CHEMOPREVENTIVE AGENTS ON A MOUSE SKIN CARCINOGENESIS MODEL: AN INTEGRATIVE REVIEW

Ana Paula da Silva[1]
Bruna Luiza de Graaf Kamchen[2]
Giovana Hamerski Trombetta[3]
Elizangela dos Anjos Silva[4]

SILVA, A. P. da.; KAMCHEN, B. de. G.; TROMBETTA, G. H.; SILVA, E. dos. A. Chemopreventive agents on a mouse skin carcinogenesis model: an integrative review. **Arquivos de Ciências da Saúde da UNIPAR**. Umuarama. v. 26, n. 3, p. 546-568, set./dez. 2022.

ABSTRACT: Squamous cell carcinoma (SCC) is a non-melanoma skin cancer, with chronic sun exposure as the main risk factor. Excisional surgery is the most indicated treatment; however, patients can suffer functional, aesthetic, and psychological damage depending on the lesion site. Topical administration of 7,12-dimethylbenz[*a*]anthracene (DMBA) and 12-O-Tetradecanoylphorbol-13-acetate (TPA) induce to the appearance of benign skin tumors in mice, some of which develop into SCC. This protocol has been used to analyze the effects of many chemopreventive agents that may block or inhibit the mechanisms of action of chemical carcinogenesis. We compared the effects of chemopreventive agents in an induced skin carcinogenesis animal model. In the Scopus, PubMed, and EMBASE databases, we searched for manuscripts published between June 16, 2011, and June 16, 2021. We excluded studies conducted *in vitro* or on transgenic mice; in addition, studies without drug dosage, route of administration, or tumor incidence were excluded. We selected 26 studies and analyzed their main characteristics and the outcomes of tumorigenesis analysis. Most chemopreventive agents have shown excellent potential to inhibit the development of skin tumors. This review also discusses the standardization of studies in animal models to ensure better responses and future randomized clinical trials for cancer treatment and prevention.
KEYWORDS: Chemoprevention; Phytochemical; Induced skin carcinogenesis; Animal model; Skin Cancer.

AGENTES QUIMIOPREVENTIVOS EM UM MODELO DE CARCINOGÊNESE DE PELE EM CAMUNDONGOS: UMA REVISÃO INTEGRATIVA

RESUMO: O carcinoma espinocelular cutâneo (CEC) é um câncer de pele não melanoma, com a exposição solar crônica como o principal fator de risco. A cirurgia excisional é o tratamento mais indicado; entretanto, os pacientes podem sofrer danos funcionais, estéticos e psicológicos dependendo do local da lesão. A administração tópica de 7,12-dimetilbenz[*a*]antraceno (DMBA) e 12-O-Tetradecanoilforbol-13-acetato (TPA) induz ao aparecimento de tumores cutâneos benignos em camundongos, alguns dos quais evoluíram para CEC. Este protocolo tem sido utilizado para analisar os efeitos de muitos agentes quimiopreventivos que podem bloquear ou inibir os mecanismos de ação da carcinogênese química. Comparamos os efeitos de agentes quimiopreventivos em um modelo animal que foi induzido à carcinogênese de pele. Nas bases de dados Scopus, PubMed e EMBASE, buscamos manuscritos publicados entre 16 de junho de 2011 e 16 de junho de 2021. Excluímos

DOI: 10.25110/arqsaude.v26i3.8802
[1] Doutorado, Departamento de Ciências Básicas da Saúde pela Universidade Federal de Ciências da Saúde de Porto Alegre. E-mail: paulasilvabio@ufcspa.edu.br Orcid: https://orcid.org/0000-0003-4163-4977
[2] Graduanda do Curso de Biomedicina pela Universidade Federal de Ciências da Saúde de Porto Alegre. E-mail: brunagk@ufcspa.edu.br Orcid: https://orcid.org/0000-0001-8550-3392
[3] Graduanda do Curso de Biomedicina pela Universidade Federal de Ciências da Saúde de Porto Alegre. E-mail: giovanaht@ufcspa.edu.br Orcid: https://orcid.org/0000-0002-7840-5791
[4] Doutora, Instituto de Biociências pela Universidade Federal do Mato Grosso do Sul. E-mail: elizangela.anjos@ufms.br Orcid: https://orcid.org/0000-0001-9470-1948

estudos realizados *in vitro* ou em camundongos transgênicos; além disso, estudos sem dosagem de drogas, via de administração ou incidência de tumores foram excluídos. Selecionamos 26 estudos e analisamos suas principais características e os resultados da análise da tumorigênese. A maioria dos agentes quimiopreventivos tem demonstrado excelente potencial para inibir o desenvolvimento de tumores cutâneos. Esta revisão também discute a padronização de estudos em modelos animais para garantir melhores respostas e futuros ensaios clínicos randomizados para tratamento e prevenção do câncer.
PALAVRAS-CHAVE: Quimioprevenção; Fitoquímicos; Carcinogênese da pele induzida; Modelo animal; Câncer de pele.

AGENTES QUIMIOPREVENTIVOS EN UN MODELO DE CARCINOGÉNESIS CUTÁNEA EN RATÓN: UNA REVISIÓN INTEGRADORA

RESUMEN: El carcinoma de células escamosas (CCE) es un cáncer de piel no melanoma, cuyo principal factor de riesgo es la exposición crónica al sol. La cirugía de escisión es el tratamiento más indicado; sin embargo, los pacientes pueden sufrir daños funcionales, estéticos y psicológicos dependiendo de la localización de la lesión. La administración tópica de 7,12-dimetilbenz[a]antraceno (DMBA) y 12-O-Tetradecanoilforbol-13-acetato (TPA) inducen a la aparición de tumores cutáneos benignos en ratones, algunos de los cuales se convierten en CCE. Este protocolo se ha utilizado para analizar los efectos de muchos agentes quimiopreventivos que pueden bloquear o inhibir los mecanismos de acción de la carcinogénesis química. Comparamos los efectos de los agentes quimiopreventivos en un modelo animal de carcinogénesis cutánea inducida. En las bases de datos Scopus, PubMed y EMBASE, se buscaron los manuscritos publicados entre el 16 de junio de 2011 y el 16 de junio de 2021. Se excluyeron los estudios realizados in vitro o en ratones transgénicos; además, se excluyeron los estudios sin dosis de fármacos, vía de administración o incidencia tumoral. Se seleccionaron 26 estudios y se analizaron sus características principales y los resultados del análisis de la tumorigénesis. La mayoría de los agentes quimiopreventivos han mostrado un excelente potencial para inhibir el desarrollo de tumores cutáneos. Esta revisión también analiza la estandarización de los estudios en modelos animales para garantizar mejores respuestas y futuros ensayos clínicos aleatorios para el tratamiento y la prevención del cáncer.
PALABRAS CLAVE: Quimioprevención; Fitoquímica; Carcinogénesis cutánea inducida; Modelo animal; Cáncer de piel.

---

1. INTRODUCTION

Skin cancer is a public health problem because it is one of the most common diseases worldwide. The incidence of non-melanoma skin cancer was 11,980,73 in 2020 for all ages and sexes (BRAY *et al.*, 2018). Squamous cell carcinoma (SCC) is a non-melanoma skin cancer type; its main risk factors include age, fair skin, immunosuppression, and chronic sun exposure. The failure of DNA repair mechanisms in keratinocytes increases the proliferation of damaged cells (ARMSTRONG; KRICKER, 2001; SÁNCHEZ-DANÉS *et al.*, 2016). The SCC mortality rate is low; however, its recurrence and morbidity may increase the risk of metastasis and death. Excisional surgery is the primary treatment for individuals diagnosed with SCC. However, as it is an invasive procedure, patients may suffer severe deformities depending on the lesion size and site (QUE; ZWALD; SCHUMULTS, 2018; STRATIGOS *et al.*, 2020).

In a classical mouse model, 7,12-dimethylbenz[a]anthracene (DMBA) and 12-O-tetradecanoylphorbol-13-Acetate (TPA) are chemical carcinogens which mimic the carcinogenesis

stages in humans, namely initiation, promotion, and progression. DMBA is an initiating agent, and a single dose causes irreversible cell damage. This substance produces DNA adducts, leading to mutations in proto-oncogenes and tumor suppressor genes, especially those that control the cell cycle, DNA repair, and cell death pathways (DIGIOVANNI, 1992; KUBO *et al.*, 2014). Morphological changes occur only in the promotion stage, characterized by clonal expansion of initiated cells. TPA is a well-known tumor promoter that increases the expression of inflammatory factors and produces reactive oxygen species (ROS). The promotion stage is reversible when TPA treatment is discontinued. Papillomas are benign skin tumors induced in murine models and occur a few weeks after repeated exposure to TPA. These tumors present exophytic wart-like growth and can regress or progress. Papillomas in mice that progress into SCC, histologically appear similar to those found in humans (ABEL *et al.*, 2009; AOTO *et al.*, 2018).

Preventive medicine has investigated new phytochemicals or synthetic derivatives through dietary supplements or topical treatments (DUNN; UMAR; RICHMOND, 2016; SURH, 1999). The mechanisms of action of the chemopreventive agents can block or suppress tumors during the initiation or promotion stages (MELO *et al.*, 2018). Blockage prevents the interaction between initiating agents and critical cellular targets, such as DNA, RNA, or proteins. The suppressive action inhibits the expression of initiated cells and acts in both the promotion and progression stages. Although DMBA/TPA is a classic two-stage tumorigenesis model, its limitation is that papillomas are heterogeneous tumors; each follows a different path within the same animal. Nonetheless, different variables can be assessed using animal models (AOTO *et al.*, 2018; COHEN; ARNOLD, 2011; REEVES *et al.*, 2018).

The main variables in skin carcinogenesis are tumor incidence, tumor multiplicity (the average number of tumors per mouse), tumor latency period, and tumor size; however, this is not a consensus. Some studies have shown only molecular or biochemical results related to cell proliferation, cell death, DNA repair mechanisms, inflammation, or oxidative stress pathways (ABEL *et al.*, 2009; DIGIOVANNI, 1992). This review performed a critical analysis of chemopreventive agents in a mouse skin carcinogenesis model. We aimed to assess whether chemopreventive agents could prevent the appearance of tumors; therefore, tumor incidence was the main inclusion criterion in this study. We also show other effects of chemopreventive agents, such as tumor multiplicity, latency period, and signaling pathways related to skin cancer prevention. This review highlights chemopreventive agents that may be candidates for future randomized clinical trials to prevent skin cancer.

2. METHODS

2.1 Search strategy

This integrative review followed the Preferred Reporting Items for Systematic Reviews and

Meta-Analyses (PRISMA) guidelines (MOHER *et al.*, 2009). We searched for articles published between June 16, 2011, and June 16, 2021, using the electronic databases PubMed, Scopus, and Embase. The descriptors were chemoprevention OR neoplastic agents AND carcinogenesis AND 9,10-dimethyl-1,2-benzanthracene AND tetradecanoylphorbol acetate AND skin neoplasms in PubMed (MeSH) and Scopus. Embase (Emtree) terms were chemoprotective agent OR antineoplastic agent AND chemical carcinogenesis AND 7,12-dimethylbenz[a]anthracene AND phorbol 13-acetate 12 myristate AND skin tumor.

2.2 Selection criteria

Studies were included (i; ii; iii; iv) or removed (v; vi) based on the following criteria: (i) studies that investigate the effects of chemopreventive agents; (ii) studies with mice chemically induced by DMBA and TPA; (iii) only studies that reported the tumor incidence; (iv) only manuscripts that were written in English; (v) duplicate studies; (vi) reviews, *in vitro* studies, case reports, book chapters, comments to the editor, knockout or transgenic mice, and other types of skin cancer.

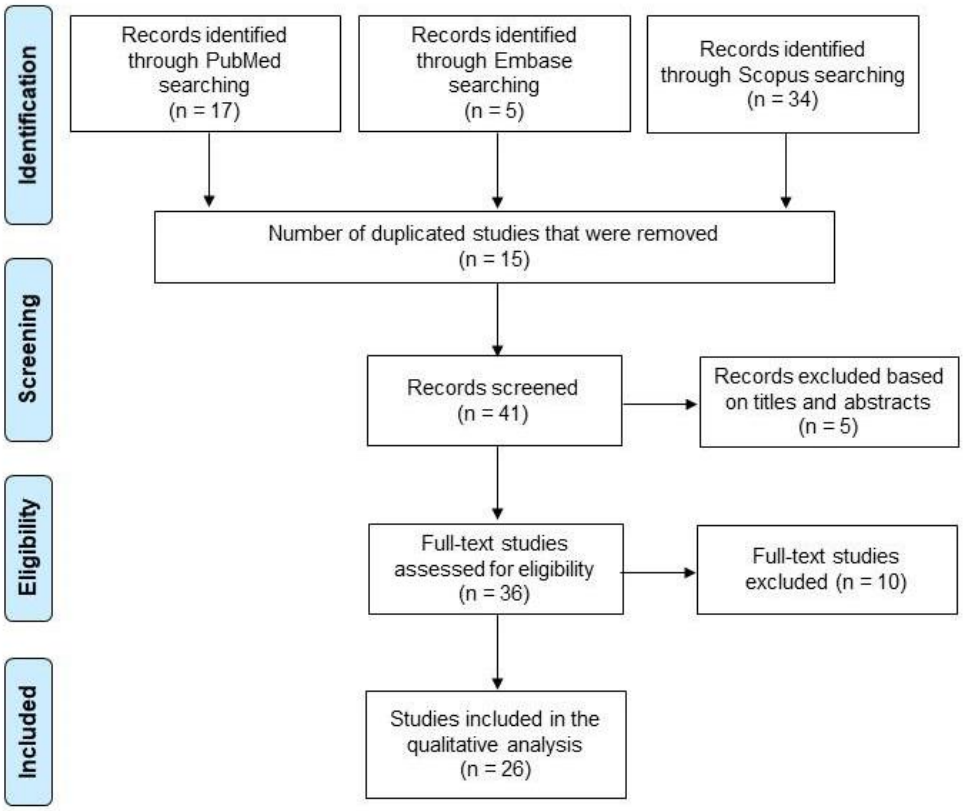
2.3 Data extraction

Two independent authors scanned the manuscripts based on the titles and abstracts. Then, the full text was read, and a third reviewer decided in case of disagreement. We excluded studies that did not use the two-stage skin carcinogenesis protocol or failed to analyze the tumor incidence. The data consisted of countries where the studies were conducted, name and a brief description of chemopreventive agents, the number of experiments in each study, variables such as doses or different compounds, route of administration, frequency, treatment times, and whether the chemopreventive agents were applied before DMBA, before TPA, or before both carcinogenic agent administration. We also extracted information about the mice, such as strain, number of animals per group, sex, initial weight, and age. Tumorigenesis analyses included tumor latency period, tumor multiplicity, and tumor incidence. In addition, other *in vivo* or *in vitro* results were reported.

3. RESULTS

The PRISMA diagram illustrates the selection process (Figure 1). We found 56 studies, of which 15 were duplicates. We excluded five studies in the first screening: one was a review, one used rats, and three used genetically manipulated animals. At the full-text screening, ten articles were excluded, eight of them did not provide tumor incidence, and two did not assess the effects of chemopreventive agents.

Figure 1. Study selection according to PRISMA guidelines.



Most studies (n = 21) used compounds extracted from parts of medicinal plants, such as roots, bark, flowers, seeds, or leaves. Chemopreventive agents were also extracted from bacteria (n = 1), algae (n = 1), oil from animals (n = 1), and synthetic drugs (n = 2). Only *Scutellaria baicalensis* and *Camellia sinensis* had different compounds extracted from them and were included in two studies each. Eight studies analyzed a single chemopreventive agent with different doses, one evaluated three chemopreventive agents, and five assessed other derivative compounds. Seven studies examined only a single variable, while four tested two variables. The route most administered was topical (n = 20), followed by oral (n = 4), topical and oral (n = 1), and intraperitoneal injections (n = 1). The most frequency and treatment times were twice a week (n = 18) for 20 weeks (n = 15).

Eighteen studies applied chemopreventive agents before TPA, four used them before DMBA/TPA administration, and two used them before DMBA administration. In addition, two studies analyzed two variables related to carcinogenic agents; one of them had a group that received the chemopreventive agent before TPA, and the other group received it before DMBA/TPA. One study administered a chemopreventive agent before DMBA administration and another before DMBA/TPA administration. Most studies used ICR mice (n = 13), followed by ICE (n = 3), and Swiss albino mice (n = 3). Most studies included only female mice (n = 16), five used males, two both sexes, and three did not provide this information. The smallest number of mice per group was

ten animals (n = 7), and the highest number was 20 (n = 4). The most frequent weight was 25–30 g (n = 4), however, twelve manuscripts did not report this information. Studies frequently used 6-week-old animals (n = 10); four studies did not mention the age.

For tumorigenesis analysis, we examined only results from the control group (DMBA/TPA) and the treated group (DMBA/TPA + chemopreventive agent). Twenty-four studies reported the appearance of the first tumor. The tumors in the control groups ranged from 4 to 9 weeks, and in the treated groups ranged from 6 to 17. Among the 68 experiments, 55 delayed the tumor latency period after chemopreventive treatment. The average papillomas per mouse ranged from 2 to 38 and 0 to 24 in the control and treated groups, respectively. Among 68 experiments, the tumor incidence ranged from 0 to ≤30% (n = 5), 31 to ≤60% (n = 24), 61 to ≤90% (n = 31), and ≥91% (n = 8). Rapamycin at doses of 200, 100, 50 (0%), and 20 nmol (8%) had the most significant effects on tumor incidence, followed by Peracetylated (-)-Epicatechin-3-gallate (AcEGCG) at a dose of 5 nmol (approximately 20%). The triterpene-quinone fraction from *Ardisia crispa* root hexane at 100 mg/kg and D-limonene at both doses (50 and 100 mg/kg) did not have any effect on tumor incidence. In addition, the *in vivo* and *in vitro* studies showed that chemopreventive agents decreased hyperplasia (n = 8) and the expression of inflammatory mediators such as COX-2 (n = 8) and NF-κB (n =7). Table 1 characterizes the 68 experiments performed in 26 studies.

Table 1. Characteristics of studies included in this integrative review

References	**Name and a brief description of chemopreventive agents**	**Number of experiments and Treatment variables:** a. Compounds (if any) b. Dose c. Route of administration d. Frequency e. Treatment times (week) f. Relationship with carcinogenic agents	**Samples:** a. Strain b. Number of mice per group c. Sex d. Initial weight (g), e. Initial age (wks)	**Tumor latency (wk):** Control (C) and Treated (T) Groups	**Tumor multiplicity (mean ± SEM or SD):** Control (C) and Treated (T) Groups	**Tumor incidence (%):** Control (C) and Treated (T) Groups	**Other results *in vivo* or *in vitro***
Akazawa *et al.* (2012)	Compounds were isolated from Hop (*Humulus lupulus* L.). Two of them were tested with DMBA/TPA protocol.	**1.** a. Compound 2 (lupulone C) b. 85 nmol c. Topical d. 2X/wk e. 20 wks f. Before TPA	a. ICE b. N = 15 c. — d.— e.—	C = 7 T = 8	C = 8.6 ± 1.6 T = 3.8 ± 1.4	C = 100 T = 87	↓TPA-induced ear inflammation ↓effects on Epstein-Barr virus early antigen (EBV-EA) activation
		2. a. Compound 14 (6-prenylnaringenin) *		T = 8	T = 3.2 ± 1.3	T = 87	
Akihisa *et al.* (2012)	Three prenylated chalcones were isolated from *Angelica keiskei*. The compounds were transformed by the fungus *Aspergillus saitoi*. Only compound 13 was tested with DMBA/TPA protocol.	**3.** a. Compound 13 b. 85 nmol c. Topical d. 2X/wk e. 20 wks f. Before TPA	a. ICE b. N = 15 c. — d. — e.—	C = 6 T = 8	C = 8.0 ± 1.3 T = 4.2 ± 0.6	C = 100 T = 87	↑cytotoxicity (Hoechst 33342) ↑cell death (Annexin V/Propidium Iodide double staining, nuclear fragmentation, and chromatin condensation) ↓effects on EBV-EA activation
Ali *et al.* (2014)	This research examined three plant extracts: seeds of *Trigonella foenumgraecum*, leaves of *Eclipta alba*, and flowers of *Calendula officinalis*	**4.** a. *Trigonella foenumgraecum* b. 10 mg/kg c. Topical d. 2X/wk e. 32 wks f. Before TPA	a. Swiss b. N = 20 c. Female d. 20−25 g e. —	—	C = 12.3 ± 2.4 T = 7.8 ± 2.5	C = 90 T = 65	↓Proliferating Cell Nuclear Antigen (PCNA) ↓lipid peroxidation level: malondialdehyde (MDA) ↑antioxidant enzyme levels: glutathione (GSH), glutathione peroxidase (GSH-Px), glutathione reductase (GR) ↓biomarkers of inflammation (myeloperoxidase activity
		5. a. *Eclipta alba* b. 10 mg/kg *			T = 9.3 ± 2.3	T = 70	

		6. a. *Calendula officinalis* b. 7 mg/kg *			T = 10.3 ± 3.2	T = 70	[MPO], nuclear factor-kappa B (NF-κB), and cyclooxygenase-2 (COX-2) activity ↑ p53 expression
Arimoto-Kobayashi *et al.* (2013)	The polyphenolic compound caftaric acid was isolated from the ethyl acetate fraction of Y-grape juice from *Vitis coignetiae* Pulliat.	**7.** b. × 2 c. Topical d. 2X/wk e. 20 wks f. Before TPA	a. SENCAR b. N = 12–16 c. Male d. 16−18 g e. 6 wks	C = 6 T = 7	C = 12.3 ± — T = 2.9 ± —	C = 100 T = 75	↓mutagenic activity of DMBA ↓TPA-induced ear inflammation ↓COX-2 activity
		8. b. × 10 c. Topical *		T = 9	T = 0.7 ± —	T = 56.3	
		9. b. × 2 c. Oral *	a. SENCAR b. N = 12 –14 c. Male	T = 8	C = 5.9 ± — T = 1.2 ± —	T = 86.7	
		10. b. × 10 c. Oral *	d. 16−18 g e. 6 wks	T = 10	T = 0.4 ± —	T = 37.5	
Bhatia *et al.* (2012)	The tamoxifen (TAM) is a synthetic non-steroidal estrogen modulator. The authors examined three liposomal formulations: elastic liposomes in gel (TAM-EL), conventional liposomes in gel (TAM-CL), and hydro-ethanolic solution (TAM-SOL).	**11.** a. TAM–EL b. 0.1% w/w c. Topical d. — e. 20 wks f. Before DMBA/TPA	a. LACA b. N = 10 c. Female d. 20−25 g e. 4–6 wks	C = 8 T = 11	—	C= 100 T = 48.7	↓keratinocyte pearls ↓abnormalities in skin structures
		12. a. TAM–CL *		T = 10		T = 71.6	
		13. a. TAM–SOL *		T = 9		T = 82.4	
		14. a. TAM–EL b. 0.1% w/w c. Topical d. — e. 21 wks f. Before TPA		T = 8		T = 58.3	
		15. a. TAM–CL *		T = 8		T = 77.7	
		16. a. TAM–SOL *		T = 8		T = 89.8	
Chaudhary *et al.* (2012)	D-limonene has been extracted from the essential oils of oranges.	**17.** b. 50 mg/kg c. Topical d. 3X/wk e. 21 wks f. Before TPA	a. Swiss b. N = 20 c. Female d. — e. 6–8 wks	C = 4 T = 8	C = 20.6 ± 2.6 T = 15.1 ± 1.8	C = 100 T = 100	↓TPA-induced ear inflammation ↓hyperplasia ↓COX-2 expression ↓ornithine decarboxylase (ODC) activity

		18. b.100 mg/kg *		T = 9	T = 11.2 ± 1.1	T = 100	↓[3H] thymidine incorporation. ↑antioxidant enzyme levels: GSH, GSH-Px, GR, glutathione S-transferases (GSTs), and catalase (CAT) ↑MDA ↓Ras and Raf expression ↓phosphorylation of extracellular signal-regulated protein kinase 1/2 (ERK 1/2) and ↓anti-apoptotic protein expression: Bcl-2 ↑pro-apoptotic signals: Bax
Checkley *et al.* (2011)	Rapamycin was initially discovered as an antifungal metabolite produced by *Streptomyces hygroscopicus*	**19.** b. 200 nmol c. Topical d. 2X/wk e. 25 wks f. Before TPA	a. FVB/N c. N =20 d. Female e. — f. 7–8 wks	C = ~9 T = 0	C = ~8 ± — T = 0	C = ~90 T = 0	↓hyperplasia ↓biomarkers of cell proliferation: PCNA and cyclin D1 expression ↓number of inflammatory cells: macrophages, T cells, neutrophils, and mast cells ↓phosphorylation of mammalian target of rapamycin (mTOR) and the mammalian target of rapamycin complex 1 (mTORC1) downstream targets ↑protein kinase B (Akt) phosphorylation
		20. b. 100 nmol *		T = 0	T = 0	T = 0	
		21. b. 50 nmol *		T = 0	T = 0	T = 0	
		22. b. 20 nmol *		T = ~17	T = ~1 ± —	T = 8	
		23. b. 5 nmol *		T = ~13	T = ~5 ± —	T = 51	
Chiou *et al.* (2013)	(-)-Epicatechin-3-gallate (EGCG) is a natural polyphenolic compound of green tea (*Camellia sinensis*). Peracetylated EGCG (AcEGCG) was synthesized to increase the lipophilicity and membrane permeability	**24.** a. EGCG b. 1 nmol c. Topical d. 2X/wk e. 20 wks f. Before TPA	a. ICR b. N = 12 c. Male/Female d. — e. 6 wks	C = 7 T = 11	C = ~30 ± — T = ~5 ± —	C = 100 T = ~80	↓p53 expression ↓expression of cell cycle biomarkers: p21, c-Myc, cyclin B1, p-cyclin-dependent kinase 1 (p-CDK1), protein kinase D1 (PKD1), and Cdc25A) ↑ERK1/2 ↓CD34+ expression ↓NF-κB ↓cyclic adenosine 3′,5′-monophosphate-responsive element-binding protein (CREB) ↓CCAAT enhancer-binding protein (C/EBPs) activation ↓c-Jun-N-terminal kinase ½ (JNK) ↓p38 phosphorylation ↓phosphatidylinositol 3-kinase
		25. a. EGCG b. 5 nmol *		T = 13	T = ~3 ± —	T = ~60	
		26. a. AcEGCG b. 1 nmol *		T = 11	T = ~4 ± —	T = ~70	
		27. a. AcEGCG b. 5 nmol *		T = 15	T = ~0.6 ± —	T = ~20	

							(PI3K)/Akt downstream targets ↓nitric oxide synthase (iNOS), COX-2, ODC ↓vascular endothelial growth factor (VEGF).
Enoki *et al.* (2012)	Agaro-oligosaccharides are produced when agarose, the main component of polysaccharides in agar, is treated with moderate acid conditions. Agar is a cell wall component of many red algae	**28.** b. 3% w/v c. Oral d. *Ad libitum* (water) e. 3 wks f. Before DMBA	a. ICR b. N = 10 c. Male d. — e. 7–9 wks	C = 8 T = 12	C = 19.2 ± — T = 2.3 ± —	C = 100 T = 70	↓nitric oxide (NO) levels ↓prostaglandin E2 ↓COX-2 and ↓microsomal PGE synthase-1
García-Fernández; Pérez-Martínez; García-Iglesias (2014)	Retinoic acid is a natural metabolite of circulating vitamin A. This compound has been extracted from animal oils	**29.** b. 30 mg/kg c. Oral d. 1X e. On day 11.5 of gestation f. Before DMBA/TPA	a. NMRI b. N = 19–20 c. Male/Female d. — e. 2 wks	—	C = 8.5 ± 4.5 T = 5.4 ± 3.7	C = 89.5 T = 80	↑cell proliferation Keratins (K) related to cell proliferative capacity: ↑K5 an early marker of malignant progression: ↑K13 Cell differentiation: ↑K1
Huang *et al.* (2020)	Oroxylin A has been extracted from *Scutellaria baicalensis*	**30.** b. 10 mg/kg c. Topical d. 5X/wk e. 20 wks f. Before TPA	a. ICR b. N = 10 c. Female d. 25–30 g e. 6 wks	C = 6 T = 10	C = ~20 ± — T = ~12 ± —	C = 100 T = ~80	↓hyperplasia ↓cell proliferation and ↓expression of inflammatory mediators: tumor necrosis factor α (TNF-α), interleukins (IL) IL-1β, IL-6, and IL-18) ↓SHC SH2 domain-binding protein 1 (SHCBP1)
		31. b. 40 mg/kg *		T = 12	T = ~4 ± —	T = ~60	
Hung *et al.* (2012)	Dimethyl dicarboxylate biphenyl is a synthetic analog of schizandrin C isolated from fructus *Schizandrae chinensis*	**32.** a. Compound 13 b. 85 nmol c. Topical d. 2X/wk e. 15 wks f. Before TPA	a. ICR b. N = 15 c. Female d. — e. 6 wks	C = 6 T = 8	C = 6.3 ± — T = 4.6 ± —	C = 100 T = 73.3	↓EBV-EA activation
		33. a. Compound 15 *		T = 8	T = 5.1 ± —	T = 73.3	
		34. a. Compound 19 *		T = 8	T = 4.4 ± —	T = 66.6	
Kapadia *et al.* (2013)	Lawsone is a reddish-orange pigment artifact formed during the extraction or preparation of the dye from *Lawsonia inermis* L. (Henna).	**35.** b. 0.015 mg/ml c. Topical d. Daily e. 20 wks f. Before DMBA	a. ICR b. N = 15 c. Female d. — e. 6 wks	C = 6 T = 7	C = 8.0 ± 0.9 T = 6.0 ± 0.1	C = 100 T = 93	↓EBV-EA activation ↓tumor incidence and multiplicity in another experimental model in vivo UV-B-inducted
Kong; Xu	Salidroside is a phenylpropanoid glycoside	**36.** b. 20 mg/kg	a. ICR	C = 8	C = ~38 ± —	C = 100	↓expression of inflammatory

(2018)	extracted from the root of *Rhodiola rosea* L.	c. Topical d. 5X/wk e. 20 wks f. Before TPA	b. N = 20 c. Female d. ~24 g e. 6–7 wks	T = 9	T = ~30 ± —	T = ~70	mediators: TNF-α, NF-κB, IL-1β, IL-18, IL-6, COX-2, and transforming growth factor β-1 (TGF-β1)
		37. b. 40 mg/kg *			T = 10 ± —	C = ~18 T = ~60	↑cell death (TUNEL assay and caspase-3 cleavage) ↑p53, ↑p21, ↑pro-apoptotic signals: PUMA and Bax
Lee *et al.* (2021)	The 3′-Hydroxypterostilbene is a natural pterostilbene analog isolated from *Sphaerophysa salsula*	**38.** b. 3 μmol c. Topical d. 2X/wk e. 10 wks f. Before DMBA	a. ICR b. N = 12 c. Female d. — e. 4–5 wks	C = 8 T = 8	C = ~8 ± — T = ~5 ± —	C = 83.3 T = ~70	↓hyperplasia ↓leukocyte infiltration ↓expression of inflammatory mediators: COX-2 and ODC ↓matrix metalloprotein-9 expression
		39. e. 20 wks f. Before DMBA/TPA *		T = 11	T = ~1 ± —	T = 58.3	↓cell cycle biomarkers (PCNA, Cyclin B1, CDK1) ↓p38 phosphorylation ↓signal transducer and activator of transcription phosphorylation (p-STAT-3) signaling pathways ↓P450 1A1 (CYP1A1) and cytochrome P450 1B1 (CYP1B1)
Liu *et al.* (2015)	Menthol is natural cyclic terpene alcohol of plant origin.	**40.** b. 20 mg/kg c. Topical d. 2X/wk e. 20 wks f. Before TPA	a. ICR b. N = 12 c. Female d. 25–30 g e. 6 wks	C = 4 T = 8	C = ~22 ± — T = ~10 ± —	C = 83.3 T = ~70	↓hyperplasia and ↓inflammatory mediators: COX-2 and NF-κB) ↓Erk ↓p38
		41. b. 80 mg/kg *		T = 9	T = ~4 ± —	T = 58.3	↓ MDA ↑antioxidant enzyme levels (GSH, CAT, GSH, GPx, and GST)
Ma *et al.* (2013)	Baicalein is one of the four significant flavonoids extracted from the root of *Scutellaria baicalensis*	**42.** b. 25 mg/kg c. Topical d. 3X/wk e. 30 wks f. Before TPA	a. C57BL/6 b. N = 18 c. Female d. 19–21 g e. 6–8 wks	C = 8 T = 14	C = 4.2 ± 0.6 T = 0.7 ± 0.2	C = 100 T = 61	↓hyperplasia ↓cell cycle biomarkers (Ki-67) ↑cell death (TUNEL assay) ↓number of inflammatory cells: mast cells, neutrophils, and macrophages ↓leukocyte infiltration
Md Roduan; Abd Hamid; Mohtarrudin (2019)	Annonacin is a mono-tetrahydrofuran acetogenin extracted from *Annona muricata* leaf and seed and other plants from the Annonaceae family	**43.** b. 85 nmol c. Topical d. 2X/wk e. 22 wks	a. ICR b. N = 10 c. Female d. 20–30 g	C = 6 T = 7	C = 2.2 ± 0.4 T = 1.6 ± 0.3	C = 88.6 T = 66.6	↓hyperkeratosis and dermal papillae ↓keratin pearl formation ↓gene expression of AKT, ERK,

		f. Before TPA	e. 6–7 wks				mTOR, p38, and Src ↑gene expression of PTEN
Nakajima; Nakae; Yasukawa (2013)	Cannabinoids are classified into three types: derived from *Cannabis sativa* L.; endogenous, and synthetic. Three synthetic cannabinoids were assessed.	**44.** a. Compound 3 from JWH-018 b. 0.02 µM, c. Topical d. 2X/wk e. 20 wks f. Before TPA	a. ICR b. N = 15 c. Female d. — e. 7 wks	C = 5 T = 7	C = 14.0 ± 9.9 T = 5.1 ± 4.9	C = 100 T = 60	↓TPA-induced ear inflammation
		45. a. Compound 3 from JWH-018 b. 0.2 µM *		T = 8	T = 1.5 ± 2.2	T = 33	
		46. a. Compound 5 from JWH-122 b. 0.2 µM *		T = 7	T = 6.3 ± 6.0	T = 60	
		47. a. Compound 5 from JWH-122 b. 2 µM *		T = 8	T = 2.3 ± 3.1	T = 40	
		48. a. Compound 6 from JWH-210 b. 0.2 µM *		T = 7	T = 7.0 ± 7.1	T = 60	
		49. a. Compound 6 from JWH-210 b. 2 µM *		T = 7	T = 3.2 ± 4.5	T = 40	
Sati *et al.* (2016)	Silibinin is the most active flavonolignans component of Silymarin, a polyphenol extracted from the milk thistle plant (*Silybum marianum).*	**50.** b. 500 mg/kg c. Oral d. 3X/wk e. 22 wks f. Before DMBA/TPA	a. LACA b. — c. Male d. 25–30 g e. 10–12 wks	C = 6 T = 7	C = ~4.2 ± — T = ~2.1 ± —	C = 88.6 T = 66.6	↑MDA ↑antioxidant enzyme levels as GSH levels ↓GR activity = GPx, CAT, and Superoxide Dismutase (SOD) activity ↑cell death (TUNEL assay)
Shebaby *et al.* (2017)	Three doses were tested using the F2 fraction from *Daucus carota* ssp. (wild carrot) oil extracts.	**51.** b. 10 mg/kg c. Intraperitoneal injections d. It was unclear e. It was unclear f. Before TPA	a. BALB/c b. N = 10 c. Male d. 18–21 g e. 6 wks	C = 6 T = 6	C = 13.3 ± — T = 10.9 ± —	C = 100 T = 80	↑cytotoxicity ↓anti-apoptotic protein expression: Bcl-2 ↑pro-apoptotic signals: Bax ↓gene expression of AKT and ERK phosphorylation
		52. b. 50 mg/kg *		T = 7	T = 9.6 ± —	T = 60	

		53. b. 200 mg/kg *		T = 7	T = 7.7 ± —	T = 50	
Tanaka *et al.* (2011)	The 3α-methoxyserrat-14-en-21β-ol (PJ-1) and 3β-methoxyserrat-14-en-21β-ol (PJ-2) are triterpenoid constituents from the bark of two *Picea* plants	**54.** a. Compound 11 from PJ-1 b. 85 nmol c. Topical d. 2X/wk e. 20 wks f. Before TPA	a. ICR b. N = 15 c. Female d. ~30 g e. 6 wks	C = 6 T = 8	C = 8 ± — T = 3.8 ± —	C = 100 T = 80	↓ effects on EBV-EA activation
		55. a. Compound 17 from PJ-1 *		T = 8	T = 4.8 ± —	T = 93	
		56. a. Compound 10 from PJ-2 *		T = 8	T = 4.1 ± —	T = 86	
Tsai *et al.* (2012)	Pterostilbene is a natural analog of resveratrol extracted from blueberries	**57.** b. 1 μmol c. Topical d. 2X/wk e. 20 wks f. Before TPA	a. ICR b. N = 12 c. Female d. 25–30 g e. 5–6 wks	C = 7 T = 7	C = 38 ± — T = 19 ± —	C = 100 T = ~80	↓expression of inflammatory mediators: iNOS, COX-2, and NF-κB ↓IkBα phosphorylation ↓p65 phosphorylation
		58. b. 5 μmol *		T = 7	T = 24 ± —	T = 100	↓ERK1/2 ↓p38 mitogen-activated protein kinase (MAPK) ↓JNK 1/2 ↓PI3K ↓Akt
Vyas *et al.* (2011)	EGCG is one of the main catechins of green tea, derived from the leaves of *Camellia sinensis*. Compounds 2–6 from O-Acyl-Substituted EGCG were tested	**59.** a. Compound 2 b. 50 mg/kg c. Oral d. 2X/wk e. 19 wks f. Before DMBA/TPA	a. Swiss b. N = 10 c. Female d. 18–22 g e. 6 wks	C = 5 T = 9	—	C = 100 T = 34	↓AP-1 (c-Jun), ↓NF-κB (p65) ↓p53 expression
		60. a. Compound 3 *		T = 9		T = 39	
		61. a. Compound 4 *		T = 9		T = 44	
		62. a. Compound 5 *		T = 7		T = 48	
		63. a. Compound 6 *		T = 7		T = 58	
Yeong *et al.* (2015)	A triterpene-quinone fraction was isolated from *Ardisia crispa* root hexane	**64.** b. 10 mg/kg, c. Topical d. 2X/wk e. 20 wks	a. ICR b. N = 10 c. Male d. 20–30 g	C = 9 T = 14	C = 2.0 ± — T = 1.0 ± —	C = 60 T = 33.3	↓MDA ↓cell death (TUNEL assay) ↓NF-κB expression ↓cell proliferation

		f. Before DMBA/TPA	e. 6–8 wks				
		65. b. 30 mg/kg *		T = 11	T = 2.3 ± —	T = 77.8	
		66. b. 100 mg/kg *		T = 14	T = 7.3 ± —	T = 100	
Zhang *et al.* (2012a)	Two compounds of cucurbitane triterpenoids were isolated from *Momordica charantia* leaves	**67.** a. Compound 1 b. 85 nmol c. Topical d. 2X/wk e. 20 wks f. Before TPA	a. ICE b. N = 15 c. — d. — e. —	C = 7 T = 8	C = 8.6 ± — T = 4.4 ± —	C = 100 T = 93	↓effects on EBV-EA activation. ↑cytotoxic (compounds 2, 5–7, 9, and 14)
		68. Compound 11 *		T = 9	T = 3.9 ± —	T = 93	

The symbol * means that the other variables are the same as in the previous experiment. The symbol — means that the data was not available.

4. DISCUSSION

Most chemopreventive agents effectively delay the tumor latency period, reduce the multiplicity of tumors, and decrease tumor incidence. We found that high doses of rapamycin reduce tumor incidence because this drug prevents the development of any tumors. Checkley *et al.* (2011) topically administered rapamycin 30 min prior to each TPA application. Rapamycin also reduced the number of inflammatory cells. Therefore, high doses increased Akt phosphorylation. A hypothesis assumes that compensatory mechanisms activate cell survival and growth (LI; KIM; BLENIS, 2014).

Tumor promotion causes morphological and biochemical changes, such as epidermal hyperplasia, inflammation, increased keratinocyte proliferation, and oxidative stress (SLAGA, 1983; SONG; BALMAIN, 2015). Phosphorylation is one of the most critical processes in the post-translational modification of proteins because it alters the interaction with other molecules, activating or inactivating proteins (CHAMCHEU *et al.*, 2019). TPA induces phosphorylation by activating the PI3K/Akt/mTOR and Ras/Raf/MAPK pathways; Akt phosphorylation activates the downstream targets Bad, Bax, caspases, and mTOR associated with cell death. Ras activation acts through the cascade phosphorylation of Raf, MEK, ERK, p38, and NF-κB. Both pathways play an essential role in cell survival, differentiation, proliferation, inflammation, metabolism, apoptosis, and motility. Dysregulation of both pathways is related to the development of SCC. Thus, skin cancer treatments seek to inhibit both signaling pathways (CAMALIER *et al.*, 2010; MENDOZA; ER; BLENIS, 2011; OZES *et al.*, 1999).

Rapamycin acts in the mTOR signaling pathway, particularly by inhibiting the mTORC1 complex, which functions as a nutrient sensor and regulates metabolism and cell growth (LI; KIM; BLENIS, 2014; PÓPULO; LOPES; SOARES, 2012). Rapamycin also delayed tumor progression and prolonged animal survival in a head and neck SCC genetic mouse model (SUN *et al.*, 2012). However, unfavorable pharmacokinetic characteristics have led to the development of analogs (rapalogs) to improve the treatment of some cancer types. Rapalogs such as everolimus, temsirolimus, and ridaforolimus confer the exact mechanism of action, as found in rapamycin. These drugs improve the sensitivity to chemotherapy and radiotherapy; moreover, when combined with other substances, they act as a therapeutic option (LI; KIM; BLENIS, 2014; LIAO; KIM; YEN, 2011). Randomized clinical trials have demonstrated that everolimus prolongs survival in patients with advanced renal cell carcinoma and pancreatic tumors (MOTZER *et al.*, 2008; YAO *et al.*, 2011).

Green tea derivatives also significantly reduced tumor incidence. Chiou *et al.* (2013) synthesized an analog compound, AcEGCG, and compared its effects with those of (-)-Epicatechin-3-gallate (EGCG). Fewer tumors developed in the AcEGCG group than in the EGCG group. In addition, AcEGCG decreased cell proliferation and induced cell cycle arrest in the G2/M phase by inhibiting the cyclin B1/CDK1 complex. Vyas *et al.* (2011) used O-Acyl-substituted EGCG that

delayed tumor latency and reduced tumor incidence. Green tea has been extensively consumed worldwide; it contains catechins, the primary polyphenol compounds derived from *Camellia sinensis*. EGCG is one of the most beneficial compounds for health because of its antioxidative, anti-inflammatory, and anticancer activities; its disadvantages correspond to its hydrophilic characteristics and low absorption. Analogs of EGCG have improved their pharmacological properties *in vivo* tissues (LANDIS-PIWOWAR *et al.*, 2013; LI *et al.*, 2018).

A randomized controlled trial was conducted for 12 months of green tea supplementation in healthy postmenopausal women who received four capsules daily. The percentage of mammographic density, a predictor of breast cancer risk, remained unchanged after treatment. In younger women, the percentage of mammographic density was reduced, indicating an age-dependent effect (SAMAVAT *et al.*, 2017). Other research shows that green tea was more effective when administered topically rather than orally in both inflammatory and non-inflammatory lesions of acne vulgaris (KIM *et al.*, 2021). EGCG can induce cell death by apoptosis, independent apoptosis pathways, and autophagy in human cancer cells, probably by mechanisms involving lysosomal membrane permeabilization related to high ROS production (ZHANG *et al.*, 2012b). New tests with the peracetylated EGCG analog could clarify whether the effects improved during oral administration.

A lower dose of triterpene-quinone fraction isolated from *Ardisia crispa* root extract significantly reduced the tumor incidence. However, the highest dose maintained the value of tumor incidence; moreover, tumors appeared three weeks earlier in the treatment group compared with the control group (YEONG *et al.*, 2015). The hexane fraction isolated from the same plant effectively reduced tumor incidence at low doses (ROSLIDA; FEZAH; YEONG, 2011), and higher doses inhibited tumors in the initiation stage on mice skins (HAMID *et al.*, 2013). A quinone-rich fraction from *Ardisia crispa* at lower doses exerted more significant effects during the promotion than the initiation stage (SULAIMAN *et al.*, 2012). The hydromethanolic extract and ethyl acetate extract isolated from *Ardisia crispa* were cytotoxic to the MCF-7 breast cancer cell line (NORDIN *et al.*, 2018). This plant can be an alternative preventative measure for skin cancer and other cancer types; however, further investigations are needed to reveal the effects of different doses and derivatives to suppress carcinogenesis.

Topical administration of D-limonene left tumor incidence unchanged in both doses; however, this chemopreventive agent decreased the expression of the Ras pathway and Bcl-2 anti-apoptotic protein and increased the expression of Bax pro-apoptotic protein. D-limonene also reduced tumor multiplicity and delayed the tumor latency period (CHAUDHARY *et al.*, 2012). D-limonene is a monocyclic monoterpene found in citrus plants. Monoterpenes belong to the terpenoid category, and their main benefits are related to their anticancer, antioxidative, and anti-inflammatory properties (ANANDAKUMAR; KAMARAJ; VANITHA, 2021). D-limonene showed significant anti-

inflammatory effects in a rat model of colitis (D'ALESSIO *et al.*, 2013). In a clinical trial, patients with advanced solid tumors revealed low toxicity after a single and repetitive dose by oral administration during 11 months of treatment (VIGUSHIN *et al.*, 1998).

Patients used D-limonene 2–6 weeks before breast cancer surgery; their concentrations were higher in breast tissue than in blood plasma. In contrast, the primary metabolite, perillic acid, was not concentrated in the target tissue, indicating low bioactivity. In addition, D-limonene decreased cyclin D expression; however, it had minimal effects on cell proliferation and apoptosis markers (MILLER *et al.*, 2013). The effects of chemopreventive agents may vary; as reported by García-Fernández; Pérez-Martínez; García-Iglesias (2014) prenatal exposure to retinoic acid decreases tumor multiplicity, reduces tumor incidence, and delays tumor latency. However, other analyses showed tumor progression to the malignant stage.

Each experiment compared the tumorigenesis criteria between the control group (DMBA/TPA) and the treated group (chemopreventive agent/DMBA/TPA). Even under various DMBA/TPA protocols, in most studies, mice received a single dose of DMBA, and after one week, they received twice or thrice weekly doses of TPA for 20 weeks. The advantage of this protocol is that it recognizes the effects of chemopreventive agents in both the initiation and promotion stages. DMBA is an initiation agent that causes mutations in the cell genome. TPA is a tumor promoter that increases the proliferation of damaged cells, recruits inflammatory cells, produces ROS, and reduces DNA repair mechanisms (NEAGU *et al.*, 2016; SONG; BALMAIN, 2015). Some papillomas may progress to the malignant stage; in carcinomas, rupturing the basement membrane leads to tumor cells invading the underlying tissues (CHANG; CHAUDHURI, 2019). The role of chemopreventive agents is to block initiating agent activity or inhibit the promoting agent's effects (MELO *et al.*, 2018).

Two studies (ENOKI *et al.*, 2012; KAPADIA *et al.*, 2013) used chemopreventive agents before DMBA treatment; the agaro-oligosaccharides and lawsone reduced the tumor incidence by 30% and 7%, respectively. Four manuscripts (GARCÍA-FERNÁNDEZ; PÉREZ-MARTÍNEZ; GARCÍA-IGLESIAS, 2014; SATI *et al.*, 2016; VYAS *et al.*, 2011; YEONG *et al.*, 2015) reported using chemopreventive agents (retinoic acid, silibinin, compounds of O-Acyl-substituted EGCG, and the lowest dose of *Ardisia crispa*) before DMBA and TPA. These substances reduced tumor incidence ranging from 22% to 66%. Lee *et al.* (2021) revealed that tumor incidence and multiplicity results were more evident in the anti-initiation/anti-promotion group than in the anti-initiation group. In most studies, chemopreventive agents were administered 30 min or one hour before TPA. This finding supports that chemopreventive agents have been more effective in inhibiting the effects of TPA than blocking DMBA actions (DUNN; UMAR; RICHMOND, 2016).

Our study limitation was the lack of values regarding the tumor latency period, multiplicity, and size in all studies. Moreover, some studies did not mention the animal strain, the number of

animals in the group, sex, age, and weight. However, these data should be present in all animal studies because these factors affect the results. We standardized our criteria so that all studies showed tumor incidence to examine whether a chemopreventive agent could block the development of tumors. However, we know that a single criterion cannot determine whether a chemopreventive agent will be effective. Therefore, we included the other available results. Seventeen studies had tumor multiplicity values; in seven of them, we approximated the values by examining the graphs or calculating the mean when the authors provided the total number of tumors, and only two studies did not mention it. Chemopreventive agents in humans may not produce the same effects as those obtained in the experimental model; however, standardizing tumorigenesis analyses may reduce the bias of studies.

5. CONCLUSION

The therapeutic properties of chemopreventive agents have been studied for a long time. We showed that high doses of rapamycin prevented the development of papillomas. In addition, green tea derivatives showed a significant reduction in tumor incidence. Compounds of *Ardisia crispa* and D-limonene did not alter papilloma incidence. In this review, we also show other characteristics to evaluate the effectiveness of these substances, such as reducing tumor multiplicity and delaying the tumor latency period. In addition, most chemopreventive agents reduce cell proliferation biomarkers, increase cell death, reduce inflammatory mediators, and elevate antioxidant enzyme levels. We expect that this review will be crucial to improving standardization in animal models using the two-stage skin carcinogenesis protocol and highlighting potential candidates for randomized clinical trials for future skin cancer prevention and treatment research.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

ABEL, E. L. *et al.* Multi-stage chemical carcinogenesis in mouse skin: Fundamentals and applications. **Nat Protoc**, v. 4, n. 9, p. 1350–1362, 2009. DOI: 10.1038/nprot.2009.120.

AKAZAWA, H. *et al.* Anti-inflammatory and anti-tumor-promoting effects of 5-deprenyllupulonol C and other compounds from hop (*Humulus lupulus* L.). **Chem Biodivers**, v. 9, n. 6, p. 1045–1054, 2012. DOI: 10.1002/cbdv.201100233.

AKIHISA, T. *et al.* Cytotoxic activities and anti-tumor-promoting effects of microbial transformation products of prenylated chalcones from *Angelica keiskei*. **Chem Biodivers**, v. 9, n. 2, p. 318–330, 2012. DOI: 10.1002/cbdv.201100255.

ALI, F. *et al.* Assessment of augmented immune surveillance and tumor cell death by cytoplasmic stabilization of p53 as a chemopreventive strategy of 3 promising medicinal herbs in murine 2-stage skin carcinogenesis. **Integr Cancer Ther**, v. 13, n. 4, p. 351–367, 2014. DOI: 10.1177/1534735413513831.

ANANDAKUMAR, P.; KAMARAJ, S.; VANITHA, M. K. D-limonene: A multifunctional compound with potent therapeutic effects. **J Food Biochem**, v. 45, n. 1, p. e13566, 2021. DOI: 10.1111/jfbc.13566.

AOTO, Y. *et al.* Time-Series analysis of tumorigenesis in a murine skin carcinogenesis model. **Sci Rep**, v. 8, n. 1, p. 12994, 2018. DOI: 10.1038/s41598-018-31349-x.

ARIMOTO-KOBAYASHI, S. *et al.* Chemopreventive effects of the juice of *Vitis coignetiae* pulliat on two-stage mouse skin carcinogenesis. **Nutr Cancer**, v. 65, n. 3, p. 440–50, 2013. DOI: 10.1080/01635581.2013.767916.

ARMSTRONG, B. K.; KRICKER, A. The epidemiology of UV induced skin cancer. **J Photochem Photobiol B**, v. 63, n. 1–3, p. 8–18, 2001. DOI: 10.1016/s1011-1344(01)00198-1.

BHATIA, A. *et al.* Tamoxifen-loaded novel liposomal formulations: Evaluation of anticancer activity on DMBA-TPA induced mouse skin carcinogenesis. **J Drug Target**, v. 20, n. 6, p. 544–50, 2012. DOI: 10.3109/1061186X.2012.694887.

BRAY, F. *et al.* Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. **CA Cancer J for Clin**, v. 68, n. 6, p. 394–424, 2018. DOI: 10.3322/caac.21492.

CAMALIER, C. E. *et al.* Elevated phosphate activates N-ras and promotes cell transformation and skin tumorigenesis. **Cancer Prev Res (Phila)**, v. 3, n. 3, p. 359–370, 2010. DOI: 10.1158/1940-6207.CAPR-09-0068.

CHAMCHEU, J. C. *et al.* Role and therapeutic targeting of the PI3K/Akt/mTOR signaling pathway in skin cancer: A review of current status and future trends on natural and synthetic agents therapy. **Cells**, v. 31, n. 8 (8), p. 803, 2019. DOI: 10.3390/cells8080803.

CHANG, J.; CHAUDHURI, O. Beyond proteases: Basement membrane mechanics and cancer invasion. **J Cell Biol**, v. 218, n. 8, p. 2456–2469, 2019. DOI: 10.1083/jcb.201903066.

CHAUDHARY, S. C. *et al.* D-Limonene modulates inflammation, oxidative stress and Ras-ERK pathway to inhibit murine skin tumorigenesis. **Hum Exp Toxicol**, v. 31, n. 8, p. 798–811, 2012. DOI: 10.1177/0960327111434948.

CHECKLEY, L. A. *et al.* Rapamycin is a potent inhibitor of skin tumor promotion by 12-O-tetradecanoylphorbol-13-acetate. **Cancer Prev Res**, v. 4, n. 7, p. 1011–1020, 2011. DOI: 10.1158/1940-6207.CAPR-10-0375.

CHIOU, Y. S. *et al.* Peracetylated (-)-epigallocatechin-3-gallate (AcEGCG) potently prevents skin carcinogenesis by suppressing the PKD1-dependent signaling pathway in CD34+ skin stem cells and skin tumors. **Carcinogenesis**, v. 34, n. 6, p. 1315–1322, 2013. DOI: 10.1093/carcin/bgt042.

COHEN, S. M.; ARNOLD, L. L. Chemical carcinogenesis. **Toxicol Sci**, v. 120, n. 1, p. 76–92, 2011. DOI: 10.1093/toxsci/kfq365.

D'ALESSIO, P. A. *et al.* Oral administration of d-limonene controls inflammation in rat colitis and displays anti-inflammatory properties as diet supplementation in humans. **Life Sci**, v. 92, n. 24–26, p. 1151–1156, 2013. DOI: 10.1016/j.lfs.2013.04.013.

DIGIOVANNI, J. Multistage carcinogenesis in mouse skin. **Pharmacol Ther**, v. 54, n. 1, p. 63–128, 1992. DOI: 10.1016/0163-7258(92)90051-z.

DUNN, B. K.; UMAR, A.; RICHMOND, E. Introduction: Cancer chemoprevention and its context. **Semin Oncol**, v. 43, n. 1, p. 19–21, 2016. DOI: 10.1053/j.seminoncol.2015.11.002.

ENOKI, T. *et al.* Anti-tumor-promoting activities of agaro-oligosaccharides on two-stage mouse skin carcinogenesis. **Biol Pharm Bull**, v. 35, n. 7, p. 1145–1149, 2012. DOI: 10.1248/bpb.b12-00188.

GARCÍA-FERNÁNDEZ, R. A.; PÉREZ-MARTÍNEZ, C.; GARCÍA-IGLESIAS, M. J. In vivo long-term effects of retinoic acid exposure in utero on induced tumours in adult mouse skin. **Vet Dermatol**, v. 25, n. 6, p. 538–546, 2014. DOI: 10.1111/vde.12149.

HAMID, R. A. *et al.* Chemopreventive effect of *Ardisia crispa* hexane fraction on the peri-initiation phase of mouse skin tumorigenesis. **Med Princ Pract**, v. 22, n. 4, p. 357–361, 2013. DOI: 10.1159/000346622.

HUANG, H. *et al.* Oroxylin A inhibits carcinogen-induced skin tumorigenesis through inhibition of inflammation by regulating SHCBP1 in mice. **Int Immunopharmacol**, v. 80, p. 106123, 2020. DOI: 10.1016/j.intimp.2019.106123.

HUNG, H. Y. *et al.* Cancer preventive agents 11. Novel analogs of dimethyl dicarboxylate biphenyl as potent cancer chemopreventive agents(†). **Pharm Biol**, v. 50, n. 1, p. 18–24, 2012. DOI: 10.3109/13880209.2011.608078.

KAPADIA, G. J. *et al.* Chemoprevention of skin cancer: effect of *Lawsonia inermis* L. (Henna) leaf powder and its pigment artifact, lawsone in the Epstein- Barr virus early antigen activation assay and in two-stage mouse skin carcinogenesis models. **Anticancer Agents Med Chem**, v. 13, n. 10, p. 1500–1507, 2013. DOI: 10.2174/18715206113139990096.

KIM, S. *et al.* The effects of green tea on acne vulgaris: A systematic review and meta-analysis of randomized clinical trials. **Phytother Res**, v. 35, n. 1, p. 374–383, 2021. DOI: 10.1002/ptr.6809.

KONG, Y. H.; XU, S. P. Salidroside prevents skin carcinogenesis induced by DMBA/TPA in a mouse model through suppression of inflammation and promotion of apoptosis. **Oncol Rep**, v. 39, n. 6, p. 2513–2526, 2018. DOI: 10.3892/or.2018.6381.

KUBO, Y. *et al.* Molecular tumorigenesis of the skin. **J Med Invest**, v. 61, n. 1–2, p. 7–14, 2014. DOI: 10.2152/jmi.61.7.

LANDIS-PIWOWAR, K. *et al.* Novel epigallocatechin gallate analogs as potential anticancer agents: a patent review (2009 - present). **Expert Opin Ther Pat**, v. 23, n. 2, p. 189–202, 2013. DOI: 10.1517/13543776.2013.743993.

LEE, P. S. *et al.* 3'-Hydroxypterostilbene inhibits 7,12-Dimethylbenz[a]anthracene (DMBA)/12-O-tetradecanoylphorbol-13-acetate (TPA)-induced mouse skin carcinogenesis. **Phytomedicine**, v. 81, p. 153432, 2021. DOI: 10.1016/j.phymed.2020.153432.

LI, F. *et al.* Perspectives on the recent developments with green tea polyphenols in drug discovery. **Expert Opin Drug Discov**, v. 13, n. 7, p. 643–660, 2018. DOI: 10.1080/17460441.2018.1465923.

LI, J.; KIM, S. G.; BLENIS, J. Rapamycin: one drug, many effects. **Cell Metab**, v. 19, n. 3, p. 373–379, 2014. DOI: 10.1016/j.cmet.2014.01.001.

LIAO, Y. M.; KIM, C.; YEN, Y. Mammalian target of rapamycin and head and neck squamous cell carcinoma. **Head Neck Oncol**, v. 3, n. 22, 1–7, 2011. DOI: 10.1186/1758-3284-3-22.

LIU, Z. *et al.* Chemopreventive efficacy of menthol on carcinogen-induced cutaneous carcinoma through inhibition of inflammation and oxidative stress in mice. **Food Chem Toxicol**, v. 82, n. 1, p. 12–18, 2015. DOI: 10.1016/j.fct.2015.04.025.

MA, G. Z. *et al.* Baicalein inhibits DMBA/TPA-induced skin tumorigenesis in mice by modulating proliferation, apoptosis, and inflammation. **Inflammation**, v. 36, n. 2, p. 457–467, 2013. DOI: 10.1007/s10753-012-9566-y.

MD RODUAN, M. R.; ABD HAMID, R. A.; MOHTARRUDIN, N. Modulation of cancer signalling pathway(s) in two-stage mouse skin tumorigenesis by annonacin. **BMC Complement Altern Med**, v. 19, n. 238, p. 1–16, 2019. DOI: 10.1186/s12906-019-2650-1.

MELO, F. H. M. *et al.* Cancer chemoprevention: Classic and epigenetic mechanisms inhibiting tumorigenesis. What have we learned so far? **Front Oncol**, v. 8, n. 644, p. 1–15, 2018. DOI: 10.3389/fonc.2018.00644.

MENDOZA, M. C.; ER, E. E.; BLENIS, J. The Ras-ERK and PI3K-mTOR pathways: cross-talk and compensation. **Trends Biochem Sci**, v. 36, n. 6, p. 320–328, 2011. DOI: 10.1016/j.tibs.2011.03.006.

MILLER, J. A. *et al.* Human breast tissue disposition and bioactivity of limonene in women with early-stage breast cancer. **Cancer Prev Res (Phila)**, v. 6, n. 6, p. 577–584, 2013. DOI: 10.1158/1940-6207.CAPR-12-0452.

MOHER, D. *et al.* Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. **Ann Intern Med**, v. 151, n. 4, p. 264–269, 2009. DOI: 10.7326/0003-4819-151-4-200908180-00135.

MOTZER, R. J. *et al.* Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo-controlled phase III trial. **Lancet**, v. 372, n. 9637, p. 449–456, 2008. DOI: 10.1016/S0140-6736(08)61039-9.

NAKAJIMA, J.; NAKAE, D.; YASUKAWA, K. Structure-dependent inhibitory effects of synthetic cannabinoids against 12-O-tetradecanoylphorbol-13-acetate-induced inflammation and skin tumour promotion in mice. **J Pharm Pharmacol**, v. 65, n. 8, p. 1223–1230, 2013. DOI: 10.1111/jphp.12082.

NEAGU, M. *et al.* Chemically induced skin carcinogenesis: Updates in experimental models (Review). **Oncol Rep**, v. 35, n. 5, p. 2516-2528, 2016. DOI: 10.3892/or.2016.4683.

NORDIN, M. L. *et al.* In vitro investigation of cytotoxic and antioxidative activities of *Ardisia crispa* against breast cancer cell lines, MCF-7 and MDA-MB-231. **BMC Complement Altern Med**, v. 18, n. 87, p. 1–10, 2018. DOI: 10.1186/s12906-018-2153-5.

OZES, O. N. *et al.* NF-kappaB activation by tumour necrosis factor requires the Akt serine-threonine kinase. **Nature**, v. 401, n. 6748, p. 82–85, 1999. DOI: 10.1038/43466.

PÓPULO, H.; LOPES, J. M.; SOARES, P. The mTOR signalling pathway in human cancer. **Int J Mol Sci**, v. 13, n. 2, p. 1886–1918, 2012. DOI: 10.3390/ijms13021886.

QUE, S. K. T.; ZWALD, F. O.; SCHMULTS, C. D. Cutaneous squamous cell carcinoma: Incidence, risk factors, diagnosis, and staging. **J Am Acad of Dermatol**, v. 78, n. 2, p. 237–247, 2018. DOI: 10.1016/j.jaad.2017.08.059.

REEVES, M. Q. *et al.* Multi-color lineage tracing reveals clonal dynamics of squamous carcinoma evolution from initiation to metastasis. **Nat Cell Biol**, v. 20, n. 6, p. 699–709, 2018. DOI: 10.1038/s41556-018-0109-0.

ROSLIDA, A. H.; FEZAH, O.; YEONG, L. T. Suppression of DMBA/croton oil-induced mouse skin tumor promotion by *Ardisia crispa* root hexane extract. **Asian Pac J Cancer Prev**, v. 12, n. 3, p. 665–669, 2011.

SAMAVAT, H. *et al.* A randomized controlled trial of green tea extract supplementation and mammographic density in postmenopausal women at increased risk of breast cancer. **Cancer Prev Res (Phila)**, v. 10, n. 12, p. 710–718, 2017. DOI: 10.1158/1940-6207.CAPR-17-0187.

SÁNCHEZ-DANÉS, A. *et al.* Defining the clonal dynamics leading to mouse skin tumour initiation. **Nature**, v. 536, n. 7616, p. 298–303, 2016. DOI: 10.1038/nature19069.

SATI, J. *et al.* Pro-oxidant role of silibinin in DMBA/TPA induced skin cancer: 1H NMR metabolomic and biochemical study. **PLoS One**, v. 11, n. 7, p. e0158955, 2016. DOI: 10.1371/journal.pone.0158955.

SHEBABY, W. N. *et al.* Wild carrot pentane-based fractions suppress proliferation of human HaCaT keratinocytes and protect against chemically-induced skin cancer. **BMC Complement Altern Med**, v. 17, n. 149, p. 1–14, 2017. DOI: 10.1186/s12906-017-1661-z.

SLAGA, T. J. Overview of tumor promotion in animals. **Environ Health Perspect**, v. 50, n. 1, p. 3–14, 1983. DOI: 10.1289/ehp.83503.

SONG, I. Y.; BALMAIN, A. Cellular reprogramming in skin cancer. **Semin Cancer Biol**, v. 32, n. 1, p. 32–39, 2015. DOI: 10.1016/j.semcancer.2014.03.006.

STRATIGOS, A. J. *et al.* European interdisciplinary guideline on invasive squamous cell carcinoma of the skin: Part 1. epidemiology, diagnostics and prevention. **Eur J Cancer**, v. 128, n. 1, p. 60–82, 2020. DOI: 10.1016/j.ejca.2020.01.007.

SULAIMAN, H. *et al.* Anti-tumor effect of *Ardisia crispa* hexane fraction on 7, 12-dimethylbenz[α]anthracene-induced mouse skin papillomagenesis. **J Cancer Res Ther**, v. 8, n. 3, p. 404–410, 2012. DOI: 10.4103/0973-1482.103521.

SUN, Z. J. *et al.* Chemopreventive and chemotherapeutic actions of mTOR inhibitor in genetically defined head and neck squamous cell carcinoma mouse model. **Clin Cancer Res**, v. 18, n. 19, p. 5304–5313, 2012. DOI: 10.1158/1078-0432.CCR-12-1371.

SURH, Y. Molecular mechanisms of chemopreventive effects of selected dietary and medicinal phenolic substances. **Mutat Res**, v. 428, n. 1–2, p. 305–327, 1999. DOI: 10.1016/s1383-5742(99)00057-5.

TANAKA, R. *et al.* Conjugates of 3α-methoxyserrat-14-en-21β-ol (PJ-1) and 3β-methoxyserrat-14-en-21β-ol (PJ-2) as cancer chemopreventive agents. **Eur J Med Chem**, v. 46, n. 8, p. 3368–3375, 2011. DOI: 10.1016/j.ejmech.2011.04.062.

TSAI, M. L. *et al.* Pterostilbene, a natural analogue of resveratrol, potently inhibits 7,12-dimethylbenz[a]anthracene (DMBA)/12-O-tetradecanoylphorbol-13-acetate (TPA)-induced mouse skin carcinogenesis. **Food and Function**, v. 3, n. 11, p. 1185–1194, 2012. DOI: 10.1039/c2fo30105a.

VIGUSHIN, D. M. *et al.* Phase I and pharmacokinetic study of D-limonene in patients with advanced cancer. Cancer Research Campaign Phase I/II Clinical Trials Committee. **Cancer Chemother Pharmacol**, v. 42, n. 2, p. 111–117, 1998. DOI: 10.1007/s002800050793.

VYAS, S. *et al.* Potential O-acyl-substituted (-)-Epicatechin gallate prodrugs as inhibitors of DMBA/TPA-induced squamous cell carcinoma of skin in Swiss albino mice. **Chem Biodivers**, v. 8, n. 4, p. 599–613, 2011. DOI: 10.1002/cbdv.201000219.

YAO, J. C. *et al.* Everolimus for advanced pancreatic neuroendocrine tumors. **N Engl J Med**, v. 364, n. 6, p. 514–523, 2011. DOI: 10.1056/NEJMoa1009290.

YEONG, L. T. *et al.* Low dose triterpene-quinone fraction from *Ardisia crispa* root precludes chemical-induced mouse skin tumor promotion. **BMC Complement Altern Med**, v. 5, n. 431, p. 1–11, 2015. DOI: 10.1186/s12906-015-0954-3.

ZHANG, J. *et al.* Cucurbitane triterpenoids from the leaves of *Momordica charantia*, and their cancer chemopreventive effects and cytotoxicities. **Chem Biodivers**, v. 9, n. 2, p. 428–440, 2012a. DOI: 10.1002/cbdv.201100142.

ZHANG, Y. *et al.* (-)-Epigallocatechin-3-gallate induces non-apoptotic cell death in human cancer cells via ROS-mediated lysosomal membrane permeabilization. **PLoS One**, v. 7, n. 10, p. e46749, 2012b. DOI: 10

Recebido em: 25/06/2022
Aceito em: 27/09/2022