Oral phytochemicals as photoprotectants in UVR exposed hairless mice

A study of hesperidin methyl chalcone, phloroglucinol, and syringic acid

Pihl, Celina; Bendtsen, Katja M. S.; Jensen, Henrik E.; Andersen, Flemming; Bjerring, Peter; Haedersdal, Merete; Lerche, Catharina M.

Published in:
Journal of Photochemistry and Photobiology, B: Biology

DOI (link to publication from Publisher):
10.1016/j.jphotobiol.2023.112760

Publication date:
2023

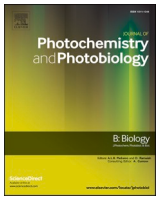
Document Version
Publisher's PDF, also known as Version of record

Journal of Photochemistry & Photobiology, B: Biology 246 (2023) 112760

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol



Oral phytochemicals as photoprotectants in UVR exposed hairless mice: A study of hesperidin methyl chalcone, phloroglucinol, and syringic acid

Celina Pihl [a,f,*], Katja M.S. Bendtsen [b], Henrik E. Jensen [b], Flemming Andersen [c,d], Peter Bjerring [d], Merete Haedersdal [a,e], Catharina M. Lerche [a,f]

[a] Dept of Dermatology, Copenhagen University Hospital – Bispebjerg and Frederiksberg, Copenhagen, Denmark
[b] Dept of Veterinary and Animal Sciences, Copenhagen University, 1870 Frederiksberg, Denmark
[c] Dept of Dermatology, Private Hospital Molholm, 7100 Vejle, Denmark
[d] Dept of Dermatology, Aalborg University Hospital, 9100 Aalborg, Denmark
[e] Dept of Clinical Medicine, University of Copenhagen, 2400 Copenhagen, Denmark
[f] Dept of Pharmacy, University of Copenhagen, 2400 Copenhagen, Denmark

ARTICLE INFO

Keywords:
Ultraviolet radiation
Hairless mice
Skin cancer
Oral delivery
Photoprotection
Phytochemicals

ABSTRACT

Ultraviolet radiation is the primary risk factor for keratinocyte carcinoma. Because of increasing incidence rates, new methods of photoprotection must be explored. Oral supplementation with photoprotective compounds presents a promising alternative. Phytochemical compounds like hesperidin methyl chalcone, phloroglucinol, and syringic acid are particularly of interest because of their antioxidant properties. Our primary outcome was to evaluate the effects of oral phytochemicals on photocarcinogenesis with time until tumour onset as the primary endpoint. A total of 125 hairless C3.Cg-*Hr*^{hr}/TifBom Tac mice were randomised to receive tap water supplemented with either 100 mg/kg hesperidin methyl chalcone, phloroglucinol, or syringic acid, 600 mg/kg nicotinamide as a positive control, or no supplementation. The mice were irradiated with 3.5 standard erythema doses thrice weekly to induce photocarcinogenesis. Supplementation with the phytochemicals phloroglucinol and syringic acid and nicotinamide delayed tumour onset from a median of 140 days to 151 ($p = 0.036$), 157 days ($p = 0.02$), and 178 ($p = 2.7{\cdot}10^{-5}$), respectively. Phloroglucinol and nicotinamide supplementation reduced tumour number. Nicotinamide increased UV-induced pigmentation and reduced oedema formation, while phloroglucinol supplementation reduced epidermal thickness. These results indicate that oral supplementation with phloroglucinol and syringic acid protects against photocarcinogenesis in hairless mice, but not to the same extent as nicotinamide.

1. Introduction

Keratinocyte carcinoma (KC) consisting primarily of basal cell carcinoma and squamous cell carcinoma (SCC) is the most common malignancy affecting white populations [1]. While basal cell carcinoma makes up 70% of the incidences, SCC is responsible for the majority of KC-related deaths [2,3]. Despite different aetiologies, ultraviolet radiation (UVR) is a common risk factor contributing to 90% of KC cases [4]. UVR consists of UVA (315–400 nm), UVB (280–315 nm), and UVC (100–280 nm) [5]. While UVA represents the majority of UV rays reaching the earth (~95%), UVB is easily absorbed by the skin where it contributes to photocarcinogenesis [6]. Albeit with a smaller contribution, UVA adds to the carcinogenic effects of UVR exposure primarily through oxidative stress, whereas UVC is absorbed by the ozone layer and does not reach the earth [7].

Elderly and immunocompromised people represent high-risk patient groups for developing keratinocyte carcinoma [8]. Furthermore, some lifestyles and careers require a sustained and repeated outdoor presence, and with the increase in UVR exposure as a consequence of global climate change [9], alternative approaches to reduce or prevent photocarcinogenesis must be identified to halt increasing KC incidence rates. The first line of photoprotection consists of protective clothing and

Abbreviations: 15-PDGH, 15-hydroxyprostaglandin dehydrogenase; CPD, cyclobutane pyrimidine dimer; KC, keratinocyte carcinoma; NAD^+, nicotinamide-adenine dinucleotide; PDRN, polydeoxyribonucleotide; $PGE2$, Prostaglandin E_2; SCC, squamous cell carcinoma; UVR, ultraviolet radiation..

* Corresponding author.
E-mail addresses: Celina.Pihl@regionh.dk (C. Pihl), kab@sund.ku.dk (K.M.S. Bendtsen), elvang@sund.ku.dk (H.E. Jensen), fan@molholm.dk (F. Andersen), email@peterbjerrig.dk (P. Bjerring), mhaedersdal@dadlnet.dk (M. Haedersdal), catharina.margrethe.lerche@regionh.dk (C.M. Lerche).

https://doi.org/10.1016/j.jphotobiol.2023.112760
Received 28 June 2023; Accepted 21 July 2023
Available online 22 July 2023

the use of topically applied sunscreen to absorb or reflect UVR. But current formulations of sunscreen are applied too infrequently or insufficiently to provide proper protection against the sun [10].

The use of photoprotective compounds – referred to as photoprotectants – has emerged as a promising approach to improve photoprotection [11]. Topically applied photoprotectants have demonstrated significant protection against photocarcinogenesis [12–14], but do not address insufficient application prior to sun exposure. Oral administration bypasses this constraint and ensures a consistent dose of photoprotection with each administration.

Plant-derived compounds represent the majority of proposed photoprotectants. Water-soluble phytochemicals are particularly of interest because of their antioxidant properties [15,16]. Phloroglucinol derived from brown algae species has demonstrated extensive protection against UVR-induced oxidative stress and cyclobutane pyrimidine dimer (CPD) formation in murine skin [17,18]. Administration of the citrus-derived compound hesperidin methyl chalcone to mice led to similar effects with the addition of anti-inflammatory properties [19,20]. Syringic acid is a common plant metabolite found to protect against photocarcinogenesis in mice following topical application [21], but the potential of oral delivery has not been investigated.

Vitamin B_3 has two major forms known as nicotinamide and nicotinic acid, often referred to as niacin. In the context of skin carcinogenesis, photoprotection following both topical nicotinamide [23] and oral niacin administration [24] have been reported in mice. Nicotinamide was included in this study to act as a positive control, as well as to elucidate the properties of oral nicotinamide treatment in hairless mice.

In the present study, we determined the photoprotective potential of three phytochemicals through oral delivery. Our primary outcome was to evaluate their effects on photocarcinogenesis with time until tumour onset as the primary endpoint. Furthermore, we investigated the phytochemicals' photoprotective effects measured by erythema, pigmentation, oedema formation, and epidermal thickness.

2. Materials and Methods

2.1. Animals

Female immunocompetent C3.Cg-*Hr*^{hr}/TifBomTac hairless mice of 24 to 32 weeks of age were purchased from Taconic (Ry, Denmark). Animals were housed in a temperature-controlled facility (23–24 °C) with a 12:12-h light-dark cycle and free access to water and standard laboratory food (Altromin 1324 maintenance feed containing 36 mg niacin/kg feed, Altromin Spezialfutter GmbH & Co. KG, Lage, Germany). The study was carried out in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of The Danish Animal Experiments Inspectorate. All protocols were approved by the Danish Animal Experiments Inspectorate (permit number 2021-15-0201-00905) and our Institutional Animal Care and Use Committee.

2.2. UV Radiation

The mice were exposed to 3.5 standard erythema doses thrice weekly through the tops of wire lid cages. The cages were rotated each time to ensure uniform exposure to the UV lamps. The lamps consisted of a UV6 tube (Waldmann, Wheeling, IL, USA) located between five Bellarium-S SA-1-12 tubes (Wolff System, Atlanta, Georgia, USA) with a maximum UVR dose at 365 nm, and 5.9% of the energy output from the UVB range as measured by a spectroradiometer (Solatell Sola-Hazard 4D Controls Ltd., Cornwall, UK) [25].

2.3. Study Design and Outcome Measures

Totally, 125 mice were anaesthetised with 0.05 mL HypDorm (0.158 mg/mL fentanyl citrate, 5 mg/mL fluanisone, 2.5 mg/mL midazolam), tattooed with an individual identification number on the abdomen, and randomised to one of the five treatments. Groups of 25 mice each received standard tap water supplemented with either 100 mg/kg hesperidin methyl chalcone, phloroglucinol, or syringic acid, 600 mg/kg nicotinamide serving as the positive control, or a non-supplemented UV control. Animals were given freshly prepared, supplemented water each morning for two weeks prior to UV radiation and throughout the study period.

The photoprotective abilities of the three phytochemicals were determined on two levels: photoprotective effects and photocarcinogenesis. Photoprotective effects were examined by erythema, pigmentation, oedema formation measured by ear punch weight, and epidermal thickness. The effects on photocarcinogenesis were evaluated by time until tumour onset as the primary endpoint, as well as tumour number and size, and histopathological tumour grading. Fig. 1 presents an overview of the study design with included outcome measures.

2.4. Erythema

Erythema was determined weekly for the first eight weeks on the dorsal skin of the mouse, whereafter development of pigmentation made it difficult to assess. Erythema scores were evaluated on a scale adapted from the OECD test (the Draize scale) with the following scores: 0 – no erythema, 1 – mild erythema, 2 – moderate erythema, 3 – severe erythema, 4 – very severe erythema [26,27].

2.5. Pigmentation and Weight

UV-induced pigmentation and weight were evaluated monthly. Pigmentation was assessed using a protocol adapted from Hansen et al. [28]. In brief, the mouse was placed under six Philips TL08 fluorescent UVA tubes (Philips, Eindhoven, The Netherlands) in a dark room. Dorsal pigmentation was scored by comparison with a Kodak Gray Scale of 20 different shades from white to black.

2.6. Tumour Measurements and Endpoints

Mice were examined weekly for the appearance of new tumours. Tumours with a diameter of 1 mm or above were mapped for each animal and tracked until an endpoint was reached. Tumours that reached a diameter of 4 mm or above were included in tumour onset calculations, defined as the median time between the start of UVR protocol until detection of the first, second, and third tumour. Mice were euthanised according to protocol after 365 days, or upon the development of 1 12 mm tumour, or 3 tumours with diameters of 4 mm each [29,30]. Upon euthanasia, tumour number and size of tumours with a diameter of at least 4 mm were recorded, and tumour area was calculated with the formula: tumour area $= \pi \bullet \left(\frac{\text{tumour length}}{2}\right) \bullet \left(\frac{\text{tumour width}}{2}\right)$.

2.7. Ear Punch Weight

To determine oedema formation following long-term UVR exposure, ear punch weights were determined. Following euthanasia, the ears of the mice were removed by scissors. Three 4-mm diameter punch biopsies were taken from each ear (a total of six punches from each mouse) and immediately weighed.

2.8. Histopathology

The dorsal skin of animals was removed. Excised tumours were fixed in 4% buffered formaldehyde for five days, dehydrated, and embedded in paraffin wax. Sections of 2–4 μm were cut and stained with haematoxylin and eosin (HE). Five tumours from each group were randomly selected for histopathological evaluation, and it was confirmed that the tumours represented SCCs in all cases. Each tumour was graded blindly by a trained pathologist based on the following categories: SCC in situ,

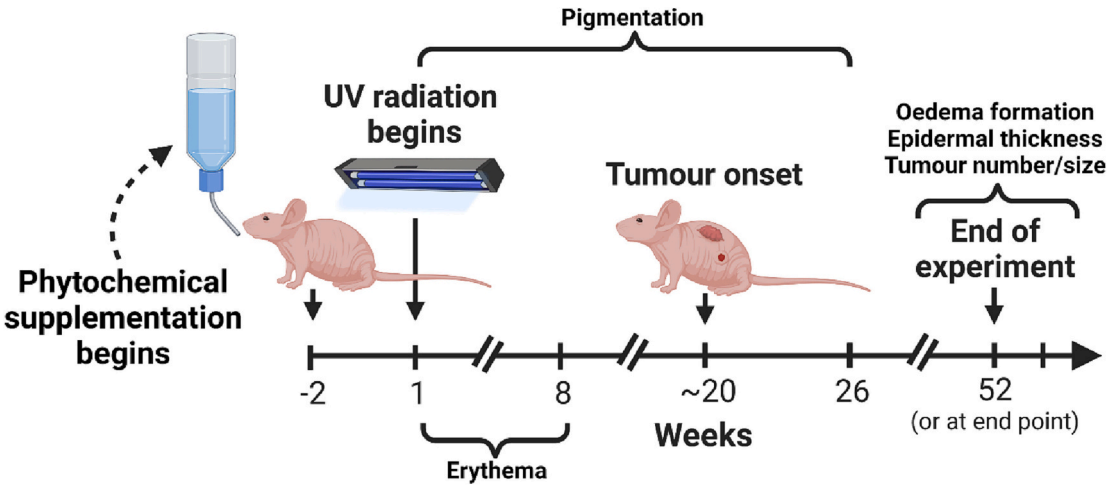


Fig. 1. Study design and associated outcome measures.

micro-invasive SCC with distinguishable (B1) or invaded dermis (B2), micro-invasive/invasive SCC, or invasive conventional SCC [31–36]. Sections of non-tumour skin were also HE stained, and the thickness of the epidermis was measured thrice on five mice from each group.

2.9. Statistics

Tumour onset data were visualised by Kaplan-Meier plots [37], and comparisons between the UV control group and phytochemical groups were made using the log-rank test (Mantel-Cox). The remaining data were assessed either by Student's *t*-test or non-parametric Mann-Whitney *U* Test depending on the normality of the data. A Fisher's exact test was used to compare independence between treatment and tumour grading. Differences were considered significant when the *p*-value was observed to be less than 0.05. All analyses were carried out using R version 4.1.0 [38].

3. Results

3.1. Photoprotective Effects Following Phytochemical Supplementation

All mice developed erythema and pigmentation following UVR exposure (Figs. 2A and B). Phytochemical and nicotinamide supplementation did not affect the erythema score following eight weeks of UVR as measured by the area under the curve (Fig. 2C). Supplementation with nicotinamide, but none of the phytochemicals, increased pigmentation to a median area under the curve of 22.5 compared to the UV control median of 17 (Fig. 2D). Representative pictures of pigmentation at week 21 are shown in Fig. 2E. No difference was observed following hesperidin methyl chalcone (Hesperidin MC), phloroglucinol, or syringic acid supplementation.

To assess the anti-inflammatory capacity of phytochemical supplementation, UV-induced oedema was measured. Based on punch biopsies taken from the ears, it was found that supplementation with nicotinamide significantly reduced oedema (Fig. 3A). Non-supplemented mice had a median ear punch weight of 3.87 mg, which was decreased to 3.67 mg following nicotinamide treatment. Supplementation with phloroglucinol had no effect on oedema formation but significantly reduced epidermal thickness from a mean of 86.74 μm of the non-supplemented control to 65.21 μm (Fig. 3B and Table 1). Representative HE-stained sections are presented in Fig. 4.

3.2. Delay of Tumour Onset by Phytochemical Supplementation

All groups developed tumours in response to UVR. Non-supplemented mice demonstrated a median of 140 days until the onset of the first tumour. Supplementation with phloroglucinol, syringic acid, and nicotinamide delayed the onset to medians of 151, 157, and 178 days, respectively (Fig. 5A). Supplementation with nicotinamide further delayed the onset of the second and third tumour compared to non-supplemented mice (Figs. 5B and C). Hesperidin methyl chalcone supplementation did not delay tumour onset. The median number of days until tumour onset is presented in Table 2. No difference was found in the body weight of animals (data not shown).

3.3. Evaluation of Tumour Development Following Phytochemical Supplementation

Supplementation with phloroglucinol and nicotinamide significantly reduced the number of tumours (Fig. 6A). Non-supplemented mice demonstrated a median of 4 tumours, which was reduced to 3 tumours following both phloroglucinol and nicotinamide treatment. No effect on tumour area was determined (Fig. 6B, Table 3). Histopathological grading of five tumours from each group is presented in Table 4. No dependency between phytochemical supplementation and tumour grading was found using a Fisher's exact test ($p = 0.06157$).

4. Discussion

Oral administration of photoprotective compounds presents a promising alternative to classical photoprotection measures. In this study, we investigated the abilities of three phytochemical compounds to act photoprotective following oral delivery. We found that supplementation with the phytochemicals phloroglucinol and syringic acid as well as the positive control nicotinamide significantly reduces photocarcinogenesis in hairless mice, in which no dependency on tumour grading was found.

Topical application of phloroglucinol is reported to reduce the formation of CPD lesions through the induction of the DNA repair machinery [18]. Phloroglucinol's photoprotection may also be attributed to its antioxidant capacity [17,39], possibly due to induction of Nrf-2 which governs antioxidant expression [40]. Phloroglucinol supplementation effectively delayed tumour onset and reduced the number of tumours but had no effect on tumour size (Fig. 6). These results suggest that phloroglucinol delays the onset of photocarcinogenesis through the induction of DNA repair and antioxidants but does not prevent further progression when malignancy has been induced.

The photoprotective potential of syringic acid has already been reported, demonstrating a reduction in tumour incidence and number of tumours following topical administration [21]. In the present study, we

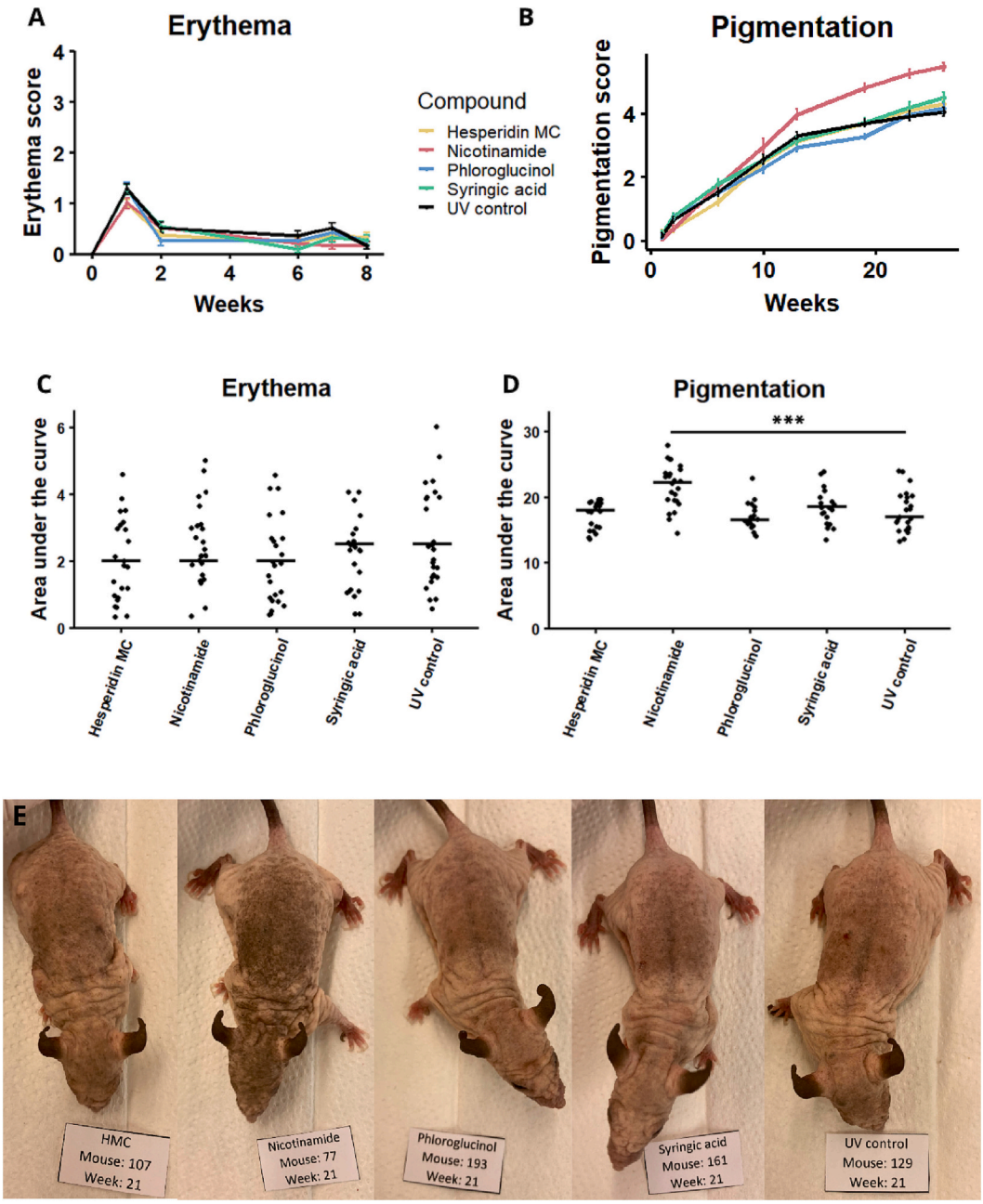


Fig. 2. Effects of phytochemical supplementation on UVR-induced erythema and pigmentation. A: dorsal erythema scores over the first eight weeks of UV radiation. B: development of dorsal pigmentation over 26 weeks of radiation. C and D: area under the curve from A (C) and B (D). E: Representative pictures of dorsal pigmentation at week 21. Values shown as means ± SEM (A-B) or individual data points with the median score presented as a line (C and D). HMC: hesperidin methyl chalcone. *** signifies a *p*-value lower than 0.001.

found a significant delay in the onset of the first tumour (Fig. 5), but no effect on either tumour number or size (Fig. 6). From experience working with the present mouse model (C3.Cg-*Hr*^{hr}/TifBomTac), we have found that tumours must be of a certain size to ensure that they are SCCs and not benign neoplasms. Therefore, only tumours with a diameter of 4 mm or above were included in determination of tumour number and size in the current study. Although Ha et al. [21] provided no protocol, it seems from the images supplied that all visible tumours were considered when determining the number of tumours [21], possibly impacting the estimation of protection. Given these reports, oral syringic acid supplementation reduces photocarcinogenesis, likely due to cyclooxygenase-2 inhibition mediated by reduced MAPK pathway activation as observed following topical administration [21].

While these results provide evidence for a photoprotective role of phloroglucinol and syringic acid, the observed effects are not as comprehensive as what we report for the positive control nicotinamide. In the present study, oral nicotinamide supplementation significantly delayed tumour onset and reduced the number of tumours (Figs. 5 and 6A). Nicotinamide photoprotection is proposed to be mediated through immunomodulation and maintenance of nicotinamide-adenine dinucleotide (NAD^+) content [23,24]. We also found that nicotinamide supplementation protected against UVR-induced oedema in hairless mice (Fig. 3A). Prostaglandin E_2 (PGE_2) expression is induced in response to UV radiation and governs many aspects of UVR-induced skin inflammation, including oedema formation [41,42]. Nicotinamide was recently found to reduce UVB-induced inflammation in human epidermal keratinocytes and skin, measured by decreased expression of pro-inflammatory mediators PGE_2, IL-4, and IL-8 [43]. PGE_2 is degraded

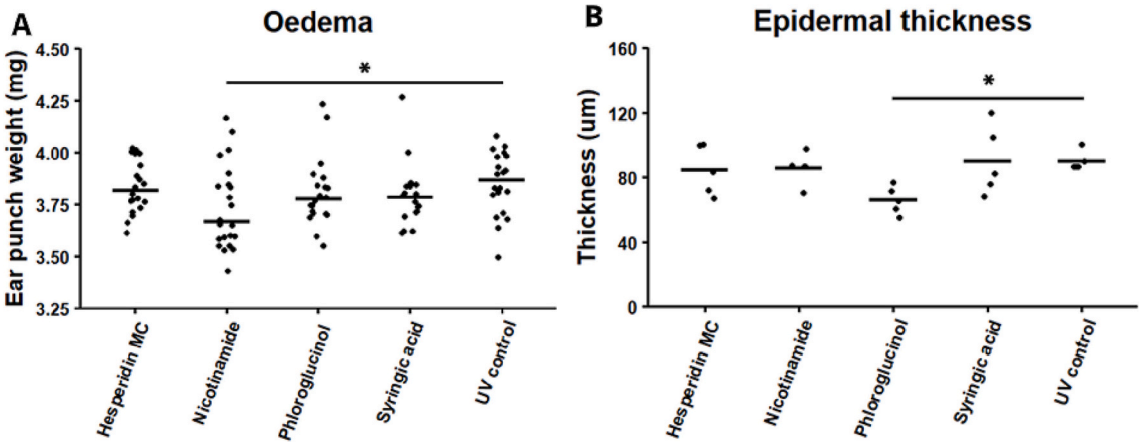


Fig. 3. Inhibitory effects of phytochemical supplementation on oedema formation and epidermal thickness. A: ear punch weight measured 24 h after the last UVR dose. B: epidermal thickness determined by dorsal HE-stained skin sections from five mice from each group at the end of the study period. Data shown as individual data points with the median (A) or mean (B) score presented as a line. * signifies a *p*-value lower than 0.05.

Table 1
Photoprotective effects following phytochemical supplementation. Effects of phytochemicals on erythema, pigmentation, oedema formation, and epidermal thickness.

		Hesperidin MC	Nicotinamide	Phloroglucinol	Syringic acid	UV control
Erythema	Median	2	2	2	2.75	3
	p-value	0.163	0.072	0.329	0.465	
Pigmentation	Median	18	22.5	16.5	18.5	17
	p-value	0.625	$5{\cdot}10^{-4}$	0.494	0.386	
Oedema formation	Median	3.82 mg	3.67 mg	3.78 mg	3.78 mg	3.87 mg
	p-value	0.579	0.036	0.184	0.092	
Epidermal thickness	Mean	83.35 µm	86.59 µm	65.21 µm	81.98 µm	86.74 µm
	p-value	0.528	0.617	0.0101	0.9859	

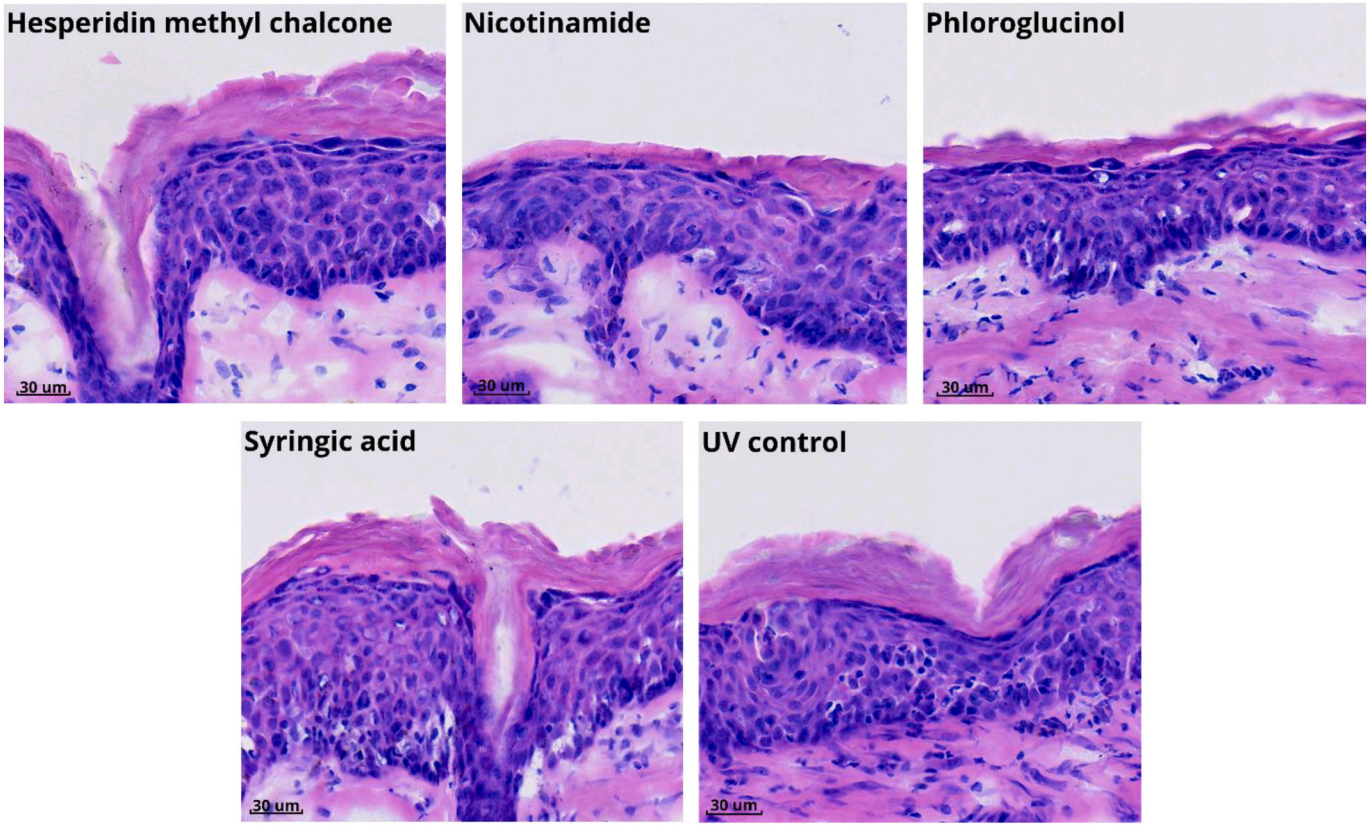


Fig. 4. Influence of phytochemical supplementation on epidermal thickness. Representative images from five dorsal skin sections stained with HE from each group.

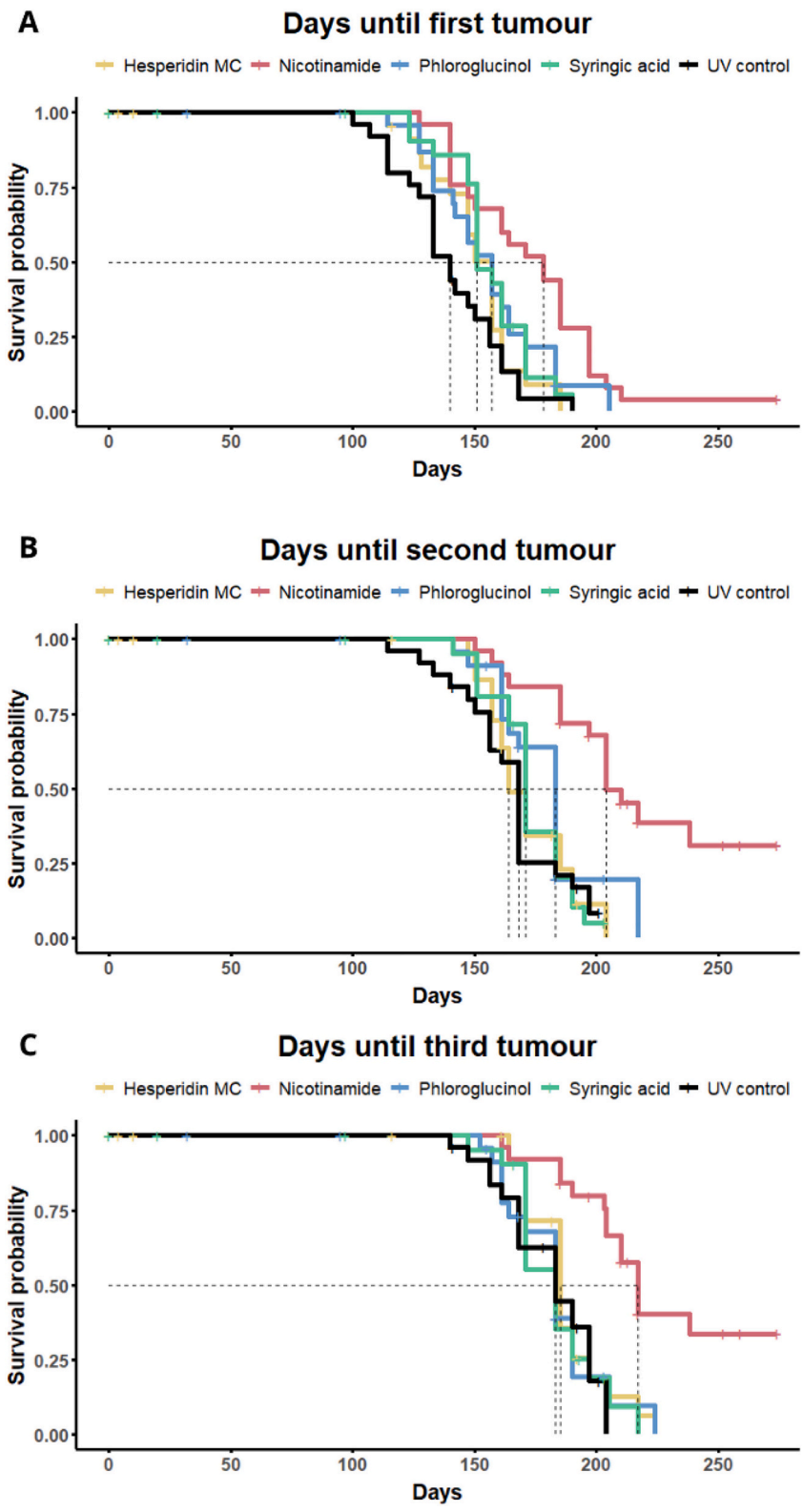


Fig. 5. Photoprotection following phytochemical supplementation. Kaplan-Meier plots presenting the probability of survival without one (A), two (B), or three (C) tumours with a diameter of 4 mm or above.

by NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase (15-PDGH) [44], which is suppressed following UV radiation [45]. These data indicate that nicotinamide supplementation increases NAD^+ content, which in turn may induce 15-PDGH activity to degrade PGE_2 and prevent UVR-induced inflammation.

C3.Cg-^{Hrhr}/TifBomTac mice develop uniform pigmentation located in the epidermis [46,47]. Nicotinamide supplementation increased dorsal pigmentation in the present mouse model (Figs. 2B, D and E). UVR-induced pigmentation protects against subsequent UVR exposure by absorbing UV rays reaching the epidermis [48,49]. Although the

Table 2
Days until the onset of the first, second, and third tumour with a diameter of 4 mm or above.

Tumour onset		Hesperidin MC	Nicotinamide	Phloroglucinol	Syringic acid	UV control
1st tumour	Median days	157	178	151	157	140
	p-value	0.216	$2.7{\cdot}10^{-5}$	0.036	0.02	
2nd tumour	Median days	164	204	183	171	168
	p-value	0.668	$1.5{\cdot}10^{-5}$	0.106	0.423	
3rd tumour	Median days	185	217	183	183	183
	p-value	0.49	$5.6{\cdot}10^{-6}$	0.81	0.74	

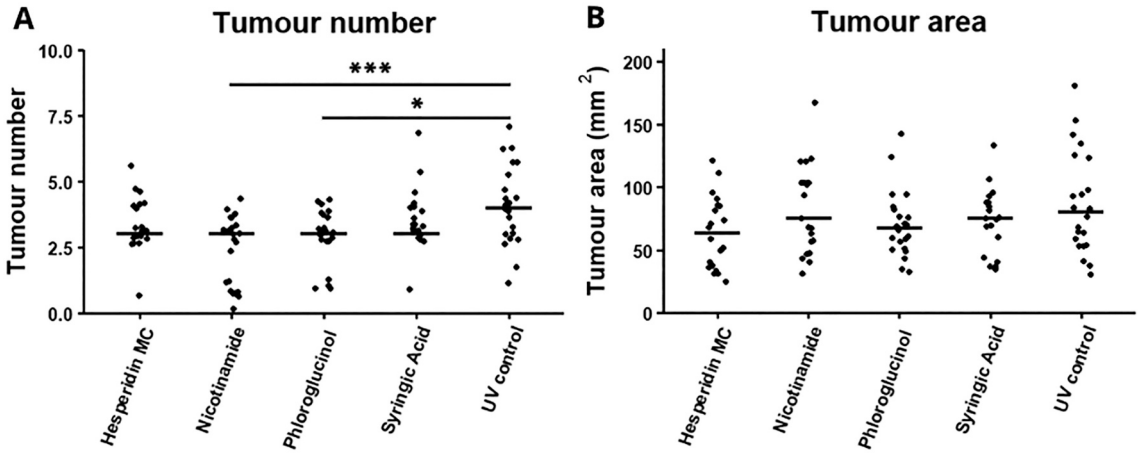


Fig. 6. Influence on tumour development following phytochemical supplementation. Tumour number (A) and tumour area (B) of tumours with a diameter of 4 mm or above. Data shown as individual data points with the median score presented as a line. * and *** signify *p*-values lower than 0.05 and 0.001, respectively.

Table 3
Evaluation of tumour development. Effects of phytochemicals tumour number and size.

		Hesperidin MC	Nicotinamide	Phloroglucinol	Syringic acid	UV control
Tumour number	Median	3	3	3	3	4
	p-value	0.1	$4.2{\cdot}10^{-4}$	0.016	0.199	
Tumour size	Median	63.62 mm^2	75.40 mm^2	67.54 mm^2	75.40 mm^2	80.11 mm^2
	p-value	0.064	0.75	0.218	0.41	

Table 4
Histopathological grading of tumours following phytochemical supplementation. SCC tumours were graded based on the following categories: SCC in situ, micro-invasive SCC with distinguishable (B1) or invaded dermis (B2), micro-invasive/invasive SCC, or invasive conventional SCC.

SCC grading	SCC in situ	Micro-invasive (B1)	Micro-invasive (B2)	Micro-invasive/ invasive	Invasive
Hesperidin MC	0	0	2	0	3
Nicotinamide	0	1	0	0	4
Phloroglucinol	0	3	0	0	2
Syringic acid	0	0	1	3	1
UV control	0	1	1	0	3

exact chromophore for pigmentation remains unidentified, p53 activation following DNA damage has been proposed to be important [50]. p53 directly increases transcription of the pro-opiomelanocortin gene, processed in part to the α-melanocyte-stimulating hormone, which in turn stimulates melanogenesis [50]. Nicotinamide treatment increases p53 expression in UV-irradiated keratinocytes [51], indicating a possible mechanism for the observed pigmentation.

However, a combination of nicotinamide, vitamin C, and polydeoxyribonucleotide (PDRN) delivered through a microneedle system was recently shown to significantly reduce UVR-induced melanin content in murine skin [52]. As PDRN has been demonstrated to supress melanin content independently [53], and no data was shown on the effects of the individual components, the observed effect is likely due to PDRN's inherent anti-melanogenic properties with little effect from nicotinamide.

With increasing incidence rates of keratinocyte carcinoma [54,55], the need for alternative method of photoprotection has gained attention. The use of topical phytochemical formulations has a limited potential due to an efficacy dependent on proper application, which is often not achieved [10]. Oral photoprotection has proven effective in several studies [56,57], but few has been translated to human trials focusing on keratinocyte carcinoma prevention. Oral nicotinamide supplementation has demonstrated good protection against the appearance of new actinic keratoses and keratinocyte carcinomas in non-immunosuppressed people [58]. But a recent study showed that daily nicotinamide supplementation did not reduce the incidence of new lesions in immunosuppressed transplant recipients [59]. This lack of robust photoprotection in high-risk patient groups may be an indication that monotherapies are not sufficient prevention methods. Instead, identification of photoprotective compounds when used in combinations provide a stronger, synergistic protection should be prioritised.

In summary, we found that oral supplementation with the phytochemicals phloroglucinol and syringic acid, but not hesperidin methyl chalcone, reduces photocarcinogenesis in hairless mice, albeit not quite to the effect of nicotinamide. Furthermore, we have demonstrated that oral nicotinamide treatment increases dorsal pigmentation and reduces UVR-induced oedema in response to long-term UVR exposure. These

results suggest that oral supplementation with phloroglucinol and syringic may contribute to an effective alternative for photoprotection.

CRediT authorship contribution statement

Celina Pihl: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Katja M.S. Bendtsen:** Methodology, Investigation, Writing – review & editing. **Henrik E. Jensen:** Methodology, Validation, Writing – review & editing. **Flemming Andersen:** Conceptualization, Writing – review & editing, Funding acquisition. **Peter Bjerring:** Conceptualization, Writing – review & editing, Funding acquisition. **Merete Haedersdal:** Conceptualization, Resources, Writing – review & editing, Funding acquisition. **Catharina M. Lerche:** Conceptualization, Methodology, Resources, Writing – review & editing, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The authors would like to thank Catrine Fischer Goldschmidt and Diana Høeg (Department of Dermatology, Copenhagen University Hospital) for their help with animal care and histology. The work was executed and funded as a part of the Danish Research Center for Skin Cancer (www.researchcenterforskincancer.org), a public-private research partnership between the Private Hospital Molholm, Aalborg University Hospital and Copenhagen University Hospital, Bispebjerg and Frederiksberg, as well as the Skin Cancer INnovation Clinical Academic Group (SCIN CAG), Greater Copenhagen Health Science Partners (GCHSP). The funding source was not involved in any step of the study creation nor submission.

References

[1] A. Lomas, J. Leonardi-Bee, F. Bath-Hextall, A systematic review of worldwide incidence of nonmelanoma skin cancer, Br. J. Dermatol. 166 (2012) 1069–1080, https://doi.org/10.1111/j.1365-2133.2012.10830.x.

[2] U. Leiter, T. Eigentler, C. Garbe, Epidemiology of skin Cancer, in: J. Reichrath (Ed.), Sunlight Vitam. Skin Cancer, Springer, New York, New York, NY, 2014, pp. 120–140, https://doi.org/10.1007/978-1-4939-0437-2_7.

[3] V. Barton, K. Armeson, S. Hampras, L.K. Ferris, K. Visvanathan, D. Rollison, A. J. Alberg, Nonmelanoma skin cancer and risk of all-cause and cancer-related mortality: a systematic review, Arch. Dermatol. Res. 309 (2017) 243–251, https://doi.org/10.1007/s00403-017-1724-5.

[4] Y. Kim, Y.-Y. He, Ultraviolet radiation-induced non-melanoma skin cancer: regulation of DNA damage repair and inflammation, Genes Dis. 1 (2014) 188–198, https://doi.org/10.1016/j.gendis.2014.08.005.

[5] B. Dale Wilson, S. Moon, F. Armstrong, Comprehensive review of ultraviolet radiation and the current status on sunscreens, J. Clin. Aesthetic Dermatol. 5 (2012) 18–23.

[6] A. Slominski, J. Pawelek, Animals under the sun: effects of ultraviolet radiation on mammalian skin, Clin. Dermatol. 16 (1998) 503–515, https://doi.org/10.1016/s0738-081x(98)00023-6.

[7] F. El Ghissassi, R. Baan, K. Straif, Y. Grosse, B. Secretan, V. Bouvard, L. Benbrahim-Tallaa, N. Guha, C. Freeman, L. Galichet, V. Cogliano, A review of human carcinogens–part D: radiation, Lancet Oncol. 10 (2009) 751–752, https://doi.org/10.1016/s1470-2045(09)70213-x.

[8] G. Tessari, G. Girolomoni, Nonmelanoma skin cancer in solid organ transplant recipients: update on epidemiology, risk factors, and management, Dermatol. Surg. 38 (2012) 1622–1630, https://doi.org/10.1111/j.1524-4725.2012.02520.x.

[9] A. Amaro-Ortiz, B. Yan, J.A. D'Orazio, Ultraviolet radiation, aging and the skin: prevention of damage by topical cAMP manipulation, Mol. Basel Switz. 19 (2014) 6202–6219, https://doi.org/10.3390/molecules19056202.

[10] D.M. Holman, Z. Berkowitz, G.P.J. Guy, N.A. Hawkins, M. Saraiya, M. Watson, Patterns of sunscreen use on the face and other exposed skin among US adults, J. Am. Acad. Dermatol. 73 (2015) 83–92.e1, https://doi.org/10.1016/j.jaad.2015.02.1112.

[11] C. Pihl, K. Togsverd-Bo, F. Andersen, M. Haedersdal, P. Bjerring, C.M. Lerche, Keratinocyte carcinoma and Photoprevention: the protective actions of repurposed pharmaceuticals, phytochemicals and vitamins, Cancers. 13 (2021), https://doi.org/10.3390/cancers13153684.

[12] S.K. Katiyar, N.J. Korman, H. Mukhtar, R. Agarwal, Protective effects of silymarin against photocarcinogenesis in a mouse skin model, J. Natl. Cancer Inst. 89 (1997) 556–566, https://doi.org/10.1093/jnci/89.8.556.

[13] A. Mittal, C. Piyathilake, Y. Hara, S.K. Katiyar, Exceptionally high protection of photocarcinogenesis by topical application of (−)-epigallocatechin-3-gallate in hydrophilic cream in SKH-1 hairless mouse model: relationship to inhibition of UVB-induced global DNA hypomethylation, Neoplasia N. Y. N. 5 (2003) 555–565, https://doi.org/10.1016/s1476-5586(03)80039-8.

[14] K.-D. Tsai, J.-C. Lin, S.-M. Yang, M.-J. Tseng, J.-D. Hsu, Y.-J. Lee, J.-M., Cherng, curcumin protects against UVB-induced skin cancers in SKH-1 hairless mouse: analysis of early molecular markers in carcinogenesis., Evid.-based complement, Altern. Med. ECAM. 2012 (2012), 593952, https://doi.org/10.1155/2012/593952.

[15] Z. Abotorabi, M. Khorashadizadeh, M. Arab, M. Hassanpour Fard, A. Zarban, Jujube and green tea extracts protect human fibroblast cells against UVB-mediated photo damage and MMP-2 and MMP-9 production, Avicenna J. Phytomedicine 10 (2020) 287–296.

[16] S.K. Katiyar, F. Afaq, K. Azizuddin, H. Mukhtar, Inhibition of UVB-induced oxidative stress-mediated phosphorylation of mitogen-activated protein kinase signaling pathways in cultured human epidermal keratinocytes by green tea polyphenol (−)-Epigallocatechin-3-gallate, Toxicol. Appl. Pharmacol. 176 (2001) 110–117, https://doi.org/10.1006/taap.2001.9276.

[17] M.J. Piao, M.J. Ahn, K.A. Kang, K.C. Kim, J. Zheng, C.W. Yao, J.W. Cha, C.L. Hyun, H.K. Kang, N.H. Lee, J.W. Hyun, Phloroglucinol inhibits ultraviolet B radiation-induced oxidative stress in the mouse skin, Int. J. Radiat. Biol. 90 (2014) 928–935, https://doi.org/10.3109/09553002.2014.911990.

[18] M.J. Piao, M.J. Ahn, K.A. Kang, K.C. Kim, J.W. Cha, N.H. Lee, J.W. Hyun, Phloroglucinol enhances the repair of UVB radiation-induced DNA damage via promotion of the nucleotide excision repair system in vitro and in vivo, DNA Repair. 28 (2015) 131–138, https://doi.org/10.1016/j.dnarep.2015.02.019.

[19] R.M. Martinez, F.A. Pinho-Ribeiro, V.S. Steffen, C.V. Caviglione, J.A. Vignoli, M. M. Baracat, S.R. Georgetti, W.A. Verri, R. Casagrande, Hesperidin methyl chalcone inhibits oxidative stress and inflammation in a mouse model of ultraviolet B irradiation-induced skin damage, J. Photochem. Photobiol. B 148 (2015) 145–153, https://doi.org/10.1016/j.jphotobiol.2015.03.030.

[20] R.M. Martinez, F.A. Pinho-Ribeiro, V.S. Steffen, C.V. Caviglione, D. Pala, M. M. Baracat, S.R. Georgetti, W.A. Verri, R. Casagrande, Topical formulation containing hesperidin methyl chalcone inhibits skin oxidative stress and inflammation induced by ultraviolet B irradiation, Photochem. Photobiol. Sci. 15 (2016) 554–563, https://doi.org/10.1039/c5pp00467e.

[21] S.J. Ha, J. Lee, J. Park, Y.H. Kim, N.H. Lee, Y.E. Kim, K.-M. Song, P.-S. Chang, C.-H. Jeong, S.K. Jung, Syringic acid prevents skin carcinogenesis via regulation of NoX and EGFR signaling, Biochem. Pharmacol. 154 (2018) 435–445, https://doi.org/10.1016/j.bcp.2018.06.007.

[23] H.L. Gensler, Prevention of photoimmunosuppression and photocarcinogenesis by topical nicotinamide, Nutr. Cancer 29 (1997) 157–162, https://doi.org/10.1080/01635589709514618.

[24] H.L. Gensler, T. Williams, A.C. Huang, E.L. Jacobson, Oral niacin prevents Photocarcinogenesis and Photoimmunosuppression in mice, Nutr. Cancer 34 (1999) 36–41, https://doi.org/10.1207/S15327914NC340105.

[25] C.M. Lerche, M. Sepehri, J. Serup, T. Poulsen, H.C. Wulf, Black tattoos protect against UVR-induced skin cancer in mice, Photodermatol. Photoimmunol. Photomed. 31 (2015) 261–268, https://doi.org/10.1111/phpp.12181.

[26] E. Wenande, J. Tam, B. Bhayana, S.K. Schlosser, E. Ishak, W.A. Farinelli, A. Chlopik, M.P. Hoang, O.R. Pinkhasov, P. Caravan, R. Rox Anderson, M. Haedersdal, Laser-assisted delivery of synergistic combination chemotherapy in in vivo skin, J. Control. Release 275 (2018) 242–253, https://doi.org/10.1016/j.jconrel.2018.02.019.

[27] OECD, Test No. 404: Acute Dermal Irritation/Corrosion. https://www.oecd-ilibrary.org/content/publication/9789264070622-en, 2002.

[28] A.B. Hansen, N. Bech-Thomsen, H.C. Wulf, In vivo estimation of pigmentation in ultraviolet-exposed hairless mice, Photodermatol. Photoimmunol. Photomed. 11 (1995) 14–17, https://doi.org/10.1111/j.1600-0781.1995.tb00131.x.

[29] H.C. Wulf, R.N. Al-Chaer, M. Glud, P.A. Philipsen, C.M. Lerche, A skin cancer prophylaxis study in hairless mice using methylene blue, riboflavin, and methyl aminolevulinate as photosensitizing agents in photodynamic therapy, Pharm. Basel Switz 14 (2021), https://doi.org/10.3390/ph14050433.

[30] F.E. Pinto, P. Olsen, M. Glud, H.C. Wulf, C.M. Lerche, Topical Brimonidine delays ultraviolet radiation-induced squamous cell carcinoma in hairless mice, Photochem. Photobiol. 98 (2022) 1390–1394, https://doi.org/10.1111/php.13622.

[31] F. Benavides, T.M. Oberyszyn, A.M. VanBuskirk, V.E. Reeve, D.F. Kusewitt, The hairless mouse in skin research, J. Dermatol. Sci. 53 (2009) 10–18, https://doi.org/10.1016/j.jdermsci.2008.08.012.

[32] V.R. Yanofsky, S.E. Mercer, R.G. Phelps, Histopathological variants of cutaneous squamous cell carcinoma: a review, J. Skin Cancer 2011 (2011) 210813, https://doi.org/10.1155/2011/210813.

[33] C.A. Mintie, A.K. Musarra, C.K. Singh, M.A. Ndiaye, R. Sullivan, J.C. Eickhoff, N. Ahmad, Protective effects of dietary grape on UVB-mediated cutaneous damages and skin tumorigenesis in SKH-1 mice, Cancers. 12 (2020), https://doi.org/10.3390/cancers12071751.
[34] I.M. Carrara, G.P. Melo, S.S. Bernardes, F.S. Neto, L.N.Z. Ramalho, P.C. Marinello, R.C. Luiz, R. Cecchini, A.L. Cecchini, Looking beyond the skin: cutaneous and systemic oxidative stress in UVB-induced squamous cell carcinoma in hairless mice, J. Photochem. Photobiol. B 195 (2019) 17–26, https://doi.org/10.1016/j.jphotobiol.2019.04.007.
[35] P.Y. Huang, A. Balmain, Modeling cutaneous squamous carcinoma development in the mouse., cold spring Harb, Perspect. Med. 4 (2014), a013623, https://doi.org/10.1101/cshperspect.a013623.
[36] M. Kiupel, M.H. Goldschmidt, R. Klopfleisch, J.S. Munday, J.L. Sruggs, Surgical Pathology of Tumors of Domestic Animals Volume 1: Epithelial Tumors of the Skin, Davis Thompson Foundation, 2018.
[37] A. Kassambara, Biecek Marcin Kosinski, Przemyslaw, survminer: Drawing Survival Curves using "ggplot2". https://CRAN.R-project.org/package=survminer, 2021.
[38] R Core Team, R: A Language and Environment for Statistical Computing. https://www.R-project.org/, 2021.
[39] K.C. Kim, M.J. Piao, S.J. Cho, N.H. Lee, J.W. Hyun, Phloroglucinol protects human keratinocytes from ultraviolet B radiation by attenuating oxidative stress, Photodermatol. Photoimmunol. Photomed. 28 (2012) 322–331, https://doi.org/10.1111/phpp.12010.
[40] K. Itoh, T. Chiba, S. Takahashi, T. Ishii, K. Igarashi, Y. Katoh, T. Oyake, N. Hayashi, K. Satoh, I. Hatayama, M. Yamamoto, Y. Nabeshima, An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements, Biochem. Biophys. Res. Commun. 236 (1997) 313–322, https://doi.org/10.1006/bbrc.1997.6943.
[41] C.C. Miller, P. Hale, A.P. Pentland, Ultraviolet B injury increases prostaglandin synthesis through a tyrosine kinase-dependent pathway. Evidence for UVB-induced epidermal growth factor receptor activation, J. Biol. Chem. 269 (1994) 3529–3533.
[42] K. Kabashima, M. Nagamachi, T. Honda, C. Nishigori, Y. Miyachi, Y. Tokura, S. Narumiya, Prostaglandin E2 is required for ultraviolet B-induced skin inflammation via EP2 and EP4 receptors, Lab. Investig. 87 (2007) 49–55, https://doi.org/10.1038/labinvest.3700491.
[43] J.C. Bierman, T. Laughlin, M. Tamura, B.C. Hulette, C.E. Mack, J.D. Sherrill, C.Y. R. Tan, M. Morenc, S. Bellanger, J.E. Oblong, Niacinamide mitigates SASP-related inflammation induced by environmental stressors in human epidermal keratinocytes and skin, Int. J. Cosmet. Sci. 42 (2020) 501–511, https://doi.org/10.1111/ics.12651.
[44] C.M. Ensor, H.-H. Tai, 15-Hydroxyprostaglandin dehydrogenase, J. Lipid Mediat. Cell Signal. 12 (1995) 313–319.
[45] B.L. Judson, A. Miyaki, V.D. Kekatpure, B. Du, P. Gilleaudeau, M. Sullivan-Whalen, A. Mohebati, S. Nair, J.O. Boyle, R.D. Granstein, K. Subbaramaiah, J.G. Krueger, A. J. Dannenberg, UV radiation inhibits 15-hydroxyprostaglandin dehydrogenase levels in human skin: evidence of transcriptional suppression, Cancer Prev. Res. Phila. Pa. 3 (2010) 1104–1111, https://doi.org/10.1158/1940-6207.CAPR-10-0089.
[46] C.M. Lerche, K. Togsverd-Bo, P.A. Philipsen, H.C. Wulf, Impact of UVR exposure pattern on squamous cell carcinoma-a dose-delivery and dose-response study in pigmented hairless mice, Int. J. Mol. Sci. 18 (2017), https://doi.org/10.3390/ijms18122738.
[47] N. Agar, A.R. Young, Melanogenesis: a photoprotective response to DNA damage? Mutat. Res. 571 (2005) 121–132, https://doi.org/10.1016/j.mrfmmm.2004.11.016.
[48] B.A. Gilchrest, M.S. Eller, DNA Photodamage stimulates Melanogenesis and other Photoprotective responses, J. Investig. Dermatol. Symp. Proc. 4 (1999) 35–40, https://doi.org/10.1038/sj.jidsp.5640178.
[49] N. Kobayashi, A. Nakagawa, T. Muramatsu, Y. Yamashina, T. Shirai, M. W. Hashimoto, Y. Ishigaki, T. Ohnishi, T. Mori, Supranuclear melanin caps reduce ultraviolet induced DNA photoproducts in human epidermis, J. Invest. Dermatol. 110 (1998) 806–810, https://doi.org/10.1046/j.1523-1747.1998.00178.x.
[50] R. Cui, H.R. Widlund, E. Feige, J.Y. Lin, D.L. Wilensky, V.E. Igras, J. D'Orazio, C. Y. Fung, C.F. Schanbacher, S.R. Granter, D.E. Fisher, Central role of p53 in the suntan response and pathologic hyperpigmentation, Cell. 128 (2007) 853–864, https://doi.org/10.1016/j.cell.2006.12.045.
[51] G. Sivapirabu, E. Yiasemides, G.M. Halliday, J. Park, D.L. Damian, Topical nicotinamide modulates cellular energy metabolism and provides broad-spectrum protection against ultraviolet radiation-induced immunosuppression in humans, Br. J. Dermatol. 1951 (161) (2009) 1357–1364, https://doi.org/10.1111/j.1365-2133.2009.09244.x.
[52] H.J. Park, K.-A. Byun, S. Oh, H.M. Kim, M.S. Chung, K.H. Son, K. Byun, The combination of Niacinamide, vitamin C, and PDRN mitigates Melanogenesis by modulating nicotinamide nucleotide transhydrogenase, Mol. Basel Switz. 27 (2022), https://doi.org/10.3390/molecules27154923.
[53] T.K. Noh, B.Y. Chung, S.Y. Kim, M.H. Lee, M.J. Kim, C.S. Youn, M.W. Lee, S. E. Chang, Novel anti-Melanogenesis properties of Polydeoxyribonucleotide, a popular wound healing booster, Int. J. Mol. Sci. 17 (2016), https://doi.org/10.3390/ijms17091448.
[54] F. Birch-Johansen, A. Jensen, L. Mortensen, A.B. Olesen, S.K. Kjær, Trends in the incidence of nonmelanoma skin cancer in Denmark 1978-2007: rapid incidence increase among young Danish women, Int. J. Cancer 127 (2010) 2190–2198, https://doi.org/10.1002/ijc.25411.
[55] H.W. Rogers, M.A. Weinstock, S.R. Feldman, B.M. Coldiron, Incidence Estimate of Nonmelanoma Skin Cancer (Keratinocyte Carcinomas) in the U.S. Population, 2012, JAMA Dermatol. 151 (2015) 1081–1086, https://doi.org/10.1001/jamadermatol.2015.1187.
[56] M. Gu, R.P. Singh, S. Dhanalakshmi, C. Agarwal, R. Agarwal, Silibinin inhibits inflammatory and angiogenic attributes in photocarcinogenesis in SKH-1 hairless mice, Cancer Res. 67 (2007) 3483–3491, https://doi.org/10.1158/0008-5472.CAN-06-3955.
[57] S.M. Meeran, S. Akhtar, S.K. Katiyar, Inhibition of UVB-induced skin tumor development by drinking green tea polyphenols is mediated through DNA repair and subsequent inhibition of inflammation, J. Invest. Dermatol. 129 (2009) 1258–1270, https://doi.org/10.1038/jid.2008.354.
[58] A.C. Chen, A.J. Martin, B. Choy, P. Fernández-Peñas, R.A. Dalziell, C.A. McKenzie, R.A. Scolyer, H.M. Dhillon, J.L. Vardy, A. Kricker, G. St George, N. Chinniah, G. M. Halliday, D.L. Damian, A phase 3 randomized trial of nicotinamide for skin-Cancer chemoprevention, N. Engl. J. Med. 373 (2015) 1618–1626, https://doi.org/10.1056/NEJMoa1506197.
[59] N.C. Allen, A.J. Martin, V.A. Snaidr, R. Eggins, A.H. Chong, P. Fernandéz-Peñas, D. Gin, S. Sidhu, V.L. Paddon, L.A. Banney, A. Lim, E. Upjohn, H. Schaider, A. D. Ganhewa, J. Nguyen, C.A. McKenzie, S. Prakash, C. McLean, A. Lochhead, J. Ibbetson, A. Dettrick, A. Landgren, K.J. Allnutt, C. Allison, R.B. Davenport, B. P. Mumford, B. Wong, B. Stagg, A. Tedman, H. Gribbin, H.A. Edwards, N. De Rosa, T. Stewart, B.J. Doolan, Y. Kok, K. Simpson, Z.M. Low, T. Kovitwanichkanont, R. A. Scolyer, H.M. Dhillon, J.L. Vardy, S.J. Chadban, D.G. Bowen, A.C. Chen, D. L. Damian, Nicotinamide for skin-Cancer chemoprevention in transplant recipients, N. Engl. J. Med. 388 (2023) 804–812, https://doi.org/10.1056/NEJMoa2203086.