC Advances. 2015;5(45):35608-21.
17. Mohan S, Thiagarajan K, Chandrasekaran R. In vitro evaluation of antiproliferative effect of ethyl gallate against human oral squamous carcinoma cell line KB. Natural product research. 2015;29(4):366-9.
18. Huu Tung N, Du GJ, Wang CZ, Yuan CS, Shoyama Y. Naphthoquinone components from Alkanna tinctoria (L.) Tausch show significant antiproliferative effects on human colorectal cancer cells. Phytotherapy research : PTR. 2013;27(1):66-70.
19. Mohan S, Thiagarajan K, Chandrasekaran R. Evaluation of ethyl gallate for its antioxidant and anticancer properties against chemical-induced tongue carcinogenesis in mice. The Biochemical journal. 2017;474(17):3011-25.

20. Nair GG, Nair CK. Radioprotective effects of gallic acid in mice. BioMed research international. 2013;2013:953079.
21. Anbuselvam C, Vijayavel K, Balasubramanian MP. Protective effect of Operculina turpethum against 7,12-dimethyl benz(a)anthracene induced oxidative stress with reference to breast cancer in experimental rats. Chemico-biological interactions. 2007;168(3):229-36.
22. Prasad KN, Kumar A, Kochupillai V, Cole WC. High doses of multiple antioxidant vitamins: essential ingredients in improving the efficacy of standard cancer therapy. Journal of the American College of Nutrition. 1999;18(1):13-25.
23. Zubair H, Khan HY, Sohail A, Azim S, Ullah MF, Ahmad A, et al. Redox cycling of endogenous copper by thymoquinone leads to ROS-mediated DNA breakage and consequent cell death: putative anticancer mechanism of antioxidants. Cell death & disease. 2013;4:e660.
24. Liu Z, Li D, Yu L, Niu F. Gallic acid as a cancer-selective agent induces apoptosis in pancreatic cancer cells. Chemotherapy. 2012;58(3):185-94.
25. Ahmed HH, Galal AF, Shalby AB, Abd-Rabou AA, Mehaya FM. Improving Anti-Cancer Potentiality and Bioavailability of Gallic Acid by Designing Polymeric Nanocomposite Formulation. Asian Pac J Cancer Prev. 2018;19(11):3137-46.
26. Faried A, Kurnia D, Faried LS, Usman N, Miyazaki T, Kato H, et al. Anticancer effects of gallic acid isolated from Indonesian herbal medicine, Phaleria macrocarpa (Scheff.) Boerl, on human cancer cell lines. Int J Oncol. 2007;30(3):605-13.
27. Inoue M, Suzuki R, Koide T, Sakaguchi N, Ogihara Y, Yabu Y. Antioxidant, gallic acid, induces apoptosis in HL-60RG cells. Biochem Biophys Res Commun. 1994;204(2):898-904.
28. You BR, Kim SZ, Kim SH, Park WH. Gallic acid-induced lung cancer cell death is accompanied by ROS increase and glutathione depletion. Molecular and cellular biochemistry. 2011;357(1-2):295-303.
29. Chia YC, Rajbanshi R, Calhoun C, Chiu RH. Anti-neoplastic effects of gallic acid, a major component of Toona sinensis leaf extract, on oral squamous carcinoma cells. Molecules. 2010;15(11):8377-89.
30. Guimaraes TA, Farias LC, Fraga CA, Feltenberger JD, Melo GA, Coletta RD, et al. Evaluation of the antineoplastic activity of gallic acid in oral squamous cell carcinoma under hypoxic conditions. Anti-cancer drugs. 2016;27(5):407-16.
31. da Rocha RG, Santos EMS, Santos EM, Gomes ESB, Ramos GV, Aguiar KM, et al. Leptin impairs the therapeutic effect of ionizing radiation in oral squamous cell carcinoma cells. Journal of oral pathology & medicine : official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology. 2019;48(1):17-23.
32. Hermersdörfer H. R. I. Freshney: Culture of Animal Cells. A Manual of Basic Technique. Third Edition. 486 Seiten, zahlr. Abb. und Tab. Wiley-Liss, a John Wiley and Sons, Inc., Publication. New York, Chichester, Brisbane, Toronto. Singapore 1994. Preis: 69.95 US $. Food / Nahrung. 1995;39(2):184-5.
33. Wang JS, Wang HJ, Qian HL. Biological effects of radiation on cancer cells. Mil Med Res. 2018;5(1):20.
34. Weng SW, Hsu SC, Liu HC, Ji BC, Lien JC, Yu FS, et al. Gallic acid induces DNA damage and inhibits DNA repair-associated protein expression in human oral cancer SCC-4 cells. Anticancer research. 2015;35(4):2077-84.
35. Inoue M, Suzuki R, Sakaguchi N, Li Z, Takeda T, Ogihara Y, et al. Selective induction of cell death in cancer cells by gallic acid. Biological & pharmaceutical bulletin. 1995;18(11):1526-30.
36. Kuo CL, Lai KC, Ma YS, Weng SW, Lin JP, Chung JG. Gallic acid inhibits migration and invasion of SCC-4 human oral cancer cells through actions of NF-kappaB, Ras and matrix metalloproteinase-2 and -9. Oncology reports. 2014;32(1):355-61.
37. Yoshioka K, Kataoka T, Hayashi T, Hasegawa M, Ishi Y, Hibasami H. Induction of apoptosis by gallic acid in human stomach cancer KATO III and colon adenocarcinoma COLO 205 cell lines. Oncology reports. 2000;7(6):1221-3.

38. Lakshmi T, Ezhilarasan D, Nagaich U, Vijayaragavan R. Acacia catechu Ethanolic Seed Extract Triggers Apoptosis of SCC-25 Cells. Pharmacognosy magazine. 2017;13(Suppl 3):S405-S11.
39. Inoue M, Sakaguchi N, Isuzugawa K, Tani H, Ogihara Y. Role of reactive oxygen species in gallic acid-induced apoptosis. Biological & pharmaceutical bulletin. 2000;23(10):1153-7.
40. Ray G, Batra S, Shukla NK, Deo S, Raina V, Ashok S, et al. Lipid peroxidation, free radical production and antioxidant status in breast cancer. Breast cancer research and treatment. 2000;59(2):163-70.
41. Kilic N, Yavuz Taslipinar M, Guney Y, Tekin E, Onuk E. An investigation into the serum thioredoxin, superoxide dismutase, malondialdehyde, and advanced oxidation protein products in patients with breast cancer. Annals of surgical oncology. 2014;21(13):4139-43.
42. Fan P, Lou H. Effects of polyphenols from grape seeds on oxidative damage to cellular DNA. Molecular and cellular biochemistry. 2004;267(1-2):67-74.
43. Gupte A, Mumper RJ. Elevated copper and oxidative stress in cancer cells as a target for cancer treatment. Cancer treatment reviews. 2009;35(1):32-46.
44. Ullah MF, Shamim U, Hanif S, Azmi AS, Hadi SM. Cellular DNA breakage by soy isoflavone genistein and its methylated structural analogue biochanin A. Molecular nutrition & food research. 2009;53(11):1376-85.

Figure list

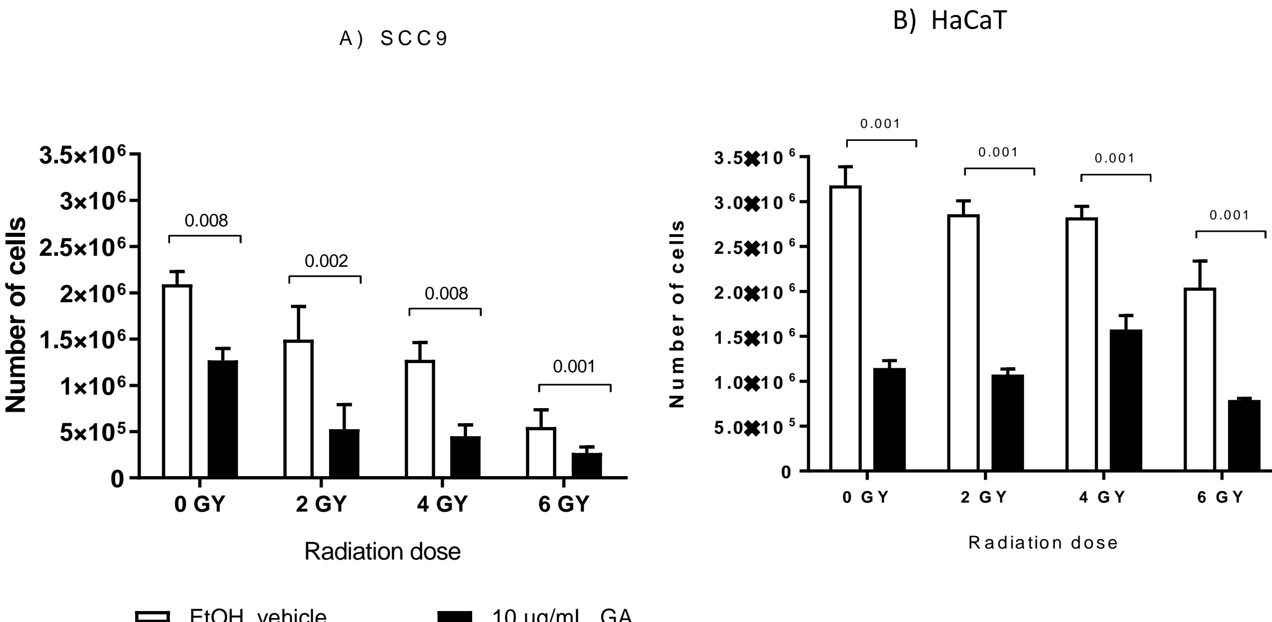


Fig. 1: Effect of gallic acid on the viability of OSCC and HaCat cells exposed to the different doses of ionizing radiation. A) SCC9 cell line. B) HaCaT cell line. GA: gallic acid, ETOH_vehicle: ethanol vehicle control. Anova Two-Way Test. Statistical significance: $p<0.05$.

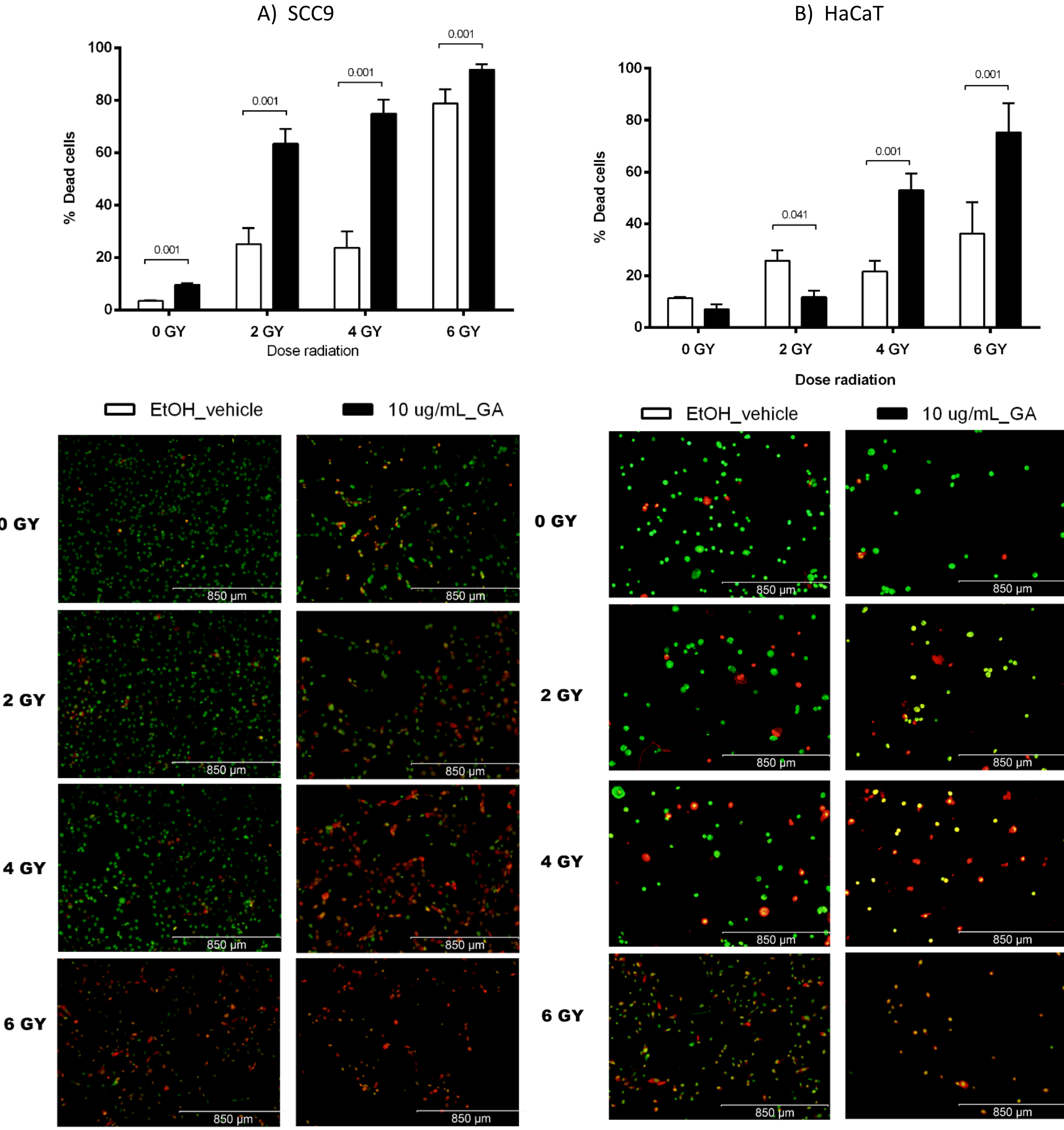


Fig. 2: Cell death/viability assay by colorimetry with AO/EB. AO penetrates into viable cells emitting green fluorescence. EB emits red fluorescence in dead cells. A) SCC9 cell line. B) HaCaT cell line. GA: gallic acid, EtOH_vehicle: ethanol vehicle control. Anova Two-Way Test and T-Test. Statistical significance: $p<0.05$.

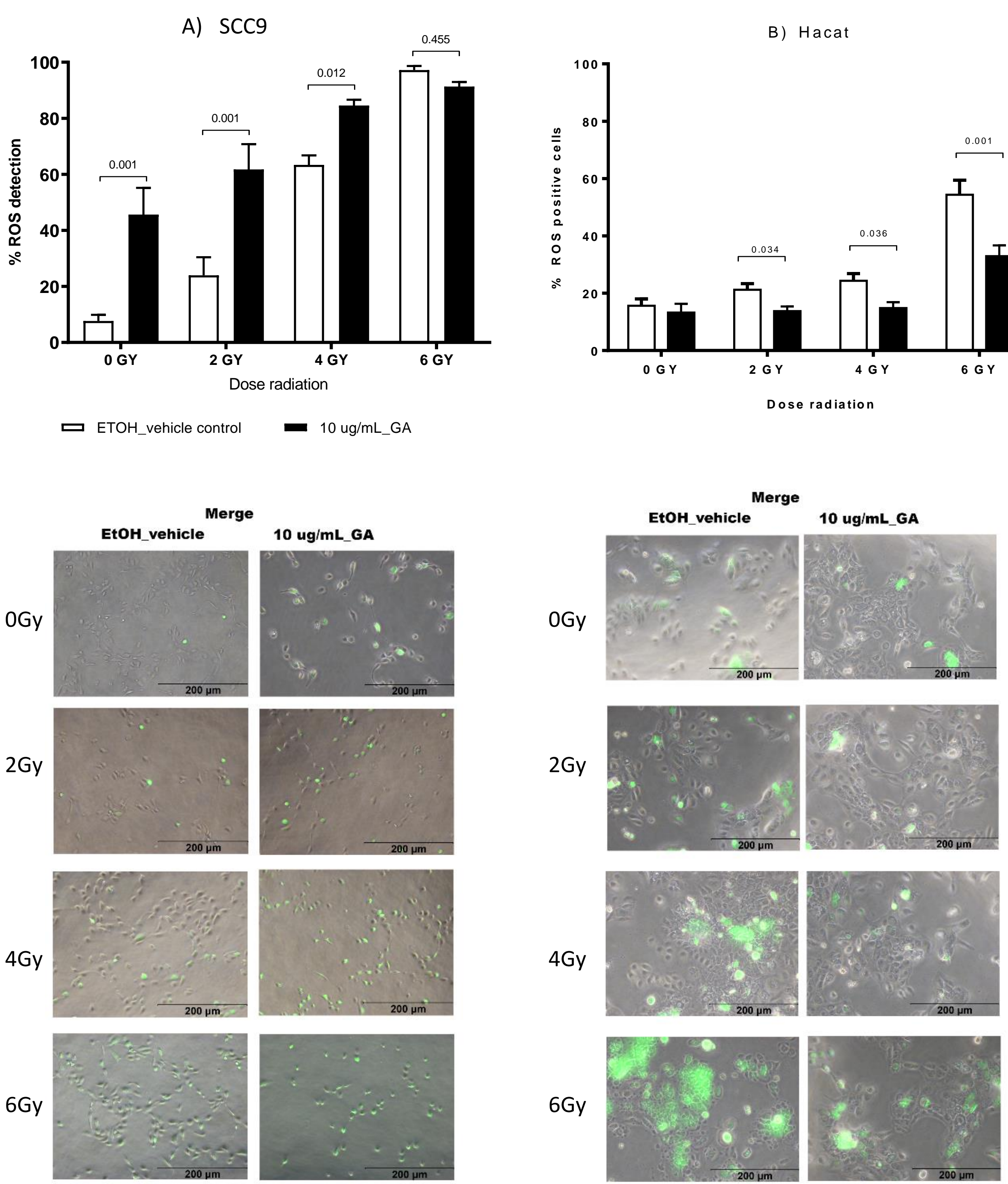


Fig. 3: Reactive oxygen species assay. A) SCC9 cell line. B) HaCaT cell line. GA: gallic acid, EtOH_vehicle: ethanol vehicle control. Anova Two-Way Test and T-Test. Statistical significance: $p<0.05$.

3.3 Produto 3:

***Pitch* para divulgação online dos resultados da tese**

Foi elaborado um vídeo de curta duração para divulgação online dos resultados do estudo, direcionado à população geral. Este produto será apresentado à banca, para posterior divulgação online. Após a avaliação do *Pitch* pela banca avaliadora, será incluído aqui o link para acesso e disponibilização online do vídeo.

4 CONSIDERAÇÕES FINAIS

O CEB representa um grave problema de saúde pública devido aos altos índices de diagnósticos realizados tardiamente e consequentemente das significativas taxas de morbimortalidade e mortalidade.

O presente estudo destacou os efeitos de antioxidantes, bem como do ácido gálico, como estratégia promissora para o controle do comportamento neoplásico. Como observado na revisão sistemática da literatura, várias destas substâncias antioxidantes, incluindo grupos de polifenóis, terpenóides ou flavonoides, extraídos principalmente de plantas, demonstraram potentes e promissores efeitos antineoplásicos contra células de CEB, controlando a proliferação ou levando à morte celular.

Os achados deste estudo apontaram o AG como uma substância promissora capaz de promover o controle das células neoplásicas, revelando seu efeito sinérgico à radiação ionizante terapêutica, promovendo redução da atividade proliferativa, aumentando a morte das células neoplásicas, explicado pelo aumento do estresse oxidativo. Um achado importante foi a indução da morte de células de CEB promovida por doses reduzidas de radiação associado ao tratamento das células com o AG. Ao contrário, em queratinócitos normais, em menores doses, exerceu efeito protetor da morte. Esse fato mostra-se relevante, uma vez que a utilização de menores doses de radiação associada a estratégias terapêuticas complementares poderá minimizar os efeitos adversos provocados pelas doses elevadas de radiação ionizante, possibilitando melhor qualidade de vida e aumento da sobrevida. Novos estudos *in vivo* e, posteriormente, ensaios clínicos são necessários para entender melhor o papel do AG e verificar suas possíveis aplicações clínicas para o tratamento de pacientes acometidos pelo câncer de boca.

REFERÊNCIAS

1. Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. Oral oncology. 2009;45(4-5):309-16.
2. Gupta B, Johnson NW, Kumar N. Global Epidemiology of Head and Neck Cancers: A Continuing Challenge. Oncology. 2016;91(1):13-23.
3. Scully C, Bagan JV. Recent advances in Oral Oncology. Oral oncology. 2007;43(2):107-15.
4. Williams HK. Molecular pathogenesis of oral squamous carcinoma. Mol Pathol. 2000;53(4):165-72.
5. Argiris A, Eng C. Epidemiology, staging, and screening of head and neck cancer. Cancer Treat Res. 2003;114:15-60.
6. Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Pineros M, et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer. 2019;144(8):1941-53.
7. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136(5):E359-86.
8. Shield KD, Ferlay J, Jemal A, Sankaranarayanan R, Chaturvedi AK, Bray F, et al. The global incidence of lip, oral cavity, and pharyngeal cancers by subsite in 2012. CA Cancer J Clin. 2017;67(1):51-64.
9. Abad-Arranz M, Moran-Rodriguez A, Mascaros Balaguer E, Quintana Velasco C, Abad Polo L, Nunez Palomo S, et al. Quantification of inaccurate diagnosis of COPD in primary care medicine: an analysis of the COACH clinical audit. Int J Chron Obstruct Pulmon Dis. 2019;14:1187-94.
10. Neville BW, Day TA. Oral cancer and precancerous lesions. CA Cancer J Clin. 2002;52(4):195-215.
11. Rodenhiser D, Mann M. Epigenetics and human disease: translating basic biology into clinical applications. CMAJ. 2006;174(3):341-8.
12. Li CC, Shen Z, Bavarian R, Yang F, Bhattacharya A. Oral Cancer: Genetics and the Role of Precision Medicine. Dent Clin North Am. 2018;62(1):29-46.
13. Feller LL, Khammissa RR, Kramer BB, Lemmer JJ. Oral squamous cell carcinoma in relation to field precancerisation: pathobiology. Cancer Cell Int. 2013;13(1):31.
14. Warnakulasuriya S, Johnson NW, van der Waal I. Nomenclature and classification of potentially malignant disorders of the oral mucosa. Journal of oral pathology & medicine : official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology. 2007;36(10):575-80.
15. Huo W, Li ZM, Pan XY, Bao YM, An LJ. Antioxidant enzyme levels in pathogenesis of oral squamous cell carcinoma (OSCC). Drug Res (Stuttg). 2014;64(11):629-32.
16. Topdag S, Aslaner A, Tataroglu C, Ilce Z. Evaluation of antioxidant capacity in lung carcinoma. Indian Journal of Thoracic and Cardiovascular Surgery. 2005;21(4):269-71.
17. Anbuselvam C, Vijayavel K, Balasubramanian MP. Protective effect of Operculina turpethum against 7,12-dimethyl benz(a)anthracene induced oxidative stress with reference to breast cancer in experimental rats. Chem Biol Interact. 2007;168(3):229-36.
18. Prasad KN, Kumar A, Kochupillai V, Cole WC. High doses of multiple antioxidant vitamins: essential ingredients in improving the efficacy of standard cancer therapy. J Am Coll Nutr. 1999;18(1):13-25.
19. Zubair H, Khan HY, Sohail A, Azim S, Ullah MF, Ahmad A, et al. Redox cycling of endogenous copper by thymoquinone leads to ROS-mediated DNA breakage and consequent cell death: putative anticancer mechanism of antioxidants. Cell Death Dis. 2013;4:e660.

20. de Faria PR, Cardoso SV, de ANS, Silva SJ, Loyola AM. Clinical presentation of patients with oral squamous cell carcinoma when first seen by dentists or physicians in a teaching hospital in Brazil. Clinical oral investigations. 2003;7(1):46-51.
21. Chatterjee A, Laskar SG, Chaukar D. Management of early oral cavity squamous cancers. Oral oncology. 2020;104:104627.
22. Dai W, Li Y, Zhou Q, Xu Z, Sun C, Tan X, et al. Cetuximab inhibits oral squamous cell carcinoma invasion and metastasis via degradation of epidermal growth factor receptor. Journal of oral pathology & medicine : official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology. 2014;43(4):250-7.
23. Vikram B. Adjuvant therapy in head and neck cancer. CA: a cancer journal for clinicians. 1998;48(4):199-209.
24. Thariat J, Hannoun-Levi JM, Sun Myint A, Vuong T, Gerard JP. Past, present, and future of radiotherapy for the benefit of patients. Nat Rev Clin Oncol. 2013;10(1):52-60.
25. Baskar R, Dai J, Wenlong N, Yeo R, Yeoh KW. Biological response of cancer cells to radiation treatment. Front Mol Biosci. 2014;1:24.
26. Hiro J, Inoue Y, Toiyama Y, Miki C, Kusunoki M. Mechanism of resistance to chemoradiation in p53 mutant human colon cancer. International journal of oncology. 2008;32(6):1305-10.
27. Cabrera AR, Yoo DS, Brizel DM. Contemporary radiotherapy in head and neck cancer: balancing chance for cure with risk for complication. Surgical oncology clinics of North America. 2013;22(3):579-98.
28. Srivastava V, Negi AS, Kumar JK, Gupta MM, Khanuja SP. Plant-based anticancer molecules: a chemical and biological profile of some important leads. Bioorganic & medicinal chemistry. 2005;13(21):5892-908.
29. Tokgun O, Akca H, Mammadov R, Aykurt C, Deniz G. Convolvulus galaticus, Crocus antalyensis, and Lilium candidum extracts show their antitumor activity through induction of p53-mediated apoptosis on human breast cancer cell line MCF-7 cells. Journal of medicinal food. 2012;15(11):1000-5.
30. Liu W, Li SY, Huang XE, Cui JJ, Zhao T, Zhang H. Inhibition of tumor growth in vitro by a combination of extracts from Rosa roxburghii Tratt and Fagopyrum cymosum. Asian Pacific journal of cancer prevention : APJCP. 2012;13(5):2409-14.
31. Song M, Park DK, Park HJ. Antrodia camphorata Grown on Germinated Brown Rice Suppresses Melanoma Cell Proliferation by Inducing Apoptosis and Cell Differentiation and Tumor Growth. Evidence-based complementary and alternative medicine : eCAM. 2013;2013:321096.
32. Borek C. Dietary antioxidants and human cancer. Integr Cancer Ther. 2004;3(4):333-41.
33. Ng TB, He JS, Niu SM, Zhao L, Pi ZF, Shao W, et al. A gallic acid derivative and polysaccharides with antioxidative activity from rose (Rosa rugosa) flowers. The Journal of pharmacy and pharmacology. 2004;56(4):537-45.
34. Kim SH, Jun CD, Suk K, Choi BJ, Lim H, Park S, et al. Gallic acid inhibits histamine release and pro-inflammatory cytokine production in mast cells. Toxicological sciences : an official journal of the Society of Toxicology. 2006;91(1):123-31.
35. Chakraborty A, Ferk F, Simic T, Brantner A, Dusinska M, Kundi M, et al. DNA-protective effects of sumach (Rhus coriaria L.), a common spice: results of human and animal studies. Mutation research. 2009;661(1-2):10-7.
36. Shahrzad S, Aoyagi K, Winter A, Koyama A, Bitsch I. Pharmacokinetics of gallic acid and its relative bioavailability from tea in healthy humans. The Journal of nutrition. 2001;131(4):1207-10.
37. Liao CL, Lai KC, Huang AC, Yang JS, Lin JJ, Wu SH, et al. Gallic acid inhibits migration and invasion in human osteosarcoma U-2 OS cells through suppressing the matrix metalloproteinase-2/-9, protein kinase B (PKB) and PKC signaling pathways. Food Chem Toxicol. 2012;50(5):1734-40.

38. Sousa JN, Paraiso AF, Andrade JMO, Lelis DF, Santos EM, Lima JP, et al. Oral gallic acid improve liver steatosis and metabolism modulating hepatic lipogenic markers in obese mice. Exp Gerontol. 2020;134:110881.
39. Ohno T, Inoue M, Ogihara Y. Cytotoxic activity of gallic acid against liver metastasis of mastocytoma cells P-815. Anticancer research. 2001;21(6A):3875-80.
40. Liu X, Huang N, Li H, Wang H, Jin Q, Ji J. Multidentate polyethylene glycol modified gold nanorods for in vivo near-infrared photothermal cancer therapy. ACS applied materials & interfaces. 2014;6(8):5657-68.
41. You BR, Moon HJ, Han YH, Park WH. Gallic acid inhibits the growth of HeLa cervical cancer cells via apoptosis and/or necrosis. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association. 2010;48(5):1334-40.
42. Lu Y, Jiang F, Jiang H, Wu K, Zheng X, Cai Y, et al. Gallic acid suppresses cell viability, proliferation, invasion and angiogenesis in human glioma cells. European journal of pharmacology. 2010;641(2-3):102-7.
43. Guimaraes TA, Farias LC, Fraga CA, Feltenberger JD, Melo GA, Coletta RD, et al. Evaluation of the antineoplastic activity of gallic acid in oral squamous cell carcinoma under hypoxic conditions. Anti-cancer drugs. 2016;27(5):407-16.
44. Lu YC, Lin ML, Su HL, Chen SS. ER-Dependent Ca++-mediated Cytosolic ROS as an Effector for Induction of Mitochondrial Apoptotic and ATM-JNK Signal Pathways in Gallic Acid-treated Human Oral Cancer Cells. Anticancer research. 2016;36(2):697-705.
45. Weng SW, Hsu SC, Liu HC, Ji BC, Lien JC, Yu FS, et al. Gallic acid induces DNA damage and inhibits DNA repair-associated protein expression in human oral cancer SCC-4 cells. Anticancer research. 2015;35(4):2077-84.
46. Kuo CL, Lai KC, Ma YS, Weng SW, Lin JP, Chung JG. Gallic acid inhibits migration and invasion of SCC-4 human oral cancer cells through actions of NF-kappaB, Ras and matrix metalloproteinase-2 and -9. Oncology reports. 2014;32(1):355-61.

ANEXOS

ANEXO A - Normas para publicação no periódico *Free Radical Biology & Medicine*

https://www.elsevier.com/journals/free-radical-biology-and-medicine/0891-5849/guide-for-authors

ANEXO B - Normas para publicação no periódico *Journal of Oral Pathology & Medicine*

https://onlinelibrary.wiley.com/page/journal/16000714/homepage/ForAuthors.html

ANEXO C - Aprovação da pesquisa pelo Comitê de Ética em Pesquisa/Unimontes

UNIVERSIDADE ESTADUAL DE MONTES CLAROS - UNIMONTES

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Ação adjuvante do ácido gálico sobre o efeito terapêutico da radiação ionizante e mecanismos de degradação proteolítica no carcinoma epidermóide de boca

Pesquisador: Lucyana Conceição Farias

Área Temática: Genética Humana:
(Trata-se de pesquisa envolvendo Genética Humana que não necessita de análise ética por parte da CONEP;);

Versão: 1

CAAE: 66610517.6.0000.5146

Instituição Proponente: Universidade Estadual de Montes Claros - UNIMONTES

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.008.010

Apresentação do Projeto:

O projeto propõe:

Cultura celular: Serão utilizadas linhagens celulares imortalizadas de carcinoma epidermóide de boca (SCC-4 e SCC-9), adquiridas comercialmente (ATCC, USA). Estas serão estocadas em ultra freezer a -80°C e criopreservadas em solução específica.

Tratamento com ácido gálico e exposição das células à radiação ionizante: As células serão tratadas com 10 mg/ml de ácido gálico (SIGMA, USA), por 72 horas. A exposição das células à radiação ionizante será realizada no dia da administração da última dose de AG, através da exposição dos cultivos de SCC-9 / SCC-4, em semiconfluência, à dose de 6 Gy de cobalto-60 (gray: unidade internacional de dose de radiação ionizante), utilizando-se um acelerador linear Telecobalto Theratron Phoenix Philips SR 7510 (Eindhoven, Holanda), a uma distância fonte-campo de 70cm.

Ensaios de comportamento celular:

- Ensaio de proliferação celular
- Ensaio de invasão celular (Transwell assay)
- Ensaio de migração celular

Endereço: Av.Dr Rui Braga s/n-Camp Univers Profº Darcy Rib
Bairro: Vila Mauricéia **CEP:** 39.401-089
UF: MG **Município:** MONTES CLAROS
Telefone: (38)3229-8180 **Fax:** (38)3229-8103 **E-mail:** smelocosta@gmail.com

- Ensaio de viabilidade celular por alaranjado de acridina e brometo de etídeo
- Ensaio clonogênico
- Ensaio de fragmentação do DNA
- Detecção de ROS utilizando carboxi-H2DCFDA
- Co-cultura de células de carcinoma e células endoteliais

Análises moleculares:

- Avaliação da expressão do mRNA do USP2, UBA, UBS, PSMD4, PSME3, CASP3, BAX, BCL2, KI67, E-CADHERIN, MMP
- Imunodetecção de proteínas através da técnica Western Blot
- Estudo imunocitoquímico: Serão investigadas as expressões celulares das proteínas USP2, UBA, UBS, PSMD4, PSME3, CASP3, BAX, BCL2,

KI67, E-CADHERIN e MMP em cada uma das amostras através da técnica de imunocitoquímica.

Estudo da angiogênese in vitro: Matrigel será descongelado em gelo e 15μl serão distribuídos em placas de 96 poços. Em seguida a placa

permanecerá por 1 h a 37ºC e CO2 5%. Células endoteliais (5x103células/poço) serão ressuspendidas em 100μl de meio RPMI suplementado com 0,2% de soro fetal bovino (SFB) e adicionado em cada poço na presença e ausência de tratamento. O ensaio será feito em quadriplicata utilizando as concentrações e tempos de tratamento com AG e radiação ionizante previamente determinados.

Análise de proteômica: Para a análise protéica será utilizado um espectrômetro de massa Q-Tof Premier (Waters). Para análise de dados, os

espectros serão adquiridos usando o software MassLynx v.4.1 e processados pelo software Mascot Distiller v.2.3.2.0, 2009, pesquisando-se contra a base de dados UniProt. Utilizando-se o programa Scaffold será preparado um diagrama de Venn para identificar proteínas expressas diferencialmente ou proteínas exclusivas para alguma das condições de estudo.

Objetivo da Pesquisa:

Analisar a ação adjuvante do AG sobre o efeito terapêutico da radiação ionizante e mecanismos de degradação proteolítica no carcinoma epidermóide de boca (CEB).

Avaliação dos Riscos e Benefícios:

Riscos:

A pesquisa será realizada a partir do cultivo de linhagens celulares imortalizadas, adquiridas comercialmente. Os riscos são àqueles relacionados à execução dos experimentos laboratoriais,

Endereço: Av.Dr Rui Braga s/n-Camp Univers Profº Darcy Rib
Bairro: Vila Mauricéia **CEP:** 39.401-089
UF: MG **Município:** MONTES CLAROS
Telefone: (38)3229-8180 **Fax:** (38)3229-8103 **E-mail:** smelocosta@gmail.com

que serão minimizados com o uso de equipamentos de proteção individual e obediência às normas de biossegurança em laboratórios.
Benefícios:
Através deste estudo, poderá ser identificado um potencial de ação adjuvante do ácido gálico ao efeito terapêutico da radiação ionizante no carcinoma epidermóide de boca.

Comentários e Considerações sobre a Pesquisa:
Pesquisa relevante na área temática carcinoma epidermoide de boca.

Considerações sobre os Termos de apresentação obrigatória:
Termos adequados, pesquisa com cultivo de linhagens celulares imortalizadas, adquiridas comercialmente.

Recomendações:
Apresentação de relatório final por meio da plataforma Brasil, em "enviar notificação".

Conclusões ou Pendências e Lista de Inadequações:
Aprovado.

Considerações Finais a critério do CEP:
O projeto respeita os preceitos éticos da pesquisa em seres humanos, sendo assim somos favoráveis à aprovação do mesmo.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_894351.pdf	03/04/2017 22:10:12		Aceito
Orçamento	Orcamento.docx	03/04/2017 22:07:48	Lucyana Conceição Farias	Aceito
Cronograma	Cronograma.docx	03/04/2017 22:04:44	Lucyana Conceição Farias	Aceito
Declaração de Instituição e Infraestrutura	DeclaracaoInfraestrutura.pdf	03/04/2017 21:59:44	Lucyana Conceição Farias	Aceito
Projeto Detalhado / Brochura	ProjetoCompleto.pdf	03/04/2017 21:47:54	Lucyana Conceição Farias	Aceito

Endereço: Av.Dr Rui Braga s/n-Camp Univers Profº Darcy Rib
Bairro: Vila Mauricéia **CEP:** 39.401-089
UF: MG **Município:** MONTES CLAROS
Telefone: (38)3229-8180 **Fax:** (38)3229-8103 **E-mail:** smelocosta@gmail.com

Investigador	ProjetoCompleto.pdf	03/04/2017 21:47:54	Lucyana Conceição Farias	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	JustificativaAusenciaTCLE.pdf	03/04/2017 21:45:27	Lucyana Conceição Farias	Aceito
Declaração de Pesquisadores	DeclaracaoCumprimentoNormasPesquisa.pdf	03/04/2017 21:36:48	Lucyana Conceição Farias	Aceito
Folha de Rosto	FolhadeRosto.pdf	03/04/2017 21:32:17	Lucyana Conceição Farias	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

MONTES CLAROS, 08 de Abril de 2017

Assinado por:
SIMONE DE MELO COSTA
(Coordenador)

Endereço: Av.Dr Rui Braga s/n-Camp Univers Profº Darcy Rib
Bairro: Vila Mauricéia **CEP:** 39.401-089
UF: MG **Município:** MONTES CLAROS
Telefone: (38)3229-8180 **Fax:** (38)3229-8103 **E-mail:** smelocosta@gmail.com

ANEXO D - Registro da Revisão Sistemática na Plataforma PROSPERO

PROSPERO
International prospective register of systematic reviews

NHS
National Institute for Health Research

Therapeutic potential of antioxidant substances in oral squamous cell carcinoma: a systematic review

Karina Marini Aguiar, Angeliny Tamiarana Lima Tabosa, Renato Sobral Monteiro-Junior, Alfredo Maurício Batista de Paula, Sérgio Henrique Sousa Santos, André Luiz Sena Guimarães, Lucyana Conceição Farias

Citation

Karina Marini Aguiar, Angeliny Tamiarana Lima Tabosa, Renato Sobral Monteiro-Junior, Alfredo Maurício Batista de Paula, Sérgio Henrique Sousa Santos, André Luiz Sena Guimarães, Lucyana Conceição Farias. Therapeutic potential of antioxidant substances in oral squamous cell carcinoma: a systematic review. PROSPERO 2018 CRD42018107206 Available from:
http://www.crd.york.ac.uk/PROSPERO/display_record.php?ID=CRD42018107206

Review question

Do antioxidants substances exert therapeutic potential in oral squamous cell carcinoma (OSCC)?

Searches

Studies will be retrieved from PubMed, Scopus, Web of Science and EBSCO.
There will be no period of restriction. We will include studies in English and use the following descriptors: oral squamous cell carcinoma OR epidermoid carcinoma of oral cavity OR squamous cell carcinoma of oral cavity OR oral epidermoid carcinoma OR squamous cell carcinoma of mouth OR epidermoid carcinoma of mouth AND antioxidant OR antioxidant effect AND treatment OR therapy OR therapeutic. These descriptors were selected according to the Medical Subject Heading (MeSH) and common terms from literature.

Types of study to be included

Randomized controlled trials and other original studies will be included to assess the effect of antioxidants for OSCC patients with OSCC human cell lines and antioxidants.

Condition or domain being studied

Therapeutic potential of antioxidant drugs on oral squamous cell carcinoma.

Participants/population

Inclusion:
1- Studies involving patients with OSCC or OSCC human cell lines.
2- Studies investigating treatments with antioxidants substances.
Exclusion:
1- Studies that assessed substances without antioxidant effect and other cancer types.
2- In vivo studies.

Intervention(s), exposure(s)

Antioxidant substances treatment with no limits to the dosage and frequency.
The intervention might be antioxidant alone or antioxidant plus other treatment protocol for OSCC cancer such as chemo- or radiotherapy.

Comparator(s)/control

OSCC patients or cell lines without treatment with antioxidant substances with no limits to the dosage and frequency.

Context

Primary outcome(s)

Antioxidant substances may exert an antineoplastic effect on OSCC.This outcome will be defined by reading

the "results" section of the included articles, which show antioxidant substances with antineoplastic effect in OSCC patients and cell lines. For randomized clinical trials, an effect size analysis and/or descriptive analysis will be performed, and for in vitro studies the antineoplastic effect on the cell lines will be evaluated through the phenotypic assays.

Secondary outcome(s)

Not applicable

Data extraction (selection and coding)

First, we will perform an inter-rater calibration by Kappa statistic for articles selection. The articles will be selected by two independent assessors (ATLT and KMA) initially based on the reading of the title, and thereafter by reading the abstracts. Full papers will be reviewed from selected abstracts. In case of disagreement between assessors as to the inclusion criteria, the title, and abstract, or the full article, these will be retained for further evaluation. Disagreements regarding the inclusion criteria, a third investigator (LCF) will be consulted. Data will be extracted and recorded independently including study results and methodological data using a standardized data collection form with the following items: treatment with antioxidant substances with no limits to the dosage and frequency.
The intervention might be antioxidant alone, or antioxidant plus other treatment protocol for OSCC cancer such as chemo- or radiotherapy.

Risk of bias (quality) assessment

The risk of bias will be assessed using the Jadad scale to classify the methodological quality of randomized controlled trial. Original in vitro studies will be assessed considering a single item: the use of one cell line only for experimental analyses.

Strategy for data synthesis

We will provide a systematic narrative synthesis of the findings from the included studies, covering the therapeutic effect of antioxidant substances on OSCC patients or cell lines. We will provide summaries about the antioxidant agent, the antineoplastic effect of that, dosage, treatment frequency, and treatment type antioxidant alone, or antioxidant plus other treatment protocol for OSCC, such as chemo- or radiotherapy, and the main outcomes.

Analysis of subgroups or subsets

None planned.

Contact details for further information

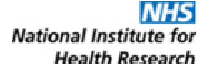
Lucyana Farias
lucyanacfarias@gmail.com

Organisational affiliation of the review

unimontes

Review team members and their organisational affiliations

Karina Marini Aguiar. Postgraduate Program in Health Sciences, Universidade Estadual de Montes Claros, Minas Gerais, Brazil
Angeliny Tamiarana Lima Tabosa. Postgraduate Program in Health Sciences, Universidade Estadual de Montes Claros, Minas Gerais, Brazil
Renato Sobral Monteiro-Junior. Postgraduate Program in Health Sciences, Universidade Estadual de Montes Claros, Minas Gerais, Brazil
Alfredo Maurício Batista de Paula. Department of Dentistry and Postgraduate Program in Health Sciences, Universidade Estadual de Montes Claros, Minas Gerais, Brazil



Sérgio Henrique Sousa Santos. Institute of Agricultural Sciences, Food Engineering College, Universidade Federal de Minas Gerais, Montes Claros, Minas Gerais, Brazil.
André Luiz Sena Guimarães. Department of Dentistry and Postgraduate Program in Health Sciences, Universidade Estadual de Montes Claros, Minas Gerais, Brazil
Lucyana Conceição Farias. Department of Dentistry and Postgraduate Program in Health Sciences, Universidade Estadual de Montes Claros, Minas Gerais, Brazil

Anticipated or actual start date
30 July 2018

Anticipated completion date
05 November 2018

Funding sources/sponsors
This study was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Brazil.

Conflicts of interest

Language
English

Country
Brazil

Stage of review
Review_Ongoing

Subject index terms status
Subject indexing assigned by CRD

Subject index terms
Antioxidants; Carcinoma, Squamous Cell; Humans; Mouth Neoplasms

Date of registration in PROSPERO
13 September 2018

Date of publication of this version
13 September 2018

Details of any existing review of the same topic by the same authors

Stage of review at time of this submission

Stage	Started	Completed
Preliminary searches	Yes	No
Piloting of the study selection process	No	No
Formal screening of search results against eligibility criteria	No	No
Data extraction	No	No
Risk of bias (quality) assessment	No	No
Data analysis	No	No

Versions

13 September 2018

PROSPERO
This information has been provided by the named contact for this review. CRD has accepted this information in good faith and registered the review in PROSPERO. CRD bears no responsibility or liability for the content of this registration record, any associated files or external websites.