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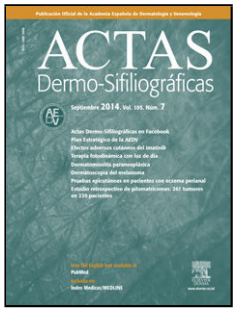

Systematic review on dietary supplements in the prevention and/or treatment of actinic keratosis and field cancerization

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Sección: revisión

Systematic review on dietary supplements in the prevention and/or treatment of actinic keratosis and field cancerization

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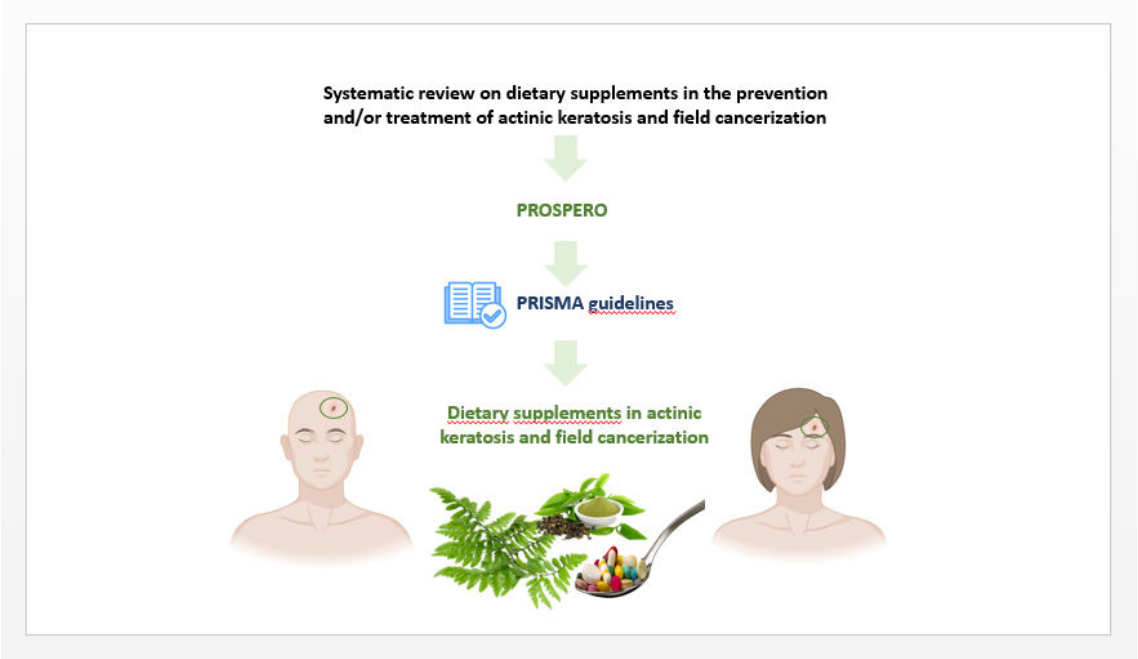
Graphical Abstract



ABSTRACT:

Background: Actinic keratoses (AKs) are chronic, recurrent precancerous lesions primarily induced by long-term sun exposure, commonly found on sun-exposed areas such as the face, neck, hands, forearms, and lower legs. AKs are prevalent, affecting millions worldwide, and pose a significant risk of transforming into invasive squamous cell carcinomas (SCCs).

Objective: This systematic review aims to update the scientific evidence on the role of oral bioactives, nutraceuticals, or dietary supplements in the treatment of AKs and field cancerization, while evaluating their safety and efficacy profile.

Methods: A review plan was pre-registered in the PROSPERO database (CRD42023485985). Following the PRISMA guidelines, we identified, selected, and included relevant studies. We screened a total of 234 articles, thoroughly reviewed 38 full texts, and ultimately included 21 articles published from 2013 through 2023 for analysis.

Results: The selected studies were categorized into 3 groups based on the chemical nature of the supplements: polyphenols (11 studies), vitamins (8 studies), and others (2 studies). The studies included preclinical (9) and clinical (12) studies. Clinical trials have demonstrated the efficacy profile of polyphenolic supplements, such as *Polypodium leucotomos* extract (PLE) and others in improving skin health and reducing the risk of

skin cancers. Preclinical studies highlighted the protective effects of polyphenols against UV-induced damage and neoplastic transformation. Vitamin supplementation studies revealed mixed results, with clear data showing benefits in reducing the risk of precancerous lesions and skin cancers after nicotinamide (NAM) treatment, while others did not demonstrate significant protective effects.

Conclusions: The review confirms the efficacy of polyphenols in preventing and treating AKs and related skin conditions. However, the role of vitamins and other supplements requires further investigation due to inconsistent and/or scarce findings. Future clinical trials should focus on diverse populations at higher risk of skin cancer and explore new ingredients as well as combinations of various ingredients to optimize therapeutic applications.

KEYWORDS

Actinic keratoses, precancerous lesions, polyphenols, vitamins, dietary supplements, skin cancer prevention, photoprotection, systematic review.

Revisión sistemática sobre suplementos dietéticos en la prevención y/o tratamiento de la queratosis actínica y el campo de cancerización

RESUMEN:

Antecedentes: Las queratosis actínicas (QA) son lesiones crónicas y recurrentes precancerosas, inducidas principalmente por la exposición solar prolongada, y que se encuentran comúnmente en áreas expuestas al sol, como la cara, el cuello, las manos, los antebrazos y las piernas. Las QA son muy frecuentes, afectando a millones de personas en todo el mundo, y presentan un riesgo significativo de transformarse en carcinomas escamosos invasivos (CEI).

Objetivo: Esta revisión sistemática tiene como objetivo actualizar la evidencia científica sobre el papel de los bioactivos orales, nutracéuticos o suplementos dietéticos en el tratamiento de las QA y el campo de cancerización, evaluando su seguridad y eficacia.

Métodos: El plan de revisión fue registrado en la base de datos PROSPERO (CRD42023485985). Siguiendo las directrices PRISMA, identificamos, seleccionamos e incluimos estudios relevantes. Se examinaron 234 artículos, revisamos exhaustivamente 38 textos completos, y finalmente se incluyeron 21 artículos publicados entre 2013 y 2023 para su análisis.

Resultados: Los estudios seleccionados se clasificaron en tres grupos según la naturaleza química de los suplementos: polifenoles (11 estudios), vitaminas (8 estudios) y otros (2 estudios). Los estudios incluyeron investigaciones preclínicas (9) y clínicas (12). Los ensayos clínicos han demostrado la eficacia de los suplementos polifenólicos, como el extracto de *Polypodium leucotomos* (PLE) y otros, en la mejora de la salud de la piel y la reducción del riesgo de cánceres cutáneos. Los estudios preclínicos destacaron los efectos protectores de los polifenoles contra el daño inducido por los rayos UV y la transformación neoplásica. Los estudios de suplementación con vitaminas mostraron resultados mixtos, con datos claros que indican beneficios en la reducción del riesgo de lesiones precancerosas y cánceres de piel tras el tratamiento con nicotinamida (NAM), mientras que otros no demostraron efectos protectores significativos.

Conclusiones: La revisión confirma la eficacia de los polifenoles en la prevención y el tratamiento de las QA y condiciones relacionadas con la piel. Sin embargo, el papel de las vitaminas y otros suplementos requiere más investigación debido a hallazgos inconsistentes y/o escasos. Los futuros estudios clínicos deberían centrarse en poblaciones diversas con mayor riesgo de cáncer de piel y explorar nuevos ingredientes, así como combinaciones de varios ingredientes para optimizar las aplicaciones terapéuticas.

PALABRAS CLAVE: Queratosis actínica, lesiones precancerosas, polifenoles, vitaminas, suplementos dietéticos, prevención del cáncer de piel, fotoprotección, revisión sistemática.

INTRODUCTION

Actinic keratoses (AKs) are chronic and recurrent cutaneous lesions mainly induced by long-term exposure to sunlight. They are frequently located on sun-exposed areas of the body, such as face, neck, dorsum of the hands, forearms and lower legs. AKs arise as intraepithelial atypical proliferations of keratinocytes and are typically characterized by flat or slightly elevated lesions with a rough, scaly texture. According to recent estimates, 40 million Americans develop a new AK every year, a trend that is expected to grow in the coming years. Moreover, the appearance of AKs affect over one-third of adults older than 60s in Europe, and up to 60% in Australia [1–3]. In Spain, the prevalence of AK is approximately 28.6% of individuals older than 45, with higher rates observed in men and older populations. This high prevalence underscores the significant burden AKs place on dermatology services in Spain[4,5].

The greatest risk associated with AKs lies in their potential to progress into invasive squamous cell carcinomas (SCC) through either a differentiated or undifferentiated pathway [6]. In fact, AKs are defined as the initial forms of SCC, with a variable risk of malignant transformation estimated to be between 0.025 and 16% each year[7,8]. Studies have demonstrated that the risk of developing SCC positively correlates with the number of AKs, since AKs are contiguous with an SCC in almost 97% of cases [8]. Moreover, AKs and SCCs share certain genetic characteristics, such as mutations in the tumor suppressor gene p53 and *NOTCH*, among others [9,10].

On the other hand, the normal-appearing, sun-exposed skin surrounding AKs is also subject to cumulative actinic damage, with subclinical lesions, and therefore could present molecular alterations similar to those of AK lesions. In this regard, the concept of “field cancerization” was first introduced by Slaughter et al. (1953) [11]. Currently, it describes the presence of precancerous and cancerous cells in a tissue close to a tumor and chronically exposed to a carcinogen [8,12]. In the context of AK, field cancerization refers to the phenomenon where keratinocytes around a visible lesion look histologically

normal but have genetic alterations identical to those in the lesion. These areas of skin with extensive UV damage are prone to develop greater numbers of AKs [13]. Taking this into account, the presence of field cancerization requires a therapeutic strategy that should not only focus on treatment of clinically visible AKs as it is the case with lesion-targetes therapies, such as cryotherapy, but also treat subclinical AKs to minimize their potential for transformation into SCC. Hence, field-directed treatments are highly recommended to address subclinical damage, reduce AK recurrence rates and potentially reduce the risk of SCC. Moreover, treatment options must allow treating large areas safeguarding patient's comfort and achieving good cosmetic outcomes [12,14–16]. Indeed, various field-directed modalities are approved for the treatment of sun-damaged areas with multiple AKs, including photodynamic therapy (PDT), and topical agents, such as 5-fluorouracil, imiquimod, tirbanibulin and diclofenac [17–20].

Consistent with this strategy, the combination of topical photoprotection and oral photoprotection is also a preventive option with potential as field-treatment for skin with severe actinic damage [21]. Therefore, since natural compounds obtained from plants or phytoconstituents are known to exert therapeutic effects mainly through their antioxidant, free-radical scavenging and anti-inflammatory properties, they can be considered to be included in field-targeted treatments along with standard pharmacological treatments. In this systematic review we aim to update the scientific evidence available on the role of oral bioactives or nutraceuticals or dietary supplements in the treatment of AKs and field cancerization, and evaluate the safety and efficacy profile reported by such treatments.

METHODS

A review plan was pre-registered in the PROSPERO database (CRD42023485985). This article was developed following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guideline, which covers identification, selection, eligibility, and inclusion [22].

Search strategy

We conducted a comprehensive literature search using various electronic databases, including but not limited to Pubmed, Google Scholar, EMBASE, The Cochrane Library, along with screening reference lists of eligible studies, and trials registers. Priority was given to Pubmed Central as the National Library of Medicine reference. Our search was limited to articles published from 2013 through 2023 (both years included), written in English, and relevant to our investigation on the effects of oral dietary supplements on skin health. Search criteria were adapted for each database

used. The detailed retrieval technique is thoroughly outlined in **Supplementary Table S1 (supplementary data)**

Eligibility criteria

The research was included following the criteria below:

1. Study type:

   Clinical studies conducted among humans with AK diagnosed through clinical and dermoscopic evaluation, including assessment of the number, grade, and dermoscopic images. Clinical studies in patients with field cancerization (as diagnosed using AK numbers, AK size, Olsen clinical classification scheme, which grades AK lesions based on their thickness and degree of hyperkeratosis, among others). *In vitro* studies with skin cells or preclinical studies using artificial skin models. The primary exclusion criteria included (a) Review studies; (b) *In vitro* studies developed with natural compounds that have no demonstrated clinical evidence on the skin. Subsequently, accepted papers needed to be peer-reviewed and have their complete text available.

2. Participants/populations being studied:

   Clinical studies involving adults aged ≥ 18 years were included. Primary exclusion criteria were (a) Studies involving children and adolescents (younger than 18 years); (b) Preclinical studies conducted on animals.

3. Type of intervention.

   A study was considered eligible if it assessed interventions for treating AKs, including individual lesion-based methods (eg,, cryotherapy) or field-targeted topical treatments (eg,, photodynamic therapy, diclofenac, 5-fluorouracil, and imiquimod). Additionally, cosmetic treatments could be used as an adjuvant to these therapies. These interventions might differ in saftery, efficacy, and cosmetic results. Therefore, oral therapies involving natural products, either alone or in combination with the above-mentioned standard pharmacological treatments could also be considered.

These parameters are indicated in table 1 following PICAR statement (**Table 1**).

Table 1: Eligibility criteria pertaining to the population and clinical areas, interventions, comparators, attributes of CPGs and recommendation characteristics (PICAR) statement.

Study selection

Two reviewers (A.R-L. and A.Z.) independently screened the documents for eligibility, initially examining titles and abstracts using a Microsoft Excel document (Microsoft), eliminating duplicates. Disagreements were resolved through consensus, with the assistance of a third reviewer (S.G). Once inclusion criteria were met, the full texts were imported into Mendeley (ELSEVIER LIMITED). Afterwards, these full texts were independently assessed by 2 researchers (A.R-L. and A.Z.) against the above-mentioned criteria to determine their eligibility.

Data mining

We extracted data into a standardized extraction form using an Excel Microsoft document (Microsoft). The characteristics of each study are shown in **Table 2,** including research specifics such as paper reference (lead author + year of publication + study location); type of study (clinical or preclinical trial); population and study characteristics; (sample size, sex; age-years; health condition); natural product intervention and control (content, daily dose); study duration; test conditions; outcome (measurement instruments and parameters) and adverse effects.

Quality assessment

The risk of bias was evaluated using the standard risk of bias assessment tool for randomized controlled trials (RCTs) recommended by the Cochrane Collaboration, with 2 reviewers independently conducting the examination [23]. This tool assesses 7 types of bias: selection bias (random sequence generation and allocation concealment), performance bias (blinding of participants and researchers), detection bias (blinding of outcome assessors), attrition bias (incomplete outcome data), reporting bias (selective reporting), and other biases (such as changes in lifestyle during the study, unstable testing conditions, and potential conflicts of interest). Each study was rated for these criteria as having a "low," "high," or "unclear" risk of bias. A criterion was considered as low risk if the study adequately reported methods to prevent bias according to Cochrane guidelines. High risk was allocated if the methods described could not eliminate bias, and unclear risk was used when information was insufficient, irrelevant, or missing [24].

RESULTS

Study characteristics

Following the screening of titles and abstracts of 234 articles and a thorough review of the full text of 38 articles, 21 articles published from 2013 through 2023 were

ultimately chosen for our analysis (see Figure 1). The studies were categorized based on the chemical nature of the agent chosen for dietary supplementation. Therefore, we selected 3 categories: administration of polyphenols (11 studies), vitamins and oligoelements (8 studies), and a third category called “others”, which includes various types of ingredients (2 studies). Type of study included preclinical (9), and clinical (12) studies (Figure 1 and Table 2).

Patients’ characteristics

Overall, our review analysis included 10463 patients, with a mean age of 52.4 years (range, 21-91 years). Based on the articles that provided this information, we determined that 51.8% (n = 5426 women out of 10493) of participants were women.

Risk of bias in research studies

The examination of each aspect of bias within the selected research was represented as percentages in Figure 2, based on the researchers' evaluation. Most trials showed strengths in their design and methods. Consequently, the risk of bias was low in most parameters analyzed: 91.7% of randomized controlled trials (RCTs) adequately mentioned randomization, with only 1 study being considered high risk (8.3%). Blinding of both subjects and researchers was implemented in 8 out of 12 studies (66.7%) and blinding of evaluators when analyzing results was also implemented in 8 out of 12 studies (66.7%). Regarding the aspect of attrition biases, most studies (8/12, 66.7%) exhibit a high risk, as many of them experienced drop out of several study subjects, mainly due to non-compliance with the oral supplement intake. In all selected studies, reporting bias posed a low risk, as most (11712, 91.7%) studies provided precise data and none of them showed a lack of information, a crucial factor for ensuring transparency and reliability in clinical research. Four studies (33.3%) were rated at high risk for "other biases," due to potential conflicts of interest and insufficient details regarding compliance with supplement consumption and maintenance of lifestyle habits.

Data extracted: Results and Discussion.

Supplementation with polyphenols

Eleven studies reported on the administration of polyphenolic compounds for the treatment of AKs, BCC and SCC and actinic ageing, as well as prevention of UVB-induced damage to the skin (CPDs formation, erythema, etc.). Five out of the 11 were clinical trials and 6 were preclinical studies.

Regarding clinical trials, *Polypodium leucotomos* extract (PLE), polyphenols from black tea, and a combination of rosemary and grapefruit polyphenols were administered as dietary supplements for the prevention of AKs, UV-induced erythema and lipid peroxidation, the improvement of signs of photoaging, and the reduction of incidence of BCCs and SCCs. Overall, these studies included a total of 1595 patients, with a mean age of 51.7 years, ranging from 28 to 85 years. Data showed that 54.4% (n = 867 women/1595) of participants were women.

Preclinical experimentation included the evaluation of PLE, flavonols (quercetin, kaempferol and galangin), and extracts obtained from the leaves of *Hamamelis virginiana* and lotus to prevent genomic UV-induced damage and formation of CPDs, to inhibit actinic aging and progression of AKs and to suppress neoplastic transformation.

Research snippets

Polypodium leucotomos extract (PLE)

A recent clinical trial analyzed the effect of PLE, orally administered in combination with topical sun protection SPF100, in subjects with severe photoaging and history of at least 3 AKs. The combination of topical and oral PLE at a daily dose of 240 mg led to an improvement in AKASI and AK-FAS parameters and reduced the number of new AKs and the need for additional intervention to a greater degree than in the case of topical treatment alone [21]. Thus, combination of topical and oral photoprotection with PLE can help prevent the onset of further lesions in sun-exposed areas of the skin [21].

Additionally, this extract has not only demonstrated the effectiveness of skin cancer prevention but also skin cancer treatment. A clinical trial assessed whether PLE could improve the effectiveness of PDT in decreasing the recurrence of AKs [25]. For this purpose, patients with at least 2 visible AKs on the scalp were categorized into 2 groups: one receiving 2 sessions of MAL PDT alone, and the other receiving the same 2 PDT sessions along with oral PLE supplementation (daily doses of 480 mg, starting 1-week after last session) for 6 months. Clinical, dermoscopic, and fluorescent image data showed that at the 6-month follow-up, PDT plus oral PLE resulted in a higher clearance rate than PDT alone. Thus, PLE supplementation appears to enhance long-term PDT effectiveness, significantly reducing the number of AKs [25].

A recent study performed by Gandarillas et al. (2023) using primary human epidermal keratinocytes suggests that PLE has the capacity to induce the repair of genomic damage induced by UV light [26]. Treatment with PLE promotes the expression of H2AX and p53—triggering DNA repair machinery—and slows down the progression of keratinocytes through G2/M transition (favoring the G2/M checkpoint control). The

authors observed that PLE treatment extended the duration of DNA repair phase in stem cells, enhancing their ability to regenerate. Additionally, increasing the DNA repair signal should decrease the number of cells undergoing differentiation after cell division. Premature differentiation of keratinocytes is a factor in skin aging. Nevertheless, cells treated with PLE showed greater resilience vs aging, maintaining a higher potential for self-renewal and proliferation after treatment. Thus, this boost to DNA repair signaling could prolong the renewal capacity of stem cells and diminish the proportion of cells undergoing differentiation post-mitotically [26].

Regarding the importance to evaluate the impact of DNA damage, Torricelli et al. (2017) also evaluated the preventive role of PLE vs UV-induced damage [27]. Their preclinical trial was performed in a model of reconstructed human epidermis (RHE) exposed to UVB and showed that treatment with PLE reduces the expression of p53 and p21, which correlated with the reduction of sunburn cells formation, and prevents the upregulation of proliferating proteins (EFG, Ki67) and formation of CPDs [27]. In connection with the formation of CPDs, Portillo-Esnaola et al. (2021) [28] analyzed the role of Fernblock® (FB; the standardized hydrophilic extract from the leaves of *P. leucotomos*) in preventing the formation of these photoproducts in UVA-exposed murine melanocytes. Results indicated that treatment with FB decreases levels of both reactive oxygen species (ROS) and reactive nitrogen species (RNS), thus reducing the formation of dark-CPDs. Both antioxidant and scavenging properties shown by FB make this compound an optimal candidate to be included in sunscreens formulations [28].

Tea polyphenols extracts

A prospective study explored whether there is a relationship between drinking black tea and the occurrence of BCC and SCC. The study estimated black tea consumption in 1,325 volunteers from 1992, 1994 and 1996 and recorded all skin cancers diagnosed in these volunteers from 1997 through 2007. Despite this comprehensive analysis, results showed no significant association between black tea consumption and the incidence of BCC or SCC. Therefore, black tea consumption cannot be considered as part of a preventive strategy to reduce the risk of skin cancer [29].

Additional polyphenol sources

Additional polyphenol sources have been investigated in various studies to explore their role in preventing UV-induced skin damage, offering promising avenues for skin protection and cancer prevention. Pérez-Sánchez et al. [30] demonstrated that a combination of rosemary and citrus bioflavonoids from grapefruit (Nutroxsun®) increased

cell survival, reduced intracellular ROS, prevented DNA damage, and decreased chromosomal aberrations in UVB-exposed human keratinocytes. Moreover, oral administration of this combination to human volunteers resulted in a significant increase in minimal erythema dose (MED), suggesting its potential as an oral photoprotection strategy [30]. Similarly, the clinical trial conducted by Nobile et al., (2016) [31] indicated that intake of the same combination of rosemary and grapefruit (Nutroxsun®) by volunteers with clinical signs of photoaging also lead to a decrease in skin redness and peroxidation of basal lipids and an increase in MED, as well as a decrease of wrinkle depth and an increase in skin elasticity [31]. In another study, Maini et al. (2015) [32] revealed the effectiveness of quercetin, kaempferol, and galangin in preventing UVR-induced CPD formation in artificial skin. Additionally, quercetin significantly reduced the secretion of MMP-1 and TNF-α. These findings suggest that these flavonols, traditionally considered antioxidants, could serve as a promising tool to prevent DNA damage associated with AK progression [32]. Regarding skin elastosis, characterized by the accumulation of abnormal elastic fibers in the skin, Pain et al. (2018) evaluated the capacity of a leaf extract from *Hamamelis virginiana* to counteract the elastin/lysyl oxidase (LOXL1) enzyme imbalance and elafin synthesis, a marker of elastotic aggregates, triggered by UV light exposure. This imbalance contributes to the accumulation of nonfunctional elastin fiber aggregates, leading to actinic aging. For that purpose, they measured LOXL1 and elafin expression in both human fibroblasts and human skin biopsies that were exposed to UVA radiation and treated with *Hamamelis virginiana* extract. The extract increased LOXL1 expression and decreased elafin synthesis, resulting in a decrease in aggregates and restoration of functional elastic fibers [33]. Phytochemicals in the diet possess chemopreventive properties that can hinder or delay the process of carcinogenesis. The lotus leaf, a traditional medicinal plant rich in numerous polyphenols, is a notable example. Lotus leaf extract, rich in phenolics and quercetin, showed strong potential in inhibiting skin carcinogenesis in murine skin JB6 P+ cells. It activated the NRF2 pathway, boosting antioxidant and detoxification enzymes like HO-1, NQO1, and UGT1A1, while also reducing DNA methylation levels. This suggests that this extract may hinder neoplastic transformation by regulating the NRF2 pathway and epigenetic processes [34] .

Thus, the exploration of polyphenolic compounds, conducted through both clinical and preclinical studies, reveals their potential in preventing and treating various skin conditions, including AKs, BCCs, SCCs, and actinic aging. Clinical trials have demonstrated the efficacy of supplements such as *Polypodium leucotomos extract*

*(*PLE), in improving skin health and reducing the risk of skin cancers. Preclinical experiments highlight the role of polyphenols in inhibiting DNA damage, suppressing neoplastic transformation, and protecting against UV-induced skin damage. However, not all polyphenols exhibit significant protective effects, as evidenced by the lack of association between black tea consumption and the incidence of BCC or SCC. Despite this, research into polyphenols from sources like rosemary, citrus bioflavonoids, lotus leaf extract, and *Hamamelis virginiana* extract offers promising avenues for developing effective photoprotective and chemopreventive strategies vs skin cancer and actinic aging.

Supplementation with vitamins and oligoelements

Eight studies reported the administration of vitamins and vitamin derivatives for the prevention and treatment of AKs, BCCs and SCCs, melanoma, oxidative stress, and UV-induced apoptosis. Of the 8 works, 6 corresponded to clinical studies and 2 were preclinical studies.

Regarding clinical trials, nicotinamide (NAM, a vitamin B3 derivative), folate (vitamin B9), an antioxidant complex (vitamin D + vitamin C + zinc), and vitamin D3, were administered as dietary supplements to prevent precancerous lesions (AKs) and non-melanocytic lesions (BCCs and SCCs) in a high-risk patient population including organ transplant recipients (OTR) patients, as well as melanoma. Overall, all these studies involved 8789 patients, with a mean age of 52.6 years, ranging from 30 to 91 years. Data indicated that 50.9% (n = 4472 female/8819) of participants were women.

Preclinical studies examined the capacity of NAM to reduce of oxidative stress associated with non-melanoma skin cancer (NMSC), and the potential role of vitamin C to inhibit UVR- induced apoptosis through modulation of DNA-methylation.

Research snippets

Nicotinamide and other vitamin B derivatives

The selected studies provide valuable insights into the potential of nicotinamide (NAM) as a preventive and therapeutic agent for skin cancer. In 2015 [35], Chen et al. conducted a clinical trial with 386 high-risk patients with a past medical history of at least two NMSCs in the previous 5 years. Participants were randomized to receive oral NAM (500 mg) or placebo twice daily for 12 months. The evaluation by dermatologists revealed promising results, with a 20% lower rate of BCCs, a 30% lower rate of new SCCs, and a 13% lower rate of new AKs in the NAM group vs the placebo group [35]. In another clinical trial, Chen et al., 2016 [36] explored the safety and efficacy profile of NAM in preventing the

appearance of BCCs and SCCs in immunosuppressed renal transplant recipients with a history of at least two NMSCs in the previous 12 months. Patients were randomized to receive oral NAM (500 mg) or placebo and skin lesions (AKs, BCCs, and SCCs) were recorded twice monthly up to 6 months by dermatologists. In this study, a reduction of BCC and SCC (35%) and of AKs (16%) were observed in the NAM vs the placebo group, yet results were not statistically significant [36]. In 2023, Allen et al, [37] also explored the efficacy of NAM in skin cancer chemoprevention in OTR patients. A total of 158 adults who had had at least two histologically confirmed keratinocyte cancers in the past 5 years and had undergone kidney, liver, or heart or lung transplantation at least 12 months previously were enrolled. These participants randomly received oral NAM (500 mg) or matched placebo twice daily for 12 months. Evaluation by dermatologists revealed no significant differences in SCC, BCC and AK counts between the NAM and placebo group, concluding that oral NAM did not prevent the appearance of new keratinocyte cancers or AKs in immunosuppressed solid OTR [37]. Camillo et al. in 2022 [38], also analyzed the potential role of NAM as chemopreventive agent from a preclinical viewpoint. They obtained skin biopsies from 30 patients with precancerous skin lesions, dysplastic nevi, NMSCs, and/or cutaneous melanoma. From these biopsies, they isolated field cancerization human primary keratinocytes (FC-HPKs) for further study. Their findings demonstrated that NAM efficiently decreased ROS levels and the expression of oxoguanine glycosylase (OGG)1 (responsible for 8-oxoG excision) in UVB-exposed FC-HPKs, thus protecting from oxidative stress and DNA damage induced by UVB irradiation. Furthermore, NAM prevented UVB-induced inflammation via modulation of the expression of proinflammatory cytokines (especially IL1b and TNFα). These results suggest that NAM could be a useful molecule for chemoprevention of NMSCs and the treatment of field cancerization [38].

All these contrasting findings about NAM highlight the complexity of the role of this compound in skin cancer prevention and underscore the need for further research to elucidate its mechanisms of action and potential therapeutic applications.

Another derivative of B vitamin is folate (vitamin B9). A prospective study performed by Donnenfeld et al. (2015) [39], aimed to investigate the impact of dietary folate intake on the risk of developing skin cancer. During the study period spanning from 1994 through 2002, 5880 volunteers completed a dietary record every 2 months, detailing all foods and beverages consumed within 24-hour periods. Throughout the trial, all diagnosed skin cancer lesions were meticulously documented. At the end of trial, a total of 144 incident skin cancer cases were diagnosed (20 melanoma, 18 SCC, and 106 BCC). Based on

these results and on erythrocyte folate analyses—which were performed on all participants at years 2 and 5—it was concluded that dietary folate intake was associated with an increased risk of BCC skin cancer. This association was more specifically observed in women [39].

Vitamin C and vitamin E

Freitas et al., (2015), conducted a study with 2 phases to investigate the effects of an antioxidant therapy (consisting of a complex with vitamin C, vitamin E and zinc) in the oxidative stress status of patients with a past medical history of NMSC. In phase I, plasma concentrations of several oxidative biomarkers were analyzed in 60 NMSC patients (previously treated with surgery) and 24 healthy volunteers. Results showed that NMSC patients had higher levels of all assessed oxidative markers vs healthy volunteers. In phase II, NMSC patients were randomized to receive oral antioxidant complex or placebo once daily for 2 months. In this case, evaluation of stress biomarkers after supplementation period did not reveal significant differences across groups. In conclusion, antioxidant therapy based on these vitamins did not significantly reduce levels of oxidative stress biomarkers [40]. Another *in vitro* study performed by Lin et al., (2014) explored the role of vitamin C on UV-induced apoptosis in cells from a human epidermoid carcinoma and p16/p21-knockout fibroblasts. As results showed, vitamin C effectively antagonized UV-induced apoptosis in skin cancer cells by promoting DNA demethylation and subsequently reactivating the activation of tumor suppressor genes p16 and p21 [41].

Vitamin D and oligoelements

Regarding vitamin D, Passarelli et al. in 2020**,** [42] examined the effect of daily oral vitamin D or calcium supplementation on the risk of developing BCC or invasive cutaneous SCC. They enrolled a total of 2259 patients diagnosed with a colorectal adenoma in this clinical trial. Participants were randomized to receive 1 of these 4 groups: (1) 1000 IU/day of vitamin D3; (2) 1200 mg/day of calcium carbonate; (3) both vitamin D3 and calcium carbonate; (4) placebo. Supplementation period was 3 or 5 years, and during that period the incidence of BCC or SCC was reported. Results indicated that while BCC incidence was not related to the supplementation administered, calcium carbonate supplementation—alone or in combination with vitamin D—seemed to reduce the incidence of SCC, suggesting the preventive role of calcium in the development of SCC [42].

Therefore, the investigation into vitamin supplementation for the prevention and treatment of various skin conditions presents diverse findings and implications. Clinical trials have explored the potential benefits of NAM, folate (vitamin B9), antioxidant complexes, and vitamin D3 in reducing the risk of precancerous lesions, NMSCs, and melanocytic lesions. Conflicting results from these trials highlight the complexity of the role of such vitamins in skin cancer prevention, particularly evident in the case of NAM, where its efficacy varies across different studies. Preclinical studies further elucidate the mechanisms underlying the protective effects of these vitamins, such as NAM's ability to reduce oxidative stress and inflammation and folate's association with an increased risk of skin cancer, especially in women. Additionally, vitamin C shows promise in modulating UV-induced apoptosis and promoting DNA demethylation in skin cancer cells. The role of vitamin D supplementation, particularly calcium carbonate, appears to have a preventive effect on the incidence of invasive cutaneous SCC. Overall, these findings underscore the importance of continued research to better understand the mechanisms of action and optimize the therapeutic potential of vitamin supplementation in skin cancer prevention and treatment.

Supplementation with other supplements

Two studies reported on the use of different compounds for inhibition of growth and development of SCCs, and the prevention of UV-induced damage to immune system. One of these 2 studies is a clinical trial and the other a preclinical trial.

Regarding clinical trial, omega-3 Polyunsaturated Fatty Acids (Ω-3 PUFAs) eicosapentanoic acid (EPA) and docosahexaenoic acid (DHA) were evaluated as dietary supplements for the prevention of UVR-induced damage to immune system. This study involved 79 women, with a mean age of 40.6 years, ranging from 21 to 60 years old.

Preclinical trial examined the capacity of EPA and DHA to inhibit the growth of cells obtained from human oral and facial SCC [43].

Research snippets

Ω-3 Polyunsaturated Fatty Acids (PUFAs): eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)

A clinical trial conducted by Pilkington et al. (2016) [43] evaluated the protective role EPA and DHA on cell-mediated immunity – the suppression of which is one of the effects of UVR exposure– through the analysis of the number of Langerhans cells (LC) and immunomodulatory mediators. For that, 79 healthy volunteers randomly received 5 capsules *per* day containing 70% EPA and 10% DHA or placebo, for 12 weeks.

Moreover, specific areas of their buttocks were exposed to UVR before and after supplementation. Analysis of collected samples did not find any differences in the number of LC and immunomodulatory mediators between EPA and placebo groups after UVR exposure. Therefore, there was no evidence that EPA reduces UVR suppression of skin immunity by changes in the number of epidermal LC or immunomodulatory mediators [43]. Within this context, Nikolakopoulou et al. (2013) [44] conducted laboratory research to assess how n-3 PUFAs affect the proliferation of premalignant and malignant keratinocytes from facial and oral human SCCs. Their findings indicated that DHA and particularly EPA specifically inhibited the proliferation of these keratinocytes by activating the ERK1/2 pathway and inducing both apoptosis and cell cycle arrest [44].

Therefore, alternative supplements like omega-3 PUFAs show potential in reducing UVR-induced damage and preventing SCC growth. While clinical trials yield mixed results, preclinical studies indicate promising effects of Ω-3 PUFAs in inhibiting the growth of malignant keratinocytes.

CONCLUSIONS

Finally, we can conclude that the reviewed studies support the potential efficacy profile of polyphenols in the prevention of cancerous lesions, particularly due to their protective effects vs DNA damage and UV-induced harm. However, of note, the limitations of the reviewed studies, including small sample sizes, variability in study designs, and the possible impact of uncontrolled variables. Moreover, it is essential to emphasize that integral photoprotection, which includes the use of topical, oral photoprotectors and the adoption of photoprotective habits, remains the most recommended strategy for skin cancer prevention. In this context, further clinical studies should aim to investigate the impact of dietary supplements, particularly in high-risk populations and explore different combinations of ingredients with varied mechanisms of action to optimize therapeutic applications.

ETHICAL APPROVAL

The study protocol was registered on PROSPERO (CRD42023485985).

CONFLICTS OF INTEREST

G.P received grants to institution from AbbVie, Almirall, Galderma, Leo, Lilly, Novartis, Pierre Fabre and Pfizer. K.P received grants from Sanofi, Novartis, Abbvie and Almirall. Consulting fees from Sanofi, honoraria for lectures and educational events from Lilly,

Sanofi and Sun Pharma, participation in advisory boards Abbvie and Almirall. Leo Pharma, Lilly, Janssen, Sanofi, Pierre Fabre, Sun Pharma, Biogen, Galderma and Philogen. P.C-P has acted as an advisory board member for AbbVie, Almirall, Cantabria, Galderma, Janssen, LEO Pharma, Novartis, Boehringer Ingelheim, Molteni and Sanofi, has received grants for talks from Almirall, AbbVie, Novartis, LEO Pharma, Sanofi, Novartis, and has acted as an investigator for Clinuvel, Mitsubishi, Novartis, Sanofi, Galderma, LEO Pharma, Amgen, Biogen, Pierre-Fabre, Regeneron, and SI Health. MV.deG has served as an investigator and speaker for Cantabria labs, La Roche-Posay, Beiersdorf, ISDIN, Pierre Fabre, Rilastil. Y.G received support as an advisor, researcher, or speaker from Galderma, Almirall, Cantabria Labs, Abbvie, Lilly, Sanofi, UCB, Isdin, PfizerRoche, and Roche-Posay. S.G. has a consultant role for Cantabria Labs.

The remaining authors declared no conflicts of interest whatsoever.

Ética de la publicación

1. ¿Su trabajo ha comportado experimentación en animales?:

No

2. ¿En su trabajo intervienen pacientes o sujetos humanos?:

No

3. ¿Su trabajo incluye un ensayo clínico?:

No

4. ¿Todos los datos mostrados en las figuras y tablas incluidas en el manuscrito se recogen en el apartado de resultados y las conclusiones?:

Sí

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PICAR Element	**Study Specific Criteria**
Population & Clinical area(s)	- Adults (aged 18 and above) diagnosed with actinic keratosis - Preclinical studies: *In vitro* studies with skin cells or preclinical studies in skin models.

PICAR Element	Study Specific Criteria
Interventions	- Compounds of natural origin that have clinical evidence in the prevention of field cancerization and actinic keratosis.
Comparators	No comparator applicable.
Attributes of eligibile studies	- **Language:** English **- Publication years:** 2013-2023 - **Type of publication**: Clinical and preclinical studies. Published papers must be peer-reviewed articles with full text. - **Publishing region:** All - **Clinical Scope:** Studies that evaluate oral compounds with established clinical evidence and focus on the pathways involved in the development of actinic keratosis or field cancerization. - **Format**: Clinical trial and *in vitro* study - **Purpose:** Reducing actinic keratosis and field cancerization - **Interventions:** individual lesion-based (eg, cryotherapy) or field-directed topical treatments (eg, diclofenac, 5-fluorouracil, imiquimod). Additionally, cosmetic treatment could be used as adjuvant therapy of these treatments. These might vary in terms of safety, efficacy, and cosmetic outcomes. Therefore, only oral therapies with natural products will be considered, also alone or in combination with cited standard pharmacological treatments.
Recommendation characteristics	- **Scope:** natural compounds that have demonstrated oral clinical efficacy in improving skin conditions by clinical evidence or modulation on the pathways involved in the development of actinic keratosis or field cancerization - **Duration of treatment:** non-applicable - **Levels of confidence:** non-applicable -**Comparators**: In clinical studies a placebo (a group of patients who will not be exposed to the natural product treatment) must be included. *In vitro* models: A control which will not be exposed to the natural product treatment. -**Locating recommendations**: non-applicable

Table 1. Eligibility criteria pertaining to the population and clinical areas, interventions, comparators, attributes of CPGs and recommendation characteristics (PICAR) statement.

Figure 1. Flow diagram for study selection process following PICAR procedure.

Figure 2. Summary of the reviewers' assessments for each domain of bias risk. Risk of bias. SB (Selection bias); PB (Performance bias); DB (Detection bias); AB (Attrition bias); RB (Reporting bias); OB (Other bias). Studies: (S1) Pellacani et al., 2023; (S2) Auriemma et al., 2015; (S3) Miura et al., 2015; (S4) Pérez-Sánchez et al., 2014; (S5) Chen et al., 2015; (S6) Chen et al., 2016; (S7) Allen et al., 2023; (S8) Freitas et al., 2015; (S9) Pasarelli et al., 2014; (S10) Pilkington et al., 2016; (S11) Donnenfeld et al., 2014; (S12) Nobile et al., 2016.

Study Location		Study Population			Intervention	Control	Study Duration	Test conditions (°C R.T.) (% R.H.)	Outcome		Adverse Effects
	Type of study	Sample size, sex	Age, years	Health Condition	Contents, daily dose	Contents, daily dose			Meaurement Instrument	Parameter (Measuring Sites)	
POLYPHENOLS											
Auriemma, 2015 Italy [25]	Clinical trial	n = 34 100% F	67-84	≥ 2 visible AKs on the scalp. Hamilton– Norwood baldness scale ≥IV	2 sessions of MAL-PDT one week apart + oral PLE supplementation (960 mg/day for 1 month and then 480 mg/day for 5 months)	2 sessions of MAL- PDT without subsequent PLE supplementation	25 w	Clearance rate	-Clinical, dermoscopic, and fluorescent images (FotoFinder Dermoscope and Medicam 800HD) -AK count	Scalp	No
Miura, 2015 Australia [29]	Clinical trial	n = 1325 44% M	35-48	Follow-up of all patients to evaluate association between black tea consumption-new BCC/SCC	Black tea consumers. Information about consumption: questionnaries 1992, 1994 and 1996.	Never drinking black tea	10 y (follow-up)	Relative risks (RRs) + 95% confidence (BCC/SCC incidence)	-Questionnaries -Diagnosed BCCs and SCCs count	Full-body skin examination	No
Pérez-Sánchez, 2014 Spain [30]	Clinical + preclinical trial	n = 10 90% F	28-52	-*Clinical trial*: healthy human volunteers -*In vitro study*: human keratinocytes (HaCaT)	**Nutroxsun®**: Citrus extract enriched in citrus bioflavonoids (22.57 ± 2.65 GAE/100 g dw) + rosemary extract containing phenolic compounds and diterpenes (57.16 ± 1.25 GAE/100 g dw) -*Clinical trial*: NutroxSun® intake (capsule 250 mg daily) -*In vitro study*:	-Clinical trial: Each subject was used as his or her own control. -*In vitro study*: UVB irradiation (800 or 1200 J/m2 dose) in the absence of extracts	24 w	*Clinical trial*: MED (after 29, 57 and 85 consecutive days of ingestion of NutroxSun®) *In vitro study*: -MTT (cell viability) -H2DCFDA (ROS generation) -Comet assay (DNA damage)	UVB lamp Bio-Link Crosslinker BLX-E312 Mexameter® (colorimetric measurements)	-	NR

					UVB irradiation (800 or 1200 J/m2 dose) in the presence of the extracts (citrus extract alone, rosemary extract alone or NutroxSun®)						
Nobile, 2016 Spain [31]	Clinical trial	n = 95	30-55	Healthy adults with mild-to-moderate chrono- or photoageing clinical signs and skin phototype I-III	**Nutroxsun®:** obtained from dried rosemary (*Rosmarinus officinalis*) and grapefruit (*Citrus paradisi*). Total phenolic standard content > 35 GAE/100g dw. Short-term test: Dose of Nutroxsun™ (100 or 250 mg) 15 min before UVB exposure (1 MED) + 2 doses of Nutroxsun™ (24 and 48 h after irradiation, respectively) Long-term test: 100 or 250 mg Nutroxsun® once daily for 2 months. UVB exposure series.	Placebo intake (maltodextrin)	8 w	Measurement of: -MED -Skin redness -Skin LPO -Wrinkle depth -Skin elasticity	-Multiport 601-300 W Solar simulator -Spectrophotometer / colorimeter CM-700D -3-D microtopography imaging system (PRIMOS 3D lite) -Skin viscoelasticity analyser (Cutometer MPA 580)	Specific sites on skin of the back	No
Pellacani, 2023 Italy [21]	Clinical trial	N = 131 84% M	60-85	≥ 3 AKs on face or scalp. Photoaging score > 16	Topical photoprotection (SPF100 twice daily) + oral photoprotection (PLE oral, 240 mg/day)	Non-specific photoprotection measures	48 w	- AKASI and AK-FAS scores - New AKs - RCM evaluation	-Direct inspection -Lesion count -Vivastack	Scalp, forehead and face	No

Maini, 2015 Canada [32]	Preclinical trial	-	-	EpiDerm™: artificial skin mimic	**EpiDerm™ samples were treated topically with**: - 50 µL and 100 µL of 26 µM quercetin (1nmol, 2 nmol respectively) in acetone. - 300 µL of 52 µM (15 nmol) flavonol (quercetin, kaempferol, galangin) in acetone for a final concentration of 4 nmol/cm^2. **EpiDerm™ samples were exposed to UVB/UVA**	Dark control	-	- HPLCAPCI MS/MS method (for CPDs analysis) -ELISA (for MMP-1 and TNF-α secretion)	-FS20T12/UVB lamp -F20T12/BL/HO UVA lamp -UVP UVX-31/36 sensors	-	-
Torricelli, 2017 Italy [27]	Preclinical trial	-	-	Reconstructed human epidermis (RHE) (Episkin) samples	PL leaves extract topically applied on RHE at 0.5 mg/mL and exposed to 300 mJ/cm^2 UVB	-RHE irradiated without PL (positive control) -RHE non-irradiated (negative control)	-	- Histology - p53, p21, and Ki-67 expression -CPDs detection -EGF production	UVB lamp	-	-
Pain, 2018 Canada [33]	Preclinical trial	-	-	-Fibroblasts from a 63 year old healthy woman -Biopsies of abdominal skin from a 27year old woman	**Fibroblasts:** -*Treatment 1*. UVA irradiation: 7.5 J/cm^2 + incubation with Hamamelis virginiana extract for 16 h -*Treatment 2*. Incubation with Hamamelis virginiana for 24 h + UVA	**Fibroblasts:** UVA-exposed fibroblasts (7.5 J/cm^2) **Biopsies:** - Dark control	-	-Evaluation of LOXL1 expression -Evaluation of elafin expression	-q-RT-PCR - Immunohistochemistry: staining quantification	-	No

					irradiation: 7.5 J/cm^2 + incubation with Hamamelis virginiana extract for 16 h. **Biopsies:** - UVA (5 J/cm^2) + 0.5% systemic Hamamelis virginiana extract	-UVA-exposed biopsies (5 J/cm^2)					
Portillo-Esnaola, 2021 Spain 28	Preclinical trial	-	-	B16-F10 mouse melanocyte cell line	Cells were treated with 0.3 and 0.75 mg/mL of Fernblock® and exposed to UVA light (94, 282, 470 and 658 mJ/cm^2)	-Cells without FB treatment and non-irradiated -Cells treated with FB but non irradiated -Cells non treated with FB but exposed to UVA	-	-Cell Viability Assay -Dark-CPD formation -NO•, $O2^-$, and $ONOO^-$ formation	-CAMAG UV lamp	-	-
Gandarillas, 2023 Spain 26	Preclinical trial	-	-	Primary human keratinocytes isolated from neonatal foreskins	Keratinocytes were treated with Fernblock® (hydrophilic extract from *P. leucotomos*) 0,8 to 2,4 mg/mL and exposed to 25 mJ/cm^2.UVB	-Keratinocytes untreated. -Keratinocytes only exposed to UVB.	-	-Clonogenicity assay -Cell cycle analysis -DNA analysis (comet assay) -Involucrin and p53 expression	-UPV CL-1000 Series UV crosslinker -CytoFLEX cytometer	-	-
Tung, 2023 Taiwan 34	Preclinical trial	-	-	Murine skin JB6 P+ cells	**<u>Lotus leaves extracts</u>:** -Water extracts (LL-WE): 6.25, 12.5, 25, and 50 mg/mL	Untreated murine JB6 P+ cells	-	-Cell viability and growth -ARE-luciferase activity -Nrf2, HO-1, NQO1 and	- Beckman microplate spectrophotometer -Beckman luminometer	-	-

					-Ethanol extracts (LL-EE): 3.125, 6.25, 12.5, 25, and 50 mg/mL -LLWE further extracted with ethanol (LL-WREE): 3.125, 6.25, 12.5, 25, and 50 mg/mL JB6 P+ cells were treated with **TPA** (to induce cell transformation) and incubated with different **lotus leaves extracts**.			UGT1A1 expression	-PCR detection system		
VITAMINS											
Chen, 2015 Australia 35	Clinical trial	n = 386 63% M	30-91	Patients with ≥ 2 keratinocyte cancers in last 5 years	**Nicotinamide (vit B3) group:** 500 mg, twice daily (coated tablets)	**Placebo:** placebo coated tables twice daily	48 w	-New NMSC -New SCC/BCC/AK	-Dermatologist skin check -Lesion count	Face, head and neck, trunk	AE (similar in both groups)
Donnenfeld, 2015 France 39	Clinical trial	n = 5880 42% M	43-56	Participants from Vitamines et Minneraux Antioxydants (SU.VI.MAX) study	**Active group:** dietary folate	-	8 y	Melanoma and NMSC	-Dietary records from participants (foods and beverages) -Count of diagnosed skin cancers (melanoma, NMSC)	Full body	NR
Freitas, 2015 Brazil 40	Clinical trial	n = 84 31% M	45-77	Patients with a history of NMSC previously treated by surgery	**Supplemented group:** daily antioxidant complex capsule (500 mg vit C, 400 IU vit D and 50 mg zinc)	**Placebo group:** daily lactose capsule	8 w	**NMSC Oxidative biomarkers:** -15-F2t-isoprostane	Oxidative markers: measurement in plasma	Plasma	NR

								-Thiobarbituric acid reactive substances (TBARS) - Nitrite - Total antioxidant capacity (TAC)			
Chen, 2016 Australia [36]	Clinical trial	n = 22 77.3% M	42-81	-Immunosuppressed renal transplant recipients	**Nicotinamide (vit B3) group:** 500 mg, twice daily (coated tablets)	**Placebo:** placebo coated tables twice daily	24 w	-New NMSC -New SCC/BCC/AK	-Dermatologist skin check -Lesion count	Face, scalp, forearms and hands	AE
Passarelli, 2020 USA [42]	Clinical trial	n = 2259 63% M	45-75	Patients who underwent polypectomy for ≥1 colorectal adenoma in last 120 d	-**Vitamin D3 group:** 1000 IU/d of vitamin D3 -**Calcium group:** 1200 mg/d of calcium -**Vitamin D3 + Calcium group:** 1000 IU/d of vitamin D3 + 1200 mg/d of calcium	Placebo supplementation	3 or 5 y	New keratinocyte cancers (BCC and SCC)	-Dermatologist skin check -Lesion count	Full body	NR
Allen, 2023 Australia [37]	Clinical trial	n = 158 59% M	49-74	-Solid-organ transplant recipients with ≥ 2 keratinocyte cancers in last 5 years	**Nicotinamide (vit B3) group:** 500 mg, twice daily (coated tablets)	**Placebo:** placebo coated tables twice daily	48 w	-New keratinocyte cancer -New SCC	-Dermatologist skin check -Lesion count	Face, head and neck, trunk	AE (similar in both groups)
Lin, 2014 China	Preclinical trial	-	-	-Human epidermoid carcinoma A431 cells	Cells were exposed to 30 J/m2 UV light and treated with either	-Normal human epidermal	-	-Apoptosis (expression of p12, p21, and	-Solar simulator (SUV100)	-	-

[41]				-p16-knockout fibroblasts -p21- knockout fibroblasts	various concentrations of vitamin C (10–200 ug/mL)	keratinocytes (NHEK)		Tet1/2/3 genes) -qRT-PCR -DNA metilation (MethylFlash Methylated DNA Quantification)			
Camillo, 2022 Italy [38]	Preclinical trial	n = 30 74% M (skin biopsies' donors)	46-91	Field cancerization human primary keratinocytes (FC-HPKs) isolated from patients with signs and personal history of intrinsic skin aging, affected by precancerous skin lesions, dysplastic nevi, NMSCs, and/or cutaneous melanoma.	FC-HPKs were treated with **nicotinamide** (NAM) (5, 25, and 50 mM) for 18, 24 and 48 h. Then, they were were irradiated (400 mJ/cm^2 UVB)	Normal human epidermal keratinocytes (NHEKs)	-	-MTT (cell viability) -DCFH2-DA assay (oxidative damage) -Measure-IT High Sensitivity Nitrite Assay (nitrite intracellular concentration). -IF, RT-PCR (expression of OGG1 and proinflammatory cytokines)	-UVB (280–320 nm) lamp VL6M -Quantum photo/radiometer (HD9021) -Victor X Multilabel Plate Readers (PerkinElmer) -q-RT-PCR	FC-HPKs were isolated from lesions on several locations (face, trunk, scalp, lower and upper limb)	-
OTHER SUPPLEMENTS											
Pilkington, 2016 UK [43]	Clinical trial	n = 79 100% F	18-60	Healthy female volunteers with phototypes I or II	**Active group:** encapsulated n-3 PUFA supplements (70% EPA and 10% DHA). Five capsules daily.	**Placebo group:** encapsulated control lipid supplement (glyceryl tricoprylate coprate). Five capsules daily.	12 w	-Epidermal Langerhans cells number -PGs and cytokines measurement	-Fluorescence microscopy (for epidermal Langerhans cell counting on skin biopsies) -Mass spectrometer (PGs and cytokines measurement)	Skin biopsies from upper buttocks	NR

Nikolakopoulou, 2013 UK [44]	Preclinical trial	-	-	Malignant and premalignant keratinocytes *Malignant cell lines*: -SCC-13 (facial epidermis) -SCC-25 (oral, tongue) *Premalignant cell lines*: -SVHFK (transformed epidermal) -D17, D19 and D20 (oral dysplasia)	Omega-3 polyunsaturated fatty acids: eicosapentaenoic acid (**EPA**) and docosahexaenoic acid (**DHA**) Incubation of cell lines with 3 µM DHA and 3 or 5 µM of EPA, 16h	Normal foreskin epidermal keratinocyte lines: -NHEK-131 -HEK-127 Normal oral keratinocyte lines: -NHOK810 -NHOK881 Non-neoplastic immortal cell lines: -OKF4 -OKF6	-	-MTT (viability) -Tritiated thymidine incorporation assay (proliferation) - DCFH2-DA and anti-8-oxo-dG assay (oxidative damage) -Apoptosis assay -EGFR, ERK1/2 and Akt pathways functioning	-	-	-

Table 2. Characteristics and research specifics of each included study: paper reference (lead author + year of publication + study location); type of study (clinical or preclinical trial); population and study characteristics; (sample size, sex; age-years; health condition); natural product intervention and control (content, daily dose); study duration; test conditions; outcome (measurement instruments and parameters) and adverse events.

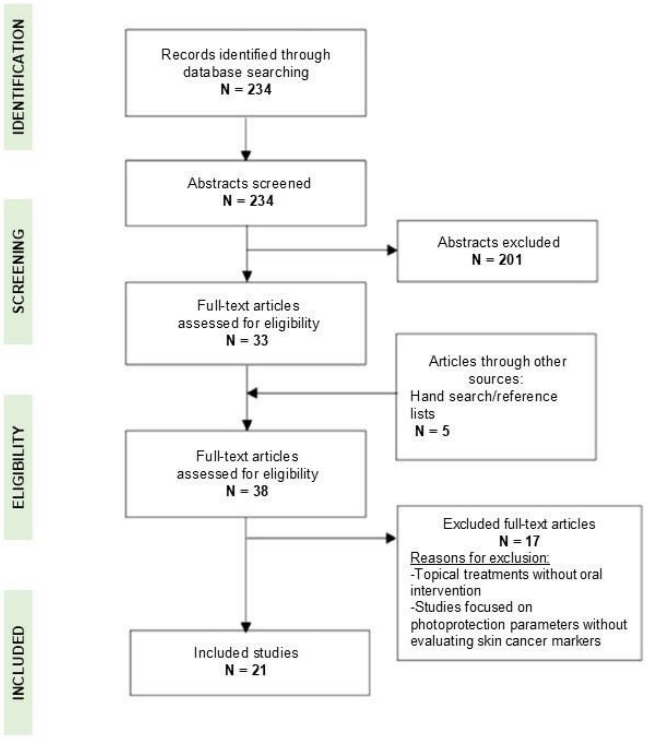

Figure 1: Flow diagram for study selection process following PICAR procedure.

Fig 1

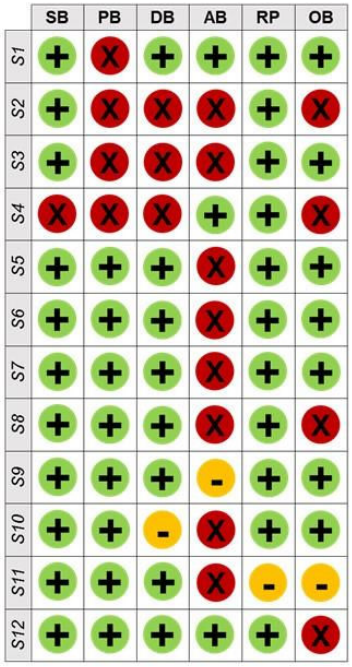

Figure 2: Summary of the reviewers' assessments for each domain of bias risk. Risk of bias. SB (Selection bias); PB (Performance bias); DB (Detection bias); AB (Attrition bias); RB (Reporting bias); OB (Other bias). Studies: (S1) Pellacani et al., 2023; (S2) Auriemma et al., 2015; (S3) Miura et al., 2015; (S4) Pérez-Sánchez et al., 2014; (S5) Chen et al., 2015; (S6) Chen et al., 2016; (S7) Allen et al., 2023; (S8) Freitas et al., 2015; (S9) Pasarelli et al., 2014; (S10) Pilkington et al., 2016; (S11) Donnenfeld et al., 2014; (S12) Nobile et al., 2016.

+: Low risk X: High risk -: Unclear risk

Fig 2